



Universitat de Girona

A MULTIAPPROACH STUDY OF SOIL ATTRIBUTES UNDER LAND USE AND COVER CHANGE AT THE CAP DE CREUS PENÍNSULA, NE SPAIN

Mohamed EMRAN KHALED ABD EL AZIZ

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Doctoral Thesis

**A multiapproach study of soil attributes under land use and cover
change at the Cap de Creus Peninsula, NE Spain**



Mohamed Emran Khaled Abd El Aziz

2012

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2012

Doctoral Programme in Experimental Sciences and Sustainability

Directed by

Dra. Maria A. Gispert

Dr. Giovanni Pardini

Memory presented for obtaining the title of Doctor for the University of Girona

Dra. Maria A. Gispert, full professor of Soil Science, Department of Chemical Engineering, Agriculture, and Food Technology, University of Girona, Spain, and

Dr. Giovanni Pardini, full professor of Soil Science, Department of Chemical Engineering, Agriculture, and Food Technology, University of Girona, Spain

Certify that:

This work, entitled “**A multiapproach study of soil attributes under land use and cover change at the Cap de Creus Peninsula, NE Spain**”, presented by **Mohamed Emran Khaled Abd El Aziz** for obtaining the title of Doctor, has been carried out under our direction, and meets the requirements to qualify for the International Mention.

Dra. Maria A. Gispert

Dr. Giovanni Pardini

Girona, October 2012

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Summary

Agricultural release progressively occurred in the last century has created, especially in areas with Mediterranean type of climate, a very disordered territory often devastated by forest fires and soil mismanagement. Abandoned areas in NE Spain have increased soil vulnerability to degradation and erosion, especially when wildfire occurs throughout the dense and disorganized scrubland cover. The fire causes a regressive dynamics in the vegetation and in soil ecosystems affecting organic matter content and structural stability, thus reducing soil resistance to erosion processes and in many cases the loss of biodiversity at the soil and plant scale may be of great concern. By contrast, areas less affected by fire, may have evolved spontaneously creating favorable conditions for the regeneration of a stable organic horizon. The studied soils, in addition, are located within an area described as a striking example of the vulnerability of the soil system after both partial and total abandonment. These pictures have arisen questions on the need of appropriate management of abandoned land and the recover of the landscape heterogeneity in order to contrast threats of desertification. Therefore, the research work was focused on the study of the most relevant physical, chemical, biological, and biochemical soil characteristics, as well as erosion survey on soils under different land use and abandonment, to establish evidence of soil quality through the study of soil carbon dynamic and interactions with other biophysical and chemical soil properties.

Soils under study described as typical Mediterranean environments, undergone changes in soil use and abandonment, and in addition were located within an area subjected to periodical changing of climatic conditions from hot summer to cold winter. Selected soils included agricultural fields (cultivated vines V and olive groves O) with low agricultural management, forests (stands of cork S and pine PI trees), abandoned scrubs (*Cistus* MC and *Erica* MB scrub) and pasture (PR). All the studied soils were chosen in a variation context of soil ecosystems such as: the agricultural fields differ in their agricultural history, both forest and scrub areas differ in the frequency of wildfire occurrence and plant colonization, and pasture is periodically receiving grazing activities in winter. It is therefore paramount to monitor these soils in order to establish their evolution under land use/cover change conditions. The investigated soils have a

common parent material, Paleozoic slates, but differ substantially in soil properties according to the history of their use and/or disuse. For their geomorphological location, the shallowness of the soil profiles is an additional factor contributing to increase the risk of degradation. Previous studies demonstrated that erosion processes may assume a considerable importance even with low erosion rates, with respect to other Mediterranean areas. Moreover, the minor amount of clay in these soils enhances the function of organic carbon in soil structure stability and nutrient cycling. At this regard dynamics of organic carbon release deserves also a special attention.

The seven soil environments investigated gave important indications of their current state according to the impact of rainfall when processing soil erosion processes. Soils with low agricultural management and those affected by repeated wildfire showed lower amount of organic carbon and higher restriction of plant species which in turn resulted in lower glomalin content, weak aggregate stability, low enzymes activity, and consequently higher carbon loss. Soils under cultivated vines resulted the most susceptible to erosion all along the period of observations and may undergo physical and chemical depletion with the current type of management. The soil susceptibility to erosion processes decreased drastically with improving soil quality conditions. Generally, the order of improvement in soil conditions was $V \rightarrow O \rightarrow PI \rightarrow S \rightarrow MC \rightarrow MB \rightarrow PR$, as to indicate the positive impact of changes in soil use and abandonment. However, the depletion of soil nutrients such as soil carbon and nitrogen by erosion was sometimes accentuated in soils with higher organic matter content like PR, without serious consequences as in V soils, leading to degradation by decreasing soil structural stability, altering cycles of water, carbon, nitrogen and other soil elements, and causing adverse impact on biomass productivity, biodiversity and the environmental quality. The losses of soil nutrients by runoff were proportionally higher in soils under forests than in soils under pasture and scrubs. The lowest losses by either runoff or erosion were found in soils under scrubs and vines though in the latter were probably more important due to the lowest content in carbon and nitrogen.

Factor analysis was carried out by using each data set obtained during the two observed periods separately, in order to emphasize the significant correlations among the studied soil parameters for evaluating soil quality and erosion processes under the natural environmental and climatic changes found between the two periods. Data of

2008 represented drier conditions of soils and data of 2009 represented the rainy or moistened conditions. The results obtained by the first three factors for each yearly data set indicated that under driest soil conditions in 2008 the soil surface was less effective in preserving stocks of organic resources when higher rainfall amount impacted the soil causing higher susceptibility to erosion processes with consequent nutrient depletion. The highest rainfall amounts recorded during 2009 showed higher losses of eroded soil and nutrients and also affected soil surface compaction to surface hydraulic processes. The overall estimation during the two observed periods indicated clear differences between soils under V, O, and PI environments, suggesting on the one hand that current cultivation practices are inappropriate and that reforestation with pines (PI) may delay the achievement of better soil conditions. On the other hand the natural succession of vegetation showed by S, MC, MB, PR environments, though with temporal perturbations like in S or MC environments suggests the achievement of better soil properties.

Soil biological activity and its interactions with soil structure along the sequence of soil use and abandonment was investigated to establish the relationships with the rainfall events and soil properties. Measurements of CO₂ emission allowed to grouping soil environments according to their susceptibility of carbon loss as per cent of organic carbon stocks, resulting the soils under pasture (PR) the less affected, hence considered suitable as carbon sink. Also the soils under *Erica* scrub (MB) (fire preserved natural succession of vegetation after abandonment) showed a high carbon storage capacity.

One of the tight relationships between biota communities and soil system is through arbuscular mycorrhizal fungi (AMF) which play an important role in soil stability and organic carbon accumulation by the production of a microbial glycoprotein named glomalin. Glomalin production differed significantly among the studied soils according to their evolution after the agricultural release and post abandonment diversity in soil physicochemical characteristics and plant associations. Glomalin concentration increased generally from cultivated soils under vines (V) and olive groves (O), to forest soils under stands of pines (PI) and cork trees (S), soils under scrubs of *Cistus monspeliensis* association (MC) and under *Erica arborea* association (MB), and soils under pasture (PR). Selected soil physical properties like bulk density (BD), mechanic impedance (MI), shear strength (SS), soil moisture (SM), water holding

capacity (WHC), together with soil chemical characteristics such as pH, electrical conductivity (EC), organic carbon (SOC), and total nitrogen (TN) showed significant impacts on microbial activity and carbon storage capacity, consequently affecting structural stability of aggregates (WSA). Statistical analysis carried out by using the k-means clustering, enabled three groups of soil environments to differ according to their low carbon accumulation and glomalin content. Soils under V, O, and PI environments (group III) were suggested to be poorly structured and prone to higher carbon loss with respect to organic carbon content against other soil environments. Soils under S, MC, MB, and PR environments were assigned to group I and II, depicting better properties and response to seasonal changes.

Glomalin and organic carbon resulted significantly more active in favoring structural stability in 2.00-5.60 mm than in 0.25-2.00 mm soil aggregates, probably indicating that a better structured soil crumb is achieved in larger aggregates with increasing stable organic compounds and glomalin content. However, organic carbon and total glomalin showed close relationships with WSA in the two investigated classes of aggregates indicating that increasing amounts of organic carbon and glomalin positively influence soil structural stability.

The susceptibility of changes in soil conditions was highly reflected in the capacity of soil response to biological and biochemical indicators such as enzymatic activities, glomalin, and CO₂ emission. Soils were catalogued for their potential carbon storage capacity under the effects of soil and environmental climatic conditions. The enzymes β -glucosidase, protease, and phosphatase showed relevant activity along the seasons in better structured soils with high organic carbon and glomalin content corroborating the same soil order of improvement in soil conditions. The principal component analysis showed significant positive correlations among the soil properties related to soil quality and enzymatic activity against carbon loss and soil compaction from soils under vines to pasture.

Similar patterns were observed in studying the structural composition of soil organic matter (SOM) carried out by pyrolysis gas chromatography. The data obtained gave relevant indications on the humification and mineralization of organic compounds carried out by soil microbial community. These processes varied significantly among

the studied soils according to the soil conditions after agricultural release and subsequent land evolution. The two indices of mineralization (FF/PY and PY/Y) informed on the decomposability of labile and stable organic substrates and resulted coherently related to the index of energy reserve (AL/AR) which was based on the ratio of aliphatic to aromatic organic compounds, confirming a rather similar soil order regarding the studied soil environments like: V→O→S→PI→PR→MC→MB soils. Moreover, the similarity index (S_{ij}), carried out by comparing each pyrolytic fragment of SOM combustion to others, showed high similarity in SOM composition for each soil group similar in their structural stability, organic content, and microbial activity. With the similarity index is possible to separate environments showing closer ($S_{ij} > 0.90$), intermediate ($S_{ij} 0.80-0.90$) or unlike ($S_{ij} < 0.80$) soil dynamics. Soils under vines (V) showed a high similarity index (> 0.90) with soils under pines (PI). The lowest similarity (< 0.80) was showed between soils under V and PR and MB environments. Soils under olive groves (O) showed a high similarity with soils under S, PI, PR, MC, and MB environments. An intermediate similarity ($0.80-0.90$) was found between soils under V environment and other environments such as O, S, and MC. Soils under PI environment showed also intermediate similarity with soils under PR, MC, and MB. Results depicted by S_{ij} index may be interpreted as the response of soil evolution. Therefore, the biochemical interactions of soil microbial activity with soil structure should be considered the main indicators of healthy soil conditions and soil quality able to differentiate among the soils under different environmental and soil conditions.

When soil erosion survey parameters were added to the previous data sets of soil parameters under study, the descriptive soil characteristics used for soil quality evaluation, i.e. clay, shear stress, soil nutrients, glomalin content, WSA, SOC, TN, bacterial and fungal populations, soil enzymes, mineralization index of labile and stable organic substrates, and energy reserve index, resulted positively correlated among them and against soil erosion and other relevant soil parameters such as sand content, soil compaction, and infiltration rates. All these parameters showed loadings higher than 0.70 and explained 49.96% of the total variance, while soil carbon loss and soil parameters like soil moisture and temperature explained only 9.18% of the total variance. This statistical evidence may indicate that despite the impacts of land use, land use change, and abandonment together with environmental climatic conditions upon the soils under study, a general enrichment of organic compounds leads to increasing the

preservation of soil organic carbon pools which in turn may minimize soil erosion processes and carbon loss. However, it must be taken into account that these soils are worth to be maintained under continuous monitoring for their fragility of profile shallowness and periodical wildfire risk.

The work enabled to establish the current dynamics of the soils under study, underlining those environments with insufficient management, i.e. V and O soils, or with higher susceptibility to fire damages, i.e. PI and MC soils, as more easily prone to be degraded. Moreover the work is useful for encouraging administration towards the management of abandoned land, aimed at reducing environmental quality decline and recovering the landscape heterogeneity. Further research is needed to establish these relationships where more complex dynamics in both soil organic carbon and structure occur.

Resumen

El abandono agrícola se produjo progresivamente en el siglo pasado creando, especialmente en zonas con clima de tipo mediterráneo, un territorio muy desordenado a menudo devastado por los incendios forestales y la mala gestión del suelo. Áreas abandonadas en el NE de España, han aumentado la vulnerabilidad del suelo a la degradación y la erosión, especialmente cuando los incendios se producen a lo largo de la cubierta de matorral denso y desorganizado. El fuego provoca una dinámica regresiva en la vegetación y en los ecosistemas del suelo que afectan el contenido de materia orgánica y estabilidad estructural, lo que reduce la resistencia del suelo a los procesos de erosión y en muchos casos la pérdida de biodiversidad en el suelo y las plantas y puede ser motivo de gran preocupación. Por el contrario, las zonas menos afectadas por el fuego, pueden haber evolucionado espontáneamente, creando condiciones favorables para la regeneración de un horizonte orgánico estable. Los suelos estudiados, además, se encuentran dentro de un área descrita como ejemplo de vulnerabilidad del sistema edáfico y botánico después del abandono parcial y/o total. Estas imágenes han generado preguntas sobre la necesidad de una gestión apropiada de las tierras abandonadas y la recuperación de la heterogeneidad del paisaje con el fin de minimizar las amenazas de degradación y desertificación. Por lo tanto, el trabajo de investigación se centra en el estudio de las características físicas, químicas, biológicas, y bioquímicas más relevantes del suelo, así como en el estudio de la erosión en ambientes bajo diferentes usos del suelo y en situaciones de abandono, para establecer algunos parámetros de calidad del suelo, estudiar la dinámica del carbono y la interacciones entre los ecosistemas del suelo en los ambientes de seleccionados.

Los suelos bajo estudio se describen como típicos del ambiente mediterráneo, incluidos dentro de un área sometida a cambios periódicos en las condiciones climáticas con veranos muy cálidos e inviernos fríos. Los suelos seleccionados incluyen campos de cultivo (viñedo, V y olivar, O) con manejo agrícola insuficiente, bosques (alcornocales, S y pineda, PI), matorrales abandonados (matorral de *Cistus*, MC y matorral de *Erica*, MB) y pastos, PR. Todos los suelos estudiados fueron elegidos en un contexto de sucesión de uso o cambio de uso de los tales como: los campos agrícolas difieren en su historia agrícola, tanto las zonas forestales y los matorrales difieren en la frecuencia de

incendios y la colonización espontánea de la vegetación después de abandono, y los pastos están sometidos periódicamente a actividades de pastoreo en invierno. La diversidad de uso y cambio de uso es fundamental para establecer su evolución y las condiciones edáficas. Los suelos investigados tienen un material de origen común, las pizarras paleozoicas, pero difieren sustancialmente en las propiedades del suelo de acuerdo con la historia de su uso y/o cambio de uso. Por su ubicación geomorfológica, la poca profundidad de los perfiles de los suelos se considera un factor adicional que contribuye a aumentar el riesgo de degradación. Estudios anteriores demostraron que los procesos de erosión pueden asumir una importancia considerable en estos suelos, incluso con bajas tasas de erosión, con respecto a otras zonas del Mediterráneo. Además, la baja cantidad de arcilla en estos suelos enfatiza la función del carbono orgánico en la estabilidad de la estructura del suelo y el ciclo de nutrientes. En este sentido la dinámica del carbono orgánico en estos suelos merece también una atención especial.

Los siete ambientes de suelos investigados dieron importantes indicaciones de su estado actual de acuerdo con el impacto de la lluvia en el tratamiento de los procesos de erosión del suelo. Los suelos con bajo manejo agrícola y aquellos afectados por los incendios forestales frecuentes mostraron una menor cantidad de carbono orgánico y una mayor restricción de las especies vegetales. Consecuentemente eso dio lugar a un menor contenido de glomalina, una más baja estabilidad de los agregados, unas menores actividades enzimáticas, todo eso causando una mayor pérdida de carbono orgánico. Los suelos de viñedo resultaron los más susceptibles a la erosión a lo largo del período de observación y pueden sufrir un agotamiento físico y químico con el tipo de gestión actual. La susceptibilidad a los procesos de erosión del suelo disminuyó drásticamente con la mejora de las condiciones de calidad del suelo. En general, el orden de mejora en las condiciones del suelo fue $V \rightarrow O \rightarrow PI \rightarrow S \rightarrow MC \rightarrow MB \rightarrow PR$, indicando un cierto impacto positivo de los cambios en el uso del suelo y el abandono. Sin embargo, la disminución de nutrientes del suelo, tales como el carbono y nitrógeno del suelo por la erosión se acentuó a veces en suelos con alto contenido de materia orgánica, como PR, sin consecuencias graves si comparado con los suelos V, en que se puede desencadenar procesos de degradación por la disminución de la estabilidad estructural del suelo, la alteración de los ciclos del agua, del carbono, nitrógeno y otros elementos nutritivos del suelo, causando efectos adversos sobre la productividad de la biomasa, la biodiversidad

y la calidad del medio ambiente. Las pérdidas de nutrientes del suelo por escorrentía fueron proporcionalmente mayores en los suelos bajo bosque que en los suelos de pastos y matorrales. Las menores pérdidas, ya sea por escorrentía o erosión fueron encontradas en los suelos bajo matorrales y viñedos, aunque en estos últimos eran probablemente más importantes, debido al menor contenido en carbono y nitrógeno.

El análisis factorial se llevó a cabo mediante el uso de cada conjunto de datos obtenidos durante los dos períodos observados por separado, con el fin de destacar las correlaciones significativas entre los parámetros del suelo estudiados, para evaluar su calidad y los procesos de erosión a lo largo de los cambios ambientales y climáticos en los dos períodos observados. Los datos de 2008 representaron condiciones más secas para los suelos mientras que los datos de 2009 representaron una época más lluviosa y condiciones del suelo más húmedas. Los resultados obtenidos por los tres primeros factores para cada conjunto de datos anuales indican que bajo condiciones más secas del suelo en el año 2008, la superficie del suelo fue menos eficaz en la preservación de los compuestos orgánicos, cuando, fuertes lluvias impactaron en el suelo provocando una mayor susceptibilidad a los procesos de erosión con la consiguiente remoción de nutrientes. Sin embargo, las mayores cantidades de precipitación registradas durante el año 2009 mostraron mayores pérdidas de sedimentos erosionados y nutrientes y la compactación del suelo fue también afectada juntamente a los procesos hidráulicos. La estimación global durante los dos períodos observados indica claras diferencias entre los suelos bajo V, S, y PI, con respecto a otros, lo que sugiere por un lado que las actuales prácticas de cultivo son inadecuadas y que la reforestación con pinos (PI) puede retrasar el logro de mejores condiciones del suelo. Por otro lado la sucesión natural de la vegetación en suelos bajo ambientes S, MC, MB, y PR, aunque con perturbaciones temporales (incendios) como en S o MC sugiere el logro de mejores propiedades del suelo.

La actividad biológica del suelo y su interacción con la estructura del suelo a lo largo de la secuencia de uso del suelo y el abandono se ha investigado para establecer las relaciones con los eventos de lluvia y las propiedades del suelo. Las mediciones de las emisiones de CO₂ permitieron agrupar los ambientes de suelo en función de su susceptibilidad a la pérdida de carbono, como porcentaje de las reservas de carbono orgánico, lo que indicó los suelos bajo pastoreo (PR) los más adecuados como sumidero

de carbono. También los suelos bajo matorral de *Erica* (MB) (preservado del fuego durante la sucesión natural de la vegetación después del abandono) mostraron una alta capacidad de almacenamiento de carbono.

Una de las estrechas relaciones entre la biota del suelo y el suelo se conoce a través de los hongos micorrízicos arbusculares (AMF) que desempeñan un papel importante en la estabilidad del suelo y la acumulación de carbono orgánico por la producción de una glicoproteína llamada glomalina. La producción de glomalina difirió significativamente entre los suelos estudiados en función de su evolución después del abandono agrícola y la sucesiva evolución de las características físico-químicas del suelo y de las especies vegetales. El aumento de la concentración de glomalina en general fue des de los viñedo (V) y olivares (O), a los suelos forestales de pineda (PI) y alcornoques (S), los suelos bajo matorrales de *Cistus monspeliensis* (MC) y de *Erica arborea* (MB), hasta los suelos bajo pastos (PR). Algunas propiedades físicas del suelo como densidad aparente (BD), impedancia mecánica (MI), resistencia al corte (SS), humedad del suelo (SM), capacidad de retención de agua (WHC), junto con las características químicas del suelo tales como pH, conductividad eléctrica (EC) , carbono orgánico (SOC), y nitrógeno total (TN) demostraron un impacto significativo sobre la actividad microbiana y la capacidad de almacenamiento de carbono, influenciando la estabilidad estructural de los agregados (WSA) en este orden. El análisis estadístico llevado a cabo mediante el uso de la k-means clustering, permitió aislar tres grupos de ambientes edáficos para en función de su menor capacidad de acumular carbono y glomalina. Los suelos bajo V, O, y PI fueron incluidos en el grupo III, sugiriendo una peor estructura y una más marcada tendencia a la pérdida de carbono con respecto a otros ambientes. Los suelos bajo los ambientes S, MC, MB, y PR fueron asignados al grupo I y II, que representan suelos con mejores propiedades y mejor respuesta a los cambios estacionales.

La glomalina y el carbono orgánico resultaron significativamente más activos en favorecer la estabilidad estructural en la clase de agregados 2.00-5.60 mm respecto a la clase 0.25-2.00 mm, probablemente indicando que con el aumento de los compuestos orgánicos estables y el contenido de glomalina se puede obtener un estado de agregación más fuerte en agregados de mayor tamaño. De todas formas, el carbono orgánico y glomalina total generalmente mostraron una estrecha relación con la

estabilidad de agregados (WSA) en las dos clases de agregados investigadas, el que indican que cada vez más cantidades de carbono orgánico y glomalina pueden influir positivamente en la estabilidad estructural del suelo.

La susceptibilidad de los cambios en las condiciones del suelo se refleja altamente en la capacidad de respuesta del suelo a los indicadores biológicos y bioquímicos, tales como las actividades enzimáticas, la glomalina y las emisiones de CO₂. Los suelos fueron catalogados por su capacidad potencial de almacenamiento del carbono según las condiciones del suelo así como las variaciones climáticas estacionales. Los enzimas β -glucosidasa, proteasa y fosfatasa mostraron una actividad relevante y con un claro efecto estacional en los suelos mejor estructurados y con alto contenido de carbono orgánico y glomalina, corroborando el mismo orden de mejora en las condiciones del suelo. El análisis de componentes principales mostró correlaciones positivas y significativas entre las propiedades del suelo relacionadas con la calidad del suelo y la actividad enzimática frente a la pérdida de carbono y la compactación del suelo en el orden suelo bajo viñedo hasta suelos bajo pasto.

Patrones similares se observaron en el estudio de la composición estructural de la materia orgánica del suelo (SOM) llevada a cabo por pirolisis/cromatografía de gases. Los datos obtenidos dieron indicaciones relevantes sobre los procesos de humificación y mineralización de los compuestos orgánicos llevados a cabo por la comunidad microbiana del suelo. Estos procesos variaron significativamente entre los suelos estudiados de acuerdo a las condiciones del suelo después del abandono agrícola y la posterior evolución. Los dos índices de mineralización (FF/PY y PY/Y) informaron sobre la descomposición de sustratos orgánicos fácilmente metabolizables o estables y resultaron coherentemente relacionados con el índice de reserva energética (AL/AR), que se basa en la ratio hidrocarburos alifáticos/aromáticos de los compuestos orgánico del suelo, lo que confirma la series de suelos en función de sus mejores propiedades: V→S→PI→S→MC→MB→PR. Por otra parte, el índice de similitud (S_{ij}), llevado a cabo mediante la comparación de cada fragmento pirolítico procedente de la combustión de la materia orgánica de un suelo con otros de otros suelos, mostraron una alta similitud en la composición de materia orgánica en cada grupo de suelos con propiedades edáficas similares como estabilidad estructural, contenido de materia orgánica, y actividad microbiana. Con el índice de similitud es posible separar

ambientes que muestran más similitud ($S_{ij} > 0.90$), similitud intermedia ($S_{ij} 0.80-0.90$) o poca similitud ($S_{ij} < 0.80$) en la dinámica global del suelo. Los suelos de viñedo (V) mostraron un índice de similitud alto (> 0.90) con los suelos bajo pineda (PI). La menor similitud (< 0.80) se mostró entre los suelos bajo V contra los suelos MB y PR. Los suelos bajo olivares (O) mostraron una gran similitud con los suelos bajo S, PI, PR, MC, MB. Una similitud intermedia ($0.80-0.90$) se encontró entre los suelos bajo V y otros ambientes, como O, S, y MC. Los suelos bajo el ambiente PI también mostró similitud intermedia con suelos bajo PR, MC y MB. Los resultados representados por el índice S_{ij} pueden interpretarse como la respuesta de la evolución del suelo. Por lo tanto, las interacciones bioquímicas de la actividad microbiana del suelo con la estructura del suelo deben ser considerados como los principales indicadores de las condiciones de salud del suelo y la calidad del mismo capaces de diferenciar entre los estados evolutivos del manto edáfico.

Cuando los parámetros relativos a los procesos de erosión se han añadido al conjunto de datos anteriores, la arcilla, el esfuerzo cortante, los nutrientes del suelo, el contenido de glomalina, WSA, SOC, TN, las poblaciones de bacterias y hongos, los enzimas del suelo, los índice de mineralización de los sustratos orgánicos lábiles y estables, y el índice de reserva de energía, mostraron una correlación positiva entre ellos y contra la erosión del suelo y otros parámetros relacionados tales como el contenido de arena, la compactación del suelo, y las tasas de infiltración. Todos estos parámetros mostraron cargas superiores a 0.70 y explicaron el 49.96% de la varianza total en las variables analizadas, mientras que la pérdida de carbono del suelo y los parámetros del suelo como la humedad y la temperatura explicaron sólo el 9.18% de la varianza total. Esta evidencia estadística puede indicar que a pesar de los impactos del uso de la tierra, el cambio de uso de la tierra, y el abandono, junto con las condiciones climáticas y ciertas perturbaciones, un enriquecimiento general de compuestos orgánicos conduce a incrementar la preservación del carbono orgánico del suelo, que a su vez puede minimizar los procesos de erosión del suelo y la pérdida de carbono. Sin embargo, debe tenerse en cuenta que estos suelos tienen que ser sometidos a monitoreo continuo por su fragilidad y el riesgo de incendios periódicos.

El trabajo permitió establecer la dinámica actual de los suelos bajo estudio, destacando los ambientes con una gestión insuficiente, es decir, suelos V y O, o con una mayor

susceptibilidad a los daños de incendio, es decir, PI y MC, que pueden ser más vulnerables a procesos de degradación. Además, el trabajo es útil para alentar a la administración a una mejor gestión de las tierras abandonadas, con el objetivo de reducir el declino de la calidad edáfica y ambiental y la recuperación de la heterogeneidad del paisaje. Sin embargo, se necesitan más investigaciones para establecer las complejas relaciones entre la dinámica del carbono y la estructura del suelo.

CHAPTER I. INTRODUCTION

1.1. Land use, land use change and land abandonment

Land use, Land use change and land abandonment have been relevant topics of study for their impact on soil quality. Especially in the last decades the soil system, a no short term renewable resource, has been subjected to many changes mainly affecting its most important component: the organic matter. Soil organic matter builds up at different land use (Genxu et al., 2006; Osuji et al., 2010) and it is considered as a driving force to contrast the fragility of soil from degradation processes because of its role in the formation of soil aggregates and structure. Moreover, soil organic carbon is the framework of organic materials and comprises 58% of soil organic matter (Lal, 2004). Fragility of soil means the excessive sensitivity to degradation and erosion processes. On the one hand, well managed lands may succeed soil stable steady state conditions and be able to resist changes. On the other hand, fragile lands may degrade to a new steady state under stress, and the altered state may be unable to support plant growth and to follow the environmental regulatory functions (Eswaran et al., 2001). Food, water, and air quality have received much attention because of their direct relationship to human health. By contrast soils have been traditionally considered as the 1.5 m layer of the earth's surface that is taken for granted when considering its importance for human kind and environment.

Land degradation is reported from scientists as one of the problem triggering worse life condition in different regions around the world. During the last centuries soil degradation has been accentuated in the Mediterranean region mainly due to farm land abandonment. Land degradation may occur in many different contexts: in cool and warm dry environments; in very arid, semi-arid and humid climates; in traditional and technological agriculture and different type of soils; in different societies: ancient and modern; rich and poor; capitalist and socialist and so on. For these reasons land degradation is a major ecological and environmental issue (Dunjó, 2004). Soil erosion is considered the main land degradation process that may lead to the progressive inability of the vegetation and soils to regenerate, exceeding the resilience status of these ecosystems and causing desertification (Dunjó et al., 2004; Wei et al., 2007).

Soil is a very heterogeneous and complex system, and hardly succeeds in reaching its climax completion, being easily subjected to degradation depending on its

use and management. Land use change and more evidently land abandonment, represent a threat to soil quality, especially in the Mediterranean region with its changing climatic conditions (Dunjó et al., 2003). Soil loss is then considered the main soil indicator for erosion potential and degradation hazard that may really affect the quality of soil. Erosion is the visible part of degradation, where the forces of gravity, water flow or wind transport soil particles and minerals downhill (Barrow, 1994). Soil environments of the Mediterranean region are characterized by:

- Fragile natural ecosystems (irregular terrain with steep slopes).
- Long-term human exploitation (extensive deforestation and intensive cultivation of sloping lands).
- Land misuse, mismanagement, and abandonment.
- Adverse climatic conditions (irregular rainfall, wind, wildfire).

Matching these characters for long periods, a serious decline in environmental resources, resulting in land degradation, has been experienced (Kosmas et al., 2000).

1.1.1. Land abandonment in the Mediterranean region

Historical evidence shows that in the Mediterranean region serious and extensive land degradation processes have occurred over several centuries following farmland abandonment. The intensity of degradation has generally increased in areas where hillside clayey soils occurred with the formation of badlands producing severe gully erosion (Poesen et al., 2003). Many examples of this post abandonment land evolution may be found in Greece (Koulouri and Giourga, 2007), Italy (Alexander, 1982) and Spain (Wise et al., 1982), even though in Spain and France we can describe other type of land degradation after abandonment mainly due to wildfire occurrence and the presence of shallow sandy soils (Brochot, 1993; Regüés et al., 2000). The process of land abandonment has been mainly recorded in marginal mountainous, semi-mountainous, or difficult to access areas, where traditional agriculture was exploited until recent years with low inputs and high human labor intensity (Loumou and Giourga, 2003). Therefore, soil type and management have been defining characteristics after abandonment inferring strong changes to soil properties. In some clear instances, soils with the same textural class have shown highly contrasting properties because of differences in soil management before abandonment.

After farmland abandonment, the evolution of soil properties depends on:

- The type of soil
- The condition of the soil at agricultural release
- The sequence of spontaneous colonization of vegetation
- The vegetation succession in the presence of natural or induced perturbations

Often, the shallow nature of soils and periodical perturbations (wildfire, heavy downpours, etc.) in the absence of proper land management steeply increase soil erosion and degradation (Pardini and Gispert, 2006). Therefore, post abandonment management to survey soil and ecosystem quality is paramount to guarantee environmental quality.

Nevertheless, land abandonment may result in soil properties improvement when soil organo-mineral complexes are preserved from erosion and degradation in a natural vegetation succession after agricultural release (Dunjó, 2004). Accumulation of organic carbon in soil as carbon pool is a balance between organic matter inputs, primarily from above ground biomass production, and decomposition by microbial mineralization of plant biomass. Particularly, the abandonment of cultivated land may be an effective way for restoring soil organic carbon pools (Zhang, 2010; Zhang et al., 2010). After longer period of abandonment, organic materials accumulating on soil surface can be used as source materials to predict organic compounds incorporation to soil, also according to C/N ratio of decaying organic debris. Moreover, the soil is considered the main terrestrial reservoir of nutrients, such as nitrogen, phosphorus, and organic carbon, being soil carbon the major component present in sediments removed by soil erosion due to its accumulation at the soil surface humic horizon. However, the translocation and burial of eroded soil may reduce decomposition of this soil organic carbon, leading to long-term carbon storage (Batjes, 1999; Quinton et al., 2010). For example, buried soils after erosion processes may constitute important soil nutrient and carbon pools, thereby increasing primary productivity and carbon uptake, and in turn reducing potential erosion. But this process is likely to occur in most plane areas, whilst sloping areas should also be taken into account by monitoring their potential for erosion.

1.1.2. Land degradation

The different forms of land degradation are listed in Figure 1. Land degradation is mainly caused by the progressive changes in physical, chemical, and biological soil processes due to soil mismanagement, neglecting soil system equilibrium.

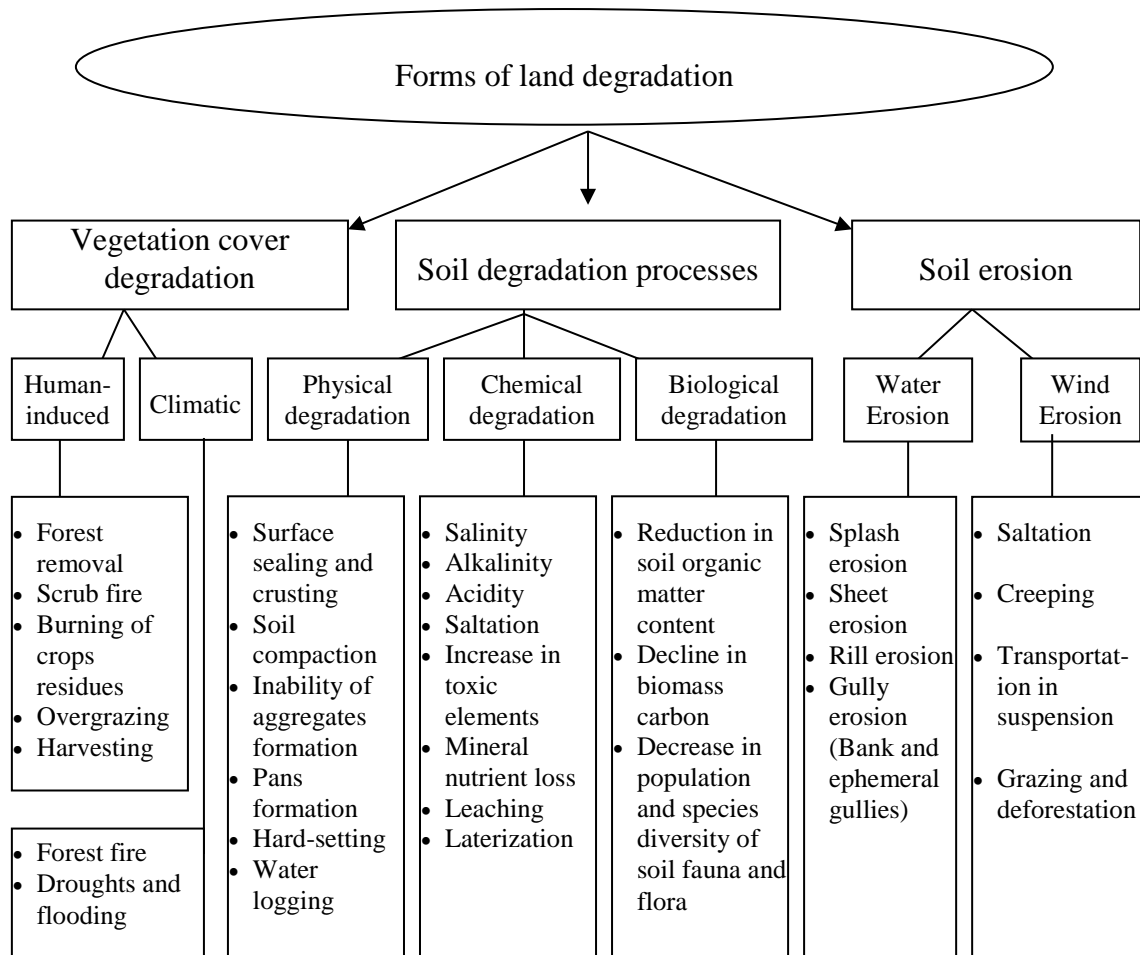


Figure 1. Main forms of land degradation adapted from Lal et al. (1989) and Dunj6 (2004).

1.1.2.1. Vegetation cover degradation

The vegetation cover degradation is a phenomenon occurring mutually with soil degradation caused by the restriction in plant species and may have climatic or anthropic origin or both (Poesen, 1995). Some of human induced causes leading to vegetation cover degradation processes are forest removal, scrub fires, burning of crop residues, overgrazing and harvesting. Natural extremely dry climatic conditions may also affect plants. Droughts and drastic seasonal climate changes may also cause strong

water stress state and a substantial reduction in the vegetation cover. Soil conditions and soil properties such as depth and organic matter content have a direct relationship with the vegetation cover (Kirkby and Kosmas, 1999). Koulouri and Giourga (2007) and Lesschen et al. (2008) reported that after abandonment vegetation is subjected to changes and the spontaneous colonization of plants in normal conditions will increase plant density, dendritic distribution and have beneficial effects on soil properties.

1.1.2.1.1. Human induced

Forest fire is known to be human induced at 90% of cases. Its occurrence constitutes serious environmental problems not only due to the devastation of vegetation but also because degradation may extend to soil with strong changes in its properties. Wildfire can extremely modify the biotic and abiotic characteristics of soil, i.e. soil structure, chemical and physicochemical properties, carbon content and nutrient levels. The degree of the alteration produced depends on the frequency and severity of fire, all these modifications being particularly important at the soil surface horizons (González-Pérez et al., 2004).

1.1.2.1.2. Climatic

Plant species and distribution may be affected by climate change altering rainfall and temperature regimes in ecosystems over long term periods causing imbalance in soil water regimes, soil surface structure, soil habitats, and microbial activities, thus reducing plant productivity and soil quality because of the changes in the phenology of the microbial and faunal soil species (Cynthia and Daniel, 2000).

1.1.2.2. Soil degradation processes

Soil degradation processes may also be a combination of anthropogenic and natural factors, altering the natural soil functions and creating disturbance along the soil system life.

1.1.2.2.1. Physical degradation

Soil physical degradation leads to changes in soil mechanical and hydrological properties which have a negative effect on the environmental quality (Lal et al., 1989). Other changes occur in soil porosity, bulk density, structural stability, and permeability, and include processes leading to surface sealing, crusting, and compaction (Poesen and Nearing, 1993). Indeed the change in soil structure is the principal effect of physical degradation which has direct effects on soil biological properties, mainly soil organic matter content and microbial activity. When soil structure is disrupted physical degradation is evidenced by surface sealing, compaction, and crusting following natural or/and human induced effects. They are generally described as natural features in arid and semi-arid soils. Compaction and crusting tend to be greater in soils with low organic matter and expanding clay type. Their effects on the acceleration of soil erosion by reducing soil infiltration rates lead to increasing water runoff and decrease amount of available soil water. Increasing the potential for surface water erosion implies depleting the soil of nutrients and organic materials.

Soil hard-setting may produce physical problems such as high soil surface strength, very low porosity, and extreme poor infiltration rates (Ley et al., 1989). Moreover fine laminations of soil material have been recorded in the upper few centimeters further affecting soil properties (Lal et al., 1989). These formations may decrease the content of organic matter and inhibit the role of soil microbes delaying biodegradation of organic materials. Generally, only resistant scrubs (*Cistus monspeliensis*) are able to colonize these environments with very scarce nutrients content in soil. Mullins et al. (1987) and Chan and Sivapragasam (1996) hypothesized that the increases in strength of hard setting soils are caused by the formation of bridging cations in fine materials as silt and clay. Through this electrostatic mechanism hard layers are progressively formed.

Around the Mediterranean regions, one third of the agricultural surface is affected by land degradation (Hurni et al., 2008). In particular, sparse cultivated soils on steep slopes coupled with semi-arid Mediterranean climate characterized by irregular heavy rains and the occurrence of periodical seasonal droughts provide favorable conditions for erosion and desertification. Water is the principal cause of overland flow

erosion even though wind erosion may play relevant role in those mostly exposed areas. Generally, soil degradation by water or wind erosion represents a serious threat to both agriculture and forestry and to the Mediterranean landscape in general.

1.1.2.2.2. Chemical degradation

Soil chemical degradation is an undesirable deterioration in soil chemical properties such as composition of cation exchange complex, organic matter content, mineral nutrients, producing element imbalance, salinity, alkalinity, and acidity of soil. Changes in one or more of these properties often have direct or indirect adverse effects on the chemical fertility of soils, which may lack in regulating nutrient dynamics thus decreasing soil productivity and fertility (Derici, 2002).

The most widespread types of chemical degradation in soils are an excessive decrease or increase in pH (acid or basic reaction), an increase in soluble salt content (salinity), a decrease in organic matter content, and a loss of mineral nutrients through leaching or crop off take. Salinity and alkalinity may vary from place to place affecting soils even over short distances, depending on tillage, local soil attributes, local topography and distance from surface to the water table (Oldeman et al., 1990).

Soils are continuously undergoing natural chemical changes as a result of mineral weathering. The combination of the weathering process with other factors such as parent material, climate, biota, and topography mark the soil type evolution. The factors and processes affecting soil formation are always changing, so that in the soil system a permanent steady state is never achieved.

1.1.2.2.3. Biological degradation

Soil biological degradation is the direct result of unsuitable soil management. When the soil biota are inhibited in their function of processing organic materials to form stable structure and promote nutrient enrichment, a decline in soil organic compounds occurs causing a general soil impoverishment. At a large extent in time and space the reduction of general microbial activity in soil may affect fertility and quality. Arid and semiarid soil environments with low organic matter and fragile structure may

therefore shift to progressive degradation of their quality and productivity (Caravaca et al., 2002).

Biological degradation is generally related to some physicochemical effects such as a lower transportation capacity of mineral and organic materials within the soil profile, and drastic changes in the soil micropore system. The lacking of this kind of mechanisms delays the formation of humus complexes along the profile due to the difficulties of soil fauna to metabolize fresh organic debris. The persistence of partially decayed organic debris at soil surface implies the decreasing of two major functions: To supply nutrients to soil itself and plants upon mineralization, that is: carbon, nitrogen, phosphorus, and sulphur, among others; To form stable aggregates that would reduce erosion and compaction. Therefore, humic organic compounds are essential substances increasing soil porosity, infiltration rates, aggregate stability, water holding capacity, and nutrient cycling. Particularly, organic matter is reported by many authors as the most important soil component which regulates directly and indirectly the majority of soil functions (Gregorich et al., 1997; Piccolo and Mbagwu, 1999; Carter, 2002).

1.1.2.3. Soil erosion

Soil erosion is the physical detachment of soil particles and their subsequent mobilization downslope by over-land flow. It may be activated following the alteration of soil physical, chemical, and biological properties under the influence of environmental or anthropogenic factors, irreversibly affecting, in some cases, soil quality. Poesen and Nearing (1993) described the soil erosion as one of the main desertification inducing processes. Generally, the main cause of erosion is a reduction of organic matter at the soil surface that in turn weakens down the stability of aggregate, increasing their vulnerability to raindrops kinetic energy (Darmody and Norton, 1994). Soil erosion can be caused by either natural or accelerated erosion (Lal, 2003; Montgomery, 2007). Natural erosion is a geological process caused by natural events, such as rainfall events, wind, tide, raises of sea level, or even natural wildfire occurrence, causing deterioration in soil surface structure and consequently nutrient depletion. The other concept of soil erosion that is caused by human activities is generally known as accelerated erosion. It may be caused by human-induced events such as vegetation removal for land development, over grazing, changes in water

regimes, fire caused by humans, intensive agriculture, timber harvesting, road construction, mining, or other similar human activity.

In most Mediterranean lands, erosion rates have been influenced by human activity since early prehistoric times (Inbar, 1992). During the last centuries and especially the last decades the lands of the Mediterranean basin have been subjected to deep transformations due to economic and social changes. In particular the abandonment of agricultural lands has had a strong impact on soil erosion with huge net soil loss in $\text{Tons ha}^{-1} \text{ year}^{-1}$. In south Spain, Wise et al. (1982) and Clarke and Rendell (2006) reported erosion rates of $2000 \text{ Tons ha}^{-1} \text{ year}^{-1}$ after the agricultural release, especially due to accelerated erosion in Pliocene or Plio-Pleistocene marine clays producing extended badland areas. In south Italy, Alexander (1982) recorded erosion rates of 1 mm year^{-1} which correspond approximately to $15 \text{ Tons ha}^{-1} \text{ year}^{-1}$. Furthermore, in France huge erosion rates of $16 \times 10^5 \text{ Tons ha}^{-1} \text{ year}^{-1}$ were recorded by Brochot (1993) in the Draix catchment with very incoherent materials. However, land abandonment may have positive or negative impacts on soil protection from erosion (Koulouri, and Giourga, 2007). In the Mediterranean basin and areas with high erosion risk, land abandonment is generally followed by colonization of natural vegetation which also favors soil profile regeneration, resulting in a decrease of soil erosion processes (Grove and Rackham, 2001; Nunes et al., 2010). Diverse kinds of plant species forming scrubland are reported to be more effective in soil protection (Francis and Thornes, 1990), through the improvement of soil properties such as organic matter content, soil structure and infiltration rates. Soils with longer age of abandonment and little disturbance are characterized by the decreasing in soil erosion rates because of the changes in vegetation cover characteristics and improvement in soil properties (Kosmas et al., 2000).

The process of land abandonment is widely spread all over the Mediterranean regions and also in lands with Mediterranean type of climate. It is estimated that only in the Mediterranean countries there are 300,000 hectares of abandoned lands of which almost 80% represent old cultivated fields in sloping areas controlled by dry stone terraces. With these practices agriculture was extending also in mid-mountain areas. Nevertheless, these practices needed more attention through field work and the creation of a drainage-network in order to reduce erosion down slope. The interval between

agricultural release and re-colonization by natural vegetation is important, as it is important the soil condition at agricultural release. Many factors like slope, rainfall, plant cover, and soil properties may drive the response of soil after abandonment. The slope gradient is one of the main factors controlling erosion. Depending on the steepness level, soil erosion may increase significantly due to higher dragging capacity of water at the soil surface. Moreover, if dry stone terraces are strongly degraded, the short distances between one another may reduce their effect of cut-slope to minimize erosion, lowering the stability of soil surface aggregates to overland flow (Koulouri, and Giourga, 2007).

1.1.2.3.1. Water erosion

During rainfall events part of the water flows over the soil surface. The laminar flux of water may be more or less thick depending of the soil infiltration capacity and the slope gradient. Soil nutrients and soluble organic compounds are generally carried by water runoff. To know the sediment yield and nutrient depletion at each rainfall events, closed or open plots are generally installed in the field. With high rainfall intensities, soil infiltration capacity may be reduced and depending on the structural stability of aggregates, detachment of soil particles may occur to a large extent according to the drop size distribution and raindrop impact on soil surface (Nunes et al., 2010). Moreover when erosion rates increase, beneath horizons may be affected, and sediment yields may contain relatively high amount of particulate organic carbon further declining soil resistance. Therefore soil erosion is a major factor depleting pools of soil organic carbon (SOC). Kimble et al. (2001) reveal that a large range of SOC lost by erosion occurs in the top 25 cm, equally 19-51% for Mollisols and 15-65% for Alfisols. In other types of soils the losses are lower but may be more relevant due to the initial low SOC content. For instance in Lithic Xerorthents the SOC loss range may be around 5-35% in the first 10-15 cm and may results in a heavy impoverishment of the upper horizons.

The installation of plant cover decreases the raindrop impact severity on previous bare soils and minimizes the extent of nutrient loss and soil erosion. The importance of vegetation in erosion control is attributed to two main effects: on the one hand, the direct mechanical protection of the soil surface by the canopy and litter cover

that intercept rainfall and consequently reduce the detachment of soil particles caused by raindrop impact at the soil surface; on the other hand the indirect improvement of the soil physical and chemical properties, essentially by the incorporation of organic matter (Dunjó et al., 2004; Nunes et al., 2010).

Within the effect of raindrop impact on soil surface, splash may be the most aggressive over soil surface aggregates. The disrupting force of individual raindrop depends on the drop diameter and the rainfall intensity. The kinetic energy of rainfall is difficult to calculate, though there are models that allow this calculation. Generally, rain simulation trials enable the correct calculation of kinetic energy as the rainfall intensity, the drop diameter and the rainfall height are previously established by the operators. In natural conditions splash erosion may be calculated by weighing the splashed particles per unit of surface and relating this amount with rainfall. When the raindrops collide with soil surface, clods are successively detached into smaller aggregates or individual particles. According to the kinetic energy of the rain drop impact and the aggregate status, soil particles may be displaced some meters away. Later on, splashed material may be removed from the collision area by runoff. The steeper the slope of soil surfaces, the highest the possibility that splashed materials became eroded down slope. Splash erosion decreases after the soil saturation with low intensity and large duration rainfall especially in well-structured soils. Plant residues and undergrowth exert a protecting action of the soil surface and stabilize the splashed soil particles. It is common that the amount of splashed soil is higher than that of eroded soil, mainly due to the low transport capacity of surface runoff. It has been reported by Pardini and Gisbert (2006) that the ratio of splashed to eroded soil gives an indication of the maximum susceptibility to erosion of that given soil, and may be used as an indicator of the real amount of soil that will be transported downslope with high rainfall intensity, steep slope and weak soil structure.

1.1.2.3.2. Wind erosion

It is the removal or deposition of soil materials by wind action. According to Dunjó (2004), wind erosion can occur when 1) The soil is loose, dry, and finely divided on a smooth surface; 2) The vegetation cover is sparse or non-existent; 3) The surface is sufficiently extended to be eroded by wind; 4) Wind velocity is high enough to move

soil particles. According to wind velocity and movement of soil particles, wind erosion can cause:

- Creeping: at low wind velocity.
- Saltation: at high wind velocity.
- Transportation in suspension: when the soil particles are suspended in air until rainfall occurs.
- Abrasion and plant damages: when the wind speed is able to strongly affect plant communities.

1.1.3. Effects of land degradation on soil biological ecosystem

Land degradation may reduce soil fertility through changes in physical, chemical, and biological soil properties. Among land degradation forms, soil erosion is the most widespread, and it is considered the primary force causing detrimental effects on soil biological functions (Mabuhay et al., 2004). At a planetary scale, the land area globally affected by water erosion has been reported to be 1,094 millions ha of which 751 millions ha are severely affected (Lal, 2003). The consequences are particularly strong especially on soil fertility due to the importance of biological activity on nutrient cycling (Carpenter et al., 2001). Accordingly, the effects of erosion on soil biology deserve special attention (Cuenca et al., 1998) in order to better understand the degree of biological decline and soil response to erosion processes. A satisfactory comprehension of these interactions is considered a main tool to deepen into soil functions, and a primary component for soil quality evaluation.

1.1.3.1. *Soil microbial community*

Soil microorganisms may be sensitive indicators in soil ecosystem. When sheet rill or gully erosion is activated by overland flow the eroded mass is also formed of nutrients, organic debris, and a diversity of dead or living organisms including the soil microbiota, which are carried away elsewhere. The topsoil is a reservoir of bacterial and fungal spores and other propagules of organisms important for decomposition, nutrient cycling and mycorrhizal formation (Sieverding, 1991). Soils support critical processes such as hydrological and biogeochemical cycling, involving a wide array of

microorganisms, and providing nutrients and water availability, crucial both for above- and below-ground organism survival (Neary et al., 1999). In turn, the metabolic activity of soil living population attempts to many functions related to debris decaying, humus formation and soil properties improvement. The microbial community includes a wide range of individual species that may behave very heterogeneously to changes occurring in the environment. Indeed, microorganisms can lose their resilience to ecosystem disturbances and become no longer able to perform their normal processes of nutrient cycling and maintaining soil structure and quality (Brady and Weil, 1999).

1.1.3.1.1. Soil fungi

Most plants require arbuscular mycorrhizal fungi (AMF) to gather nutrients, especially phosphorus (P), from soil that is often already nutrient's poor (Cooperband et al., 1994; Janos, 1996). AMF also improve soil aggregation thereby allowing water and nutrients movements (Burns and Davies, 1986), because fungal mycelium through its extension within soil particles emphasizes the role of fungi in forming cementing substances of soil aggregates and reducing erosion effects produced by meteoric or anthropogenic agents. In addition, the diversity of AMF may determine the productivity and diversity of plant communities (Carpenter et al., 2001).

1.1.3.2. Soil respiration

Soil respiration is the oxidation of soil carbon by metabolic processes of soil biota. The primary oxidation is occurring at the soil surface through the progressive transformations of decaying organic debris into nutrients, carbon dioxide, water and other elements or compounds. Whilst a part of carbon is maintained in the soil for its resistance to oxidation, more labile carbon forms are lost as CO₂ to the atmosphere. If the ratio between CO₂ losses and soil organic carbon pool is high, soil itself may lack in stability because of aggregation failure and degradation may be increasing as a result of soil surface erosion (Pimentel et al., 1995; Evans and Lindsay, 2010).

1.1.4. Land management practices for minimizing soil degradation

Obviously, soil degradation and erosion is not just a problem in our current time. A lot of it is inherited from past activities. Any action should be directed to improve soil quality for future generations. A general objective to do not trigger further soil degradation should be achieved at worldwide scale in order to maintain land use potential (Fu et al., 1994). We need to learn from the past and put in practice solutions to protect the future environment.

As it is well known, adding crops residues, manures, compost, or any other organic compounds to the soil contributes to maintain favorable soil fertility and a stable structure. Therefore, the most effective way to maintain soil fertility, soil structure and biological activity is to provide enough soil organic matter to soil and favor the mineralization/humification process. Despite that, organic carbon pool alone is not sufficient to maintain a stable structure and support crop production or standing plants. The addition of supplementary organic matter may be needed to balance the demand of crop production and provide good soil structure and biodiversity in the soil carbon cycle (Studdert et al., 1997; Chen et al., 1998).

1.1.5. Impact of land management on terrestrial carbon sinks

Atmospheric carbon dioxide concentrations vary between 0.036% (360 ppm) and 0.039% (390 ppm), depending on the location. The concentration of CO₂ in the atmosphere increased from 285 ppm at the end of the nineteenth century, before the industrial revolution, to about 366 ppm in 1998 (equivalent to a 28 percent increase) as a consequence of anthropogenic emissions of about 405±60 gigatons of carbon into the atmosphere (IPCC, 2001). This increase was the result of fossil-fuel combustion and cement production (67 percent) and land use change (33 percent). Acting as carbon sinks, the marine and terrestrial ecosystems have absorbed 60 percent of these emissions while the remaining 40 percent has resulted in the observed increase in atmospheric CO₂ concentration (Lal, 2004; IPCC, 2007; Smith et al., 2008; Eneje and Obasi, 2011). Figure 2 presents the different carbon pools and fluxes of the global carbon balance.

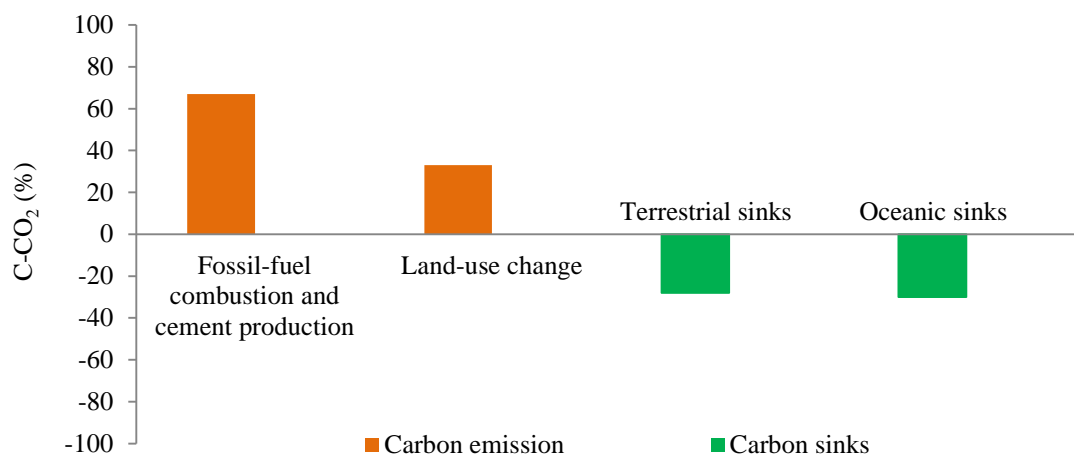


Figure 2. Global carbon dynamics between carbon emission as CO₂ and carbon sinks (adapted from IPCC, 2007).

Soil organic matter is the major reservoir of carbon in terrestrial ecosystems, storing some 1,500 Pg of carbon in the upper parts of mineral soils (Trumbore, 2000). Soil organic carbon in active exchange with the atmosphere constitutes approximately two-thirds of the carbon in terrestrial ecosystems and two to three times as much carbon as atmospheric carbon dioxide (Trumbore et al., 1996). The annual transfer of carbon dioxide from soils to the atmosphere has been estimated at 60-80 Pg C year⁻¹ (Raich and Potter, 1995), 12-16 times the annual rate of addition of fossil fuel CO₂ to the atmosphere (Trumbore, 2000). Above all this, any alteration in the soil properties affects soil carbon and the exchangeability with the atmospheric carbon dioxide. Soil moisture, temperature, structure (aggregates and porosity), biological activity (root development and microbial activity), and climatic change may be used to control the carbon dioxide efflux (Emran et al., 2012a; Suseela et al., 2012).

The presence of organic matter in soil is fundamental to balance consumption by metabolic activity of microorganisms and CO₂ emission. Land use, land use change and abandonment may be therefore major processes for releasing carbon dioxide (CO₂) to the atmosphere when imbalance of C inputs and outputs occurs. In fact, depletion of soil organic carbon pool has contributed as much as 78±12 Pg C to the atmosphere. The depletion of soil C is accentuated by soil degradation and exacerbated by land misuse and soil mismanagement. Thus, adoption of a restorative land use and recommended management practices in agricultural and abandoned soils may reduce the rate of enrichment of atmospheric CO₂ emitted from soil while having positive impacts (Lal, 2004).

1.2. Soil quality evaluation

Along the research work to define soil quality, the literature reports several sentences. When an assessment of the performance of soil functions to be essential for human requirements and environment is carried out, we are evaluating soil quality. The term soil quality is not only related to the improvement of some physical, chemical, or biological soil properties but also with water and air quality and must be seen in a global change context under perspectives of sustainability (Acton and Gregorich, 1995; Warkentin, 1995; Johnson et al., 1997). Accordingly, van Bruggen and Semenov (2000) defined soil quality as “the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health”. Karlen et al. (1997) stated that soil quality is the “capacity of a specific soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation”. Warkentin (1995) postulated that soil quality is “a dynamic interaction between various physical, chemical and biological soil properties, which are influenced by many external factors such as land use, land management, the environment and socio-economic priorities”. The same author inferred that soil quality is considered “a key element of sustainable agriculture because it is essential to support and sustain crop, range and woodland production and contribute to maintain other natural resources such as water, air and wildlife habitat”. Therefore, an integrated soil quality index based on the contribution of weighed individual soil properties to evaluate soil quality for different land uses should be used.

1.2.1. Soil quality indicators

Assessment of soil quality is the basis for assessing sustainable soil management. It is particularly difficult to select factors of soil quality for degraded or polluted soils. Appropriate indicators are needed to show whether those requirements are being met. Some soil variables which may define resource management domains are soil texture, drainage, slope and land form, effective soil depth, water holding capacity, cation exchange capacity, organic carbon, soil pH, salinity or alkalinity, surface stoniness, fertility parameters, and other limited properties (Eswaran et al., 1998). The utility of each variable is determined by several factors, including weather changes that

can be measured over time, sensitivity of the data to these changes, relevance of information to the local situation, and statistical tools which can be employed for processing information.

Larson and Pierce (1994) proposed a minimum data set (MDS) of soil indicators for assessing soil quality (Table 1). These indicators should be useful across a range of ecological and socio-economic situations (Doran and Jones, 1996). The minimum data set is the smallest set of soil properties or indicators needed to measure or characterize soil quality that are sensitive to changes in soil functions. Each minimum data set is tailored to a particular region or soil type and includes only those properties that are relevant to soil types, farming system, and land uses of the areas being evaluated (NRCS, 2001).

Our knowledge of soil quality is based primarily on quantitative analysis of individual physical, chemical, and biological soil properties. Moreover, the interaction of these quantitative aspects determines the levels of soil quality. Integrative tools are needed by researchers, farmers, regulators, and others to evaluate changes in soil quality from human activity at a local and global level. Therefore, an index is needed to be adaptable to local or regional conditions. For example, the parameters needed to determine changes in soil quality might differ between a semi-arid wheat field and a rice paddy. The present challenge is to integrate a suite of soil tests into a meaningful index that correlates with productivity, environmental, and health goals.

Standard methodologies and procedures must be established in the assessing of soil quality indicators to:

- Correlate well with natural processes in the ecosystem.
- Integrate soil physical, chemical, and biological properties.
- Be relatively easy to use under field conditions, so that both specialists and producers can use them to assess soil quality.
- Be sensitive to variations in land management and climate. The indicators should be sensitive enough to reflect the influence of management and climate on long-term changes in soil quality, but not so sensitive that they are influenced by short-term weather patterns.
- Be the components of existing soil databases where possible.

Table 1. Proposed minimum data set (MDS) of physical, chemical, and biological indicators for screening the condition, quality, and health of soils.

Indicators and units	Relationship to soil quality
PHYSICAL	
Soil texture (%)	Retention and transport of water and nutrients, habitat for microbes, and level of soil erosion.
Soil aggregation (%)	Microbial exudates preservation, soil stability, water infiltration, potential for soil erosion, soil erosion, soil resistance and resilience, and organic matter content.
Depth of soil and rooting (cm)	Potential for productivity, compaction, and erosion rates.
Infiltration (mm h ⁻¹)	Water movement, air exchange, and soil structure.
Bulk density (g cm ⁻³)	Porosity, organic materials, infiltration rate, and hydraulic conductivity.
Water holding capacity (%)	Water storage and availability, water available for plant and soil biota.
Soil temperature (°C)	Promote biological activity.
CHEMICAL	
pH	Biological and chemical activity, nutrient availability, and limits for plant growth and microbial activity.
Electrical conductivity (dS m ⁻¹)	Plant and microbial activity, salt tolerance, and limits for plant growth and microbial activity.
Available nitrogen (mg kg ⁻¹)	Plant available nutrients, potential for N and P loss, productivity, and environmental quality indicators.
Available phosphorus (mg kg ⁻¹)	
Available potassium (mg kg ⁻¹)	
BIOLOGICAL	
Soil organic matter (mg g ⁻¹)	Soil fertility, structure, stability, nutrient retention, level of soil erosion, potential productivity, and available water capacity.
Soil microbiota (mg g ⁻¹)	Microbial catalytic potential and repository for C and N. Soil productivity and nitrogen supplying potential.
Potentially mineralizable nitrogen (mg g ⁻¹)	
Soil respiration (mg g ⁻¹)	Microbial activity and carbon loss.
Glomalin production (mg g ⁻¹)	Microbial activity, soil structure and stability, nutrient availability, and repository for C and N.

Adapted from Larson and Pierce (1994), Hseu et al. (1999), and NRCS (2001).

Indicators can be assessed by qualitative or quantitative techniques. Measurements can be evaluated by looking for patterns and comparing results to measurements taken at a different time or sites. It is suggested that soil quality indicators might be divided into two major groups, analytical and descriptive. Experts often prefer analytical indicators, while farmers and the public users are often using descriptive indicators.

1.3. Aim and objectives

The aim of this work was to study the dynamics of soil properties and erosion processes with particular emphasis to organic carbon (SOC) pools in fragile Mediterranean soil environments under different land use and age of abandonment. Monitoring of spatial and temporal variations of the main soil characteristics, critical to land use change and land abandonment, was scheduled along 2008 and 2009 to achieve a clear picture of the soil environments dynamics with the following priorities:

1. To establish the effects of current land use and abandonment on soil erosion and degradation processes in the selected soil environments;
2. To investigate the relationships between the soil susceptibility to erosion, plant cover and type and soil properties along the period of study;
3. To study the effect of land use, land use change and land abandonment on soil organic carbon stocks in the selected soil environments;
4. To evaluate the CO₂ emission and carbon loss from the selected soil environments along the period of study;
5. To clarify the contribution of soil organic carbon (SOC) to soil structural stability and microbial activity in the soils under study;
6. To analyze the relationships between glomalin, soil organic carbon, and soil structural stability in the studied soil environments with diverse plant associations;
7. To estimate the content and role of SOC pools and easily extractable (EE-BRSP) and total glomalin (BRSP) in different fractions of soil aggregates;
8. To investigate the relationships between SOC pools, glomalin stocks and C-CO₂ loss in the selected soil environments;
9. To establish the potential of carbon storage along the soil sequence under study;
10. To state the potential of abandoned areas for carbon storage capacity and emphasize its relevance in future land management;
11. To understand the interaction mechanisms between soil biological activity and structure through the biochemical interactions of soil enzymes act on organic substrates and through the cycling of soil nutrients;
12. To study the effects of soil use and abandonment on β -glucosidase, phosphatase, and protease activities, and the relationships with carbon, phosphorus, and nitrogen dynamics;

13. To identify the chemical structural composition of soil organic matter in the selected soil environments;
14. To validate statistically the results obtained in order to contribute to a better management of the soils under study;
15. To suggest land management practices for future carbon sequestration scenarios.

CHAPTER II. METHODOLOGY

2.1. Description of the study area

2.1.1. Characteristics of the area of study

2.1.1.1. Geology

The area of study belongs to a mid-mountainous region (North-East part of Pyrenees) formed by a Paleozoic basement made of metamorphic silicic materials such as Cambrian-Ordovician schists, slates, gneiss, and granite intercalated by Carboniferous-Permian (Tomas et al., 1987). The depressions are filled with quaternary sediments due to erosion and deposition processes (Figure 3). It is named the Cap de Creus Peninsula and has a significant value expressed by striking geological features, with unique examples of maritime and littoral terrestrial environments (Franquesa, 1995).

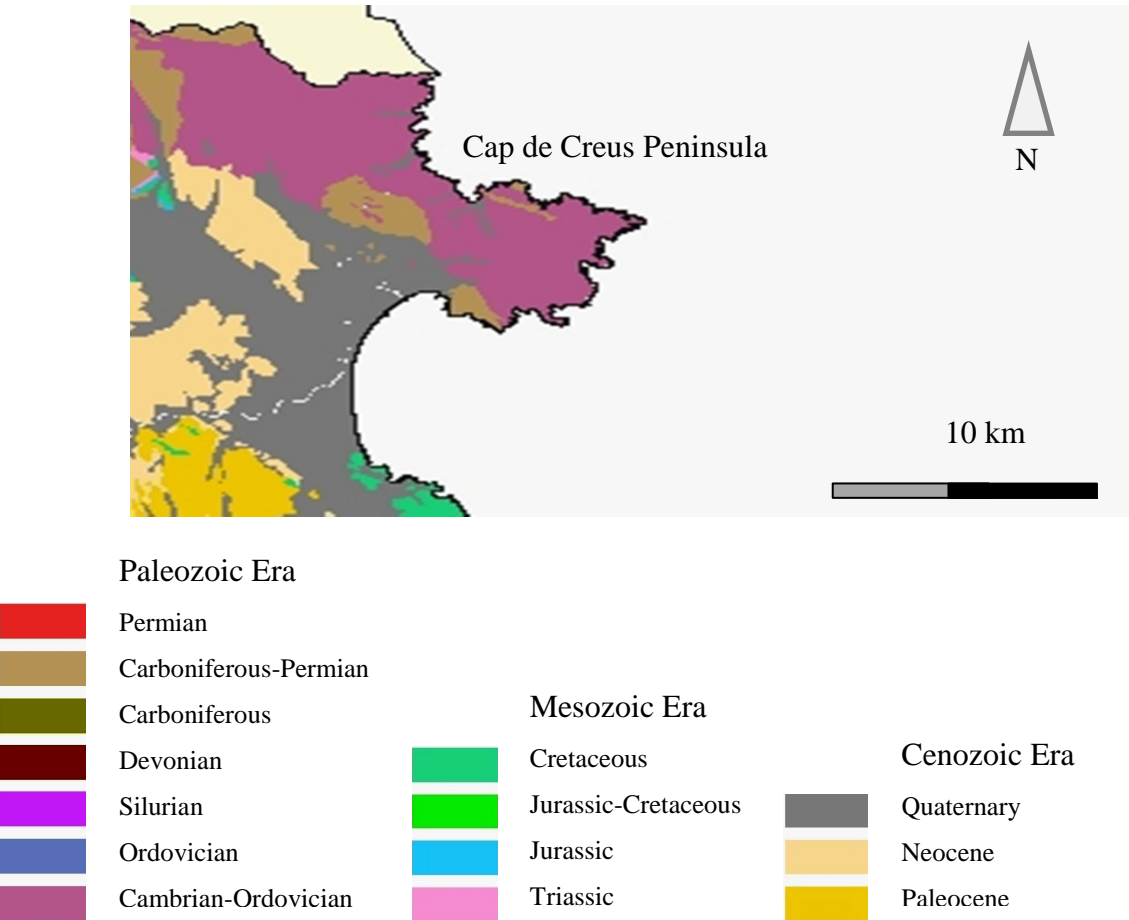


Figure 3. Geologic map showing the distribution of the geological areas of Alt Empordà including the area of Cap de Creus Peninsula.

2.1.1.2. Geography

The study area is located in the Romanya catchment (42°18'N; 3°13'E), Cap de Creus Peninsula, Province of Girona, Catalunya, North East Iberian Peninsula (Figure 4). It is enclosed in the Natural Park of “Cap de Creus” and occupies an area of 30 km² ranging from 60-300 m asl (Dunjó et al., 2003).



Figure 4. Location of the Cap de Creus in the Iberian Peninsula and topographic map 1:250,000.

The terrains of the natural Park of “Cap de Creus” are protected by special laws to guarantee the sustainable development of this particular area. Three different protection levels are established (in increasing order of protection): natural park zone, natural sites of national interest, and integral reserve. Protection and preservation measures specifically for the terrestrial environments are related to the geological, botanical, faunal, and landscape values, as well as the different elements of cultural interests in the area, and are legally punished in case of negligence. The area of study is bounded by the Mediterranean Sea in the NE, Roses bay in the SW, Albera Mountains in the W, and Empordà plain area in the S. It represents a typical Mediterranean ecosystem (Dunjó, 2004).

The studied soils environments: soils under cultivated vines (V), soils under olive groves (O), soils under stands of cork (S) trees, soils under stands of pine (PI)

trees, soils under pasture (PR), soils under *Cistus* scrub (MC), and soils under *Erica* scrub (MB), are located within the delimited area of the Natural Park of Cap de Creus (Figure 5).

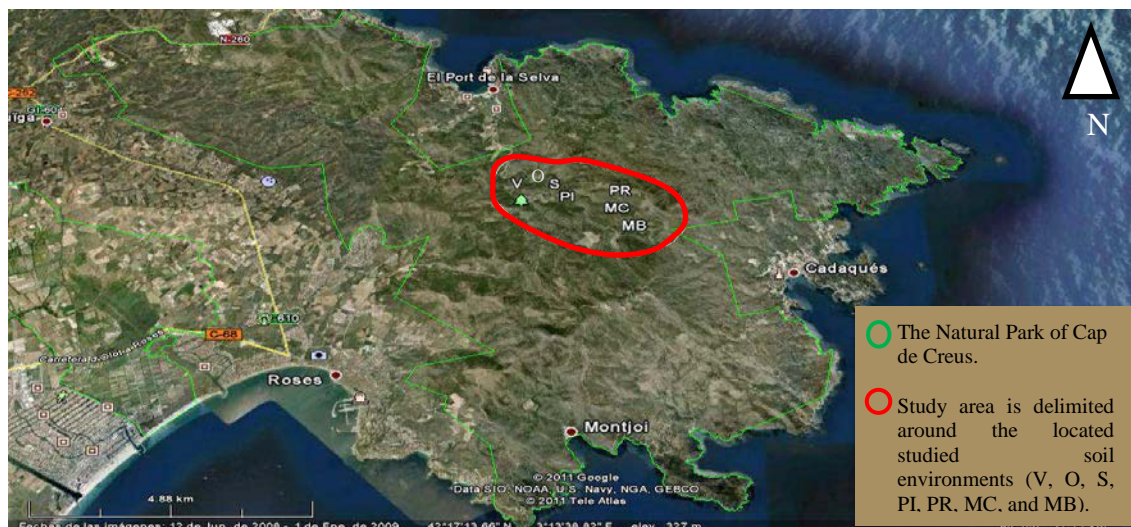


Figure 5. Limits of the Natural Park of Cap de Creus and location of the selected soil environments at the study area.

The area of study has a Mediterranean Xerotheric climate. The mean annual average temperature is set between the isotherms of 15-16 °C with seasonal oscillations, with hot summers and mild winters (Franquesa, 1995; Dunjó et al., 2003). In this mid-mountain environment the hottest month is July and the coldest is January, with a mean annual temperature around 16 °C, maxima of 36 °C and minima of 4 °C (Dunjó, 2004). The rainfall is seasonally irregular and the mean annual precipitation is around 450 mm. Interannual variability shows peaks increasing in spring and autumn (Franquesa, 1995; Dunjó et al., 2003) as observed in Figure 6.

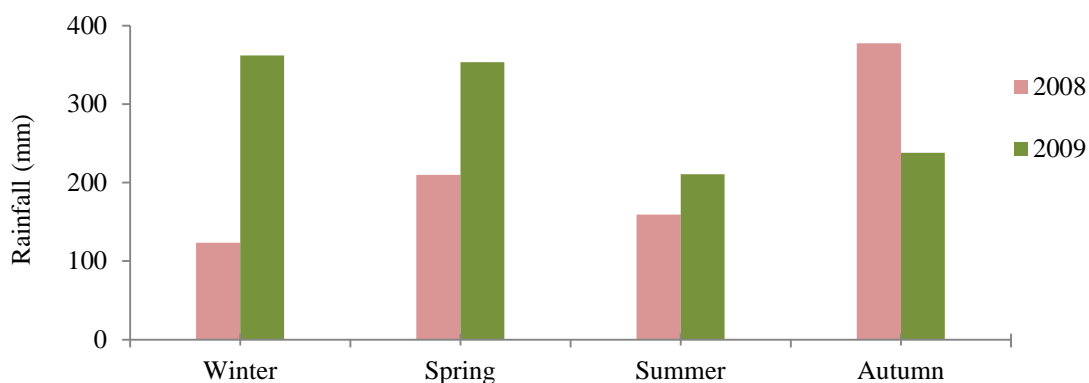


Figure 6. The mean seasonal precipitation of rainfall during 2008 and 2009 in the area of the selected soil environments.

Rainfall regimes have changed in the last years, and torrential rainstorms occur frequently in summer (Franquesa, 1995). The irregular precipitation patterns and distribution over the year determine the type of vegetation growing in this area which has to adapt to the scarcity of water during some periods of the year. The climate of the area of study is severely affected by the *Tramuntana* wind, coming from the North. It is a cold and dry wind normally achieving high speeds, up to 120 km h^{-1} (Pascual and Callado, 2010) and consequently strongly decreases the soil water content. It is considered a bioclimatic factor of relevant importance in this area. Moreover, the *Tramuntana* wind is often contributing to fire propagation in the very extended scrubland difficulting the spontaneous regeneration of vegetation (Dunjó, 2004).

2.1.2. Soil classification at the area of study

The soils of the Cap de Creus Peninsula proceed from a very complex geological system and its formation has been clarified through the mineralogical composition of the parent material, the geographical and topographical position and the history of land use and management. The fundamental aspect to be taken into account is the soil evolution and the physicochemical characteristics of the soil horizons that have been progressively changed, such as the depth, the textural class, the structure and the nutrient availability, that have a direct influence on water retention capacity and the capacity to support a stable vegetation cover. The majority of land surface is covered by shallow soils formed on slates. The soils generally present Ap, C/R horizon development. In some instances, forest areas soils may present a Bw horizon. The same evolution has been found in top plain areas where agricultural exploitations lasted for many years and are now transformed into pastureland. However, all the study area is covered by terraced soils used during many years for agricultural purposes. Original slopes have been therefore modified by anthropic intervention in order to maintain little portions of plain areas to be cultivated, keeping drainage networks and dry stone walls to prevent erosion. Nevertheless, after the progressive land abandonment soils have become shallower, dry and with higher stoniness percentage, and vegetation has become adapted to this new ecosystem. Soils are prevalently classified as Lithosols (Xerorthents) for the periodical accumulation of subsequent layers of materials transported from higher parts of the catchment, sometimes differentiated by the

mineralogical or structural composition, without presenting a marked process of horizon development (Soil Survey Staff, 1992).

2.1.3. Land use change and abandonment at the area of study

The agricultural exploitation of the area of study was carried out during the last centuries until 1960 for cereals, wine and olive oil production. The cultivation of olive trees and especially vineyards continued until the 19th century when the phylloxera destroyed all the vineyards of the region in 1865. The *Daktulosphaira vitifoliae* is an aphid-like gall-forming parasite of grapevines and forms damaging root galls on the European species *Vitis vinifera* (Kellow et al., 2004). This destruction had serious social repercussions on the population which was obligated to abandon the terraced soils in sloping fields during decades. Later on 1950, farmers were reorganized to replant new vines (from USA) in the best plots in plane areas. This was also possible by the mechanization of agriculture which further induced the almost complete abandonment of vines cultivation in terraces. Although the agricultural exploitation decreased enormously in the terraced soils, some patches of vines and olive plantations remained. Olive groves constituted an important crop for oil production until February 1956 when extremely cold temperatures eliminated the majority of the olive trees, leading to the general abandonment of olive cultivation. Cork trees (*Quercus suber*) were also introduced in substitution of olive groves, but this cultivation was abandoned as well around the sixties. Nowadays the cultivated surface is less than 5% of the total terraced territory. The economical alternative for the habitants of these rural areas is tourism (Dunjó, 2004) even though the completely disorganized territory covered by scrubland should be managed to reduce wildfire occurrence.

2.1.3.1. Grazing activities

Grazing was also one of the most important activities in the Cap de Creus territory. In this area, grazing has been carried out since ancient time with cattle and herds coming down from Pyrenees from November to March. In this area pasture was abundant with extended meadows. The progressive abandonment of grazing and rural activity led to a significant decrease of meadows, currently invaded by scrub. Nowadays, only few herds and cattle are brought from the Ripollés region in late

autumn until the end of spring. Grazing activity may have twofold aspects: 1) increase of soil nutrients due to manure addition, which may enrich soil with organic matter, 2) increase soil surface compaction by overgrazing (because of progressively reduced surface), decreasing porosity and altering soil surface structure with consequent relatively higher susceptibility to erosion, especially in steeper slopes (Dunjó, 2004).

2.1.3.2. *Occurrence of wildfire*

Forest fires have occurred frequently over the abandoned areas during the last decades even causing the disappearance of cultivated fields. Nowadays, most of the current vegetation species are adapted to fire conditions, due to seed capacity to withstand high temperature. As a result of frequent wildfire occurrence, the vegetation succession seems to undergo more regressive stages, with restriction of species causing impoverishment in plant heterogeneity. However, due to the incidental occurrence of fire, some abandoned areas with spontaneous vegetation remain unburned for years allowing the establishment of a natural vegetation succession (Pardini et al., 2003).

2.1.4. *Sequence of land use and abandonment*

In the area of study, almost 90% of the old terraced territory has been progressively covered with different stages of scrubs as may be observed in Figure 7 and Figure 8.

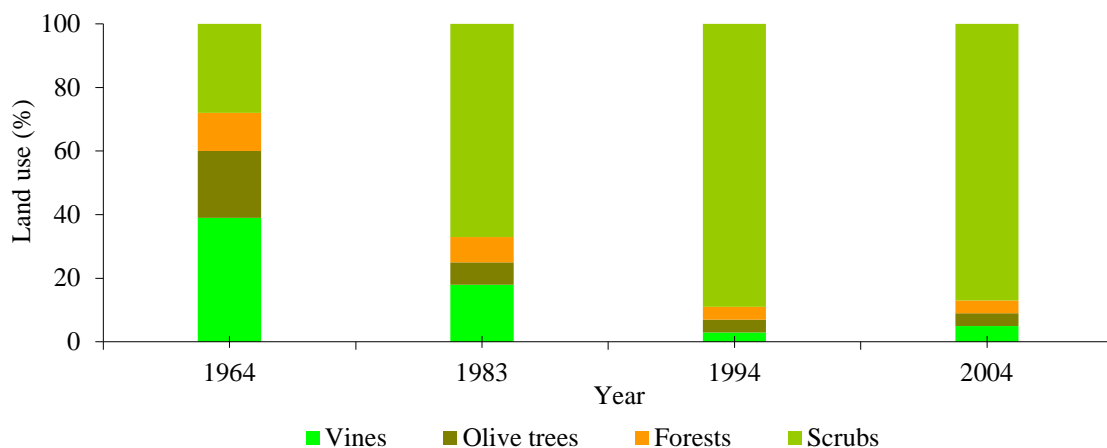


Figure 7. Sequence of land use and abandonment along different periods.

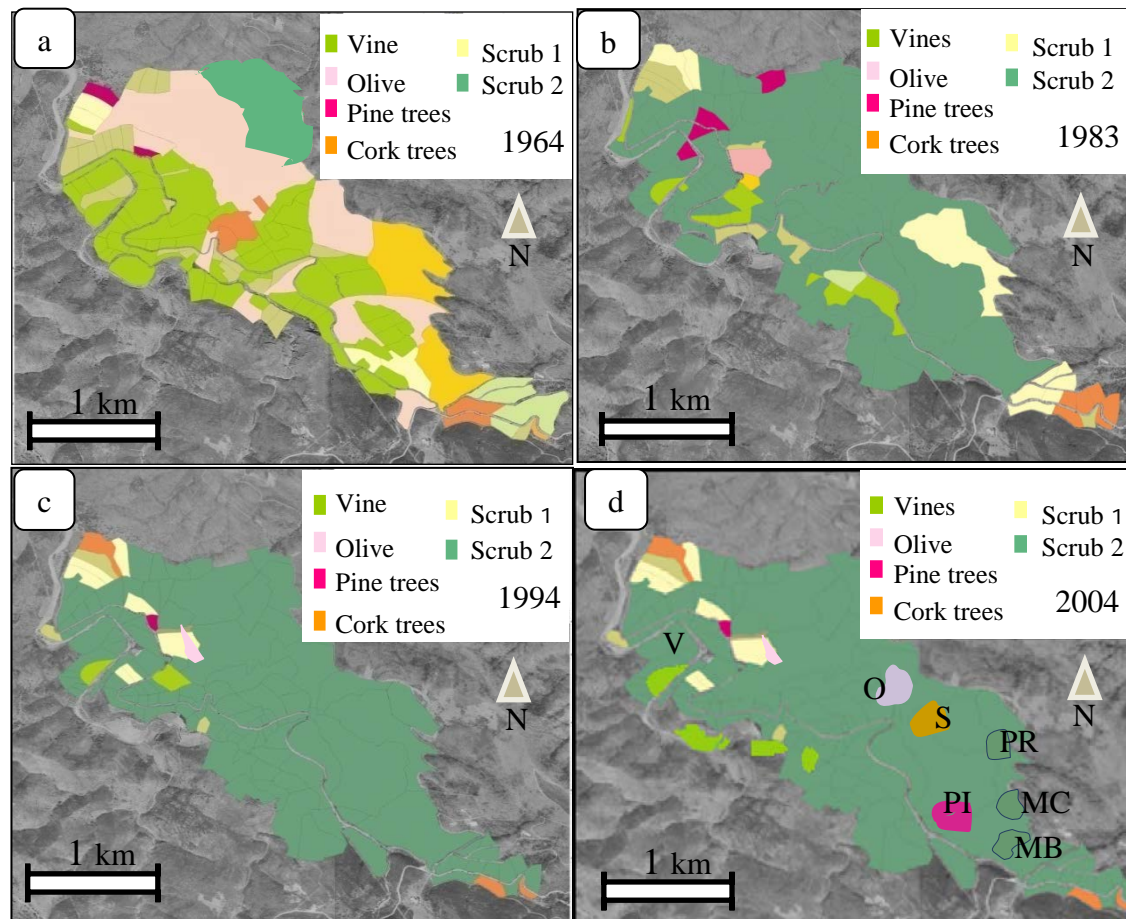


Figure 8. Chronological sequence of land use and abandonment along different periods.

2.1.5. Soils under study

Seven environments were selected (Figure 8d), ranging from current cultivated soils at minimal agricultural management, soils under stands of pine and cork trees, soils under pasture and soils under different stages of natural vegetation succession after abandonment:

2.1.5.1. Soils under cultivated vines (*V* environment)

This soil environment is currently cultivated with vines (*Vitis vinifera*) maintained at very low agricultural management. The vineyard is exploited by the municipality of Port de la Selva to encourage the recovery of abandoned fields and create spatial landscape heterogeneity to combat wildfire occurrence (Figure 9).



Figure 9. General view of the soils under cultivated vines surrounded by abandoned scrubland.

Tillage is the only agricultural practice periodically applied in soil under the vines (Figure 10). Sometimes chemical fertilizers and herbicides are applied for nutrients increase and to combat the red spiders invasion. The monitored area of soils under vines have an extension of 0.5 ha, terraces with 15% slope and 6% of canopy cover, and the soil profile is 52 cm deep. The vegetation inventory conducted by delimiting an area of 10×10 m is reported in Table 2.



Figure 10. Tillage practices in soils under cultivated vines.

Table 2. Relative abundance of vegetation cover in V soil environment.

Vegetation cover	Specie	Family name	Plants (N°)	Specie/ plant cover (%)	Occupied surface (%)		
					Aerial	Under-growth	Bare soil
Tree	<i>Vitis vinifera</i>	Vitaceae	10	89.29	6		
Herbaceous	<i>Brachypodium retusum</i>	Poaceae	4	10.71		1.2	
Total			14	100	6	1.2	94

Soil classification

Soil Taxonomy: Xerorthent lithic; FAO: Lithosol

Location

It is located at 42° 18' 36" North, 3° 12' 42" East according to the Universal Transverse Mercator (UTM) geographic coordinate system.

Situation

Plain area with large terraces (6 m width one another) and stony soils cultivated with vines (*Vitis vinifera*) situated at 65 m asl. The soil profile has been excavated in a representative site with NE orientation and a smooth slope (around 15%). In the moment of description the soil was dry and well drained.

Soil profile (Figure 11)

- Ap (28 cm): Horizon with scarce presence of organic matter. The texture is sandy loam and stones of different shape and size are mixed with the fine fraction. The soil has a single grain structure and clods are easily disrupted by applying a weak pressure. Not plastic. A relatively high porosity is visible within soil particles. No biological activity is detected but roots of various sizes. No reaction with HCl is observed. The limit with the underneath horizon is regular. The color of the Ap soil profile is pale brown (10YR 6/3) according to Munsell (1992).
- C/R (28-52 cm): Dense stony layer with fragmented slates rocks not chemically altered. Fissures are generally filled with sandy fine material migrated from the upper parts of the profile. No biological activity is detected. Very well drained horizon.

Comments

Still cultivated vineyards in the area are generally maintained at low agricultural management. Application of organic matter is scarce and only some agrochemicals and herbicides are periodically applied.



Figure 11. Example of Ap, C/R horizon sequence typical of the soil environment under vines (V).

2.1.5.2. Soils under olive groves (O environment)

This environment is covered by olive trees (*Olea europaea*) in terraced soils. It is maintained at low agricultural practices for both soil and plants. The area delimited in soils under olive groves was of 0.5 ha, with 18% slope, 15% of canopy cover and the total soil depth was 45 cm (Figure 12). The vegetation inventory conducted by delimiting an area of 10×10 m is reported in Table 3.



Figure 12. General view of soils under olive groves.

Table 3. Relative abundance of vegetation cover in O soil environment.

Vegetation cover	Specie	Family name	Plants (N°)	Specie/ plant cover (%)	Occupied surface (%)		
					Aerial	Under-growth	Bare soil
Tree	<i>Olea europaea</i>	Oleaceae	3	76.53	15.00		
Scrub	<i>Lavandula stoechas</i>	Lamiaceae	9	12.76		2.50	
	<i>Brachipodium retusum</i>	Poaceae	8	6.12		1.20	
	<i>Pistacia lentiscus</i>	Anacardiaceae	5	2.59		0.45	
	<i>Helichrysum stoechas</i>	Lamiaceae	6	2.00		0.45	
Total			23	100	15	5	85

Soil classification

Soil Taxonomy: Xerorthent lithic; FAO: Lithosol

Location

It is located at 42° 18' 33" North, 3° 13' 16" East according to the Universal Transverse Mercator (UTM) geographic coordinate system.

Situation

Terraced area (terrace's width 6 m one another) and stony soils cultivated with olives (*Olea europae*) situated at 175 m asl. The soil profile has been excavated in a representative site with NE orientation and a slope around 18%. At the moment of description the soil was dry and well drained.

Soil profile (Figure 13)

- Ap (25 cm): Horizon presenting a certain amount of organic matter mixed with mineral matter. The texture is loamy sand and the soil is slightly plastic. Stones of different shape and size are mixed with the fine fraction. The structure is granular and blocky and clods are slightly resistant to pressure. A relatively high porosity is visible within soil particles. Biological activity is often detected. No reaction with HCl is observed. The limit with the underneath horizon is regular. The color of the Ap soil profile is grey (10YR/5/1) according to Munsell (1992).
- C/R (25-45 cm): Dense stony layer with fragmented slates rocks not chemically altered. Fissures are generally filled with sandy fine material migrated from the upper parts of the profile. No biological activity is detected. Fairly well drained horizon.

Comments

Still cultivated olive groves in the area are generally maintained at very low agricultural management. No application of organic matter is provided. Undergrowth formed mainly by *Lavandula stoechas* and *Brachipodium retusum*.

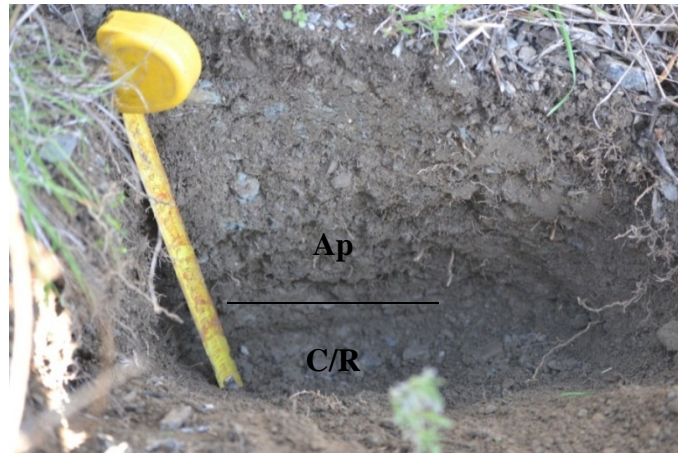


Figure 13. Example of Ap, C/R horizon sequence typical of the soil environment under olive groves (O).

2.1.5.3. *Soils under stands of cork trees (S environment)*

Terraced soils under stands of cork trees (S) are representing residuals of ancient cultivation of *Quercus suber* for cork production. Probably this area had previously been cultivated with vines and cork trees were successively introduced. Nowadays cork trees are approximately 4-5 m tall and mixed with other type of undergrowth (Figure 14).



Figure 14. General view of soil environment under stands of cork trees.

Wildfires have affected this environment repeatedly and the last wildfire occurrence was recorded in July 2008 (Figure 15). The area surveyed in soils under cork trees had 0.5 ha extension, 15% slope, 60% of plant canopy, and a total soil depth of 10-30 cm. The vegetation inventory conducted by delimiting an area of 10×10 m is reported in Table 4.



Figure 15. Terraced soils under stands of cork trees after the wildfire in July 2008.

Table 4. Relative abundance of vegetation cover in S soil environment.

Vegetation cover	Specie	Family name	Plants (N°)	Specie /plant cover (%)	Occupied surface (%)		
					Aerial	Under-growth	Bare soil
Tree	<i>Quercus suber</i>	Fagaceae	5	40.46	25.00		
Scrub	<i>Erica arborea</i>	Ericaceae	4	3.24		2.00	
	<i>Cistus monspeliensis</i>	Cistaceae	22	17.80		11.00	
	<i>Cistus salviifolius</i>	Cistaceae	4	3.24		2.00	
	<i>Asparagus acutifolius</i>	Liliaceae	16	2.07		1.28	
	<i>Lavandula stoechas</i>	Lamiaceae	3	0.34		0.21	
	<i>Pistacia lentiscus</i>	Anacardiaceae	5	3.24		2.00	
	<i>Euphorbia dendroides</i>	Euphorbiaceae	4	3.88		2.40	
	<i>Thymelaea tinctoria</i>	Thymelaeaceae	5	3.64		2.25	
	<i>Sideritis angustifolia</i>	Lamiaceae	2	0.65		0.40	
	<i>Calicotome spinosa</i>	Fabaceae	13	9.47		5.85	
	<i>Euphorbia peplus</i>	Euphorbiaceae	2	3.24		2.00	
Herbaceous	<i>Brachypodium retusum</i>	Poaceae	10	1.62		1.00	
	<i>Verbascum thapsus</i>	Scrophulariaceae	7	1.70		1.05	
	<i>Helictotrichon filifolium</i>	Poaceae	3	1.21		0.75	
	<i>Thapsia villosa</i>	Apiaceae	4	0.65		0.40	
	<i>Iberis ciliata</i>	Cruciferae	6	0.97		0.60	
	<i>Echinops ritro</i>	Lamiaceae	5	1.86		1.15	
	<i>Dactylis glomerata</i>	Poaceae	3	0.73		0.45	
Total			74	100	25	37	40

Soil classification

Soil Taxonomy: Xerorthent lithic; FAO: Lithosol

Location

It is located at 42° 18' 34" North, 3° 13' 22" East according to the Universal Transverse Mercator (UTM) geographic coordinate system.

Situation

Terraced area (terrace's width 6 m one another) and stony soils anciently exploited for cork (*Quercus suber*) situated at 175 m asl. The soil profile has been excavated in a representative site with NE orientation and a slope around 15%. In the moment of description the soil was dry and well drained.

Soil profile (Figure 16)

- Ap (5 cm): Shallow horizon with humified organic matter mixed with mineral matter. Loamy sand and slightly plastic. Stones of different shape and size are mixed or intercalated with the fine fraction. The structure is granular/blocky and clods show relative resistance to pressure. The soil is somewhat plastic and adherent. A relatively high porosity is visible within soil particles. Biological activity is often detected. No reaction with HCl is observed. The limit with the underneath horizon is regular. The color of the Ap soil profile is dark gray (10YR/4/1) according to Munsell (1992).
- C/R (5-30 cm): Dense stony layer with fragmented slates rocks not chemically altered. Fissures are filled with fine material migrated from the upper parts of the profile. Some biological activity is detected. Slow drained horizon.

Comments

Abandoned cork trees. Undergrowth vegetation mainly composed by some *Cistus monspeliensis*, *Erica arborea*, *Lavandula stoechas*, *Brachipodium retusum*. No management is provided. This environment was burnt in 2008.

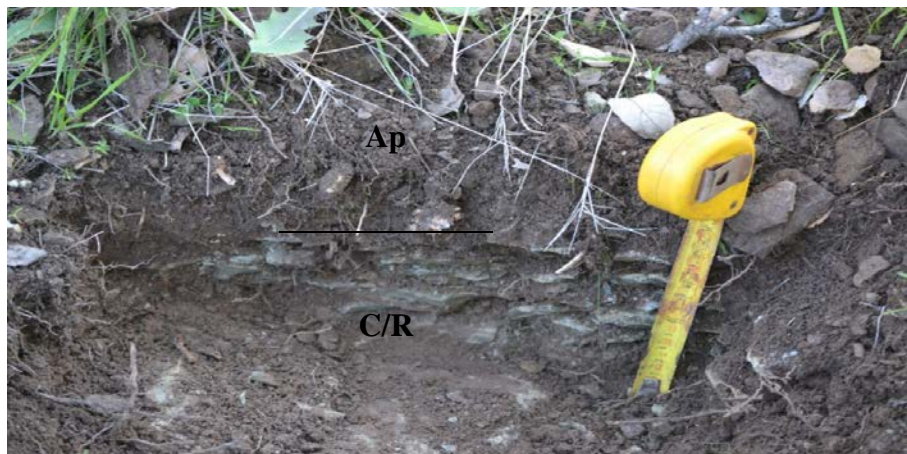


Figure 16. Example of Ap, C/R horizon sequence typical of the soil environment under stands of cork trees (S).

2.1.5.4. Soils under stands of pine trees (PI environment)

Terraced soils under stands of pine trees was anciently planted with *Quercus suber* and then reforested after it was devastated by wildfire in 1955 with Aleppo pine (*Pinus halepensis*) for its rapid growth and wood value. Pine trees are approximately 5-6 m tall (Figure 17). The area of soils under PI environment covered 0.5 ha, slope was 18%, and had 70% of canopy cover, with a soil profile 30 cm deep. The vegetation inventory conducted by delimiting an area of 10×10 m is reported in Table 5.



Figure 17. General view of soils under stands of pine trees.

Table 5. Relative abundance of vegetation cover in PI soil environment.

Vegetation cover	Specie	Family name	Plants (N°)	Specie/ Plant cover (%)	Occupied surface (%)		
					Aerial	Under- growth	Bare soil
Tree	<i>Pinus halepensis</i>	Pinaceae	8	71.13	66.40		
	<i>Olea europaea</i> (sprouts)	Oleaceae	3	1.13	9.00		
Scrub	<i>Erica arborea</i>	Ericaceae	1	12.70		0.80	
	<i>Asparagus acutifolius</i>	Liliaceae	9	5.08		3.60	
	<i>Lavandula stoechas</i>	Lamiaceae	6	0.59		0.42	
	<i>Polypodium vulgare</i>	Polypodiaceae	3	0.42		0.30	
	<i>Cistus salviifolius</i>	Cistaceae	4	2.26		1.60	
	<i>Braquipodium retusum</i>	Poaceae	1	0.85		0.60	
Herbaceous	<i>Brachypodium sylvaticum</i>	Poaceae	1	0.64		0.45	
	<i>Vicia tenuifolia</i>	Leguminosae	5	1.41		1.00	
	<i>Euphorbia characias</i>	Euphorbiaceae	4	1.13		0.80	
	<i>Euphorbia hyberna</i>	Euphorbiaceae	3	0.97		0.69	
	<i>Ranunculus auricomus</i>	Ranunculaceae	2	0.99		0.70	
	<i>Scorzonera hispanica</i>	Asteraceae	2	0.28		0.20	
	<i>Silene latifolia</i>	Caryophyllaceae	3	0.42		0.30	
Total			55	100	75	11	70

Iovieno et al. (2006) postulated that pine trees may have a negative effect on soil microbial community due to acid pH caused by resins and the hardly decomposable pine needles forming a considerable layer at soil surface. Therefore, soil properties like organic matter and nutrients cycling may be affected. Consequently a less favorable environment may be able to decrease the proliferation of herbaceous vegetation as indicated by the low proportion of undergrowth.

Soil classification

Soil Taxonomy: Xerorthent lithic; FAO: Lithosol

Location

It is located at 42° 18' 23" North, 3° 13' 28" East according to the Universal Transverse Mercator (UTM) geographic coordinate system.

Situation

Terraced area (terrace's width 6 m one another) and stony soils situated at 190 m asl. Pines (*Pinus halepensis*) were planted in the fifties after a big wildfire. The soil profile has been excavated in a representative site with NE orientation and a slope around 18%. In the moment of description the soil was dry and well drained.

Soil profile (Figure 18)

- Ao (3 cm): Thin layer of pine needles partially decomposed.
- A (3-10 cm): Shallow horizon with partially humified organic matter mixed with mineral matter. Not plastic. Stones of different shape and size are mixed with the fine fraction. The structure is blocky and clods show low resistance to pressure. Pores within soil particles. Biological activity is scarcely detected. No reaction with HCl is observed.
- Bw (10-20 cm): Weathering horizon showing the presence of iron oxides. The color of the Ao, A, and Bw soil profile is brownish yellow (10YR/6/6) according to Munsell (1992).
- C/R (20-40 cm): Stony layer with fragmented slates chemically altered. Fissures are filled with fine material migrated from the upper parts of the profile. Slow drained horizon.

Comments

Abandoned pine trees. Undergrowth vegetation mainly composed by some *Lavandula stoechas*, *Brachipodium retusum*, *Asparagus acutifolius*, *Polypodium vulgare*, *Brachypodium sylvaticum*, and *Vicia tenuifolia*. No management is provided.



Figure 18. Example of Ao, A, Bw, C/R horizons typical of the soil environment under stands of pine trees (PI).

2.1.5.5. Soils under pasture (PR environment)

Terraced soils under pasture (PR) are mainly covered with *Brachypodium retusum*, *Trifolium stellatum*, *Dactylis glomerata*, *Lavandula stoechas*, and *Ulex parviflorus*. This environment is currently in transition to scrubland for gradual abandonment of grazing activity (Figure 19).



Figure 19. General view of soils under pasture.

The therophyte annual plants are able to survive during the unfavorable dry season in the form of seeds and complete their life-cycle during more favorable climatic

conditions. A very dense root system distributed within few centimeters of the Ap horizon may allow isohumic processes to enrich the upper horizon with organic matter increasing the humus content thus decreasing the susceptibility to erosion. Although the organic soil horizon is only 10 cm depth over C/R horizon, it is structurally stable due to higher organic matter content in comparison with other environments. However, such shallow soils may be easily affected by compaction due to grazing and strong rainfall events producing particles detachment, runoff erosion and nutrient depletion (Dunjó, et al., 2003). The monitored area was 0.5 ha, with 18% slope, 60% of canopy covers, and a soil profile 34 cm deep. The vegetation inventory is reported in Table 6.

Table 6. Relative abundance of vegetation cover in PR soil environment.

Vegetation cover	Specie	Family name	Plants (N°)	Specie/ plant canopy (%)	Occupied surface (%)	Bare soil
					Under-growth	
Herb	<i>Lavandula stoechas</i>	Lamiaceae	140	25.81	42.00	
	<i>Ulex parviflorus</i>	Fabaceae	120	22.12	36.00	
	<i>Euphorbia dendroides</i>	Euphorbiaceae	15	3.69	6.00	
	<i>Euphorbia cyparissias</i>	Euphorbiaceae	17	3.13	5.10	
	<i>Helichrysum stoechas</i>	Asteraceae	12	1.47	2.40	
Herbaceous	<i>Trifolium stellatum</i>	Fabaceae	120	7.37	12.00	
	<i>Brachypodium retusum</i>	Poaceae	111	5.46	8.88	
	<i>Brachypodium distachyon</i>	Poaceae	69	2.97	4.83	
	<i>Lagurus ovatus</i>	Poaceae	44	2.16	3.52	
	<i>Trifolium cherleri</i>	Fabaceae	36	1.99	3.24	
	<i>Ornithopus compressus</i>	Fabaceae	44	1.89	3.08	
	<i>Trifolium campestre</i>	Fabaceae	34	1.88	3.06	
	<i>Vicia monantha</i>	Fabaceae	43	1.85	3.01	
	<i>Lupinus micranthus</i>	Fabaceae	47	1.73	2.82	
	<i>Dactylis glomerata</i>	Poaceae	55	1.69	2.75	
	<i>Plantago coronopus</i>	Plantaginaceae	34	1.67	2.72	
	<i>Aira cupaniana</i>	Poaceae	48	1.47	2.40	
	<i>Mentha pulegium</i>	Lamiaceae	34	1.46	2.38	
	<i>Cerastium arvense</i>	Caryophyllaceae	46	1.41	2.30	
	<i>Jasione crispa</i>	Campanulaceae	20	1.35	2.20	
	<i>Scirpus cernuus</i>	Cyperaceae	25	1.23	2.00	
	<i>Trifolium angustifolium</i>	Fabaceae	50	0.92	1.50	
	<i>Dactylorhiza sambucina</i>	Orchidaceae	72	0.88	1.44	
	<i>Ranunculus parviflorus</i>	Ranunculaceae	12	0.88	1.44	
	<i>Gaudinia fragilis</i>	Poaceae	60	0.74	1.20	
	<i>Thymus vulgaris</i>	Lamiaceae	10	0.74	1.20	
	<i>Bellis annua</i>	Asteraceae	56	0.69	1.12	
	<i>Filago gallica</i>	Asteraceae	55	0.68	1.10	
	<i>Silene ciliata</i>	Caryophyllaceae	53	0.65	1.06	
Total			1482	100	163	40

Soil classification

Soil Taxonomy: Xerorthent lithic; FAO: Lithosol

Location

It is located at 42° 18' 27" North, 3° 14' 14" East according to the Universal Transverse Mercator (UTM) geographic coordinate system.

Situation

Smooth sloping area with stony soils situated at 260 m asl anciently exploited for cereals and successively as rangeland. The soil profile has been excavated in a representative site with NE orientation and a slope around 18%. In the moment of description the soil was dry and well drained.

Soil profile (Figure 20)

- Ap (10 cm): Shallow horizon with humified organic matter mixed with mineral matter. Plastic. Stones of different shape and size are mixed with the fine fraction. The structure is granular/blocky and clods show resistance to pressure. Pores within soil particles are evident. Biological activity is detected. Consistent presence of fine and very fine roots is recorded. No reaction with HCl is observed. The limit with the underneath horizon is regular. The color of the Ap soil profile is dark yellowish brown (10YR/4/4) according to Munsell (1992).
- C/R (10-34 cm): Stony layer with fragmented slates not chemically altered. Fissures are filled with fine material migrated from the upper parts of the profile. Slow drained horizon.

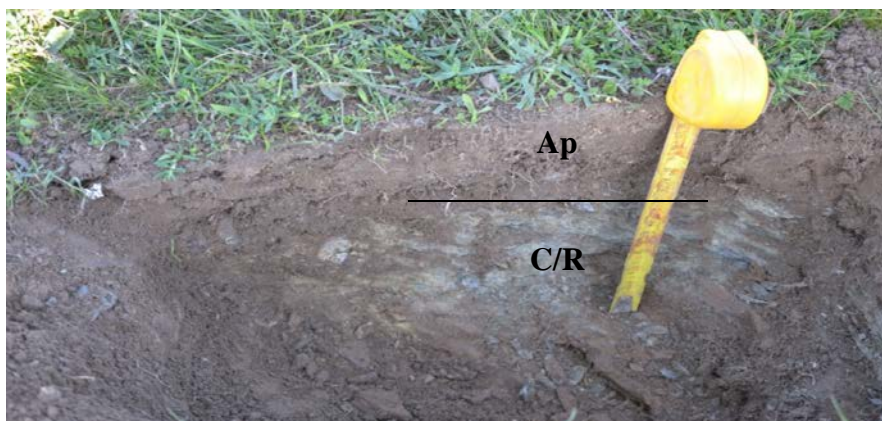


Figure 20. Example of Ap, C/R horizons typical of the soil environment under pasture (PR).

Comments

Abandoned pasture. Undergrowth vegetation mainly composed by *Lavandula stoechas*, *Dactylis glomerata*, *Ulex parviflorus*, *Thymus vulgaris*, *Rosmarinus officinalis*, *Brachipodium retusum*. No management is provided.

2.1.5.6. *Soils under Cistus scrub (MC environment)*

This area was agriculturally exploited during many decades and then abandoned. The area is dominated by *Cistus monspeliensis*, *Calicotome spinosa* and some patches of *Brachipodium retusum*, and was devastated by wildfire in 1988, 1990, 1994, and 2000, though is satisfactorily regenerated by different type of scrub (Figure 21).



Figure 21. General view of soils under *Cistus* scrub.

The Cistaceae associations have shown a noticeable ability to colonize abandoned areas devastated by wildfire (Trabaud, 2002). The high concentrations of aliphatic hydrocarbons (mainly turpentine and camphor oil) in *Cistus* scrubs may exert allelopathic effects on plants and animals (Darwish and Stewart, 1974), further increasing its persistence after wildfire (Pardini et al., 2004b). The representative monitored area of this soil environment was 0.5 ha, with 21% slope, 50% of plant canopy, and a soil profile 40 cm deep. The vegetation inventory is reported in Table 7.

Soil classification

Soil Taxonomy: Xerorthent lithic; FAO: Lithosol

Location

It is located at 42° 18' 11" North, 3° 14' 11" East according to the Universal Transverse Mercator (UTM) geographic coordinate system.

Table 7. Relative abundance of plant vegetation cover in MC soil environment.

Vegetation cover	Specie	Family name	Plants (N°)	Specie/ plant cover (%)	Occupied surface (%)	
					Under-growth	Bare soil
<i>Scrub</i>	<i>Cistus monspeliensis</i>	Cistaceae	85	33	34.00	
	<i>Cistus salviifolius</i>	Cistaceae	30	12	12.00	
	<i>Cistus populifolius</i>	Cistaceae	22	7	7.70	
	<i>Calicotome spinosa</i>	Fabaceae	74	14	14.80	
	<i>Genista anglica</i>	Fabaceae	24	6	6.00	
	<i>Genista scorpius</i>	Fabaceae	25	6	5.75	
	<i>Cistus crispus</i>	Cistaceae	19	6	5.70	
	<i>Genista pilosa</i>	Fabaceae	35	5	5.25	
	<i>Lavandula stoechas</i>	Lamiaceae	40	4	4.00	
	<i>Erica arborea</i>	Ericaceae	13	4	3.90	
	<i>Brachypodium retusum</i>	Poaceae	29	2	2.32	
<i>Herbaceous</i>	<i>Asparagus horridus</i>	Asparagaceae	20	2	1.60	
	Total		416	100	103	50

Situation

Sloping area with stony pavement frequently affected by fire, situated at 250 m asl anciently exploited for cereals and successively abandoned. The soil profile has been excavated in a representative site with NE orientation and a slope around 21%. In the moment of description the soil was dry and well drained.

Soil profile (Figure 22)

- Ap (14 cm): Shallow horizon with humified organic matter mixed with mineral matter. Low plasticity. Stones of different shape and size are mixed with the fine fraction. The structure is granular/blocky and clods show resistance to pressure. Pores within soil particles are evident. Biological activity is detected. Presence of fine and very fine roots is recorded. No reaction with HCl is observed. The limit with the underneath horizon is regular. The color of the Ap soil profile is brown (10YR/5/3) according to Munsell (1992).
- C/R (14-40 cm): Stony layer with fragmented slates not chemically altered. Fissures are filled with fine material migrated from the upper parts of the profile. Slow drained horizon.

Comments

Abandoned scrubs. Undergrowth vegetation mainly composed by *Cistus monspeliensis*, *Calicotome espinosa*, *Brachypodium retusum*. No management is

provided. Frequent wildfire occurrence during the last twenty years restricted the vegetation species.



Figure 22. Example of Ap, C/R horizons typical of the soil environment under *Cistus* scrub (MC).

2.1.5.7. Soils under *Erica* scrub (MB environment)

Terraced soils under *Erica* scrub (MB) are mainly covered by *Quercus coccifera*, *Erica arborea*, *Lavandula stoechas*, and *Brachipodium retusum*. This environment was not affected by fire since 1984 and showed the more ancient vegetation (Figure 23). The representative area selected in this soil environment was 0.5 ha, with 17% slope, 55% of plant canopy, and a total soil depth of 40 cm. The vegetation inventory conducted by delimiting an area of 10×10 m is reported in Table 8.

Soil classification

Soil Taxonomy: Xerorthent lithic; FAO: Lithosol

Location

It is located at 42° 17' 55" North, 3° 14' 25" East according to the Universal Transverse Mercator (UTM) geographic coordinate system.

Situation

Sloping area with terraces not frequently affected by fire, situated at 230 m asl, anciently exploited for cereals and successively abandoned. The soil profile has been excavated in a representative site with NE orientation and a slope around 17%. In the moment of description the soil was dry and well drained.



Figure 23. General view of soils under *Erica* scrub environment.

Table 8. Relative abundance of plant vegetation cover in MB environment.

Vegetation cover	Specie	Family name	Plants (N°)	Specie/ plant cover (%)	Occupied surface (%)	
					Under-growth	Bare soil
Tree	<i>Quercus coccifera</i>	Fagaceae	1	1.14	1.50	
Scrub	<i>Erica arborea</i>	Ericaceae	85	38.68	51.00	
	<i>Erica scoparia</i>	Ericaceae	55	12.51	16.50	
	<i>Cistus monspeliensis</i>	Cistaceae	35	7.96	10.50	
	<i>Pistacia lentiscus</i>	Anacardiaceae	26	7.89	10.40	
	<i>Calicotome spinosa</i>	Fabaceae	28	6.37	8.40	
	<i>Lavandula stoechas</i>	Lamiaceae	27	6.14	8.10	
	<i>Helichrysum stoechas</i>	Asteraceae	25	4.74	6.25	
	<i>Rosa canina</i>	Rosaceae	22	4.17	5.50	
	<i>Brachypodium retusum</i>	Poaceae	35	6.64	8.75	
Herbaceous	<i>Sedum sediforme</i>	Crassulaceae	21	2.39	3.15	
	<i>Genista pilosa</i>	Fabaceae	12	1.37	1.80	
Total			372	100	132	45

Soil profile (Figure 24)

- Ap (24 cm): Horizon with humified organic matter mixed with mineral matter. Considerable plastic. Stones of different shape and size are mixed with the fine fraction. The structure is granular/blocky and clods show resistance to pressure. Pores within soil particles are evident. Biological activity is detected. Presence of fine and very fine roots is recorded. No reaction with HCl is observed. The limit with the underneath horizon is regular. The color of the Ap soil profile is brown (10YR/4/3) according to Munsell (1992).
- C/R (24-40 cm): Stony layer with fragmented slates not chemically altered. Fissures are filled with fine material migrated from the upper parts of the profile. Slow drained horizon.

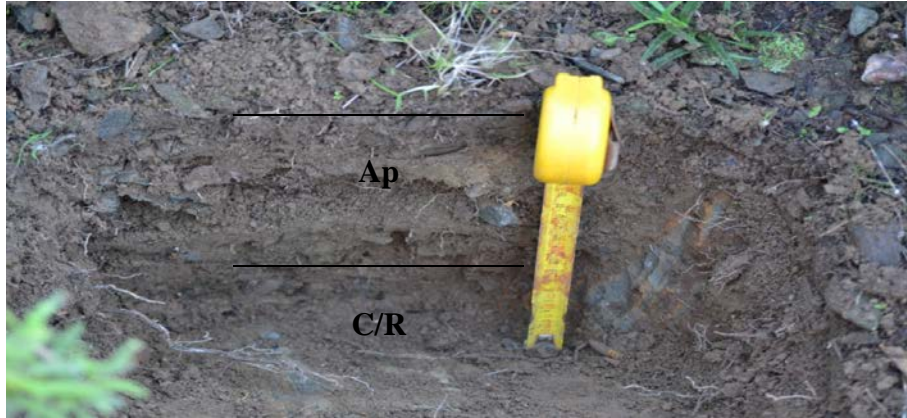


Figure 24. Example of Ap, C/R horizons typical of the soil environment under *Erica* scrub (MB).

Comments

Abandoned scrubs. Undergrowth vegetation mainly composed by *Erica arborea*, *Quercus coccifera*, *Brachipodium retusum*. No management is provided. The vegetation succession after abandonment has allowed a more heterogeneous number of species.

2.1.6. Physiographical and pedological characteristics of the soils under study

The overall physiographical and pedological characteristics for each selected soil environment are explained in the Table 9.

Table 9. Physiographical and pedological characteristics of the selected soil environments.

ENV	UTM	Textural class	Plant cover (%)	Surface (ha)	Slope (%)	Or	Horizon	Soil depth (cm)
V	42°18'36"N, 3°12'42"E	Loamy sand	6	0.5	15	NE	Ap, C/R	52
O	42°18'33"N, 3°13'16"E	Sandy loam	15	0.5	18	NE	Ap, C/R	45
S	42°18'34"N, 3°13'22"E	Sandy loam	60	0.5	15	NE	Ap, C/R	30
PI	42°18'23"N, 3°13'28"E	Sandy loam	70	0.5	18	NE	Ao, A, Bw, C/R	40
PR	42°18'27"N, 3°14'14"E	Sandy loam	60	0.5	18	NE	Ap, C/R	34
MC	42°18'11"N, 3°14'11"E	Sandy loam	50	0.5	21	NE	Ap, C/R	40
MB	42°17'55"N, 3°14'25"E	Sandy loam	55	0.5	17	NE	Ap, C/R	40

ENV: Soil environments; UTM: Universal Transverse Mercator geographic coordinate; Or: Orientation; V: Soils under vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub; NE: Northeast.

2.2. Experimental layout

Relevant soil parameters have been determined in the field and laboratory. In order to follow the soil dynamics in the selected environments we determined soil physicochemical and biological parameters on a monthly basis and the erosion processes surveyed at any rainfall events generating runoff.

2.2.1. Field physical analyses

2.2.1.1. Survey of erosion processes

Principle:

Soil degradation and erosion is the vulnerability of soil surface to anthropic or natural stresses. The susceptibility of soil to degradation and erosion is conceptually accepted as a decrease in soil quality because of soil and nutrient loss. To evaluate soil and nutrient loss a survey of eroded soil materials and sediment yields must be carried out. Soil erosion was estimated by monitoring of runoff and sediment yields after each rainfall by using erosion plots Gerlach types (Gerlach, 1976; Pardini et al., 2004a; Pardini and Gispert, 2006) installed at each environment.

Used materials and equipments:

- Gerlach plot
 - Erosion plots Gerlach type (Figure 25) were installed in each environment taking into account the orientation, vegetation cover, and slope.
 - Each plot was equipped with a central unit (tank) of 45 liters capacity armed with two lateral strips adjusted over the soil bordering the terrace and coupled with a plastic container of 10 liters capacity.
 - Water runoff and sediments are moved up slope and directed to the tank by the two lateral open strips.
 - The contributing area of each open plot was established as 36 m², considering the width of the two strips and the tank (4.5 m) and the length of the terrace (8 cm).
- Graduated cylinder 1000 ml.
- Bottles of 20 liters equipped with funnel of 33 cm diameter (rainfall gauges).

- Plastic cups (7×5 cm) for splash erosion.

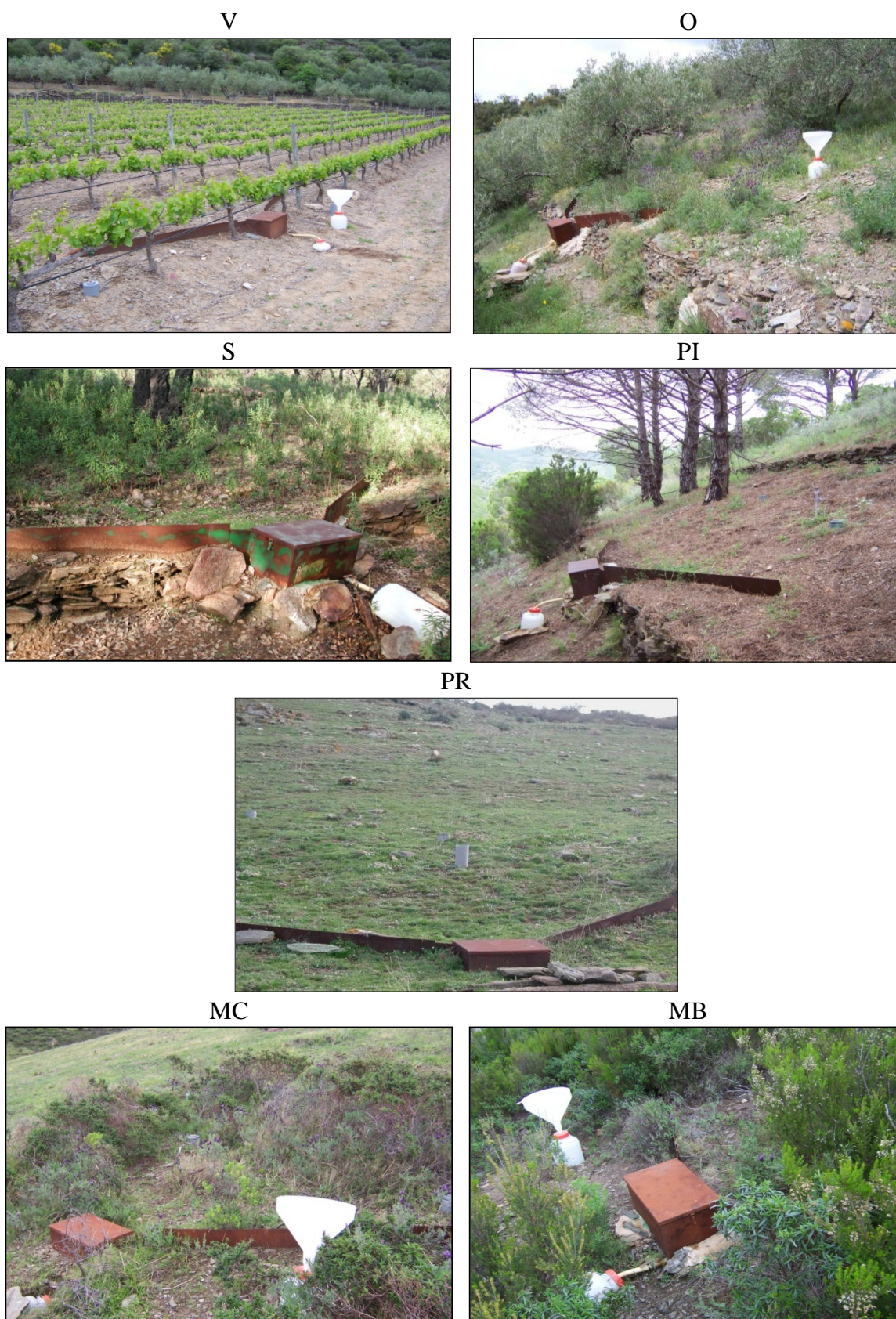


Figure 25. Erosion plots Gerlach types installed in all soil environments.

Procedure:

- Survey of water runoff and eroded materials:
 - Water runoff in the tank was measured immediately at field by using a graduated cylinder of 1000 ml. Samples of water runoff were also collected.
 - The eroded soil deposited in the bottom of the tank was collected, transferred to the laboratory, air dried, and weighed.
 - Runoff water and soil finer particles were also collected in the plastic container of 10 liters capacity, which was transported to the laboratory to decant and successively quantify both the water volume and soil passed into the plastic container after drying.
 - Total water runoff and eroded soil were then calculated at the plot scale and expressed as l m^{-2} and g m^{-2} respectively.
 - Samples of water runoff and eroded soil were stored for subsequent analyses of dissolved organic carbon (DOC) and nitrogen (DN) and eroded organic carbon (EOC) and nitrogen (EN).
- Rainfall gauges:
 - Rainfall was measured by using bottles of 20 liters equipped with funnel of 33 cm diameter installed at each plot in each environment.
 - Rainfall was measured immediately at field by using a graduated cylinder of 1000 ml, and the amount calculated with respect to the funnel area and expressed as l m^{-2} .
- Survey of splashed materials
 - Splash erosion was collected at each plot equipped at least with three splash cups (7×5 cm) to collect the splashed materials during rainfall events. Splash erosion was expressed as g m^{-2} .
- Runoff coefficient was calculated by using the runoff volume (l m^{-2}) with respect to the rainfall amount (l m^{-2}).
- Sediment concentration (the concentration of the eroded sediments in runoff volume) was also calculated and expressed as g l^{-1} .

2.2.1.2. *Mechanic impedance (MI)*

Principle:

The mechanic impedance was measured with a static penetrometer to evaluate the potential resistance of soil surface to vertical stresses causing crusting and sealing. The procedure is based on applying a constant force with the penetrometer to a depth of 1 cm using a flat-end tip of 0.6 cm upon soil surface at different sites.

Used materials and equipments:

- Static soil penetrometer UFL-031 Tenzaghi equipped with different heads for different type of texture (Figure 26).

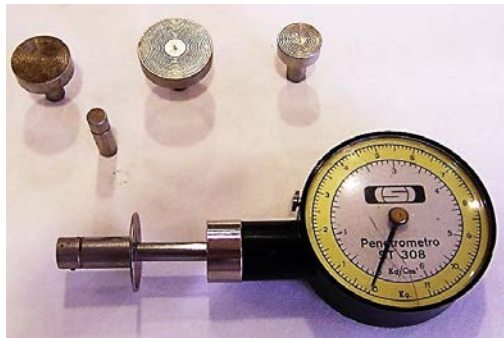


Figure 26. Static penetrometer Tenzaghi.

Procedure:

- The penetrometer equipped with the adequate head is posed on the soil surface vertically previous 0 reset.
- Soon after the apparatus is manually pressed into the soil until the rod is penetrated 1 cm and the value (kg cm^{-2}) is read on the manometer.
- The results were converted in kPa by the following equation;

$$1 \text{ kPa} = \frac{1 \text{ kg}}{\text{cm}^2} \times 98$$

2.2.1.3. *Shear strength (SS)*

Principle:

The shear strength is based on the soil resistance potential to lateral physical stresses (Das, 2008). Soil shear strength was measured by using Eijkelkamp pocket vane tester. The pocket vane tester is supplied with three vanes CL 101, CL 100, and

CL 102 with a measuring range of 0-0.2, 0-1, and 0-2.5 kg cm⁻² respectively (Figure 27).



Figure 27. Shear strength vane tester.

The values of shear strength vane tester were obtained in kg cm⁻² by using the graph showed in Figure 28 where the values read in the apparatus cadran are converted in kg cm⁻² depending on the vane type used. When one complete revolution is made with the apparatus (0-10 cadran) the maximum scale value is the conversion factor as shown in Table 10. Later the conversion of this unit to kPa is carried out.

Table 10. Maximum conversion values when 1 complete revolution has been made with each of the three vane type.

Vane type	Conversion factor (CF) (kg cm ⁻²)
CL102	2.734
CL100	1.0936
CL101	0.2186

Used materials and equipments:

- Eijkelkamp pocket vane shear strength tester Model: ASTM D 2573.

Procedure:

- The tester is equipped with an axle loaded with the vane type CL 100 used along the experiments.
- The pointer is dragged and the tester reset at 0 in the cadran.
- The blades of the vane CL 100 are inserted vertically in soil surface.
- The head of the tester is turned horizontally until the vane sheared the penetrated soil portion.
- The value of the cadran is read from the pointer and introduced in the graph in Figure 28 and converted in kg cm⁻², then in kPa.

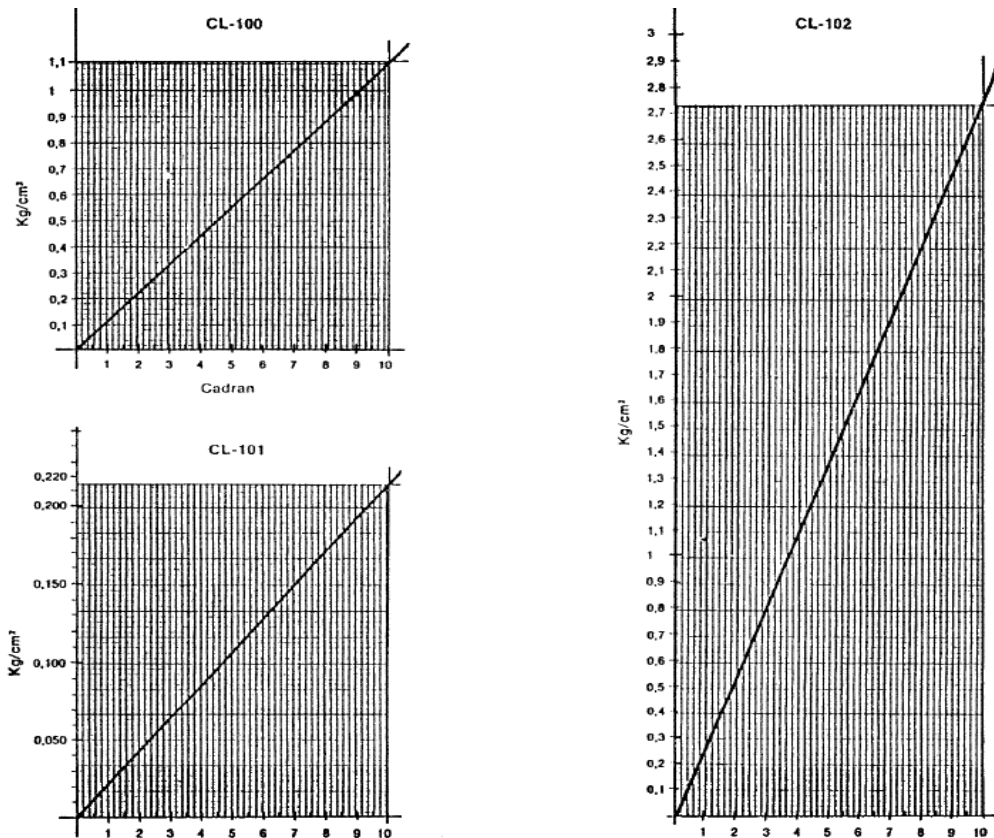


Figure 28. Graphs used to convert cadran values in kg cm^{-2} according to the used vane.

2.2.1.4. Soil infiltration rate (IR)

Principle:

The infiltration is the vertical water entry into the soil from the surface. The intensity of this process is called the infiltration rate (IR). The infiltration rate is expressed in terms of height of water entering the soil per unit of time (mm h^{-1}). This parameter can be measured directly in the field over a selected area, using the ring infiltrometer. The water infiltrating into an unsaturated soil is influenced by both capillary pressure and gravity forces, and may be forced to move more laterally than vertically. Therefore, in order to reduce this effect, the area between the two rings of the double-ring infiltrometer (Figure 29) is previously wetted to act as a “buffer zone” and measurements are only carried out in the inner ring to calculate the infiltration rate (Dunjó, 2004).

Used materials and equipments:

- Double ring infiltrometer Eijkelkamp, composed by one ring of 30 cm diameter and one ring of 40 cm diameter as shown in Figure 29.

- Scaled (cm) rod placed in the middle of the 30 cm Ø ring.
- Hammer and chronometer.

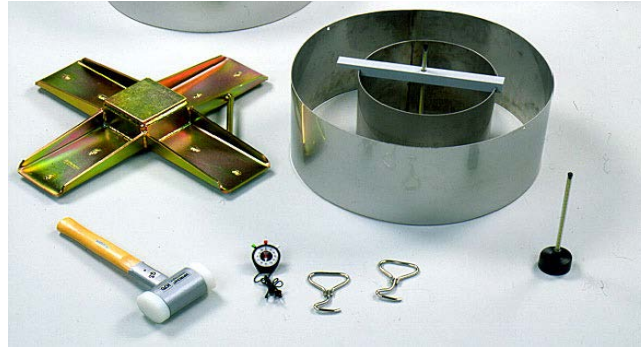


Figure 29. Eijkelkamp double ring infiltrometer.

Procedure:

- The infiltration rings were inserted 10 cm into the soil surface and the water was first added to the outer part of the 30 cm infiltration ring to ensure vertical water flow when infiltration trial would be carried out.
- The rod to count the height of water infiltrating into the soil is then adjusted to the infiltration ring (30 cm).
- As soon as water is added into the smaller ring the rod shifts up to water surface and starts lowering slowly according to the speed of water infiltration. In the rod it is possible to read the centimeters of water infiltrated, and the time for each cm may be recorded by a chronometer.
- Infiltration rate was calculated as (mm h^{-1}) from the following equation;

$$\text{IR} = \frac{\Delta Q}{A \times \Delta t}$$

where, IR is the infiltration rate (mm h^{-1}), ΔQ is the amount of water (l) at each infiltrated centimeter, A is the area of soil surface inside the infiltration ring (m^2), and Δt is the time interval of infiltration (h) at each centimeter.

2.2.1.5. *Soil hydraulic conductivity (k)*

Principle:

Soil hydraulic conductivity is the capacity of soil to conduct water into the soil, thus it reveals the dynamics of water distribution into the profile. It means the

replacement by water of air space in the soil. Soil hydraulic conductivity is measured by using the Mini-Disk infiltrometer (Zhang, 1997).

Used materials and equipments:

- A Decagon's handheld Mini-disk infiltrometer, 2.0 cm suction was used (Figure 30). The device is composed of polycarbonate graduated cylinder with a semi-permeable disk on the bottom in order to permit the exchange of air soil with water. A small holed rod which regulates the suction speed is connected at the base of the cylinder. The top of the cylinder is closed by a rubber after adjusting to 0 the total volume of water.



Figure 30. Decagon Mini-disk infiltrometer, 2.0 cm suction.

Procedure:

- At time zero, the infiltrometer is posed on soil surface assuring that the base makes complete adhesion to soil surface.
- The volume of water suctioned by the soil at the 2 cm suction constraint is recorded at fixed time intervals (every 30 seconds).
- The height of infiltrated water was calculated as the centimeters of water infiltrated into the soil through an area of 7.942 cm² at each time interval.
- The cumulative infiltration was then plotted against the square root of time.
- A polynomial regression is obtained (Figure 31).

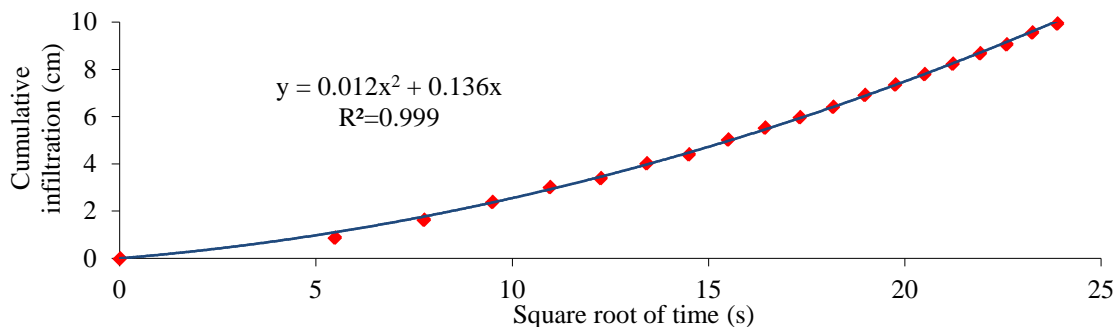


Figure 31. Example of a representative polynomial equation between cumulative infiltration (cm) and square root of time (s) when the Decagon Mini-disc infiltrometer 2.0 cm suction was used in soils under vines.

- The hydraulic conductivity cm s^{-1} is calculated as follow;

$$k = C_1/A$$

where, k is the hydraulic conductivity (cm s^{-1}), C_1 is the slope of the polynomial equation, and A is calculated according to the type of the mini-disk infiltrometer [0.5, **2.0** and 6.0 cm] suction and the Van Genuchten parameters of the textural class listed in the Table 11.

Table 11. The Van Genuchten parameters of Carsel and Parrish (1988) for 12 classes of soil texture.

Textural Class	α (cm^{-1})	n	A	A	A
			ho=0.5 cm	ho=2.0 cm	ho=6.0 cm
Sand	0.145	2.68	5.4	8.9	33.3
Loamy sandy	0.124	2.28	4.7	5.8	10.0
Sandy loam	0.075	1.89	6.3	5.2	5.1
Loam	0.036	1.56	6.8	6.0	4.1
Silt	0.016	1.37	10.2	9.3	7.2
Silt loam	0.020	1.41	9.1	8.1	6.0
Sandy clay loam	0.059	1.48	3.7	2.8	1.3
Loamy clay	0.019	1.31	7.4	6.5	4.7
Silty clay loam	0.010	1.23	10.3	9.5	7.8
Sandy clay	0.027	1.23	4.0	3.3	1.9
Silty clay	0.005	1.09	8.1	7.7	6.8
Clay	0.008	1.09	5.2	4.9	4.0

n and α : The van Genuchten parameters for the soil; A: Value relating the van Genuchten parameters for a given soil type to the suction rate and radius of the infiltrometer disk; ho: The suction at the disk.

2.2.1.6. Soil temperature

Principle:

Soil temperature plays an important role in many processes which take place in the soil such as chemical reactions and biological interactions (Davidson et al., 1998). Soil temperature varies in response to exchange processes that take place primarily through the soil surface. Soil surface temperature was measured by non-contact laser thermometer (Xu et al., 2004).

Used materials and equipments:

- Mini-Temp Comark Infra-Red Thermometer w/Laser Sighting, Model MTSFU with backlit LCD display and a hold function to freeze the display reading (Figure 32). Its measurements range is from $-30\text{ }^{\circ}\text{C}$ to $200\text{ }^{\circ}\text{C}$. It is pocket sized and easy to use.

Procedure:

- The thermometer has laser sighting to pinpoint the target area.
- The thermometer was used at a distance of 30-50 cm from soil surface and vertically oriented to soil surface.
- The values of soil temperature were immediately displayed at the backlit LCD display and recorded.
- The measurements were performed at different sites in each environment in order to represent a mean soil surface temperature.



Figure 32. Comark Infra-Red Thermometer w/ Laser Sighting.

2.2.2. Field biological analyses

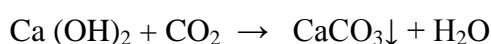
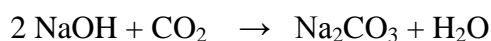
2.2.2.1. Soil respiration

Principle:

Soil respiration is the expression of CO₂ emission from soil. According to the world wide extraordinary emission of soil carbon to the atmosphere, estimations of soil carbon efflux from soil may be an indication of its contribution to global change. Different methods have been tested in field and laboratory studies to evaluate this contribution. In the studied soil environments both a static and a dynamic method were used for the estimation of CO₂ flux from surface soil horizon. The static soda lime method was used for both field and laboratory measurements whilst an Infrared Gas Analyzer apparatus for dynamic field measurements of CO₂ concentration in air and at different levels of soil depth was used. Measurements of carbon dioxide fluxed from soils were carried out periodically in the field and laboratory during 2008 and 2009.

*2.2.2.1.1. Field estimation of CO₂ by soda lime method (SLF)**Principle:*

Soda lime is commonly used as a desiccant because of its ability to absorb water vapor from the air and able to loss the water in a reversible reaction by oven drying. Water is molecularly required to form Sodium and Calcium carbonates, its formation recorded by an increase of soda lime weight. Because of its alkaline properties soda lime removes carbon dioxide very efficiently from the atmosphere according to these reactions:



Soda lime absorbs carbon dioxide and water resulting from soil respiration but only CO₂ is chemically bounded with soda lime after oven drying. Carbon dioxide respiration can be estimated by the Edwards method (Edwards, 1982) modified by Grogan (1998) and Keith and Wong (2006).

Used materials and equipments:

- Oven C. R. MARES, S. A. Model: 203.
- Glass cups for soda lime.
- PVC cylinders called Cover Box or Chambers having 11 cm diameter and 10 cm height, covering a soil surface of 0.0095 m² with tight screw lids were installed in each soil environment.
- Digital analytical balance, 4 digits (Mettler A. E. 100).

Used solutions and chemicals:

- Soda lime containing: 75% Calcium hydroxide [Ca (OH)₂], 20% water [H₂O], 3% sodium hydroxide [NaOH], and 1% potassium hydroxide [KOH]. It was used in granular form. It absorbs carbon dioxide in the presence of water vapor.

Procedure:

- Three PVC cylinders were inserted 5 cm into the soil at a distance of 15 meters one another in each soil environment under study.

- The soda lime had been previously prepared in the laboratory as follows: 15 g of pure soda lime were placed in a glass cup, oven dried at 105 °C overnight, cooled in the desiccator, and weighed with the accuracy of 0.01 mg.
- Cups were soon hermetically closed and stored.
- At field site, the cups were opened and immediately posed on the soil surface (0.0095 m^2) inside each cover box, and the upper screw top lid of the cover box immediately closed.
- Generally this operation was done in the time interval between 13:00 and 15:00 p.m.
- After 24 h the lid of each cover box was opened and the soda lime cups were immediately hermetically closed and collected.
- Later on cups were transferred to the laboratory, opened, oven dried at 105 °C for 12 h, cooled in a desiccator and then weighed with the accuracy of 0.01 mg.
- The weight of carbon dioxide absorbed by soda lime was calculated by multiplying the weight gain after oven drying by 1.69 as a correction factor. As observed each mole of CO_2 chemically bound to soda lime favors the formation of a mole of water, which is then lost by oven drying. Therefore dry mass increase before and after exposure underestimates CO_2 absorbed. The correction factor takes into account that 44 g of CO_2 react with 74 g of $\text{Ca}(\text{OH})_2$ to form 100 g CaCO_3 and 18 g H_2O . The measured increase in soda lime after oven-drying is 26 (i.e. $100-74$). Thus the correction factor of $44/26$ (i.e. 1.69) must be applied to the measured mass differences in order to obtain the true value of CO_2 absorbed (Grogan, 1998; Keith and Wong, 2006; Emran et al., 2012a).
- The weight of CO_2 absorbed by soda lime has been calculated with respect to the soil surface area inside the cylinder as following: $\text{mg CO}_2 \text{ m}^{-2} = \text{weight difference of soda lime} \times 1.69 / \text{soil surface area (m}^2\text{)}$.
- Carbon dioxide was also calculated as $\mu\text{mole CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ considering the soil surface inside the cylinder, the incubation time, and the molecular weight of CO_2 .
- C- CO_2 loss was also obtained by multiplying the CO_2 values by the 12/44 ratio being 12 the molecular weight of Carbon and 44 the molecular weight of CO_2 .

*2.2.2.1.2. Field estimation of CO₂ by infrared gas analyzer method (IRGA)**Principle:*

IRGA has also been used for measuring carbon dioxide flux from soil surface at the same sites of the installed cover boxes, in order to compare the results of the two methods in the field. IRGA is a portable infrared gas analyzer allowing dynamic CO₂ measurements. This apparatus has been designed to provide continuous monitoring of gases including carbon dioxide. The equipment is composed of a newly developed miniaturized Non-Dispersive Infrared (NDIR) sensor (Figure 33A) measuring CO₂ concentration directly in parts per million (ppm). IRGA PGM-54 Multi-Gas Analyzer is adapted to measure the carbon dioxide and is calibrated at the level of soil CO₂ efflux. It was tried in the field in three different places (at the same distance of the cover boxes) for each soil environment at two soil levels: at the soil surface and at 5 cm depth soil holes, and in the air.

Used materials and equipments:

- MultiRAE IR Monitor PGM54 (IRGA) from RAE Systems Inc., Sunnyvale, CA.

The apparatus includes an integrated sampling pump, a diaphragm pump providing about 300 cm³ minute⁻¹ flow rate. When the sampling pump is aspirating telluric air from the soil, infrared light is shone through the gas sample and the amount absorbed by the CO₂ is proportional to its concentration. The patented PID (Photoionization detector) uses an electrodeless discharge UV lamp as a high energy photon source to ionize the gas to be measured. The resultant electrical current is proportional to the gas concentration (Figure 33A). Previous calibration of the CO₂ sensor must be executed before any set of measurements. Generally it is zero calibrated with isobutylene (supplied by RAE) which is free of CO₂. Next, the CO₂ span gas is supplied, typically 5000 ppm, to calibrate the upper level of CO₂ detectable. The multigas analyzer is easily portable in the field, reaching constant and reproducible CO₂ values from air and soil air, even at different depth. It has 12 hours of continuous operation with a rechargeable Li-ion battery. The data storage capacity is up to 20,000 readings which can be downloaded to PC through RS-232 link. Moreover it can work in very extreme conditions as its temperature and relative humidity

working ranges are $-20\text{ }^{\circ}\text{C}$ to $45\text{ }^{\circ}\text{C}$ and 0% to 90% respectively. The MultiRAE IR monitor gives real time measurements and activates alarm signals whenever the exposure exceeds preset limits of calibration.

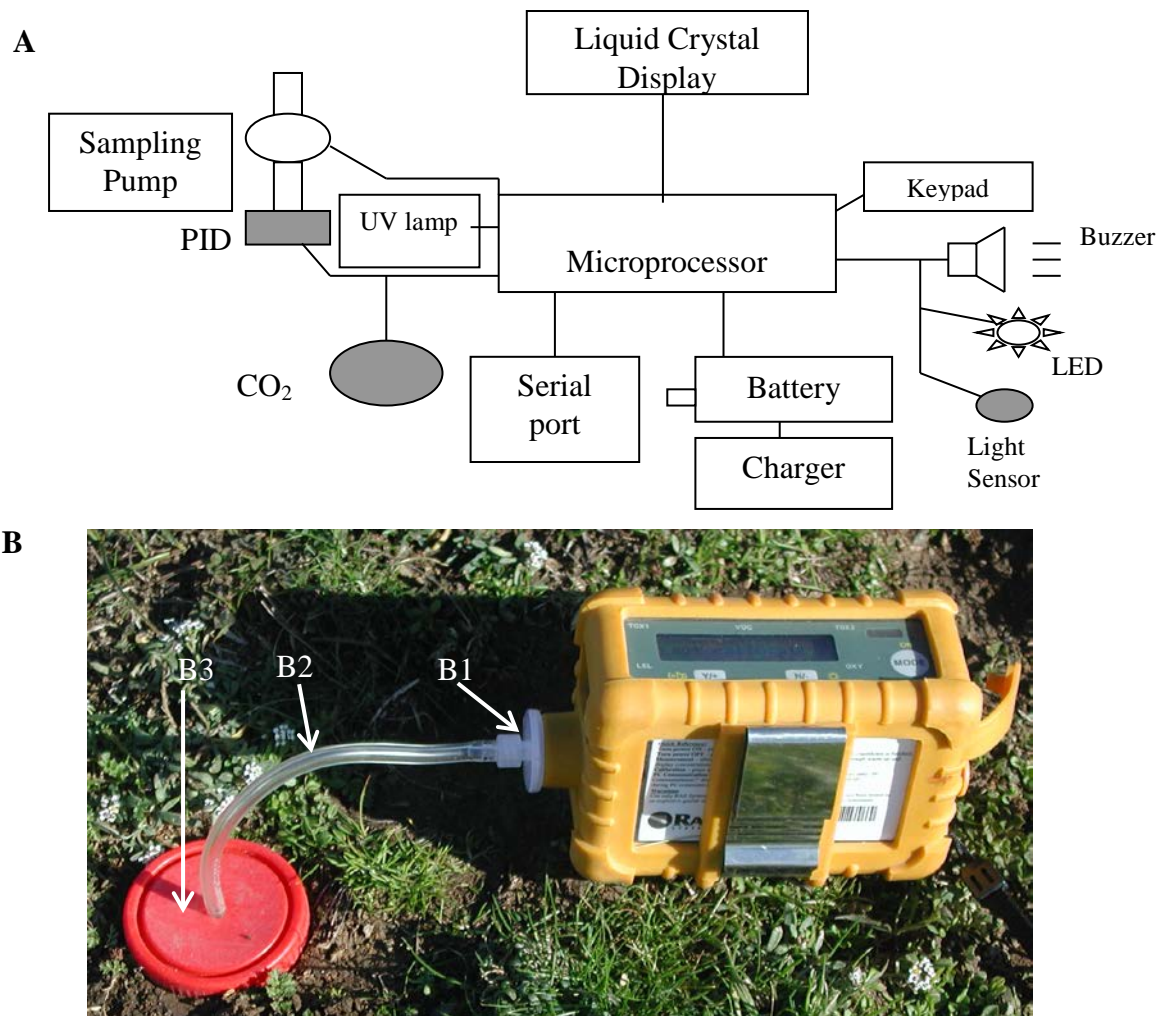


Figure 33. A) Schematic diagram of the MultiRAE IR; B) The apparatus at field. Accessories devices: B1, water and dust filter; B2, hose; B3, lid (chamber) to contact soil surface.

Procedure:

- Prior to use the battery of the apparatus must be fully charged.
- At field a filter was connected to the apparatus for retaining water and dust during measurements. A hose (0.5 cm internal diameter) was then assembled both to the filter and to the lid device (i.e. the chamber) having 10 cm diameter and 5 cm height being hermetically connected to the soil surface (Figure 33B).
- With the lid inserted a few mm into the soil surface the apparatus may provide flow rate and measure a volume of air gas emitted from the soil surface.

- Measurements were performed in the field in three different places (parallel to soda lime measurements sites) for each environment (at 15 m distance one another) between 13:00 and 15:00 p.m.
- The measurement of CO₂ in the aspirated soil air lasted until the concentration of CO₂ (expressed as ppm) was constant in the digital screen of the apparatus. Generally, this happened after 5 minutes.
- Values from IRGA measurements were reported as $\mu\text{mole CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ according to the following expression:

$$\text{CO}_2\text{flux} = (\Delta_c) \frac{Q}{V_{\text{air}} A}$$

where,

Δ_c is the difference between the air and soil chamber CO₂ concentration ($\mu\text{mole mole}^{-1}$) respectively.

Q is the volume flow rate through the chamber ($\text{m}^3 \text{ s}^{-1}$).

A is the soil surface area covered by the chamber in contact with the soil

V_{air} is the molar volume of air (moles m^{-3}).

- C-CO₂ loss was also obtained by multiplying the CO₂ values by the 12/44 ratio being 12 the molecular weight of Carbon and 44 the molecular weight of CO₂ (Emran et al., 2012a).

2.2.3. Laboratory physical analyses

Soil samples were collected at 0-15 cm depth, at three different locations from each soil environment for representative results. Organic residues and stones in soil samples were removed. In the laboratory, soil samples were air dried at room temperature, mixed and sieved at 2.00 mm diameter by using C.I.S.A. mesh apparatus. Soil samples were kept at room temperature in plastic pots until use. Portions of soil samples were used to obtain aggregates in the 0.25-2.00 mm and 2.00-5.60 mm classes for subsequent analysis. All samples were collected on monthly basis.

2.2.3.1. *Soil texture**Principle:*

The soil textural classes have been determined by using Robinson's pipette that is based on the sedimentation of soil particles by gravity. The dimensional classes are: 2000-20 μm for sand fraction, 20-2 μm for silt fraction, and <2 μm for clay fraction according to the International Society of Soil Science (ISSS). The recovery of aliquot at a given depth and time makes it possible to collect a specific class of particles when all the particles bigger than the selected diameter have decanted. The method is based on the Stoke's Law equation (Stokes, 1851) to determine the velocity of settling soil particles as follow:

$$v = \frac{2 g r^2 (d_s - d_w)}{9 v_w}$$

where, v = velocity of settling particle (cm s^{-1}).
 g = acceleration of gravity (cm s^{-2}).
 d_s = density of soil particle (g cm^{-3}).
 d_w = density of water (g cm^{-3}).
 v_w = viscosity of water ($\text{g cm}^{-1} \text{s}^{-1}$)
 r = radius of spherical particles (cm).

Used materials and equipments:

- Oven C. R. MARES, S. A. Model: 203.
- Digital balance (GF-1200).
- Rotating shaker.
- Robinson's pipette.
- Beakers 1000 ml.
- Glass cylinders 1000 ml.
- Plastic bottles 1000 ml.
- Sieves of 0.2 mm diameter.

Used chemicals and solutions:

- Sodium polyphosphate 5% $[(\text{NaPO}_3)_n]$.
- Hydrogen peroxide 35% $[\text{H}_2\text{O}_2]$.

Procedure:

- 20 g of air dried sieved soil were placed in a beaker of 1000 ml and wetted with some drops of water.
- 30 ml of 35% hydrogen peroxide were added in order to oxidize the organic carbon.
- Samples were heated at 80 °C for two hours while 10 additional ml of H₂O₂ were added after one hour from the beginning of heating.
- After reaction the suspensions were transferred into a 1 liter plastic bottle and 15 ml of sodium polyphosphate 5% were added.
- The plastic bottles were shaken in the rotating shaker overnight.
- After shaking, the content was filtered through a 0.2 mm diameter sieve contained in a Buchner funnel and the suspension collected into a glass cylinder of 1000 ml.
- Particles >0.2 mm remained in the mesh were accurately washed, oven dried at 105 °C for 12 hours, and then weighed to calculate the percentage of coarse sand [SG] fraction of 0.2-2 mm.

The SG fraction was calculated as following:

Coarse sand [SG]% = weight of particles >0.2 mm (g) × 100 / initial soil amount (g).

- The suspension containing particles <0.2 mm was vigorously agitated and then posed in a stable place for 4.48 min. Soon after 20 ml of the suspension were sampled with a Robinson's pipette at 10 cm depth from the surface.
- This volume (20 ml) was posed in appropriate cup, oven dried at 105 °C and weighed to estimate the silt (L) and clay (A) content corresponding to the fractions 0.02-0.002 and <0.002, which were found at 10 cm depth after 4.48 minutes of sedimentation. The L+A fraction was calculated as following:

[L + A] content = 1st weight (g) of particles after 4.48 min × 1000 / volume (20 ml)

[L + A]% = [L + A] (g) × 100 / soil (g)

- The suspension was again vigorously shaken and sampling of 20 ml suspension was repeated at 10 cm depth after 8 hours previous agitation.
- The second 20 ml allowed estimation of the clay content (A), fraction <0.002 mm

The A fraction was calculated as following:

A content = 2nd dry weight (g) of particles after 8 h × 1000 / volume (20 ml)

Clay [A]% = [A] (g) × 100 / soil (g)

Silt [L]% = [L + A]% – A%

Finally fine sand was calculated by difference with respect to the fractions

$$\text{Fine sand [SF]\%} = 100 - (\text{SG\%} + \text{L} + \text{A\%})$$

$$\text{Sand fraction\%} = \text{coarse sand} + \text{fine sand}$$

- The soil texture was determined for each environment from the soil texture triangle (Figure 34).

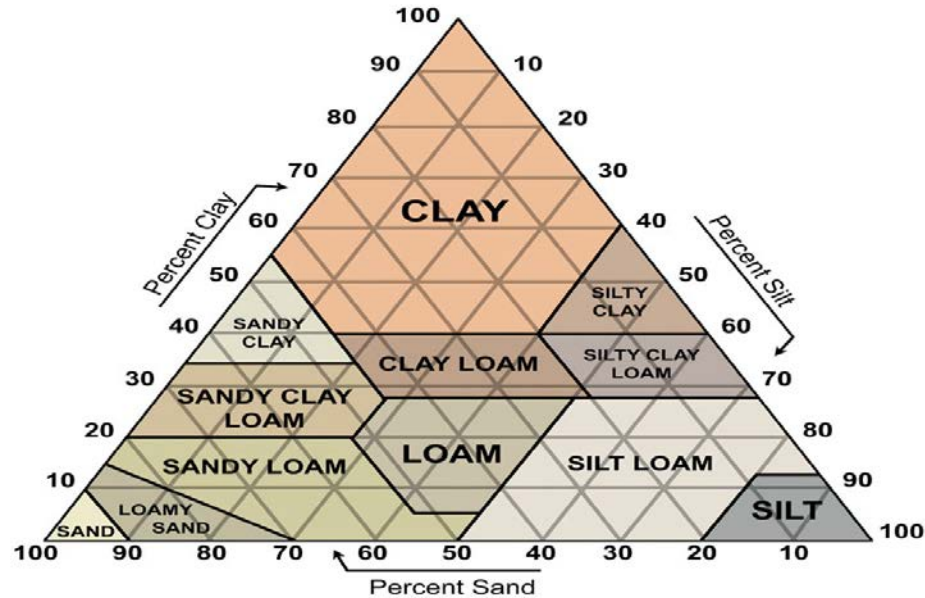


Figure 34. Triangle of soil texture to calculate the textural class.

2.2.3.2. Soil bulk density (BD) and soil moisture (SM)

Principle:

Bulk density is the weight of soil per unit volume, usually given on an oven dry (105 °C) basis. Soil moisture represents the water content within the soil profile. Bulk density and soil moisture were determined by the core method by using 5×5 cm stainless cylinders according to Porta et al. (1994).

Used materials and equipments:

- 5×5 cm stainless steel cylinder, hummer and trowel.
- Oven C. R. MARES, S. A. Model: 203 and digital balance (GF-1200).

Procedure:

- The stainless steel cylinder 5×5 cm was completely inserted into soil from surface by using a hummer with accuracy to do not alter soil structure.

- The cylinder was removed from site and cut at the edges to maintain the volume of the cylinder.
- The two edges of the cylinder were covered with their lids and the sample identified and transferred to laboratory
- In laboratory, one lid was removed and the device weighed with the soil for the total fresh weight (soil + cylinder + the base lid)
- Soil samples was then oven dried at 105 °C for 24 hours and then weighed
- Soil moisture (SM) and bulk density (BD) were calculated as follows;

$$SM (\%) = \frac{\text{Fresh soil (g)} - \text{Dried soil (g)}}{\text{Dried soil (g)}} \times 100; BD (g \text{ cm}^{-3}) = \frac{\text{Dried soil (g)}}{\text{Soil volume (cm}^3\text{)}}$$

2.2.3.3. Water stable aggregates (WSA)

Principle:

Aggregate stability is a measurement of the resistance of natural aggregates (ped) to disruptive forces of water. Water aggregate stability is the stability of soil aggregates against the water capillary forces. It is the mass of aggregated soil remaining after wet sieving as a percent of the total mass of soil used. Measurements of WSA were determined following Kemper and Rosenau (1986).

Used materials and equipments:

- Wet sieving apparatus Model: 08.13 by Eijkelkamp Inc. (Figure 35).
- Sieves of 0.25 mm and 2.00 mm diameters (Figure 35).
- Oven C. R. MARES, S. A. Model: 203 and digital balance (GF-1200).



Figure 35. Eijkelkamp wet sieving apparatus and sieves.

Procedure:

- Aliquots of air dried aggregates were placed in a 0.25 mm sieve (the 0.25-2.00 mm aggregate fraction) and in a 2.00 mm sieve (the 2.00-5.6 mm aggregate fraction).
- Soil aggregates were subjected to vertical oscillatory sieving during 3 minutes cycling of immersion-emersion in cans of deionized water.
- One immersion-emersion cycle lasted 1.67 seconds, with a total of 102 cycles in which the aggregates are subjected to the dispersive forces of water.
- Soil aggregates surviving disruption and detachment were dried at 105 °C and weighed.
- The stability of aggregates to water (WSA) was calculated for each fraction from the following equation taking into account the sand content;

$$\text{WSA (\%)} = \frac{M(a + s) - M_s}{M_t - M_s} \times 100$$

where, $M(a+s)$ is the mass of the resistant aggregates plus sand (g), M_s is the mass of the sand fraction alone (g), and M_t is the total mass of the soil sample (g).

2.2.3.4. Water holding capacity (WHC)

Principle:

Water holding capacity is the amount of water which should correspond to field capacity, that is the water retained in soil pores $<50 \mu\text{m}$, which is also defined as the hydric reserve water. The WHC is often used for microbiological measurements made after adjusting the water content at this value. This ensures similar conditions for soil microorganisms as concerns the availability of water, which is crucial for their growth and metabolic activity. WHC is the percentage of water retained in the initially saturated soil after drainage by gravity during 24 h.

Used materials and equipments:

- Graduated PVC tube of 8 cm diameter and 250 ml volume. This device is equipped with a 0.10 mm diameter mesh at the bottom. A paper filter is posed on the mesh to avoid loss of soil particles during the trial.
- Glass beaker 1000 ml.
- Glass cover for PVC container and plastic funnel Ø 8 cm.

Procedure:

- The PVC container is weighed alone.
- Approximately 150 g of air dried sieved soil (0-2 mm) is added to the PVC tube and then weighed.
- The PVC container was placed on the bottom of the beaker and water was then added to the beaker in order to let the soil absorb the deionized water by capillary action until saturation.
- After the saturation of the soil, the tube was posed with the base on the funnel and allowed to percolate the excess of water during 24 hours, covering the surface with a glass to prevent water evaporation, and then weighed.
- Water holding capacity was calculated with respect to the soil dry weight as following;

$$\text{WHC (\%)} = \frac{\text{Soil drained for 24 h (g)} - \text{Dry soil (g)}}{\text{Dry soil (g)}} \times 100$$

2.2.4. Laboratory chemical analyses

Soil samples were air dried at laboratory temperature, mixed, sieved at 2.00 mm diameter by using C.I.S.A. mesh apparatus.

2.2.4.1. Soil pH

Principle:

It is a potentiometric determination depending on the difference of the electric potential between the two sides of the electrode membrane giving the H^+ activity in the soil suspension in water or salt solution (Porta et al., 1994; Forster, 1995).

Used materials and equipments:

- Beaker of 50 ml.
- pH meter (pH/Ion510 PRODUCER).
- Stirrer (AGIMATIC-N).
- Digital balance (Mettler A. E. 100).

Used chemicals and solutions:

- Buffer solution, pH 4.00 and 7.00 for calibration.
- 0.1 N KCl (7.4551 g l⁻¹) and deionized water.

Procedure:

- pH meter should be calibrated by 4.00 and 7.00 pH buffer solutions.
- 10 g of air dried sieved soil (0-2 mm) were added to 25 ml of deionized water in a 50 ml beaker.
- Other 10 g of air dried soil were added to 25 ml of 0.1 N of KCl in another 50 ml beaker.
- Each suspension was stirred for 15 min and let to decant.
- The electrode is immersed in the solution with the cell in contact with the surface of the soil
- pH values were recorded for H₂O and KCl solution in order to evaluate the ΔpH as follow:

$$\Delta\text{pH} = \text{pH}_{\text{H}_2\text{O}} - \text{pH}_{\text{KCl}}$$

High values of ΔpH may indicate beginning of acidification processes.

2.2.4.2. Soil electrical conductivity (EC)

Principle:

Measurement of soil electrical conductivity (EC) is carried out to estimate the relationship between the amounts of total dissolved salts (TDS) in the soil extraction and their capacity to conduct electricity. EC is generally expressed as dS m⁻¹ at 25 °C.

Used materials and equipments:

- Beaker of 100 ml.
- Conductivity meter (CON510).
- Stirrer (AGIMATIC-N).
- Digital balance, 4 digits (Mettler A. E. 100).

Used chemicals and solutions:

- Buffer KCl solution for calibration (1413 $\mu\text{S cm}^{-1}$).

Procedure:

- The conductivity meter was calibrated by means of buffer solution of KCl having an EC of $1413 \mu\text{S cm}^{-1}$.
- 10 g of air dried soil sieved at 2 mm, were added to 50 ml of deionized water in a beaker of 100 ml.
- The solution was stirred for 15 min and let to separate the supernatant.
- The EC electrode was immersed into the solution up to the second ring in the electrode.
- Conductivity value has been taken from the digital screen measuring in mS cm^{-1} and expressed in dS m^{-1} .

*2.2.4.3. Cation exchange capacity (CEC)**Principle:*

Cation exchange capacity (CEC) is the measure of a soil ability to retain readily exchangeable cations which neutralize the negative charge of soils. Thus, CEC is important for maintaining adequate quantities of plant available calcium (Ca^{2+}), magnesium (Mg^{2+}) and potassium (K^{+}) in soils. Under acid conditions ($\text{pH} < 5.5$), aluminum (Al^{3+}) may also be present as an exchangeable cation. Soil CEC is normally expressed in units of charge per weight of soil. Two different, but numerically equivalent sets of units are used: $\text{meq}/100 \text{ g}$ (milliequivalents of charge per 100 g of dry soil) or $\text{cmole}_{(+)} \text{ kg}^{-1}$ (centimoles of charge per kilogram of dry soil). While a soil with a higher CEC may not necessarily be more fertile, when combined with other measures of soil fertility, CEC is a good indicator of soil quality. The CEC was determined according to Pardini et al. (2004b).

Used materials and equipments:

- Centrifuge (EBA 21 D-78532 Tuttlingen).
- Rotor shaker.
- Distillation unit (J. P. SELECTA-PRONITRO I).
- Digestion tubes of 100 ml.
- Digital analytical balance, 4 digits (Mettler A. E. 100).

Used chemicals and solutions:

- 1 N ammonium acetate ($\text{CH}_3\text{COONH}_4$).
- Ethanol 80%.
- 1 N KCl.
- 0.02 N H_2SO_4 .

Procedure:

- All used tubes have been rinsed with acid water.
- 5 g of air dried soil are shaken for two hours with 60 ml of 1N $\text{CH}_3\text{COONH}_4$ at pH 7, to saturate exchange sites with ammonium ions and then centrifuged at 600 rpm.
- Excess free ammonium ions are washed from the soil with ethanol 80%, following centrifugation.
- The remaining ammonium ions held on exchange sites are replaced by treating the soil with 1N KCl following the same procedure with ammonium acetate.
- Ammonium is determined on the leachate by automatic Kjeldahl distillator-titrator.
- The tube was adjusted in the distillation apparatus and 50 ml of NaOH 40% were automatically introduced in the digestion tube and the distillation was started.
- The distilled solution containing NH_3 was titrated with 0.02 N H_2SO_4 standard solution.
- The meq NH_3 were calculated by multiplying the milliliters of H_2SO_4 consumed for titration (ml) with the normality of H_2SO_4 .
- The results were then expressed in cmole kg^{-1} of soil.
- The capacity of the exchangeable cations for each studied soil was evaluated according to Table 12.

Table 12. Soil classification according to cation exchange capacity (CEC) range.

CEC (cmole kg^{-1})	Soil classification
<5	Poor
5-10	Low
10-15	Under normal
15-25	Super normal
25-40	High
>40	Very high

2.2.4.4. Soil exchangeable bases (ammonium acetate method)

Principle:

This method covers the determination of exchangeable bases such as calcium, magnesium, sodium, and potassium extracted from soil by saturating with ammonium acetate solution. The soil sample is extracted with a 1 M ammonium acetate solution at pH 7.00 (Suarez, 1996). The soil solution slurry is shaken for 2 h, and the solution is separated from the solid by centrifugation. The addition of NH_4^+ in excess to the soil displaces the rapid exchangeable alkali and alkaline cations from the exchange sites of the soil particles. The concentrations of Ca, Mg, K, and Na are subsequently analyzed by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry).

Used materials and equipments:

- ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry apparatus; Perkin-Elmer 3000 DV, San Jose, CA).
- Centrifuge and rotor shaker apparatus.

Used chemicals and solutions:

- 1 M ammonium acetate ($\text{CH}_3\text{COONH}_4$).

Procedure

- 20 ml 1 M ammonium acetate were added to 2 g of air dried soil (2 mm) in 50 ml centrifuge tubes and placed in the rotor shaker for extraction for two hours.
- Samples were centrifuged for 10 min at 6000 g until the samples had a clear solution.
- The supernatant was transferred into 15 ml sampling tubes.
- Samples were analyzed for Ca, Mg, K, and Na by ICP-OES.
- Standards and instrument blanks must be prepared in 1 M ammonium acetate.
- The exchangeable bases were evaluated as follows:

$$\text{Exchangeable Ca} = \frac{(a-b) \times 20 \times mcf}{10 \times 20.04 \times s}$$

$$\text{Exchangeable Mg} = \frac{(a-b) \times 20 \times mcf}{10 \times 12.15 \times s}$$

$$\text{Exchangeable K} = \frac{(a-b) \times 20 \times mcf}{10 \times 39.10 \times s}$$

$$\text{Exchangeable Na} = \frac{(a-b) \times 20 \times mcf}{10 \times 23.00 \times s}$$

where $a = \text{mg l}^{-1}$ Ca, Mg, K or Na in the extraction solution

$b = \text{mg l}^{-1}$ Ca, Mg, K or Na in the blanks

$s =$ air-dry sample weight in gram

$mcf =$ moisture correction factor

20 ml of ammonium acetate used in extraction

The atomic weights of the elements are Ca = 40.078; Mg = 24.305; K = 39.098;

Na = 24.305.

- All results were expressed in cmole kg^{-1} .

2.2.4.5. Exchangeable aluminum (Al)

Principle:

Exchangeable Al ions are most commonly displaced with an unbuffered salt solution, such as 1N KCl at low pH value able to exchange Al ions with K cations. Aluminum complex was then estimated by colorimetric method proposed by Barnhisel and Bertsch (1982).

Used materials and equipments:

- 100 ml polyethylene centrifuge tube.
- Centrifuge (EBA 21 D-78532 Tuttlingen).
- Spectrophotometer (UV-160A, Shemadzu).
- Whatman paper no. 42 filter paper.
- 50 ml Erlenmeyer flask.

Used chemicals and solutions:

- 1N potassium chloride (KCl).
- 1N HCl.
- Deionized water.
- Aluminon-acetate buffer: 120 ml of glacial acetic acid were diluted in 900 ml of deionized water. 24 g of sodium hydroxide (NaOH) were added, mixed, and 0.35 g of aluminon dissolved in the resulting solution and then all diluted to 1 liter (pH 4.2, stable for 6 month).

- Aluminum standards, 500 ppm solution. The 5 ppm diluted standard solution was prepared by diluting 10 ml of 500 ppm solution in 1 liter of deionized water.
- 1 N HCl.
- 0.5% ascorbic acid.
- Standard concentrations:

The standard dilutions should be prepared by using aliquots of 0, 2, 4, 6, and 8 ml of the diluted standards of Al (5 ppm). Two milliliters of 1N HCl were added to each solution. The deionized water was added to bring each volume to 10 ml. One milliliter of 0.5% of ascorbic acid was added to each standard solution the then heated for 30 min at 80-90 °C. Ten milliliters of aluminon-acetate buffer were added to each standard solution after cooling and then diluted to 35 ml with deionized water. The color intensity of the standard solutions was measured, after 2 hours, by spectrophotometer at 530 nm. A plot of absorbance versus Al concentration in each standard solution was fitted linearly (Figure 36).

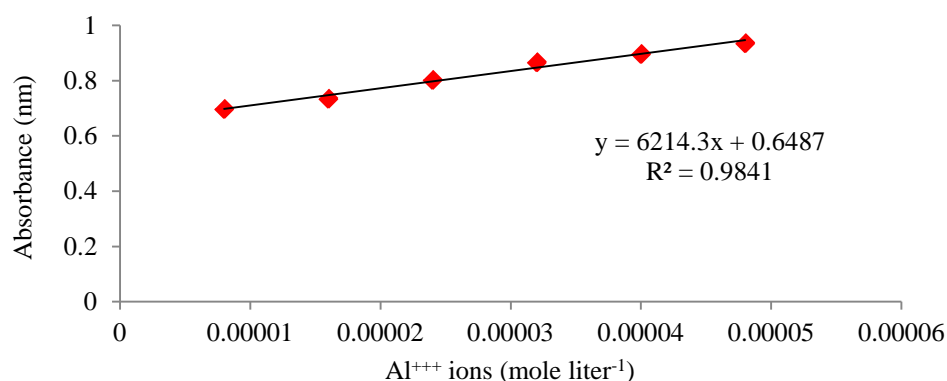


Figure 36. Linear fitting between absorbance and Al concentration of the standard solutions.

Procedure:

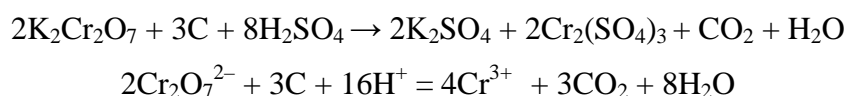
- 50 ml of 1N KCl were added to 5 g air dried soil sample in a polyethylene centrifuge tube of 100 ml and centrifuged at 2500 rpm for 10 min.
- The supernatant solution was filtered through Whatman paper no. 42 filter paper to remove floating organic matter and soil particles.
- 2-4 ml of 1N HCl were added to the supernatant for 2-3 pH range.
- An aliquot of the supernatant, containing 10 to 60 µg of Al, was pipetted to 50 ml Erlenmeyer flask and then appropriate volumes of the complementary acid or extractant solution was added to each flask.

- Deionized water was added to bring the volume to 10 ml.
- 1 ml of 0.5% ascorbic acid was added and then heated for 30 min at 80-90 °C.
- The solution was cooled and diluted to 35 ml with deionized water.
- 10 ml of aluminon-acetate buffer were added and mixed thoroughly with the solution (pH should be 3.7-4).
- After 2 hours, the color intensity of the prepared solution was measured by the spectrophotometer at 530 nm.
- Results were expressed in cmole kg^{-1} .

2.2.4.6. Soil organic carbon by dichromate oxidation

Principle:

The dichromate oxidation method is an estimation of the easily oxidizable components of organic carbon. This oxidation process is carried out by potassium dichromate as an oxidizing agent in presence of a strong acid. The reaction is the following;



The residual amount of dichromate can be titrated by a solution of ferrous ammonium sulphate in the presence of an indicator [diphenylamine] and [H_3PO_4 85%] to improve the indicator action, the end point noticed by the appearance of green color (acidic pH) (Schnitzer, 1991).

Used materials and equipments:

- Erlenmeyer of 250 ml.
- Burette of 25 ml for titration.
- Digital balance, 4 digits (Mettler A. E. 100).

Used chemicals and solutions:

- Concentrated sulphuric acid [H_2SO_4 95% - 98%].
- 1 N $\text{K}_2\text{Cr}_2\text{O}_7$ (49.04 g/l).
- Ortho-phosphoric acid [H_3PO_4 85%].

- Diphenylamine indicator solution: 0.5 g of diphenylamine dissolved in 20 ml distilled water and then added 100 ml of concentrated H₂SO₄.
- 18 N H₂SO₄: 489.13 ml of concentrated sulphuric acid diluted in 1000 distilled water.
- Titration solution [0.5 N Mohr's salt]: Ferrous ammonium sulphate [(NH₄)₂Fe(SO₄)₂·6H₂O] (196.07 g l⁻¹) dissolved in deionized water, acidified with 10 ml of 18 N H₂SO₄ to prevent oxidation of ferrous iron and then completed with deionized water to 1000 ml.

Procedure:

- 10 ml of 1 N potassium dichromate [K₂Cr₂O₇] were added to a suitable quantity of air dried soil (0-2 mm) in Erlenmeyer of 250 ml.
- 20 ml of concentrated sulphuric acid were added to promote the oxidation.
- After 30 min, 10 ml of ortho-phosphoric acid and 100-150 ml of deionized water were added.
- Before the titration with Mohr's salt solution, 5 drops of diphenylamine solution were added to facilitate the detection of the end point during titration process.
- The residual dichromate was evaluated with the titration solution until the green color appears.

Oxidizable organic carbon can be calculated from the following equation;

$$\text{SOC (\%)} = \frac{0.3(A - B) \times M \times CF}{g}$$

where, A = milliliters of Mohr's salt solution for blank (ml).

B = milliliters of Mohr's salt solution for soil sample (ml).

M = normality of Mohr's salt solution.

g = weight of soil (g).

CF = correction factor ratio: Theoretical/Real ml of Mohr salt solution consumed for the blank.

- Organic matter (%) was calculated as follow: $\text{SOC (\%)} \times (0.58)^{-1}$

2.2.4.7. Soil organic carbon by flash combustion method

Principle:

Soil organic carbon (SOC) has been determined also by the flash combustion method using the RC-412 Multiphase carbon apparatus LECO Corporation (Figure 37) according to Ceccanti et al. (2008) and Bianchi and Ceccanti (2010). The optimum temperature for the combustion process was settled from 300 °C to 400 °C. The apparatus has been calibrated to measure the SOC and the results have been obtained as a percentage of soil carbon with respect to the soil sample introduced in the apparatus. The samples weight must be adjusted in a range of 0.06-0.07 grams of dried soil. During the combustion, all forms of carbon (except some carbides like SiC) in soil sample oxidize and convert to CO₂. During oxidization, organic parts in the soil sample also produce H₂O. The presence of organic carbon may be verified by finding coincident peaks of H₂O and CO₂ in the outcome diagram (Figure 38).

Used materials and equipments:

- RC-412 MULTIPHASE CARBON Analyzer (LECO Corporation, USA).
- Digital balance, 4 digits (HM-200).



Figure 37. RC-412 Multiphase apparatus for the SOC determination.

Procedure:

- The apparatus has been previously calibrated to measure the SOC content of soil samples.
- Samples were weighed in a range of 0.06-0.07 grams of air dried soil.
- Soil sample was inserted in the sample container, and then inserted into the flash combustion chamber of the apparatus.

- The results output, obtained after 460-470 seconds, expressed the SOC content as a percentage of soil with respect to the soil sample introduced in the apparatus.
- The results and the signal curve of carbon combustion are presented graphically and data may be recorded (Figure 38).

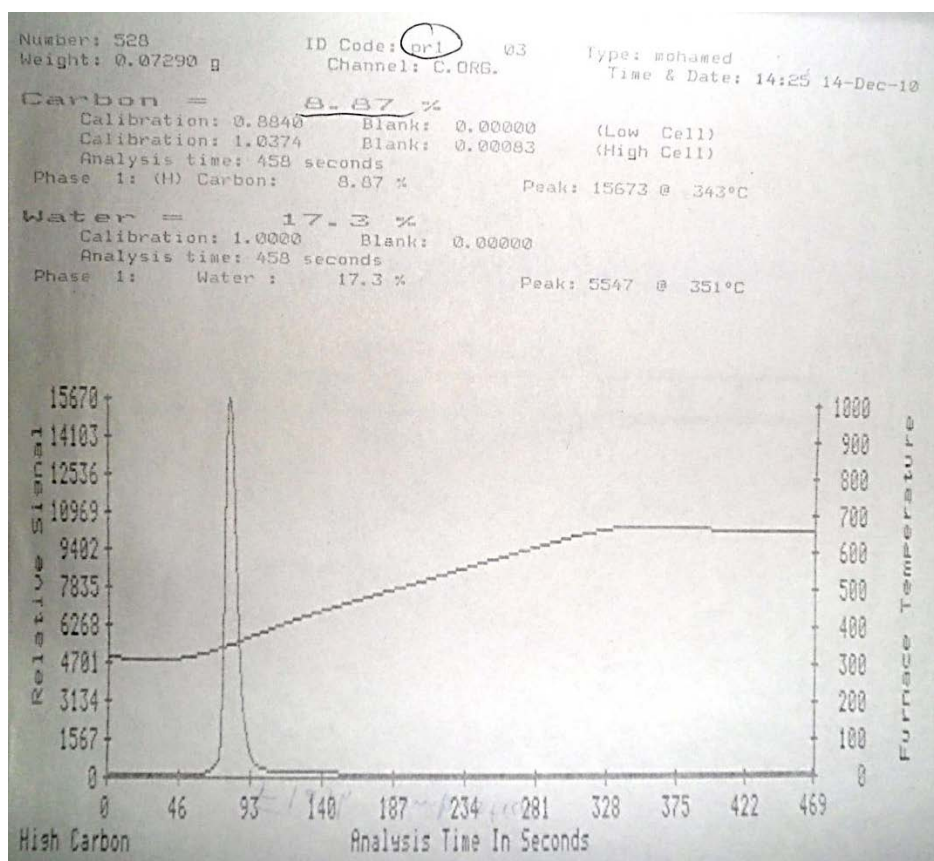


Figure 38. Diagram of RC-412 Multiphase apparatus.

2.2.4.8. Eroded organic carbon (EOC)

Principle:

Soil vulnerability to erosion leads to soil nutrients loss. Soil organic carbon under these conditions may be removed by sediment production during water erosion. Estimation of eroded organic carbon (mg) in the eroded soil (g) may be carried out by using the same oxidation method with $K_2Cr_2O_7$ as an oxidizing agent in presence of concentrated H_2SO_4 . EOC was reported as mg eroded carbon per eroded soil (g). It may be also expressed as $mg\ m^{-2}$ or even as % of the organic carbon in soil (Schnitzer, 1991).

2.2.4.9. Dissolved organic carbon (DOC)

Principle:

Overland water flow may transport the soluble organic carbon under the form of dissolved organic carbon. Dissolved organic carbon (DOC) may be estimated by the same oxidation method (dichromate in the presence of strong acid) although the reagent concentration is different as will be described hereafter (Kalembasa and Jenkinson, 1973).

Used materials and equipments:

- Digestor (MULTI-BLOC SYSTEM) provided with Aluminum blocks with holes of 40 mm diameter and 80 mm depth.
- Erlenmeyer of 250 ml.
- Test tubes of 25 ml.
- Burette of 25 ml.

Used chemicals and solutions:

- Concentrated sulphuric acid [H_2SO_4 95%-98%].
- Concentrated ortho-phosphoric acid [H_3PO_4 85%].
- 66.7 mM potassium dichromate [$\text{K}_2\text{Cr}_2\text{O}_7$] (19.6125 g l^{-1}).
- Ferroin solution Indicator [$\text{Fe}(\text{C}_{12}\text{H}_8\text{N}_2)_3\text{SO}_4$ M.W.: 692.52 (0.025 M).
- Acid mixture solution: [2 parts of H_2SO_4 :1 part of H_3PO_4].
- Titration solution [0.04 N Mohr's Salt]: 15.96 g of ferrous ammonium sulphate [$(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot \text{H}_2\text{O}$] dissolved in liter of deionized water, acidified with 20 ml of concentrated H_2SO_4 to prevent oxidation of ferrous iron then completed by deionized water to 1000 ml.

Procedure:

- 2 ml of 66.7 mM $\text{K}_2\text{Cr}_2\text{O}_7$ was added to 4 ml of water runoff in test tube followed by 7 ml of acid mixture.
- Two blank tubes were prepared by adding 2 ml of 66.7 mM $\text{K}_2\text{Cr}_2\text{O}_7$ and 7 ml of acid mixture in order to control the end point and estimation of water sample.

- The sample tubes and one blank tube were heated at 60 °C on the digester for 30 minutes to allow the oxidation of dissolved carbon by dichromate; one blank was left to react at room temperature.
- The contents of each tube was decanted and rinsed with 20-25 ml deionized water in Erlenmeyer of 250 ml.
- 4-5 drops of indicator were added to each Erlenmeyer.
- The excess of non-oxidized dichromate was titrated with the titration solution until the appearance of the end point, the conversion of orange red color to green color.
- Dissolved organic carbon was calculated by the following equation:

$$\text{DOC} = \frac{(H - S)(A \times E \times 1000)}{(C \times M \times D) \times 1000}$$

where, DOC = dissolved organic carbon (mg ml⁻¹).

H = volume of titration solution for hot blank (ml).

C = volume of titration solution for cold blank (ml).

S = volume of titration solution for sample (ml).

M = normality of K₂Cr₂O₇ (eq l⁻¹).

D = volume of K₂Cr₂O₇ (ml).

A = volume of the water runoff sample (ml).

E = conversion factor in the reduction process that occurs in the Cr⁶⁺ to Cr³⁺, the value is equal to 3.

2.2.4.10. Total nitrogen by Kjeldahl method

Principle:

The estimation of total soil nitrogen with the Kjeldahl method is carried out by previous sulphuric acid digestion and is based on serial reactions. The first reaction is the conversion of organic nitrogen forms into NH₄⁺-N by digestion of nitrogen compounds, by heating the mixture 1 h at 175 °C and 1 h 30 min. at 375 °C. The oxidation process is promoted by selenium as alkaline catalyst. The second process is the distillation of NH₄⁺-N from previous basification into ammonium (NH₃), addition of Boric acid to avoid NH₃ volatilization and titration with a standard sulphuric acid solution in presence of methylene blue indicator. The end point is reached when the original color of indicator is obtained, indicating the formation of boric acid again by reconversion of NH₃ to NH₄⁺ (Kjeldahl, 1983; Forster, 1995).

Used materials and equipments:

- Digestion Unit (J. P. SELECTA Model: 508) (Figure 39A).
- Distillation Unit (J. P. SELECTA-PRONITRO I) (Figure 39B).
- Digestion tubes of 100 ml.
- Digital analytical balance, 4 digits (Mettler A. E. 100).

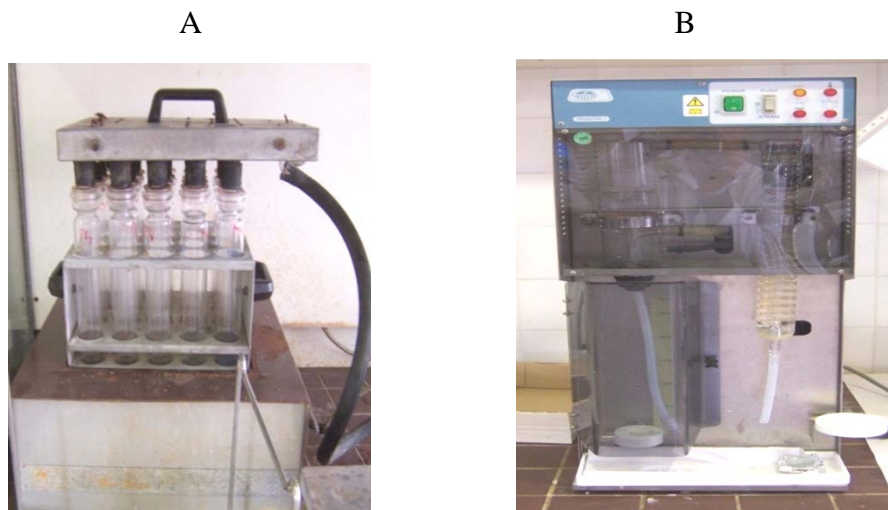


Figure 39. The digester unit and digestion tubes (A) and distillation unit (B) used for the determination of total nitrogen by Kjeldahl method.

Used chemicals and solutions:

- Concentrated sulphuric acid [H_2SO_4 95%-98%].
- Titration solution: Standard sulphuric acid solution [0.2 N H_2SO_4].
- Boric acid [H_3BO_3 4%].
- Indicator [methyl blue + methyl green].
- Sodium hydroxide [NaOH 35%].
- Kjeldahl Catalyst Tablets [99.9% Potassium sulphate + 0.1% Selenium].

Procedure:

- 5 g of Kjeldahl Catalyst and 20 ml of sulphuric acid were added to a suitable quantity of soil in a digestion tube to raise the temperature during digestion.
- The digester was adjusted at 175 °C for 1 h and then at 375 °C for 1.5 h.
- After digestion and cooling, 25 ml of distilled water and 4-5 drops of indicator were added to each tube.
- The tube was adjusted in the distillation apparatus and 50 ml of NaOH 35% were automatically introduced in the digestion tube and the distillation was started.

- 100 ml of the distilled solution was collected in a 250 ml Erlenmeyer and 25 ml of Boric acid were added.
- The distilled solution containing NH_3 was titrated with 0.2 N H_2SO_4 standard solution until the original color of indicator reappeared indicating the back formation of Boric acid.

The milligrams of nitrogen (mg N) in the soil are calculated as following:

$$\text{mg N} = A \text{ ml} \times M \times 14$$

where, A = ml of the titration solution.

M = normality of sulphuric acid (titration solution).

14 = molecular weight of nitrogen.

- The percentage of total nitrogen (%) was calculated with respect to the soil used.

$$\text{TN (\%)} = \frac{\text{mg N (mg)}}{\text{Soil (mg)}} * 100$$

2.2.4.11. Total nitrogen by flash combustion method

Principle:

Total nitrogen in soil was also determined by using flash combustion method using LECO FP-528 Protein/Nitrogen analyzer LECO Corporation (Figure 40). This method is principally depending on the combustion at 850-900 °C of previously ball-milled soil sample (Ceccanti et al., 2008; Bianchi and Ceccanti, 2010). Protein content was then estimated by internal automatic standard curve made by using ethylenediamine tetraacetic acid (EDTA) as a standard.

Used materials and equipments:

- FP-528 PROTEIN/NITROGEN DETERMINATOR (LECO Corboration, USA) (Figure 40).
- Digital balance, 4 digits (HM-200).

Procedures:

- The calibration has been run for several trials by using both EDTA as a calibration sample (0.0640-0.0680%) and soil sample with known organic carbon content (0.0666%).



Figure 40. FP-528 Protein/Nitrogen apparatus for total nitrogen determination (A) and aluminum foil for sample preparation (B).

- Soil samples have been weighed in a range of 0.06-0.07 g and coiled with a small piece of aluminum foil.
- An encapsulated sample is placed into the loading head of the FP-528 apparatus, where it is sealed and purged of any atmospheric gases that have entered during the sample loading.
- The sample is then dropped into a hot furnace at 900 °C and flushed with pure oxygen for very rapid combustion.
- By-products of combustion such as CO₂, H₂O, NO_x, and N₂ pass through the furnace filter and a thermoelectric cooler for subsequent collection in a ballast apparatus.
- These collected gases in the ballast are mixed, and a small aliquot dose is then used for further conversion of the gases.
- The remaining aliquot that has been reduced is measured by the thermal conductivity cell for Nitrogen.
- The percentage of the total nitrogen (%) content is directly obtained by a printed sheet outcome from the apparatus.

2.2.4.12. *Eroded and dissolved nitrogen*

Principle:

The estimation of total eroded nitrogen (EN) and dissolved nitrogen (DN), depleted by runoff water, was carried out following the Kjeldahl method as explained with the estimation of total nitrogen in soil (TN) (Forster, 1995).

2.2.4.13. Total phosphorus (TP)

Principle:

Total phosphorus (TP) has been determined by digestion-spectrophotometric method reported by the Murphy and Riley (1962) and Bianchi and Ceccanti (2010). All soil analyses have been analyzed in triplicate. Phosphorus is an important component of organic matter as a constituent of nucleic acids in all cells and is vital for all organisms. It is usually the limiting nutrient hindering additional biological productivity. Phosphorus is present in the soil almost exclusively as orthophosphate (PO_4^{-3}) in organic and inorganic forms. Hydrogen ions in inorganic forms of ortho-phosphoric acid may be wholly or partially replaced with metal cations and organic forms are present in one or more ester-type bonds. The determination of total phosphorus is based on the transformation of all compounds of phosphorus, organic and inorganic, to orthophosphate by mineralization with concentrated acids. Orthophosphate ions react with ammonium molybdate and potassium tartrate, in an acid media to form a heteropolyacid (a class of acidic compounds from a particular combination of hydrogen and oxygen with some metals and nonmetals) that is reduced with ascorbic acid to molybdenum blue. The intensity of blue color is related to P concentration in the specimen (Ceccanti et al., 2006).

Used materials and equipments:

- Block Digester (Technicon 40).
- Spectrophotometer (UV-VIS Model: CGA PM-2).

Used chemicals and solutions:

- Reagent preparation:
 - 1.5 g of ascorbic acid was dissolved immediately in 100 ml (correspondence of 0.375 g in 25 ml) of reagent **C** before use. The reagent is stable only for 4-5 hours and must be protected at dark because of ascorbic acid sensitivity to light.
 - Reagent **C**:
 - The reagent **C** was prepared by mixing both the solutions A and B.

- Solution A: 12.5 ml of H_2SO_4 dissolved in 40 ml of distilled water.
 - Solution B: 1 g of ammonium molybdate dissolved in 30 ml of distilled water and heated to 60 °C.
 - 10 ml of 0.5% antimony potassium tartrate was added to the mixture of solution A and B and the volume was completed to 100 ml with distilled water.
 - The solution was kept in the dark and refrigerated.
- 0.25% ρ -nitrophenol solution: 0.125g of ρ -nitrophenol dissolved in 50 ml of distilled water.
- 5 M NaOH Solution: 10 g of NaOH dissolved in 50 ml distilled water
- Calibration curve for total phosphorus:
 - Standard solution of phosphorus of 1000 ppm was prepared: 0.445 g sodium dihydrogen phosphate monohydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$) dissolved in 100 ml of distilled water.
 - A serial solution of 10 ppm was then prepared by using 1 ml of the 1000 ppm standard P solution diluted with 100 ml of distilled water.
 - From this final solution of 10 ppm, 1.2 ml, 1 ml, 800 μl , 400 μl , 200 μl , 100 μl and 50 μl were respectively transferred in flasks of 10 ml.
 - 1 ml of reagent C was added to each flask and the volume was completed to 10 ml with distilled water.
 - In each prepared solution the concentration of P was 1.2, 1.0, 0.8, 0.4, 0.2, 0.1, and 0.05 ppm respectively.
 - After 10 minutes and not more than 15 minutes the absorbance of each solution was measured spectrophotometrically at 720 nm against a blank, containing all reagents except the solution of phosphorus.
 - The concentration for each standard solution is reported in Table 13.
 - The absorbance values was plotted on x axis against the concentration values of each standard solution on y axis and the slope and intercept were obtained from the generation of the calibration equation (Figure 41).

Table 13. Standard concentrations used for the calibration curve of total phosphorus determination.

Standard solution (μl)	Absorbance (nm)	TP concentration (ppm)
50	0.034	0.05
100	0.059	0.10
200	0.120	0.20
400	0.220	0.40
800	0.410	0.80
1000	0.493	1.00
1200	0.619	1.20

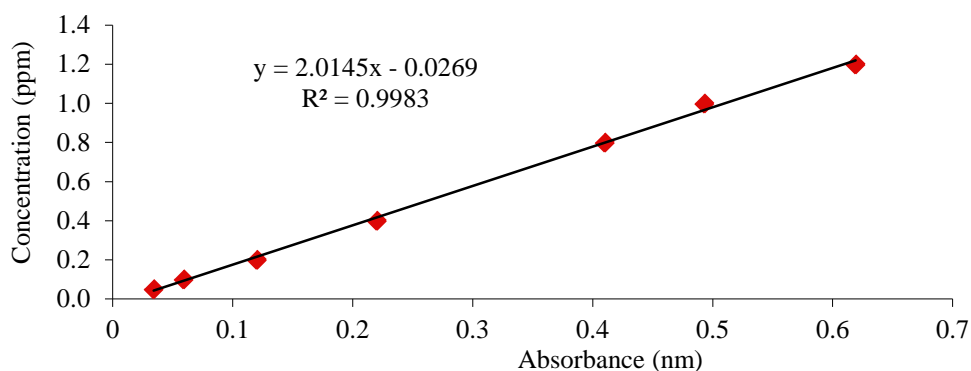


Figure 41. The linear regression equation obtained from the measurements of the standard solutions of phosphorus.

- The P concentration of soil samples was calculated using the following equation:

$$\text{TP concentration (ppm)} = 2.0145 (\text{Absorbance (nm)}) - 0.0269$$
 where 2.0145 and 0.0269 are the slope and the intercept obtained from the linear regression of the standard solutions of phosphorus.

Procedure:

- Digestion of the sample:
 - All glassware used for the analysis including those used to prepare solutions had to be thoroughly washed with hydrochloric acid or nitric acid with concentration 1:5 or 1:10 and then rinsed with deionized water.
 - 5 ml of HNO₃ 69.5% and 2 ml of HClO₄ were added to 0.5 g of air dried soil (Ø 0-2 mm) in hydrolysis tubes of 50 ml.
 - Each tube was covered by hydrolysis funnel (preferably large) to avoid loss of sample during heating and small ceramic grains or glass balls were placed inside the tubes to reduce splashing of sample or solution.

-
- The tubes were heated in a block-digester to 90-100 °C for 2 hours, 150 °C for about 2 hours and then 210 °C for 1 hour.
 - The complete oxidation of organic matter (digested samples converted to white color) is obtained by adding 1-2 ml of concentrated hydrochloric acid at cool conditions and the sample was heated for 30 minutes at 140 °C if needed.
 - Later on, once the samples have been cooled, distilled water was added until 50 ml volume, capped with parafilm, vigorously shaken and let to settle.
 - The supernatant was then transferred into plastic containers and filtered through Whatman paper n° 42.
 - Spectrophotometric determination:
 - One milliliter of the supernatant was pipetted by accuracy pipette to new tubes of 10 ml.
 - The pH of the samples was controlled by adding some drops of p-nitrophenol solution until the color became yellow and 1 ml of 5M NaOH added to return to the transparent color. Then the volume was completed to 5 ml by distilled water.
 - One milliliter of the specific reagent of ammonium molybdate and potassium tartrate was added and the volume adjusted to 10 ml.
 - After 10 minutes, the samples were measured by UV-VIS spectrophotometry at 720 nm against a blank containing all reagents with distilled water without soil extract.
 - Sample was diluted, if needed
 - The results were accepted when the absorbance did not exceed more than 0.6 nm.
 - In the cases that the absorbance exceeded 0.6 nm, sample dilution by distilled water was necessary until the limit is achieved.
 - Total phosphorus calculations:
 - The total phosphorus (TP) was determined by the following equation:

$$TP (\mu g g^{-1}) = \frac{conc \times V_1 \times V_2}{g \times p}$$

where conc = concentration calculated from the calibration curve.

V_1 = total volume in the digested tubes (50 ml).

V_2 = total volume in the digested reagent tubes (10 ml).

p = withdrawal of sample volume to the reagent tube (1 ml).

g = grams of dried soil (0.5 g).

2.2.4.14. Pyrolysis-gas chromatography of the studied soils

Principle:

The structural composition of soil organic matter (SOM) can be determined by using pyrolysis-gas chromatography (Py-GC) in order to establish which chemical components of SOM are better correlated with soil structure and to compare the soil organic matter chemical components of the organic horizons in the selected soil environments. Py-GC has been proposed as a quick and valuable technique to study the structural evolution of organic matter in soil. It is used to record the rapid decomposition of soil organic matter in the bulk soil under high temperature conditions, and does not need sample manipulation with respect to the traditional methodologies based on extraction and fractionation processes (Ceccanti et al., 2007).

Used materials and equipments:

- Chromatograph CARLO ERBA 6000 VEGA SERIES 2, equipped with a flame ionization detector (FID) (Figure 42A)

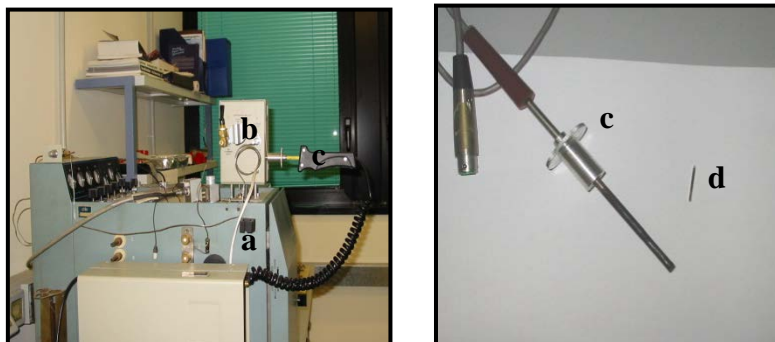


Figure 42. Gas chromatograph (a), FID (b), pyrolysis probe (c), and quartz tube (d).

- The pyrolysis unit PIROPROBE 190 (CDS chemical data system analytical Inc., P.O. Box 277, Oxford, PA U.S.A.) (Figure 42b,c) consists of;
 - Programming unit.
 - Temperature control.

- Probe equipped with a spiral of platinum (2.5×1.8 mm) supported with electric resistance (Figure 42c) to envelope the fire polished quartz sample tube (Figure 42d). The pyrolysis unit is interfaced with the chromatograph apparatus.

Procedure:

- The soil sample was air dried, sieved (0.2 mm), grinded, and inserted inside the quartz tube depending on the quantity of soil carbon content to allow the peaks of pyrolytic fragments avoiding the peaks to go out from the detector limit. Soil sample should have contained about 200-400 $\mu\text{g C g}^{-1}$ soil in order to have an acceptable level of instrument sensitivity and preferably to be checked before apparatus running.
- The quartz tube was then inserted in the electric resistance of the spiral platinum probe.
- Firstly, the column had to be cleaned by running the chromatograph unit at least one complete cycle under the same rate for the subsequent cycles at 60-225 °C at 8 °C min⁻¹.
- After cleaning cycles of the chromatograph unit, the unit was run for soil analyses using the following conditions: A separation of pyrolytic fragments was performed using 3×6 mm, 80/100 μm mesh, SA 1422 (Supelco-Sigma Aldrich-Inc., Bellefonte, PA, USA) POROPAK Q packed column; the temperature program was 60 °C, increasing to 240 °C by 8 °C min⁻¹; The mobile phase consists of nitrogen at a flow rate of 40 ml min⁻¹.
- When the program cycle of the gas chromatograph unit was ready to start, the pyrolysis probe containing the soil sample was coupled directly to a Carlo Erba 6000 gas chromatograph with a flame ionization detector (FID).
- Pyrolysis was carried out at 697 °C for 10 seconds, with a heating rate of 10 °C ms⁻¹ (millisecond) (nominal conditions).
- Then the pyrolysis probe was disconnected and let to cool to be ready for another cycle.
- During the pyrolysis, soil organic matter (SOM) was burned and destroyed producing volatile gases that can be detected by the resin inside the column and recorded according to the arrival time of the volatile gases to the column.

- The peaks were depending on:
 - Temperature inside the column.
 - Type of the resin in the column.
 - The reaction probability of the volatile gases released from the combustion of SOM and the resin.
 - The dimension of the column to divide the fragments; long column= long time for peaks appearance.
- The nominal conditions of the running cycle was summarized as follows:
 - Filament pyrolysis temperature: 700 °C for 10 sec.
 - Heating rate of probe: 10 °C ms⁻¹, where 1 ms = (1/Hz)×1000= second×1000 = the cycles of electric power frequency (number of hertz) per milliseconds (ms).
 - Injector temperature: 250 °C.
 - Gas chromatograph temperature program provided a heating schedule of 60 °C, increasing to 240 °C by 8 °C min⁻¹, before starting the cooling cycle.
- A computer program was connected to the Carlo Erba 6000 gas chromatograph unit to present the peaks of the fragments of the decomposed organic matter in the soil sample.
- Pyrograms of the relevant volatile pyrolytic fragments were interpreted by the quantification of seven peaks corresponding to: acetonitrile (E1), acetic acid (AA), benzene (B), pyrrole (PY), toluene (E3), furfural (FF), and phenol (Y) (Ceccanti et al., 2007). Figure 43 shows an example of pyrograms.

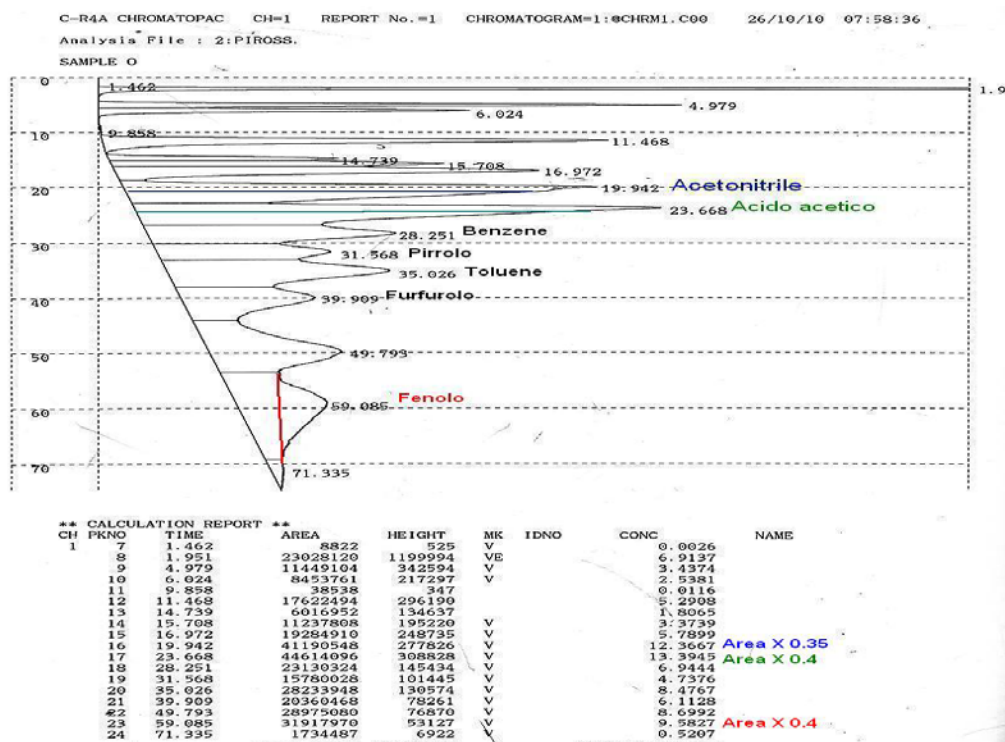


Figure 43. Pyrogram of the pyrolytic fragments of SOM at 700 °C.

2.2.5. Laboratory biological analyses

Fresh soil samples were collected and stored in sterile plastic bags for estimation of soil respiration and soil microbiota. In the laboratory, fresh soil samples were homogenized, sieved at 5.60 mm, and stored in the refrigerator at 4 °C until use. Samples for glomalin estimation were air dried, mixed and sieved as follows: 0.00-2.00 mm, 0.25-2.00 mm, and 2.00-5.60 mm soil fractions. All soil samples were kept at room temperature in plastic pots until use.

2.2.5.1. Estimation of carbon dioxide by soda lime method (SLJ)

Principle:

This method was applied in the laboratory in order to explore the soil potential for soil respiration (CO₂ emission). As the determination of CO₂ in natural conditions may be altered by root respiration, the application of SLJ method in laboratory may result more reliable. In fact, measurements were carried out on root-free soil to better approximate the CO₂ concentration to microbial activity. However, these changes may also introduce artifacts in the measurements.

Used materials and equipments:

- Oven C. R. MARES, S. A. Model: 203.
- Porcelains cups.
- 500 ml hermetic glass jar.
- Digital analytical balance, 4 digits (Mettler A. E. 100).

Used chemicals and solutions:

- Soda lime containing: 75% Calcium hydroxide [Ca (OH)₂], 20% water [H₂O], 3% sodium hydroxide [NaOH], and 1% potassium hydroxide [KOH]. It was used in granular form. It absorbs carbon dioxide in the presence of water vapor.

Procedure:

- 15 g of soda lime was previously oven dried at 105 °C overnight and weighed with the accuracy of 0.01 mg.
- 150 g of fresh soil samples at the original field moisture were placed in jars of 500 ml.
- Soda lime and soil samples were incubated during 24 h at 25 °C at dark.
- After incubation, soda lime was collected, oven dried at 105 °C for 12 h, cooled in a desiccator and weighed.
- The weight of carbon dioxide absorbed by soda lime has been calculated by multiplying the increasing weight of soda lime after oven dry by 1.69 as a correction factor (Grogan, 1998; Keith and Wong, 2006; Emran et al., 2012a).

$$\text{mg CO}_2 \text{ g}^{-1} = (\text{The weight difference of soda lime} \times 1.69) / \text{soil (g)}$$

- As observed each mole of CO₂ chemically bound to soda lime favors the formation of a mole of water, which is then lost by oven drying. Therefore dry mass increase before and after exposure underestimates CO₂ absorbed. Therefore dry mass increase before and after exposure underestimates CO₂ absorbed. The correction factor takes into account that 44 g of CO₂ react with a 74 g of Ca(OH)₂ to form 100 g CaCO₃ and 18 g H₂O. The measured increase in soda lime after oven-drying is 26 (i.e. 100–74). Thus the correction factor of 44/26 (i.e. 1.69) must be applied to the measured mass differences in order to obtain the true value of CO₂ absorbed.

- CO₂ is expressed as (mg CO₂ g⁻¹ d⁻¹) with respect to the soil weight and incubation period.
- The amount of carbon in the CO₂ released from soil [C-CO₂] was then obtained by multiplying corrected CO₂ by 12/44.

2.2.5.2. *Soil microbiota*

Principle:

Microorganisms are heterogeneously distributed in soil, mainly adsorbed and coated by organic and clay particles. Therefore, soil sample must be suspended in isotonic solution to proceed to microbial determination (Alef, 1995).

Used materials and equipments:

- Mesh sieve 5.0 mm (C.I.S.A. Mesh apparatus).
- Test tubes with Sero-taps.
- Screw bottle 250 ml.
- Vortex device (IKA VORTEX GENIUS 3 Model: VG3).
- Sterile inoculation loops.
- Petri dishes 9.0 cm.
- Horizontal shaker (NEW BRUNSWICK SCIENTIFIC CO., INC. G-33).
- Incubator (J.P. SELECTA, s.a.).

Used chemicals and solutions:

- Isotonic solution: Sterile saline solution: 8.5 g of sodium chloride (NaCl) dissolved in 1000 ml distilled water.
- Nutrient agar medium prepared by using powdered medium containing peptone (5 g), meat extracts (5 g), agar (15 g) for each 100 grams. 23.0 g of powder suspended in 1 liter of distilled water, heated until boiling, and autoclaved at 121 °C for 15 min for sterilization. After sterilization, pH was adjusted at 7.0 ± 0.2 and 0.05 g of cycloheximide fungal antibiotics was added.
- Sabouraud chloramphenicol medium prepared by using powdered medium containing casein peptone (5 g), meat peptone (5 g), D(+)glucose (40 g), chloramphenicol (0.5 g), and agar (15 g) for each 100 grams. A quantity of 65.5 g

of this powder was suspended in 1 liter of distilled water, boiled, and sterilized by autoclave at 121 °C for 15 min. pH was adjusted at 5.6 ± 0.2 after sterilization.

- The two types of media were cooled at 45 °C in order to facilitate the pouring of 15-20 g in Petri dishes of $\varnothing=9$ cm under the laminar flow hood (LAF) for aseptic conditions and stored in the refrigerator until use before 15 days.
- Dispersing solution: 180 ml of water sterilized at 121 °C for 15 minutes and mixed with tetra sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7$) (M.W.: 265.91) prepared to be at final concentration of 0.18% after sterilization.

Procedure:

- An equivalent 20 g of fresh homogenized sieved soil (5 mm) are transferred to 180 ml of the dispersing solution to disperse the soil colloids giving a dilution of 10^{-1} .
- This dilution (10^{-1}) was shaken for 15 min. in horizontal shaker and left 15 min. to settle the soil.
- 10 ml of this dilution (10^{-1}) were pipetted to 90 ml of isotonic solution and the dilution (10^{-2}) shaken with the vortex.
- Serial dilutions were prepared in test tubes ranged from 10^{-3} to 10^{-6} .
- 100 μl of 10^{-3} and 10^{-4} were inoculated in the Petri dishes with nutrient agar medium for bacterial count (3 plates per dilution).
- 100 μl of 10^{-5} and 10^{-6} were inoculated in the Petri dishes of sabouraud agar media for fungal count (3 plates per dilution).
- The inoculums' surface agar was spread by sterile inoculation loop.
- Microbial (bacterial and fungal) count was calculated in the dry soil by multiplying the plate count with the dilution factor and reported as cfu g^{-1} .

2.2.5.3. *Glomalin estimation*

Glomalin is a soil proteinaceous substance produced by arbuscular mycorrhizal fungi and deposited on soil particles forming soil aggregates (Wright and Upadhyaya, 1996). The recalcitrant characteristic and stability of this protein allow highly specific procedures for the extraction process by using citrate solution under high temperature. Under different concentrations of citrate solution and variations in autoclaving

conditions, different types of glomalin can be extracted. Recent investigations have shown that following incubations of soil samples, glomalin moves in and out of the total glomalin (BRSP) and easily extractable glomalin (EE-BRSP) pools regardless of incubation time (Steinberg and Rillig, 2003). This indicates that this procedure does not separate glomalin fractions by age, but is differentiating on operationally defined glomalin pool. By analyzing this pool some valuable information may be produced in regards to the function of glomalin on fungal hyphae or soil aggregates.

The effectiveness of citrate for glomalin extraction decreases at 110 °C (Wright and Upadhyaya, 1996), and only a small amount of glomalin is extracted at room temperature (Wright and Upadhyaya, 1996). These results show the importance of high temperature but are confusing about the importance of citrate (Wright et al., 2006). Sodium citrate (20 mM) at pH 7.0 and 121 °C was used firstly for glomalin extraction from hyphae (Wright et al., 1996) and was the only solution of many organic and inorganic solutions tested, that successfully solubilized the compound (Wright and Upadhyaya, 1996). It was subsequently used to soil and more glomalin was extracted by increasing citrate concentration under higher pH (Wright and Upadhyaya, 1996). Therefore, citrate (50 mM), pH 8.0 was agreed as a standard solution used for soil. Alkaline pH of the extractant was thought to be a necessity because solubilized glomalin flocculates slowly in a mild acid (pH < 5.5) or rapidly at pH 2.5 (Nichols and Wright, 2005; Wright et al., 2006). Wright and Upadhyaya (1998) proposed that citrate acted as a competitive chelating agent of iron associated with glomalin and thereby assisted in the solubilization of the molecule. Glomalin contains iron, but amounts differ for glomalin extracted from pot-cultured hyphae and soil. Iron content associated to glomalin in several soils reached 0.8-8.8% (Wright and Upadhyaya, 1998). Glomalin freshly produced on hyphae may reach Fe concentrations of 60.3% (Nichols, 2003). Therefore, a citrate buffer (pH 8.0) at high temperature (121 °C) is used to extract glomalin. Two extraction procedures were carried out to identify the two pools of glomalin depending on their solubility characteristics: the easily extractable glomalin (EE-BRSP) and the total glomalin (BRSP) which were successively quantified by modified method of Bradford protein assay on the extracted solutions (Wright et al., 1996; Wright and Upadhyaya, 1998).

*2.2.5.3.1. Extraction of easily extractable glomalin (EE-BRSP)**Principle:*

The extraction of easily extractable glomalin was performed by using more gentle conditions in order to extract glomalin exudates, defined as freshly produced glomalin. This protein is more weakly aggregated with soil particles in the form of soil aggregates. A part of this protein can easily be extracted from soil aggregates under high temperature in the presence of a lower concentration of sodium citrate. The EE-BRSP pool was extracted by using 20 mM citrate, pH 7.0, by one extraction autoclave cycle at 121 °C for 30 minutes (Wright and Upadhyaya, 1998).

Used materials and equipments:

- Polyethylene tubes (50 ml) with screw cap and not tightly closed to relieve pressure during autoclaving.
- Autoclave (PRESOCLAVE 30 L Selecta).
- Centrifuge (EBA 21 D-78532 Tuttlingen).
- Oven C. R. MARES, S. A. Model: 203.
- Vortex (IKA VORTEX GENIUS 3 Model: VG3).

Used chemicals and solutions:

- 20 mM trisodium citrate dihydrate [$C_6H_5O_7Na_3 \cdot 2H_2O$] (M.W.: 294.10); 5.882 g of trisodium citrate dihydrate dissolved in one liter of distilled water and pH adjusted at 8.0 with HCl solution.

Procedure:

- An equivalent one gram oven dry soil of air dried sieved (0-2 mm) soil was added to 8 ml of 20 mM sodium citrate in polyethylene tubes, shaken with vortex to have appropriate soil solution contact, and then autoclaved at 121 °C for 30 min.
- After autoclave, samples were cooled at room temperature and then immediately centrifuged at 5000g for 20 min (centrifugation is just to pellet the soil particles and may be conducted at any speed from 3000-10000g).
- G-Force of 5000g was converted to 6682 rpm by using a radius of 99.9872208 mm.

- The supernatant that contains the protein was measured by a graduated cylinder and stored in screw capped tubes at 4 °C.
- The stored solution extraction should be examined for microbial growth contamination and not exceed up to 15 days for their use.

2.2.5.3.2. *Extraction of total glomalin (BRSP)*

Principle:

Total glomalin extraction is consequence of several extraction cycles to solubilize the more stable glomalin forms adhering to particles forming soil aggregates. The total extraction of glomalin is carried out under higher citrate concentration and longer time of autoclaving. The total glomalin was extracted with 50 mM citrate, pH 8.0, by sequential autoclave extraction cycles at 121 °C for 60 minutes until the brown color became yellow pale color (Wright and Upadhyaya, 1996; Wright and Upadhyaya, 1998).

Used materials and equipments:

- The same materials are used for the extraction of easily extractable glomalin.

Used chemicals and solutions:

- 50 mM trisodium citrate dihydrate [$C_6H_5O_7Na_3 \cdot 2H_2O$] (M.W.: 294.10); 14.705 g of trisodium citrate dihydrate dissolved in one liter of distilled water and pH adjusted at 8.0 with HCl solution. Otherwise, in case of using trisodium citrate 5.5 hydrate [$C_6H_5O_7Na_3 \cdot 5.5H_2O$] (M.W.: 357.15), 17.8575 g l⁻¹ was used.

Procedure:

- An aliquot of 0-2 mm air dry soil (equivalent to 1 g oven dry soil) was added to 8 ml of 50 mM sodium citrate dihydrate in polypropylene centrifuge tubes.
- The tubes were shaken by vortex to homogenize the soil suspension and then autoclaved at 121 °C for 60 min. for the first cycle.
- Samples were centrifuged at 5000g for 20 min. and supernatant decanted and collected in screw capped tubes.

- Soil pellets were re-suspended with 8 ml of 50 mM sodium citrate, shaken, and autoclaved for the second cycle.
- The last two steps were repeated until the supernatant has a yellow pale or limpid color. Extracts were pooled together.
- Total glomalin extracts were measured with a graduated cylinder and stored at 4 °C until use.
- Glomalin-Carbon (G-C) was determined in the extraction solution by dichromate oxidation as previously described in the Subsection 2.2.4.6.

2.2.5.4. *Quantification of glomalin by Bradford protein assay*

Principle:

This modified procedure was used for the determination of protein concentrations in solutions depending on the change in absorbance that, in an acidic solution of Coomassie Blue G-250, shifts from 465 nm to 595 nm upon binding of protein (Bradford, 1976). The Bradford protein assay is a quantitative spectroscopic analytical procedure used to measure the concentration of glomalin in soil extraction solution. The current assumption in this method is that all or the vast majority of proteins were destroyed during the harsh extraction procedure except glomalin (Rosier et al., 2006).

Both hydrophobic and ionic interactions stabilize an ionic form of the dye, causing a visible color change. There is significant protein-to-protein variation in absorbance values obtained with the Bradford procedure and it is advisable to choose a protein standard that is likely to give absorbance values close to those for the protein samples of interest. Addition of protein results in a shift of the dye's absorption maximum to 595 nm. As the protein concentration increases, the absorbance of light at 595 nm increases linearly. Although the absorbance of Coomassie blue dye at 595 nm is proportional to the amount of protein bound, it is necessary to establish a correspondence between absorbance values and known amounts of protein. To do this, a series of protein standards dilutions of a protein solution of known concentration should be prepared. Once measured each standard at 595 nm one will be able to plot the A₅₉₅ as a function of the known protein content of each standard. After measuring the A₅₉₅

of unknown sample, the standard curve then can be used to determine the amount of protein corresponding to the absorbance values measured (Wright and Upadhyaya, 1998).

Used materials:

- Visible light spectrophotometer (UV-160A, Shemadzu) calibrated at 595 nm.
- Disposable cuvettes are recommended in order to avoid the coloring with reagent.
- Vortex shaker (IKA VORTEX GENIUS 3 Model: VG3).

Used chemicals and solutions:

- Bradford reagent: Bradford reagent ready-to-use should be used and can be stored at 4 °C. Alternatively, it can be made by dissolving 100 mg of Coomassie Blue G-250 (available from several sources) in 50 ml of 95% ethanol, adding 100 ml 85% (w/v) phosphoric acid to this solution, and diluting the mixture to 1 liter with water.
- Reagent solution: One volume of Bradford reagent diluted with four volumes of distilled water. The solution should appear brown and pH is 1.1. It is stable for 2 weeks in a dark bottle at 4 °C.
- Buffer solution: The same buffer concentration used for extraction procedure, i. e. 20 mM trisodium citrate dihydrate for the quantification of EE-BRSP and 50 mM for BRSP.
- Protein standard solution (0.5 mg ml⁻¹): 50 mg bovine serum albumin (BSA) dissolved in 100 ml distilled water.
- Standard concentrations: The standard dilutions should be prepared in the same buffer of the extraction procedure. A convenient standard curve (Figure 44) can be made using bovine serum albumin concentration as detailed in Table 14.

Table 14. Preparation of the BSA concentrations to obtain the standard curve.

Conc. of BSA	BSA (ml)	Buffer (ml)	Total volume (ml)
0 µg ml ⁻¹	0.00	25.00	25.00
25 µg ml ⁻¹	1.25	23.75	25.00
50 µg ml ⁻¹	2.50	22.50	25.00
100 µg ml ⁻¹	5.00	20.00	25.00
200 µg ml ⁻¹	10.00	15.00	25.00

BSA: Bovine serum albumin.

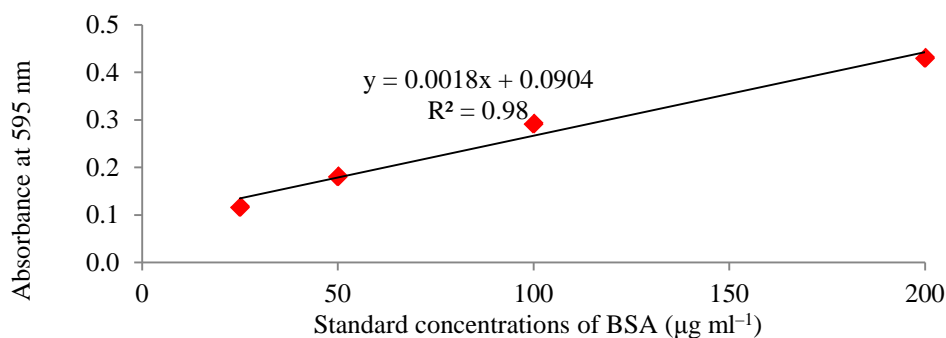


Figure 44. Representative standard curve prepared by the standard concentration of BSA.

Procedure:

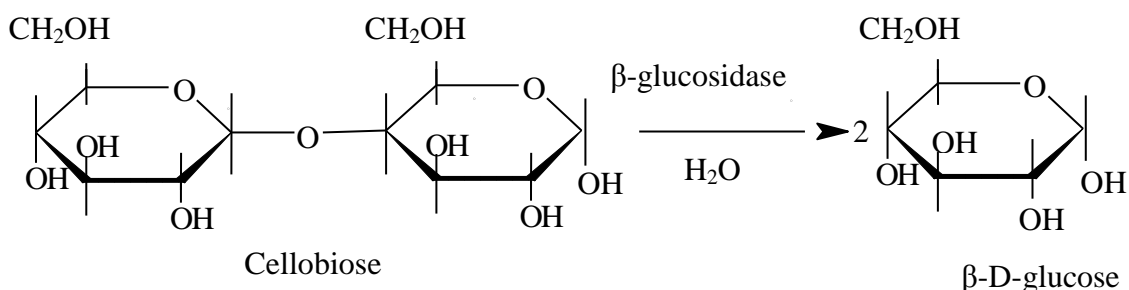
- 200 µl of each concentration of BSA were diluted with 4 ml of Bradford reagent solution.
- The assay is useful since the extinction coefficient of a dye-albumin complex solution is constant over a 10-fold concentration range (extinction coefficient of BSA is 0.667).
- Samples are diluted as the standard dilutions, 200 µl of glomalin extracted diluted with 4 ml of Bradford reagent solution.
- Standard and sample dilutions were immediately shaken and incubated for maximum 5 min. before measurements with the spectrophotometer at 595 nm.
- The standard curve of absorbance versus the concentration was prepared and the concentration equation from the curve was determined.
- Absorbance of samples was measured and the concentration (ppm) calculated from the standard concentration equation.
- Total glomalin was calculated with respect to initial soil weight.

2.2.6. Laboratory biochemical analyses

Soil samples were air dried, mixed, sieved at 2.00 mm diameter, and saved at room temperature in plastic pots until use.

2.2.6.1. *Determination of β -glucosidase activity**Principle:*

β -glucosidase is an enzyme related to carbon cycle in soil as well as it is involved in microbial degradation of cellulose to glucose (Alef and Nannipieri, 1995). β -glucosidase is generally responsible for the regulation of the whole cellulolytic process and it is often the rate-limiting factor. Degradation and mineralization of cellulose are the main processes of the carbon cycle in the soil and, therefore, this activity is considered a good indicator of biomass turnover. The β -glucosidase hydrolyses non-reducing end-groups of cellobiose (dimer resulting from the degradation of cellulose), and to some extent cello-oligosaccharides releasing β -glucose as can be seen in the following reaction:



The determination of this enzyme activity is based on the method of Masciandaro et al. (1994). It is a colorimetric method which can be measured spectrophotometrically at 398 nm. β -glucosidase activity was tested on soils using as substrate pNPG (4-nitrophenyl- β -D-glucopyranoside, 0.05 M). This assay is based on the release and detection of pNP (p -nitrophenol). The β -glucosidase is ranging from 20.9-72.6 $\mu\text{g pNP g}^{-1} \text{ h}^{-1}$ in degraded soils, while it ranges from 105-405 $\mu\text{g pNP g}^{-1} \text{ h}^{-1}$ in natural reforestation (Garcia et al., 2000).

Used materials and equipments:

- Spectrophotometric UV-VIS (Model: CGA PM-2).
- Poly propylene tubes of 10 ml (16×100 MM).
- Water Dubnoff bath at 37 °C.

Used chemicals and solutions:

- Enzyme substrate preparation: 0.05 M 4-nitrophenyl- β -D-glucopyranoside (pNPG) (Code: N7006SG) was prepared by dissolving 0.3013 g of pNPG in 20 ml of distilled water and preserved at -20°C . The preparation must be used within 2 days and should be maintained in dark bottles even during the test procedures to avoid light effect. Preliminary tests were carried out with soil samples before analyses to determine the amount of substrate to be used. Furthermore, the substrate should be added to the soil sample in higher concentration in order to allow the enzyme activity to develop in a sufficient amount of substrate.
- Preparation of 0.5 M NaOH (PM 40): 2 g of NaOH dissolved in 100 ml distilled water. The NaOH is used to increase the alkaline character of the product obtained, giving a yellow color.
- Preparation of 0.5 M CaCl_2 : 3.675 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ dissolved in 50 ml distilled water. The CaCl_2 prevents dispersion of colloids, which would have interfered with the reading.
- Preparation of 0.1 M Maleate buffer at pH 6.5, prepared as follow:
 - Two solutions were prepared (solutions **A** and solution **B**).
 - Solution **A**: 0.2 M Sodium Hydrogen Maleate was prepared by dissolving either 8 g of NaOH + 23.2 g of Maleic acid or 19.6 g of Maleic anhydride in 1000 ml distilled water.
 - Solution **B**: 0.2 M NaOH was prepared by dissolving 2 g of NaOH in 250 ml of distilled water.
 - The two solutions were mixed by using 50 ml of solution **A** with 39 ml of solution **B**, using the following table to adjust the pH at 6.5 (Table 15), and then diluted to a total volume of 200 ml.

Table 15. Scheme of different volumes of solution B that can be added to solution A to adjust the mixed solution at a specific pH ranges from 5.2 to 6.8.

Solution B (ml)	pH	Solution B (ml)	pH
7.2	5.2	33.0	6.2
10.5	5.4	38.0	6.4
15.3	5.6	39.0	6.5
20.8	5.8	41.6	6.6
26.9	6.0	44.4	6.8

- Preparation of calibration curve were done before analyses as follows:

- Stock solution of 1000 ppm p-nitrophenol (pNP) was prepared by dissolving of 100 mg of pNP in 100 ml distilled water.
- From this a serial solution with lower concentration of 100 ppm was prepared.
- Different concentrations of pNP standard solutions (0.5, 1, 2, 4, 8, 15, 30, 60, and 80 ppm) were prepared in new test tubes of 10 ml using the 100 ppm solution as a stock solution (Table 16).
- From each standard solution, 1 ml was pipetted and transferred into new tubes of 10 ml, where 2 ml Maleate buffer, 0.5 ml CaCl₂, and 2 ml NaOH were added and volume was completed to 10 ml with distilled water.
- The solutions obtained were centrifuged at 3500 rpm for 10 minutes and then measured in a spectrophotometer at a wavelength of 398 nm, against a blank containing all reagents except the solution of pNP.

Table 16. The pNP concentrations ($\mu\text{g pNP}$) and the absorbance (nm) of the prepared standard solutions (0.5-80 ppm).

Standard solutions (ppm)	Absorbance (nm)	Concentration ($\mu\text{g pNP}$)
0.5	0.131	1.0
1	0.229	2.0
2	0.360	3.0
4	0.478	4.0
8	0.619	5.0
15	0.882	7.5
30	1.213	10.0
60	1.478	12.5
80	1.819	15.0

- The standard curve for the pNP concentration versus the absorbance of prepared concentrations is obtained (Figure 45).

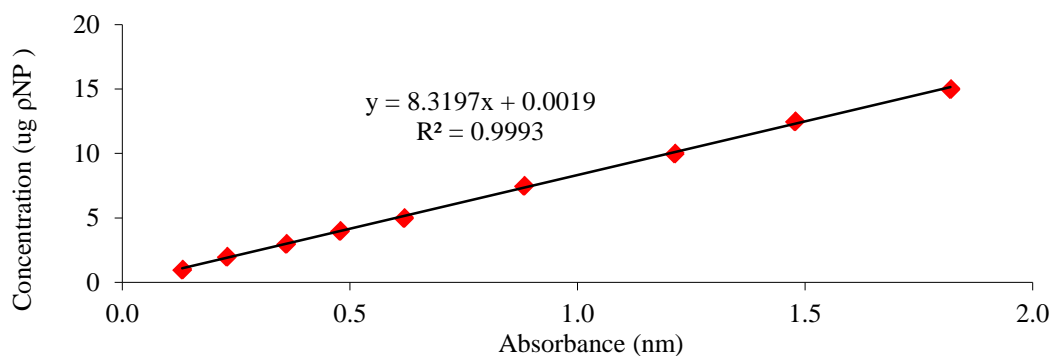


Figure 45. The standard curve for the pNP concentration ($\mu\text{g pNP}$) versus the absorbance (nm) of the standard solutions.

- Linear relationship was obtained by using the values of absorbance (nm) and concentration (μg pNP).
- The following equation was used for the calculation of pNP concentrations:

$$\text{Concentration} = (8.319 \times \text{Absorbance}) + 0.0019$$

Note: All aqueous solutions used in enzyme activities must be carefully prepared using distilled water.

Procedure:

- Soil samples were air dried and sieved at 2.0 mm.
- Both test and control samples were prepared in plastic tubes of 10 ml as following:
- Test samples:
 - 0.5 g of air dried sieved soil.
 - 2 ml of 0.1 M Maleate buffer with pH 6.5.
 - 0.5 ml of 0.05 M of the enzyme substrate, pNPG (4-nitrophenyl- β -D-glucopyranoside).
- Control samples:
 - 0.5 g of air dried soil.
 - 2 ml of 0.1 M Maleate buffer with pH 6.5.

The control preparation is used for the correction of the substrate reaction during the incubation period required by enzymatic assay.
- Blank
 - 2 ml of 0.1 M Maleate buffer with pH 6.5.
 - 0.5 ml of 0.05 M pNPG substrate (4-nitrophenyl- β -D-glucopyranoside).

Blank tubes were used to adjust the wave length (λ) of the spectrophotometer at 0.00 nm.
- Only test and control samples tubes were incubated in water bath at 37 °C for 2 hours under shaking to homogenize the substrate with soil sample.
- After incubation 0.5 ml of substrate (pNP) were added to the control tubes and then all tubes were preserved in the refrigerator at 4 °C for 10 minutes to stop the reaction.

- After cooling 0.5 ml of 0.5 M CaCl₂ and 2 ml of 0.5 M NaOH were added to samples, controls, and blank tubes and then the volume of each tube was completed to 10 ml by distilled water.
- All tubes were centrifuged for 10 minutes at 3500 rpm.
- The blank was used to set the spectrophotometer at 0.00 nm.
- The supernatant of all sample and control tubes were measured by the spectrophotometer versus the blank tube at a wavelength of 398 nm (Tabatabai and Bremner, 1969; Masciandaro et al., 1994).
- The optical densities (nm) detected by the instrument are converted into concentrations using the concentration equation obtained from the standard curve.
- The maximum value of absorbance accepted is 1.6 corresponding to the calibration curve. Otherwise, it is necessary to perform a dilution.
- β -glucosidase activity was calculated by applying the following equation:

$$\mu\text{g pNP g}^{-1} \text{ h}^{-1} = \text{conc} \cdot d \cdot \frac{V_f}{g \cdot h}$$

where, conc = concentration of the sample obtained from the calibration curve (μg).

h = hours of incubation (h).

g = grams of soil weighed (g).

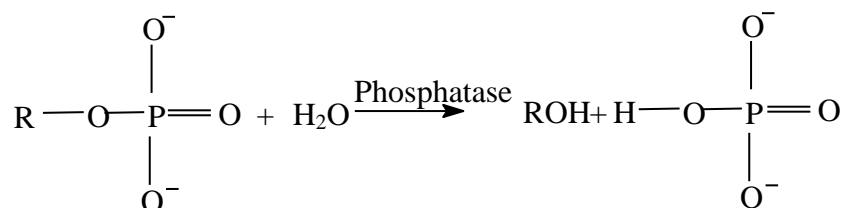
V_f = final volume of solution before spectrophotometric reading (ml).

d = any dilution made before the spectrophotometric reading (ml).

2.2.6.2. Determination of phosphatase activity

Principle:

The phosphatase catalyzes the hydrolysis of phosphate esters to phosphate as can be explained by the following reaction, therefore is an enzyme representative of the phosphorus cycle:



The method is based on a colorimetric determination at wavelength of 398 nm. ρ -nitrophenol (ρ NP) was measured spectrophotometrically after incubation of samples with para-nitrophenyl-phosphate hexahydrate (ρ NPP) for 1 hour and 30 minutes at 37 °C (Alef and Nannipieri, 1995).

Used materials and equipments:

- Spectrophotometric UV-VIS (Model: CGA PM-2).
- Polypropylene tubes of 10 ml 16×100 MM.
- Water bath at 37 °C.

Used chemicals and solutions:

- The 0.115 M ρ -nitrophenyl-phosphate hexahydrate FLUKA (ρ NPP) (PM 371.2) was prepared as needed because it tends to hydrolyze easily over time and must be protected from the light in dark bottle by wrapping with aluminum foil also during the tests. ρ NPP was prepared by adding 1.067 g of ρ NPP in 25 ml distilled water. Preliminary tests were applied to determine the amount of substrate to use. Furthermore, the substrate should be added to the sample with higher concentration in order to allow the enzyme activity sufficient amount of substrate.
- Preparation of 0.5 M NaOH (PM 40): 2 g of NaOH dissolved in 100 ml distilled water. The NaOH is used to increase the alkalinity of the product obtained, giving a yellow color.
- Preparation of 0.5 M CaCl_2 : 3.675 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ dissolved in 50 ml distilled water. The CaCl_2 prevents dispersion of colloids, which would have interfered with the reading.
- Preparation of 0.1 M Maleate buffer and calibration curve as in Section 2.2.6.1.

Procedure:

- Soil samples were air dried and sieved at 2.0 mm.
- Both test and control sample contents were prepared in plastic tubes of 10 ml as following;
- Test samples:
 - 0.5 g of air dried sieved soil.

- 2 ml of 0.1 M Maleate buffer with pH 6.5.
- 0.5 ml of 0.115 M of PNPP enzyme substrate.
- Control samples:
 - 0.5 g of air dried soil sieved.
 - 2 ml of 0.1 M Maleate buffer with pH 6.5.

The control preparation is used for the correction of the substrate reaction during the incubation period required by enzymatic assay.

- Blank:
 - 2 ml of 0.1 M maleate buffer with pH 6.5.
 - 0.5 ml of 0.05 M pNPP enzyme substrate.

Blank tubes were used to make the wave length (λ) of the spectrophotometer at 0.00 nm.

- Only test and control samples tubes were incubated in water bath at 37 °C for 1 hour and 30 minutes under shaking to homogenize the substrate with soil sample (Ceccanti et al., 2006).
- After incubation 0.5 ml of substrate (pNPP) were added to the controls and all tubes were preserved in the refrigerator at 4 °C for 10 minutes to stop the reaction.
- After cooling 0.5 ml 0.5 M CaCl₂ and 2 ml of 0.5 M NaOH were added to samples, controls, and blank tubes and then the volume of each tube was completed to 10 ml.
- All tubes were centrifuged for 10 minutes at 3500 rpm.
- The blank was used to set the spectrophotometric at 0.00 nm.
- The supernatant of all sample and control tubes was measured by the spectrophotometer versus the blank tube at a wavelength of 398 nm (Tabatabai and Bremner, 1969; Masciandaro et al., 1994).
- The optical densities (nm) detected by the instrument are converted into concentrations using a standard line, obtained with the standard concentrations of pNP.
- Maximum value of absorbance accepted is 1.6 corresponding to the calibration of the concentration curve. Otherwise, it was necessary to perform a dilution.
- Phosphatase activity was calculated by applying the following equation:

$$\mu\text{g } \rho\text{NP g}^{-1} \text{ h}^{-1} = \text{conc} \cdot d \cdot \frac{V_f}{g \cdot h}$$

where, conc = concentration of the sample obtained from the calibration curve (μg).

h = hours of incubation (h).

g = grams of soil weighed (g).

V_f = final volume of solution before spectrophotometric reading (ml).

d = any dilution made before the spectrophotometric reading (ml).

2.2.6.3. Determination of protease activity (BAA)

Principle:

Protease is an enzyme that hydrolyzes all the nitrogen forms in soil and plays an important role in the Nitrogen cycle. The activity of this enzyme is in the form of N- α -Benzoyl-L-ArgininAmide hydrochloride monohydrate protease, generally known as BAA protease, and was measured by modified method of Nannipieri et al. (1980).

Used materials and equipments:

- Spectrophotometric UV-VIS (Model: CGA PM-2).
- Polypropylene tubes of 10 ml (16×100 MM).
- Water Dubnoff bath at 37 °C.

Used chemicals and solutions:

- Substrate enzyme preparation: 0.03 M BAA was prepared by dissolving of 0.25 g of N- α -Benzoyl-L-ArgininAmide hydrochloride monohydrate protease (SIGMA) (BAA) in 25 ml of distilled water.
- Buffer preparation: 0.1 M phosphate buffer with pH 7 was prepared as follows:
 - Solution **A**: 0.2 M sodium dihydrogen phosphate was prepared by dissolving 27.6 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 1000 ml of distilled water
 - Solution **B**: 0.2 M sodium phosphate dibasic was prepared by dissolving either 71.6 g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ or 28.4 g of anhydrous Na_2HPO_4 in 1000 ml of distilled water.

The two solutions **A** and **B** were mixed; 39 ml of solution **A** with 61 ml of solution **B** using data of Table 17 to adjust the pH at 7 and the volume was completed to 200 ml of distilled water.

- Sodium salicylate/sodium hydroxide solution was prepared under the hood as follows:
 - 0.3 M sodium hydroxide NaOH solution was prepared by dissolving 3 g of NaOH in 250 ml of distilled water.
 - Sodium salicylate solution was prepared by dissolving 42.5 g of sodium salicylate ($\text{HOC}_6\text{H}_4\text{COONa}$) + 300 mg of sodium nitroprusside ($\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]\cdot 2\text{H}_2\text{O}$) (RPE-ACS) in 250 ml of distilled water.

Equal volumes of 0.3 M NaOH, sodium salicylate and distilled water were mixed. This mixed solution was prepared daily and protected from light because of its instability.

Table 17. Standard volumes for solution A and B to have specific pH value for the mixed solution.

Solution A (ml)	Solution B (ml)	pH
93.5	6.5	5.8
87.7	12.3	6.0
81.5	18.5	6.2
68.5	31.5	6.5
51.0	49.0	6.8
39.0	61.0	7.0
28.0	72.0	7.2
16.0	84.0	7.5
8.5	91.5	7.8
5.3	94.7	8.0

- 0.1% Sodium dichloroisocyanurate dihydrate ($\text{C}_3\text{Cl}_2\text{N}_3\text{NaO}_3\cdot 2\text{H}_2\text{O}$) was prepared by dissolving 0.1 g of sodium dichloroisocyanurate in 100 ml distilled water, also daily prepared.
- The calibration curve was performed from the results obtained from the standard solution of BAA that was prepared as follow:
 - Stock solution of 1000 ppm BAA was prepared by dissolving 100 mg in 100 ml distilled water
 - A serial solution with lower concentration of 100 ppm was then prepared.
 - Suitable standard concentrations of BAA solutions were prepared in new test tubes of 10 ml using the 100 ppm solution as a stock solution.
 - From each standard solution 1 ml was pipetted and transferred into new tubes of 10 ml, where 2 ml of 0.1 M Phosphate buffer, 2.5 ml of the solution

of salicylate-Na/NaOH, and 1 ml of 0.1% of sodium dichloroisocyanurate were added and the volume completed to 10 ml by distilled water.

- The solutions thus obtained were centrifuged at 3500 rpm for 10 minutes and then read in a spectrophotometer at a wavelength of 398 nm, against a blank containing all reagents except the solution of pNP (Table 18).
- Linear relationship was obtained by using the values of absorbance (nm) and concentration (ppm) (Figure 46).
- The following equation was used for the calculation of BBA concentrations:

$$\text{Concentration (ppm)} = 1.0129 (\text{Absorbance (nm)}) + 0.0128$$

Table 18. The BAA concentrations (ppm) and the absorbance (nm) of the standard prepared solutions.

Absorbance (nm)	Concentration (ppm)
0.09	0.1
0.187	0.2
0.382	0.4
0.789	0.8
1.128	1.2
1.495	1.5

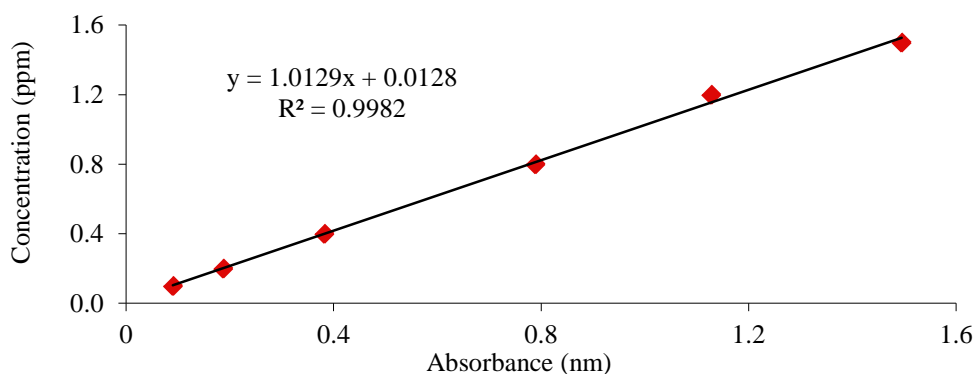


Figure 46. The standard curve for the BAA concentrations (ppm) versus the absorbance (nm) of the standard solutions.

Procedure:

- 2 ml of 0.1 M Phosphate buffer at pH 7 were added to 0.5 g of soil in both sample and control tubes.
- 0.5 ml of 0.03 M BAA substrate was added only for sample tubes.
- All samples and controls tubes were incubated at 40 °C for one hour and 30 minutes.

- After incubation 0.5 ml of the substrate was added to the control tubes and all controls and samples tubes were cooled immediately in the refrigerator at 4 °C for 10 minutes to stop the reaction.
- Sample and control volumes tubes was completed to 10 ml with distilled water and centrifuged at 3500 rpm for 10 minutes.
- After the centrifugation, 1 ml of the supernatant was transferred from all samples, controls, and blank (control tubes without soil) tubes to new tubes of 10 ml.
- Working under a hood, 2.5 ml of the solution of salicylate-Na/NaOH and 1ml of 0.1% of sodium dichloroisocyanurate were added and the volume completed to 10 ml by distilled water.
- All tubes were shaken and let to stand for 30 minutes at dark and then measured with the UV-VIS spectrophotometer at a wavelength of 690 nm.
- The optical densities (nm) detected by the instrument were converted into concentrations in (ppm) using the following equation obtained with the standard concentrations of BAA.

$$\text{Concentration (ppm)} = 1.0129 (\text{Absorbance (nm)}) + 0.0128$$

- Maximum value of absorbance accepted is 1.5 corresponding to the calibration of the concentration curve. Otherwise, it was necessary to perform a dilution.
- The following equation was used for the estimation of BAA Protease in soil:

$$\mu\text{g NH}_4^+ \text{ g}^{-1} \text{ h}^{-1} = \text{conc} \cdot d \cdot \frac{V_f}{p_c} \cdot \frac{V_e}{g \cdot h}$$

where,

conc = the sample concentration calculated from the calibration curve (ppm).

h = hours of incubation (h).

g = grams of air dried soil (g).

V_f = final volume of solution before spectrophotometric reading (ml).

p_e = ml of extract obtained after incubation (ml).

V_c = volume of the solution after the incubation period (ml).

d = any dilution made before the spectrophotometric reading (ml).

All analyses were carried out in triplicate.

CHAPTER III. RESULTS AND DISCUSSION

3.1. Characteristics of the selected soil environments**3.1.1. Lithological and physiographical characteristics**

The studied soils are classified as lithic Xerorthents according to Soil Taxonomy System (NRCS, 2010). Soils have a lithic contact mainly composed of slates with overlying Ap horizons created at the time of conversion of the steeping slopes to terraced soils, which total depth ranges from 30 to 50 cm. The Ap horizon is 10 cm depth on average with low organic matter ranging from low to considerably high according to the land use and age of abandonment. The difference in soil properties have been generally attributed to the history of soil use and abandonment and natural events after agricultural release (Dunjó et al., 2003). This assumption is mainly based on the fact that all soils were formed on the same parent material and their evolution changed according to history of use. Probably the parent material has influenced only the soil texture and pH. Textural class was mainly sandy loam but soils under vines being loamy sand, due to the low content of clay and organic matter. Soils are easily subjected to rapid wetting-drying cycles often causing water stress, structural decline and low biological activity. The slope range (15-21%) of the old hillside terraced soils may increase the susceptibility of water erosion especially when high intensity rainfall occurs.

3.1.2. Characterization of relevant soil properties

Because of the shallow nature of these soils, the Ap horizons were investigated in the field and laboratory for their importance in maintaining satisfactory soil properties. All field and laboratory determinations relevant to soil erosion process were carried out at each rainfall event or on a monthly basis during the two years of observation, from winter 2008 to autumn 2009. Nevertheless the soil textural classes were determined on yearly basis. Bulk density, soil moisture content, and soil surface compaction (mechanic impedance and shear strength) were determined at each rainfall events generating runoff and sediment yield to evaluate the impact of rainfall at the studied soil environments. Moreover, the water holding capacity, soil organic carbon, and total nitrogen were also estimated on a seasonal basis to monitor the potential capacity of the soil to sustain the environmental changes and the effects of different land

use, land use change, and age of abandonment. The soil textural classes for the selected soil environments were classified considering the proportions of sand, silt, and clay particles defined by the International Society of Soil Science (ISSS). A descriptive statistical analysis was used for the obtained data during the two observed years and can be seen in Table 19.

Table 19. Descriptive statistical analysis of the textural classes for the studied soils during the two observed years (2008 and 2009).

ENV	Textural Class	n	2008					2009				
			Min	Max	\bar{x}	σ	CV	Min	Max	\bar{x}	σ	CV
V	Clay (%)	3	2.50	5.00	4.17	1.44	35	2.50	5.00	4.17	1.44	35
	Silt (%)	3	10.00	15.00	11.67	2.89	25	10.00	12.50	10.83	1.44	13
	Sand (%)	3	82.50	85.00	84.17	1.44	2	85.00	85.00	85.00	0.00	0
O	Clay (%)	3	6.25	8.75	7.50	1.25	17	7.50	10.00	8.33	1.44	17
	Silt (%)	3	27.50	41.25	33.33	7.11	21	22.50	27.50	25.00	2.50	10
	Sand (%)	3	52.50	65.00	59.17	6.29	11	65.00	70.00	66.67	2.89	4
S	Clay (%)	3	10.00	17.50	12.50	4.33	35	10.00	12.50	10.83	1.44	13
	Silt (%)	3	22.50	35.00	27.50	6.61	24	25.00	30.00	27.50	2.50	9
	Sand (%)	3	55.00	65.00	60.00	5.00	8	60.00	65.00	61.67	2.89	5
PI	Clay (%)	3	7.50	10.00	8.75	1.25	14	7.50	15.00	11.67	3.82	33
	Silt (%)	3	17.50	26.25	22.92	4.73	21	15.00	25.00	20.83	5.20	25
	Sand (%)	3	65.00	72.50	68.33	3.82	6	65.00	70.00	67.50	2.50	4
PR	Clay (%)	3	10.00	17.50	14.17	3.82	27	15.00	22.50	17.50	4.33	25
	Silt (%)	3	12.50	25.00	17.50	6.61	38	17.50	25.00	20.00	4.33	22
	Sand (%)	3	60.00	77.50	68.33	8.78	13	60.00	67.50	62.50	4.33	7
MC	Clay (%)	3	10.00	12.50	10.83	1.44	13	10.00	10.00	10.00	0.00	0
	Silt (%)	3	15.00	37.50	24.17	11.81	49	27.50	35.00	31.67	3.82	12
	Sand (%)	3	50.00	75.00	65.00	13.23	20	55.00	62.50	58.33	3.82	7
MB	Clay (%)	3	10.00	12.50	11.67	1.44	12	10.00	15.00	12.50	2.50	20
	Silt (%)	3	22.50	27.50	25.83	2.89	11	25.00	25.00	25.00	0.00	0
	Sand (%)	3	60.00	65.00	62.50	2.50	4	60.00	65.00	62.50	2.50	4

ENV: Soil environments; n: Number of observations; Min: Minimum; Max: Maximum; \bar{x} : Mean; σ : Standard deviation; CV: Coefficient of variation; V: Soils under vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub.

All the studied soils during the two observed years resulted sandy loam except soils under vines (V) which was loamy sand. Low clay content was observed over all the studied soils. Particularly, the results showed very low clay content in soils under cultivated vines and olive groves respectively. The mean average values during the observed period of the clay soil fraction was 4%, 8%, 12%, 10%, 16%, 10%, 12%, the silt fraction was 11%, 29%, 28%, 22%, 19%, 28%, 25%, and the sand fraction was 85%, 63%, 61%, 68%, 65%, 62%, 63% for V, O, S, PI, PR, MC, and MB soil

environments respectively. Soils with the sandy loam textural class showed a high variability in the contents of sand, silt, and clay soil particles even under the same soil use. An average variation of 23%, 16%, and 4% was observed in the contents of clay, silt, and sand particles respectively in these soils. The higher clay content was found in soils under pasture, *Cistus* and *Erica* scrubs respectively. Thus, along the changes in soil use, plant canopy, and age of abandonment the textural class may be affected, and these soils formed originally under the same parent material, may be prone to variations in several soil properties.

3.1.2.1. Soil surface compaction and moisture content

Soil compaction has been reported as one of the most significant aspect of soil degradation and erosion. Therefore, the evaluation of soil surface compaction and the potential resistance of soil surface to the external stresses were evaluated by soil mechanic impedance (MI) and shear strength (SS) at the same time of the measurements of soil bulk density (BD). The compactability of soil surface is primarily affecting the water regimes within the soil profile. Soil BD, MI, SS, and SM were determined in the field at each rainfall event or on monthly basis during the observed period from March 2008 to November 2009.

3.1.2.1.1. Soils under cultivated vines and olive groves

A descriptive statistical analysis of these four parameters for soils under cultivated vines (V) and olive groves (O) can be seen in Table 20. Highly compacted Ap soil horizon in soils under cultivated vines and considerably higher in soils under olive groves were recorded. The BD data obtained per each DOY ranged from 1.4 to 1.6 g cm⁻³ with the mean annual value of 1.52 g cm⁻³ for soils under cultivated vines. While the range for soils under olive groves was 1.3-1.4 g cm⁻³ with an annual average of 1.34 g cm⁻³. The soils under vines showed a higher BD and lower surface compaction with respect to the soils under olive groves with lower BD and higher surface compaction. The surface compaction value (kPa) is obtained by measuring the 1 cm of surface and may result lower in vines being sand particles weakly consolidated at surface. Despite that, the bulk density value is measured on 5 cm depth and may result higher because of dense sand particles settlement especially after rainfall events.

Table 20. Descriptive statistical analysis of soil bulk density (BD), mechanic impedance (MI), shear strength (SS), and soil moisture (SM) per each DOY during the observed period for soils under cultivated vines and olive groves.

Year	DOY	n	BD (g cm ⁻³)			MI (kPa)			SS (kPa)			SM (%)		
			\bar{x}	σ	CV	\bar{x}	σ	CV	\bar{x}	σ	CV	\bar{x}	σ	CV
Soils under cultivated vines														
2008	64	3	1.56	0.08	5	314	110	35	193	6	3	4.02	1.11	28
	101	3	1.53	0.08	5	147	98	67	124	37	30	4.13	1.15	28
	141	3	1.46	0.09	6	163	15	9	111	30	27	7.92	2.62	33
	149	3	1.52	0.08	5	124	46	37	78	29	38	7.75	2.55	33
	158	3	1.54	0.08	5	359	28	8	144	6	4	3.73	1.00	27
	185	3	1.45	0.09	6	287	50	17	114	28	25	5.10	1.53	30
	312	3	1.40	0.10	7	204	180	88	193	97	50	5.88	1.83	31
2009	13	3	1.50	0.08	6	170	74	43	78	60	76	6.41	2.04	32
	23	3	1.45	0.09	6	150	63	42	144	36	25	3.22	0.80	25
	37	3	1.40	0.08	6	118	20	17	176	20	11	8.92	2.67	30
	51	3	1.56	0.04	3	333	55	16	121	25	20	3.42	0.50	15
	65	3	1.58	0.10	6	304	78	26	105	11	11	4.18	0.58	14
	85	3	1.54	0.03	2	294	49	17	114	28	25	2.32	0.42	18
	93	3	1.54	0.02	1	209	15	7	173	25	14	3.86	0.22	6
	118	3	1.52	0.02	1	229	75	33	163	57	35	4.12	0.27	6
	135	3	1.55	0.08	5	290	89	31	225	35	16	2.22	0.41	19
	167	3	1.58	0.06	4	372	136	36	127	61	48	1.06	0.12	11
	247	3	1.59	0.01	1	193	15	8	56	6	10	0.76	0.16	21
	279	3	1.60	0.10	6	261	28	11	154	59	38	0.80	0.27	33
	310	3	1.55	0.11	7	69	17	25	97	37	39	4.27	1.18	28
Soils under olive groves														
2008	64	3	1.35	0.08	6	523	41	8	307	30	10	6.11	1.39	23
	101	3	1.35	0.08	6	555	41	7	310	75	24	1.58	0.50	32
	141	3	1.40	0.08	6	539	49	9	418	25	6	1.60	0.51	32
	149	3	1.33	0.08	6	327	69	21	238	59	25	13.82	2.90	21
	158	3	1.32	0.08	6	549	52	9	353	52	15	8.05	1.77	22
	185	3	1.39	0.08	6	506	64	13	343	69	20	1.23	0.43	35
	312	3	1.33	0.08	6	490	52	11	287	23	8	9.42	2.04	22
2009	13	3	1.28	0.08	6	317	20	6	195	19	10	14.30	2.99	21
	23	3	1.35	0.08	6	280	65	23	250	49	20	7.40	1.64	22
	37	3	1.31	0.11	7	245	29	12	307	11	4	13.22	5.60	42
	51	3	1.36	0.06	4	461	74	16	196	20	10	5.35	1.23	23
	65	3	1.29	0.04	3	392	69	18	327	57	17	15.22	0.58	4
	85	3	1.39	0.04	3	539	49	9	392	85	22	3.54	1.44	41
	93	3	1.36	0.02	2	343	130	38	372	52	14	9.75	0.52	5
	118	3	1.32	0.02	2	523	75	14	346	49	14	8.87	1.60	18
	135	3	1.33	0.08	6	490	0	0	350	62	18	6.00	1.37	23
	167	3	1.36	0.05	4	400	68	17	359	28	8	1.82	0.13	7
	247	3	1.40	0.12	8	359	123	34	310	28	9	1.32	0.07	5
	279	3	1.38	0.06	4	376	57	15	245	49	20	1.18	0.36	31
	310	3	1.30	0.08	6	268	40	15	212	44	21	12.45	1.53	12

DOY: Day of the year; n: Number of observations; \bar{x} : Mean; σ : Standard deviation; CV: Coefficient of variation.

Conversely, the soils under olive groves, with finer particles, may present a harder surface but a lower bulk density for a different arrangement along the 5 cm depth, where a more open structure may be formed.

As the increase in BD may indicate soil surface compaction, it was postulated that these soils may be prone to low resistance to external stresses affecting the soil surface and causing crusting and sealing mechanisms. However, the mechanic impedance (MI) measurements in soil under cultivated vines ranged from 69 to 372 kPa during the two years with an annual average of 228 kPa in 2008 and 230 kPa in 2009. Similarly, shear strength (SS) determinations varied from 56 to 225 kPa, with an annual average of 136 kPa in 2008 and 133 kPa in 2009 indicating certain weakability to lateral physical stresses in this soil. Soil response to compaction and lateral stresses observed in soils under olive groves increased with respect to soils under vines and MI values ranged from 245 to 555 kPa, in the whole period with a mean annual value in 2008 of 498 kPa and 384 kPa in 2009. Likewise, the SS ranged from 195 to 418 kPa, with a mean value of 322 kPa in 2008 and 297 kPa in 2009. The increase of soil MI and SS annual mean values observed in soils under olive groves contrasted with the slight decreasing of soil BD and may be explained by the textural composition of the two soils. Within rainfall periods may have a noticeable importance in these soils with relatively low water retention. Soils under vines may rapidly loose water and particles, mainly planar sandy, may settle down forming a packed grain structure with low efficient porosity in the first centimeters when dry. For this reason we find higher BD and lower resistance of surface. In soils under olive groves we find a reverse behavior, due to a more equilibrated texture and a more efficient porosity, justifying lower BD values and higher surface resistance to penetration and lateral stresses. Moreover, the soils under vines showed low differences of MI and SS values in 2008 and 2009 indicating a more predictable response than the soils under olive groves which showed highest differences of MI and SS between 2008 and 2009. The soil moisture content observed along the studied period may have affected the soil surface compaction indicated by both parameters, MI and SS, in both soils. Higher soil moisture contents were generally recorded in soils under olive groves with respect to soils under cultivated vines. However, as above mentioned, the frequent wetting/drying cycles occurring in soils under olive groves may have been more effective in surface hardening. Along the observed period, soil moisture content ranged from 0.8 to 9% and 1 to 15% for the

whole period in soils under vines and olive groves respectively. Despite that the soils under vines showed a mean moisture values of 5.50% in 2008 and 3.50% in 2009, whereas in soils under olive groves mean values were 6.00% and 7.72% for 2008 and 2009 respectively.

These findings may be related with the general soil response in 2008 (low rainy year) and 2009 (higher rainy year) according to the textural composition. Effectively in the soil under vines (loamy sand) the moisture content was on average comparatively lower with respect to the soil under olives in 2009. However, the wetting-drying processes may also have their importance within rainfall events, thus giving higher MI and SS values in soils under olives because of higher clay content. Generally, organic matter in soil can hold up to 20 times its weight in water (Reicosky, 2005). This is particularly important for sandy soils in order to improve soil moisture conditions during dry periods. Nevertheless the water retention capacity favored by organic matter is likely to occur in the bulk soil where a more sorted porosity exist, which seem not to be the case of the soils under vines with very low organic matter content and sandy particles compacted but incoherent at the same time. Therefore, sandy soils with lower organic matter content become easily drier and tend to be prone to runoff erosion.

3.1.2.1.2. Soils under stands of cork and pine trees

A descriptive statistical analysis of soil bulk density (BD), mechanic impedance (MI), shear strength (SS), and soil moisture (SM) was tried at each rainfall event or on monthly basis during the observed period for soils under stands of cork (S) and pine (PI) trees (Table 21). These soils showed lower BD values with respect to soils under cultivated vines and olive groves. The data obtained per each DOY ranged from 1.0 to 1.3 g cm⁻³ with mean annual value of 1.15 g cm⁻³ in soils under stands of cork trees, while the range for soil under stands of pine trees was 1.0-1.4 g cm⁻³ with annual average of 1.19 g cm⁻³ for the whole period respectively.

Table 21. Descriptive statistical analysis of soil bulk density (BD), mechanic impedance (MI), shear strength (SS), and soil moisture (SM) per each DOY during the observed period for soils under stands of cork and pine trees.

Year	DOY	n	BD (g cm ⁻³)			MI (kPa)			SS (kPa)			SM (%)		
			\bar{x}	σ	CV	\bar{x}	σ	CV	\bar{x}	σ	CV	\bar{x}	σ	CV
Soils under stands of cork trees														
2008	64	3	1.01	0.16	16	395	124	31	278	32	11	17.56	5.03	29
	101	3	1.23	0.14	12	539	26	5	294	43	15	4.92	0.55	11
	141	3	1.28	0.14	11	464	190	41	307	30	10	3.68	0.11	3
	149	3	1.13	0.15	13	382	35	9	186	10	5	10.92	2.67	24
	158	3	1.09	0.15	14	297	20	7	294	0	0	16.35	4.60	28
	185	3	1.24	0.14	11	402	35	9	157	52	33	1.96	0.50	26
	312	3	1.07	0.15	14	304	29	10	232	25	11	15.60	4.33	28
2009	13	3	1.08	0.15	14	271	50	19	180	54	30	19.71	5.79	29
	23	3	1.10	0.15	14	290	73	25	225	47	21	10.21	2.42	24
	37	3	1.17	0.06	5	310	57	18	353	52	15	19.12	9.77	51
	51	3	1.22	0.16	13	340	60	18	287	11	4	10.28	1.37	13
	65	3	1.16	0.05	4	402	45	11	261	28	11	17.95	1.87	10
	85	3	1.22	0.13	10	232	76	33	376	34	9	5.49	0.65	12
	93	3	1.06	0.14	13	245	98	40	222	63	28	12.82	2.07	16
	118	3	1.08	0.12	11	405	30	7	206	35	17	13.52	1.47	11
	135	3	1.12	0.15	13	450	74	17	317	43	14	10.00	2.35	23
	167	3	1.14	0.08	7	523	57	11	408	28	7	4.41	0.82	19
	247	3	1.34	0.10	8	359	57	16	310	28	9	2.36	0.59	25
	279	3	1.18	0.04	3	578	17	3	327	57	17	2.15	0.34	16
	310	3	0.99	0.18	19	167	17	10	153	50	33	15.30	1.68	11
Soils under stands of pine trees														
2008	64	3	1.23	0.14	11	265	35	13	193	15	8	4.95	1.49	30
	101	3	1.28	0.12	10	408	118	29	225	69	30	2.62	1.02	39
	141	3	1.10	0.17	16	438	126	29	212	34	16	3.23	1.14	35
	149	3	1.26	0.13	10	363	45	12	140	40	28	9.10	2.32	26
	158	3	1.36	0.10	7	513	50	10	176	29	17	8.08	2.12	26
	185	3	1.25	0.13	10	336	40	12	111	32	28	1.60	0.81	51
	312	3	1.12	0.17	15	154	20	13	118	20	17	14.99	3.51	23
2009	13	3	1.12	0.17	15	196	98	50	150	57	38	21.34	4.79	22
	23	3	1.11	0.17	16	188	65	35	166	46	28	12.80	3.07	24
	37	3	1.11	0.08	7	180	34	19	173	25	14	19.67	4.24	22
	51	3	1.06	0.07	7	448	59	13	196	20	10	13.05	1.35	10
	65	3	1.23	0.12	9	248	49	20	235	34	14	17.60	2.76	16
	85	3	1.35	0.19	14	131	75	57	157	34	22	5.16	2.37	46
	93	3	1.29	0.02	1	310	28	9	310	57	18	7.11	0.57	8
	118	3	1.00	0.28	28	431	39	9	242	6	2	12.02	4.40	37
	135	3	1.20	0.15	12	370	88	24	271	51	19	8.00	2.10	26
	167	3	1.14	0.10	9	304	35	12	196	85	43	3.69	1.60	43
	247	3	1.35	0.07	5	229	28	12	206	35	17	1.74	0.15	9
	279	3	1.25	0.19	15	457	113	25	261	28	11	1.58	0.66	42
	310	3	1.10	0.10	9	300	79	26	230	49	21	12.69	1.82	14

DOY: Day of the year; n: Number of observations; \bar{x} : Mean; σ : Standard deviation; CV: Coefficient of variation.

The slight differences of BD values in soils under pine trees with respect to cork trees formed under the same parent material may be probably due to the different type of organic decaying debris, which under pines may be subjected to slower decomposition rates than under cork trees. The higher values recorded in both soils were found in September 2009 (DOY 247) and the lower value was recorded in soil under stands of cork trees in November 2009 (DOY 310) while in PI soil was in April 2009 (DOY 118). The mechanic impedance (MI) measurements in soils under stands of cork and pine trees ranged from 167 to 578 kPa and 131 to 513 kPa respectively during the two years. Despite that, during 2008 the MI mean values were 397 and 353 kPa for cork and pine trees soils, whereas values amounted to 351 and 297 kPa for the same soils during 2009. The same pattern was found in shear strength (SS) measurements with a range of 153-408 kPa and 111-310 kPa during whole period. The mean SS values were 249 and 167 kPa for 2008 and 278 and 214 kPa during 2009 for soils under cork and pine trees respectively. The variations in these two parameters (MI and SS) were in past induced by the dynamics of soil moisture along the observed period, which in turn is driven by rainfall events and the soil response capacity.

In fact a decrease in mechanic impedance was generally recorded in those DOY with comparatively higher moisture content that highly increased in autumn and spring (2008 and 2009) with respect to summer season with driest soil conditions. Moreover, higher soil moisture content was recorded in both soils during 2009 than 2008. Despite that, within rainfall period repeated wetting-drying cycles altered the soil response in both soils. This may be seen at the highest soil moisture contents recorded in both soils in January 2009 (DOY 13) to which does not correspond to a proportional decrease of mechanic impedance (Table 21). The soil moisture content recorded in soils under stands of cork trees was generally higher than the values recorded in soils under stands of pine trees, that are 10.14% and 11.03% and 6.36% and 10.50% in soil S and PI for 2008 and 2009 respectively. This corroborates that the soil surface layer of pine needles may temporarily retain more water without relevant benefit to soil properties for their high C/N content which may delay organic compounds incorporation into the soil.

3.1.2.1.3. *Soils under pasture*

Soils under pasture were also analyzed for the same parameters (BD, MI, SS, and SM) and results are reported in Table 22.

Table 22. Descriptive statistical analysis of soil bulk density (BD), mechanic impedance (MI), shear strength (SS), and soil moisture (SM) per each DOY during the observed period for soils under pasture.

Year	DOY	n	BD (g cm ⁻³)			MI (kPa)			SS (kPa)			SM (%)		
			\bar{x}	σ	CV	\bar{x}	σ	CV	\bar{x}	σ	CV	\bar{x}	σ	CV
2008	64	3	1.09	0.14	13	539	43	8	425	74	17	8.52	1.75	20
	101	3	1.15	0.13	11	581	11	2	393	67	17	5.37	0.41	8
	141	3	1.06	0.15	14	588	0	0	421	80	19	30.68	11.16	36
	149	3	1.10	0.14	13	580	24	4	392	67	17	20.00	6.62	33
	158	3	1.14	0.13	11	575	11	2	389	6	1	9.49	2.16	23
	185	3	1.12	0.14	12	532	59	11	365	65	18	2.72	0.72	26
	312	3	1.01	0.17	16	578	10	2	391	67	17	26.96	9.58	36
2009	13	3	0.95	0.19	20	549	39	7	304	61	20	40.59	15.37	38
	23	3	1.00	0.17	17	400	49	12	288	59	21	27.41	9.77	36
	37	3	1.10	0.14	13	261	41	16	261	57	22	48.98	17.85	36
	51	3	1.14	0.05	4	353	26	7	585	6	1	11.89	4.76	40
	65	3	1.09	0.10	8	581	11	2	565	104	18	20.51	2.04	10
	85	3	1.14	0.02	1	581	11	2	454	128	28	2.98	0.26	9
	93	3	1.13	0.20	18	366	40	11	431	17	4	22.48	2.07	9
	118	3	1.16	0.08	6	572	28	5	490	49	10	19.19	2.61	14
	135	3	1.12	0.13	12	580	24	4	392	67	17	15.00	4.50	30
	167	3	1.23	0.10	8	588	0	0	408	34	8	2.75	0.52	19
	247	3	1.17	0.08	7	555	57	10	333	17	5	2.32	0.44	19
	279	3	1.04	0.03	3	588	0	0	474	34	7	2.44	0.34	14
	310	3	1.08	0.19	18	221	65	29	184	52	28	19.58	5.27	27

DOY: Day of the year; n: Number of observations; \bar{x} : Mean; σ : Standard deviation; CV: Coefficient of variation.

Soils under pasture showed low BD values with respect to the other soils, independently of variation along DOYs which was of the order of 10% in the whole period. The BD average values in 2008 and 2009 were 1.10 g cm⁻³ indicating a rather constant trend. Higher values were recorded during 2009 than 2008. The higher value was recorded in June 2009 (DOY 167) and the lower in January 2009 (DOY 13). The soils under pasture showed much higher resistance to surface penetration and lateral stress test. In fact, it must be taken into account that they are located in the higher part of the area of study, and more exposed to climatic variations, especially windy conditions. It was observed that the variation in moisture content was higher within and between DOY observations, though these soils showed the highest content of organic

matter. The shallow profile depth may favor easier water loss when subjected to strong wind. Moreover, periodical grazing activity contributes also to increase the formation of a compacted layer of few mm depths. As showed in Table 22 this increased the soil MI values along 2008 (low rain) to averages of 568 kPa, which lowered to average values at 431 kPa (though still relatively high) in 2009 (higher rain). The SS values of soils under pasture showed a mean annual average of 396 kPa during 2008 and 2009 probably related to a minor resistance to lateral stresses. Even in these soils the moisture retention capacity is of fundamental importance.

The results in Table 22 showed that along the observed period the soil moisture content varied from 2% to 49%, with mean values of 14.82% during 2008 and 18.16% during 2009 (22% more due to increased total rainfall amount). The lowest value was recorded in September 2009 (DOY 247) and the higher value was in February 2009 (DOY 37). Although the highest and the lowest values were recorded in 2009, the high variability among the data obtained was recorded during 2008 (26%). BD, MI, and SS data in soils under pasture reflect better than other soils the lack of relationship between the surface response (few mm) to physical stress and the soil packing capacity in the first 5 cm. The soils under pasture is a striking example of a thin crust formation due to loss a water and grazing effect in relatively higher clayey conditions, over a structural arrangement capable to present BD values of 1 g cm^{-3} indicating a relatively stable structure and an adequate porosity along the profile.

3.1.2.1.4. *Soils under Cistus and Erica scrub*

Descriptive statistical analysis of soil BD, MI, SS, and SM at each rainfall event or on monthly basis during the observed period can be seen in Table 23 for soils under *Cistus* (MC) and *Erica* (MB) scrub. Regarding the BD, the MC soils showed mean value of 1.20 g cm^{-3} during 2008 and 1.17 g cm^{-3} in 2009, whereas the values were 1.02 g cm^{-3} and 0.96 g cm^{-3} for 2008 and 2009 in MB soils. As MC soils were more affected by fire in several occasions it may justify its higher BD (+17% in 2008 and +22% in 2009) than MB soils. Despite these differences, both soils showed accumulation of organic materials which, however, did not hinder to record high MI and SS values during 2008 and 2009.

Table 23. Descriptive statistical analysis of soil bulk density (BD), mechanic impedance (MI), shear strength (SS), and soil moisture (SM) during the observed period for soils under *Cistus* and *Erica* scrub.

DOY	n	BD (g cm ⁻³)			MI (kPa)			SS (kPa)			SM (%)		
		\bar{x}	σ	CV	\bar{x}	σ	CV	\bar{x}	σ	CV	\bar{x}	σ	CV
Soils under <i>Cistus</i> scrub													
64	3	1.26	0.12	10	542	30	6	399	49	12	13.81	2.39	17
101	3	1.27	0.12	10	523	28	5	376	104	28	11.29	1.93	17
141	3	1.24	0.13	10	572	28	5	343	49	14	4.95	0.78	16
149	3	1.10	0.15	14	359	60	17	229	41	18	17.70	3.09	17
158	3	1.25	0.13	10	588	0	0	356	32	9	6.92	1.14	16
185	3	1.17	0.14	12	549	52	9	343	43	12	1.78	0.21	12
312	3	1.10	0.15	14	444	75	17	343	55	16	18.33	3.21	17
13	3	1.12	0.15	13	474	34	7	314	34	11	19.96	3.50	18
23	3	1.17	0.14	12	400	71	18	288	60	21	13.15	2.27	17
37	3	1.08	0.01	1	359	41	11	389	89	23	24.70	2.75	11
51	3	1.27	0.08	6	434	89	20	307	23	7	16.39	0.65	4
65	3	1.14	0.02	2	399	108	27	392	20	5	20.25	1.28	6
85	3	1.25	0.12	9	441	49	11	457	41	9	6.00	0.85	14
93	3	1.03	0.08	8	392	49	13	333	52	16	21.05	0.27	1
118	3	1.26	0.13	10	581	11	2	372	29	8	16.01	1.93	12
135	3	1.20	0.13	11	550	55	10	375	67	18	12.00	2.06	17
167	3	1.26	0.08	6	506	102	20	350	80	23	4.66	0.38	8
247	3	1.25	0.06	6	474	34	7	343	49	14	2.90	0.59	20
279	3	1.17	0.22	19	503	11	2	415	25	6	2.49	0.68	27
310	3	0.99	0.32	32	106	37	35	118	49	41	22.14	8.89	40
Soils under <i>Erica</i> scrub													
64	3	1.00	0.14	14	464	93	20	382	35	9	13.71	4.17	30
101	3	1.10	0.04	3	552	32	6	480	52	11	4.22	0.89	21
141	3	1.07	0.06	6	565	25	4	415	106	25	2.75	0.38	14
149	3	0.99	0.15	15	222	63	28	137	59	43	11.54	3.42	30
158	3	1.09	0.05	5	588	0	0	258	123	47	4.77	1.08	23
185	3	0.95	0.19	20	588	0	0	408	28	7	2.21	0.19	9
312	3	0.94	0.20	21	457	41	9	350	59	17	19.17	6.05	32
13	3	1.03	0.11	10	372	119	32	284	35	12	19.01	5.99	32
23	3	1.00	0.14	14	250	70	28	202	74	37	14.02	4.27	30
37	3	0.92	0.19	20	180	34	19	242	49	20	26.02	5.06	19
51	3	0.83	0.22	26	434	59	14	314	35	11	19.82	5.02	25
65	3	1.07	0.04	4	369	25	7	320	45	14	21.14	3.12	15
85	3	1.02	0.00	0	425	28	7	457	102	22	7.63	1.46	19
93	3	0.81	0.26	32	261	28	11	271	74	27	26.34	13.96	53
118	3	0.96	0.08	8	408	28	7	271	40	15	20.46	0.51	3
135	3	0.97	0.17	17	380	58	15	277	74	27	14.00	4.26	30
167	3	0.94	0.09	10	369	123	33	301	50	17	5.72	1.13	20
247	3	1.05	0.11	11	359	28	8	327	28	9	3.49	0.38	11
279	3	1.04	0.33	40	503	11	2	392	20	5	3.73	1.89	51
310	3	0.82	0.25	31	216	17	8	182	74	41	22.48	8.34	37

DOY: Day of the year; n: Number of observations; \bar{x} : Mean; σ : Standard deviation; CV: Coefficient of variation.

As in soils under pasture, the presence of organo mineral complexes in the upper part of these shallow soils does not avoid the formation of thin crusts causing surface hardening. Independently of the frequent wildfire occurrence in abandoned MC soils the MI values were rather similar to those found in MB soils. In fact, mean values were 511 and 491 kPa for 2008 and 432 kPa and 348 kPa for 2009 respectively. Similarly the SS mean values were 341 kPa and 347 kPa for soils MC and MB during 2008 and 342 kPa and 295 kPa in 2009, indicating a lower surface hardening in MB soils. During 2008 the mean soil moisture (SM) values were 10.70% and 8.33% for soils MC and MB respectively whilst in 2009 the mean SM values were 13.90 and 15.70% for the same soils. The total variability of SM content along the observed period was higher in soils under *Erica* scrub (27%) than in soils under *Cistus* scrub (20%). The lowest and highest values in both soils were recorded in July 2008 (DOY 185) and February 2009 (DOY 37) respectively.

3.1.2.1.5. *Soil compaction and moisture content relationships*

Effectively data of SM content and BD obtained per each DOY (mean of three replications) depicted a significant negative correlation for each studied environment (Figure 47). As may be observed the different environments varied the bulk density values as a function of different water content along the observed period. Moreover, from the graph of each environment it is possible to detect that in many occasions different BD values correspond to the same SM value indicating that during the experimental period the structural arrangement in the 0-5 cm depth may vary according to previous natural conditions. This dynamic behavior may be explained by the ongoing modification of the pore system in the 0-5 cm layer, which may give varying BD values at the same SM value suggesting one dimensional shrinkage concept (Berndt and Coughlan, 1977). It was postulated that the soils under study were particularly sensitive to changing climatic conditions: likely, rainfall, wind, irradiation, may cause a swelling-shrinkage effect in such shallow soils. Also, different SM values showed the same BD value. For instance the soils under pasture (PR) showed a BD value of 1.1 g cm^{-3} when the soil was at 3% and 48% of soil moisture respectively, probably indicating that these soils may be subjected to weak swelling mechanisms even lacking of expandable clay minerals, maintaining a relatively stable structure without collapsing porosity. Mineralogical analyses carried out by X-Ray diffraction reported the presence of

kaolinite and illite as the main clay minerals (Dunjó, 2004), in agreement with the parent material composition, attributing a great relevance to SOC in soil reactivity dynamics, but also a higher vulnerability of the soil system.

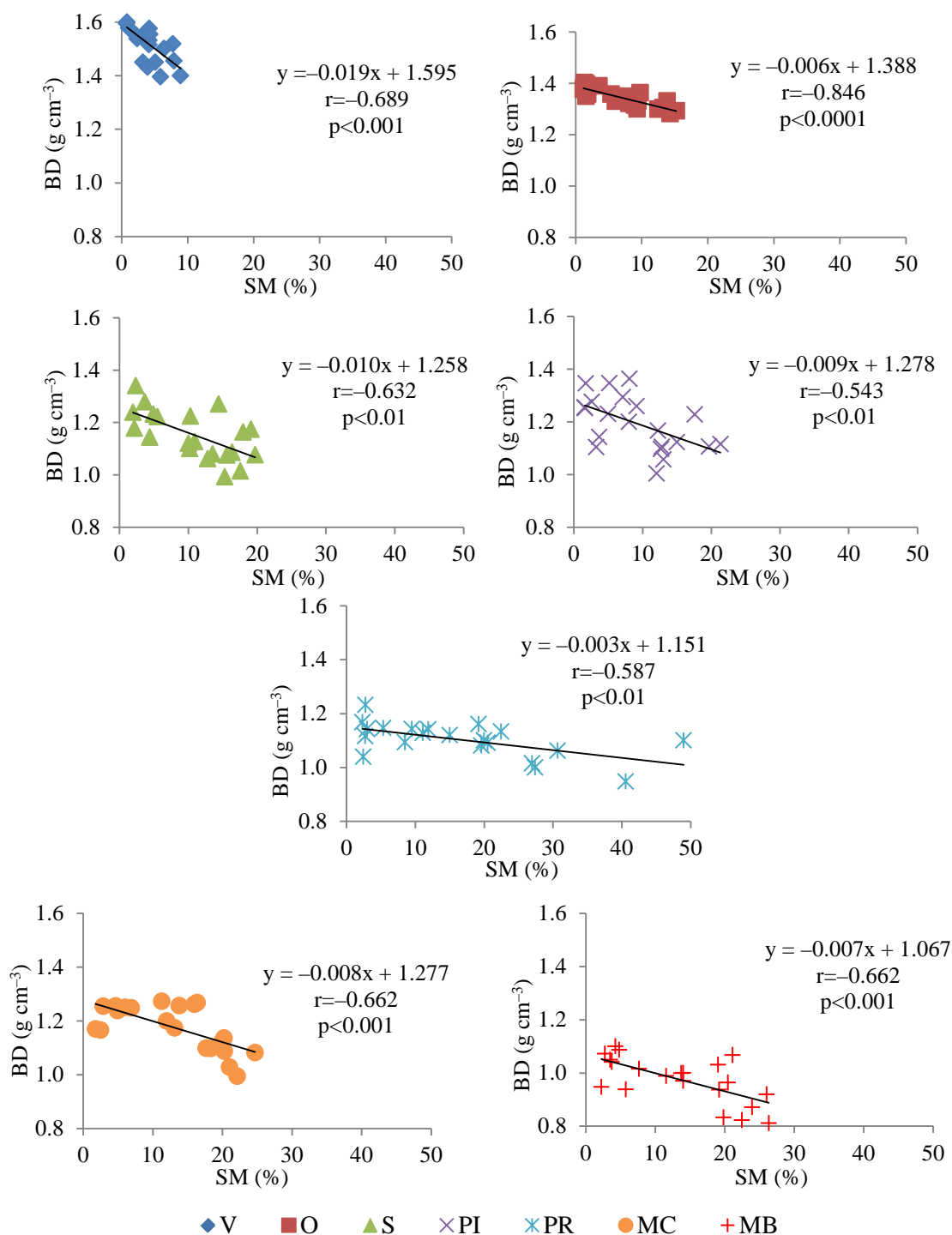


Figure 47. Linear regression equations between soil bulk density (BD) and soil moisture (SM) during the observed period for each soil environment.

According to the regression equations obtained for each soil environment (Figure 47), the SM values necessary to reach a BD value of 1 g cm^{-3} was extrapolated indicating SM amounts of 9.88%, 26.55%, 30.49%, 31.49%, 35.50%, 52.00%, and 61.62% for MB, S, PI, V, MC, PR, and O soil environments respectively. The different water percentage to reach a BD value of 1 g cm^{-3} may describe a porosity related dynamics, taking into account the individual characteristics of each soil environment in terms of water retention capacity, structural arrangement and organic matter content.

3.1.2.1.6. Soil surface resistance relationships

Soil surface behavior as a consequence of soil use and age of abandonment depends largely on the accumulation of humic compounds in the A horizons. Accordingly the relationship between mechanic impedance (MI) and shear strength (SS) was tried (Figure 48).

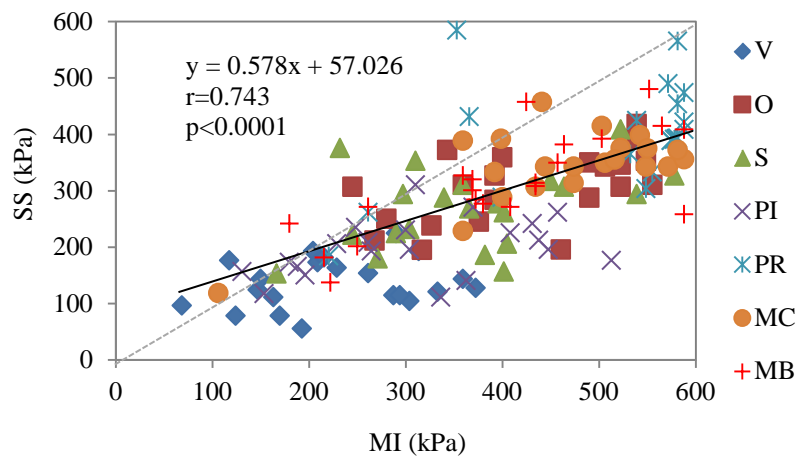


Figure 48. Linear regression equation between shear strength (SS) and mechanic impedance (MI) for the selected soil environments during the observed period.

As indicated by the 1:1 line a larger sensitivity to vertical stresses was showed in all soils. This pattern may be due to the formation of a hardened but fragile surface that is weaker to shear strength force especially in less structured soils. However, higher values of SS may be found when well-structured soil are compacted and wet (pasture soil). The scatter of points indicates the high variability of field measurements along the observed period and within each environment.

3.1.2.2. *Organic reserve*3.1.2.2.1. *Organic carbon and total nitrogen in soil*

Seasonal measurements of organic carbon were carried out during the short stage in the Institute of Ecosystems Study (ISE) in Pisa, Italy to compare the results obtained by dichromate oxidation for SOC estimation in our laboratory in the University of Girona. In the hosting Institute, SOC was determined by the flash combustion method using the RC-412 LECO Multiphase carbon apparatus (Ceccanti et al., 2008). Significant results were found between the analytical methods used (Figure 49).

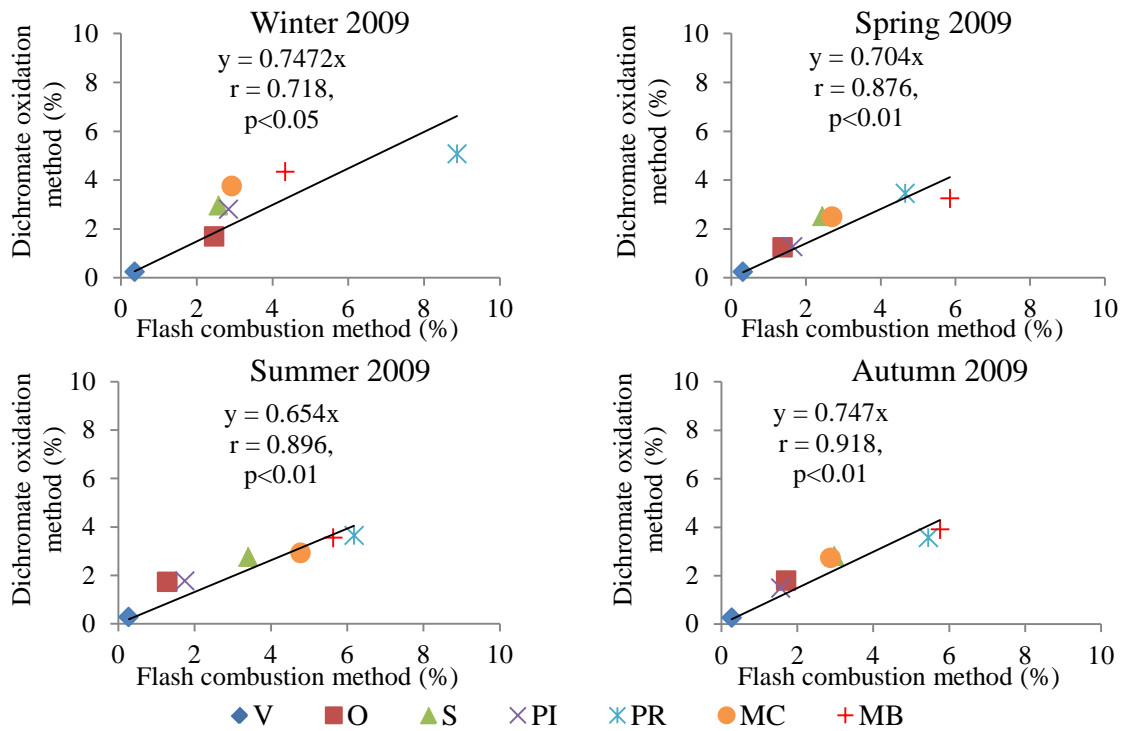


Figure 49. The linear regression equations between the dichromate oxidation method and flash combustion method for the SOC determinations during 2009.

The results by flash combustion method were always higher than the dichromate oxidation method. The equations indicate that the dichromate oxidation may quantify only 75%, 70%, 65%, and 75% with respect to the values found by flash combustion method in winter, spring, summer, and autumn respectively. In spite of this the studied soils maintained the organic carbon content trend from soils under vines to soils under pasture.

Total nitrogen (TN) was also determined in the hosting Institute by using a LECO FP-528 Protein/Nitrogen analyzer by combustion of soil samples at 850-900 °C (Ceccanti et al., 2008). TN was determined in our laboratory of Girona by Kjeldahl method (Kjeldahl, 1983). Results by flash combustion were also higher than Kjeldahl method and a good significant correlation was found with $R^2=1$ (Figure 50). The equations indicated that the Kjeldahl method may quantify 55% of the total nitrogen compared to the flash combustion method. However, data were arranged in the order V, O, PI, S, MC, MB, and PR as for SOC.

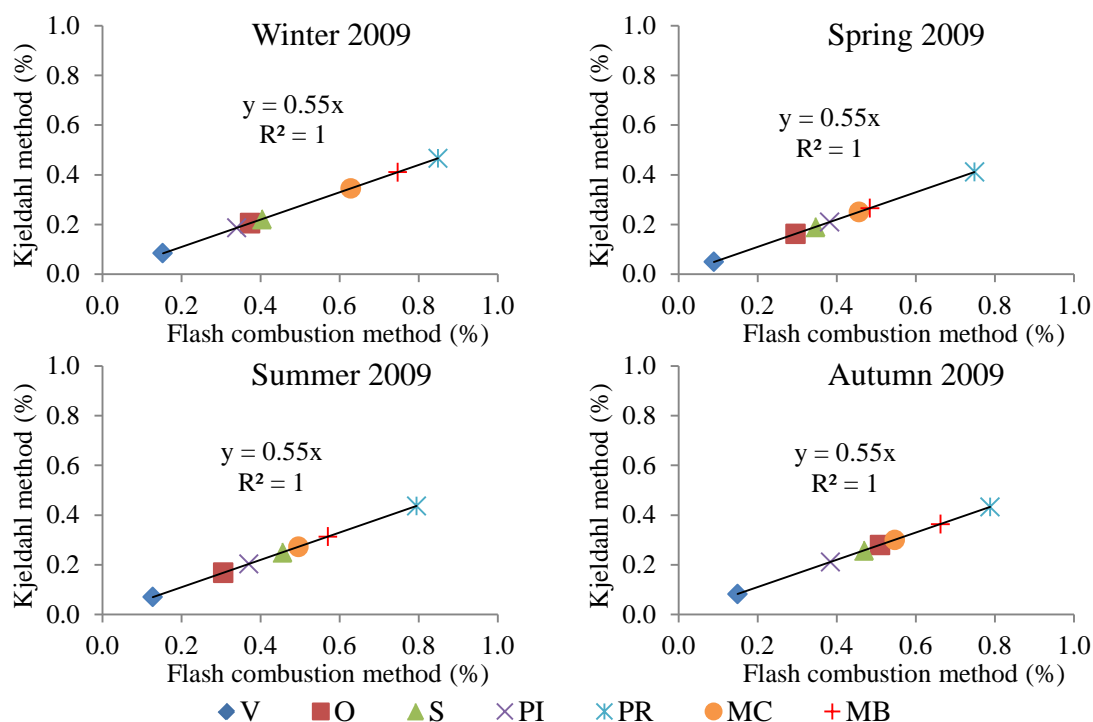


Figure 50. The linear relationship between the Kjeldahl method and flash combustion method for the TN determination.

3.1.2.2.1.1. Soils under cultivated vines and olive groves

Soil organic carbon (SOC), total nitrogen (TN), and water holding capacity (WHC) were investigated seasonally. Their descriptive statistical analysis for soils under cultivated vines and olive groves is presented in Table 24. Very low SOC and TN values were found in soils under cultivated vines along the measurement period. The SOC content in vines showed seasonal means of 0.22-0.45%, an average of 0.31% and CV of 24%. SOC increased by 393% in soils under olive groves with mean values of 1.25-1.80%, average of 1.53% and 15% of CV. TN in soils under vines ranged from

0.04% to 0.08% with a mean annual content of 0.06% (CV 26%). In soils under olive groves TN increased by 200% ranging from 0.13% to 0.28%, average of 0.18% and CV of 27%. Increasing of SOC content was always related to high TN content. WHC increased as well from soils under vines to soils under olive groves due to higher SOC content. Independently of the differences between vines and olive groves the interseasonal variability of SOC and TN data in each environment reached maximum of 100% (in vines SOC in summer 2008=0.45%; SOC in winter 2009= 0.22%); similar trend was found for TN, accounting for the irregular dynamics of these parameters. In fact the poor soil conditions in soils under vines are the consequence of lack of manuring because of giving more importance to agrochemicals. The higher SOC, TN and WHC in olive groves may be explained because of its condition of sandy loam soil, better structure, and probably more efficient turnover of organic compounds. C/N ratio also varied considerably and reached very low values especially in soils under vines.

Table 24. Descriptive statistical analysis of the seasonal values of WHC, SOC, TN, and Carbon/Nitrogen (C/N) ratio from winter 2008 to autumn 2009 for soils under cultivated vines and olive groves.

Season	WHC (%)					SOC (%)					TN (%)					C/N
	Min	Max	\bar{x}	σ	CV	Min	Max	\bar{x}	σ	CV	Min	Max	\bar{x}	σ	CV	
Soils under vines																
W08	27.39	29.67	28.53	1.61	6	0.23	0.46	0.32	0.13	39	0.06	0.06	0.06	0.00	0	5.33
Sp08	23.25	26.06	24.65	1.99	8	0.26	0.45	0.36	0.14	38	0.04	0.06	0.05	0.01	20	7.20
Su08	22.00	24.47	21.97	1.74	8	0.44	0.45	0.45	0.01	2	0.04	0.04	0.04	0.00	10	11.25
Au08	32.16	33.00	32.58	0.59	2	0.31	0.45	0.36	0.08	21	0.05	0.07	0.06	0.01	13	6.00
W09	24.75	26.18	24.35	2.05	8	0.21	0.22	0.22	0.01	4	0.08	0.08	0.08	0.00	0	2.75
Sp09	23.56	25.98	23.77	1.71	7	0.23	0.26	0.25	0.02	6	0.04	0.06	0.05	0.01	20	5.00
Su09	25.12	25.70	25.41	0.41	2	0.22	0.37	0.27	0.08	30	0.06	0.08	0.07	0.01	18	3.86
Au09	23.58	23.68	25.24	0.07	0	0.19	0.39	0.26	0.11	41	0.08	0.09	0.08	0.01	9	3.25
Soils under olive groves																
W08	46.54	49.92	48.23	2.40	5	1.42	1.79	1.63	0.19	12	0.14	0.17	0.15	0.02	11	10.87
Sp08	46.00	47.37	46.68	0.97	2	1.25	1.42	1.39	0.04	3	0.13	0.14	0.14	0.00	0	9.93
Su08	53.72	55.00	54.36	0.91	2	1.25	1.42	1.35	0.09	6	0.13	0.14	0.13	0.01	4	10.38
Au08	53.79	55.00	54.40	0.86	2	1.70	1.92	1.79	0.12	7	0.21	0.21	0.21	0.00	0	8.52
W09	52.80	54.80	52.53	2.41	5	0.85	1.62	1.32	0.41	31	0.20	0.22	0.21	0.02	8	6.29
Sp09	38.44	40.41	39.42	1.39	4	1.05	1.45	1.25	0.20	16	0.16	0.17	0.16	0.01	5	7.81
Su09	40.39	41.34	39.24	2.85	7	1.35	1.96	1.73	0.33	19	0.08	0.26	0.17	0.09	55	10.18
Au09	41.06	42.28	40.62	1.92	5	1.52	2.21	1.80	0.36	20	0.24	0.36	0.28	0.07	26	6.43

Min: Minimum; Max: Maximum; \bar{x} : Mean; σ : Standard deviation; CV: Coefficient of variation; W: Winter; Sp: Spring; Su: Summer; Au: Autumn; 08: 2008; 09: 2009.

3.1.2.2.1.2. Soils under stands of cork and pine trees

Descriptive statistical analysis of the seasonal values of WHC, SOC, and TN in soils under stands of cork and pine trees are shown in Table 25. An increasing of SOC and TN content in soils under stands of pine and cork trees respectively with respect to soils under cultivated vines and olive groves was found along the period of measurements. However values were higher in soils under corks than pines. In soils under stands of cork trees SOC content ranged from 2.54% to 3.63%, average of 2.94% and CV of 11%. Conversely soils under stands of pine trees showed SOC content ranging from 1.28% to 2.62%, average of 2.10% and CV of 24%. Similarly, TN values in soil under stands of cork trees were higher than in soils under stands of pines. A higher capacity of soils under corks to retain water within soil structure may have favored biological activity and humification processes. Effectively when the SOC and TN decreased 29% and 25% respectively in soils under stands of pine trees with respect to soils under stands of cork trees the WHC also decreased 14%.

Table 25. Descriptive statistical analysis of the seasonal values of WHC, SOC, TN, and Carbon/Nitrogen (C/N) ratio from winter 2008 to autumn 2009 for soils under stands of cork and pine trees.

Season	WHC (%)					SOC (%)					TN (%)					C/N
	Min	Max	\bar{x}	σ	CV	Min	Max	\bar{x}	σ	CV	Min	Max	\bar{x}	σ	CV	
Soils under stands of cork trees																
W08	63.50	67.88	65.69	3.09	5	2.63	2.94	2.74	0.17	6	0.25	0.28	0.26	0.02	6	10.54
Sp08	57.00	59.50	58.25	1.77	3	2.66	3.01	2.82	0.27	10	0.27	0.26	0.25	0.00	2	11.28
Su08	80.00	82.05	81.02	1.45	2	3.01	3.21	3.10	0.10	3	0.25	0.27	0.26	0.01	3	11.92
Au08	84.00	88.00	86.00	2.83	3	3.51	3.88	3.63	0.21	6	0.22	0.25	0.24	0.02	9	15.13
W09	68.33	68.33	68.09	0.33	0	2.85	3.41	3.11	0.28	9	0.22	0.22	0.22	0.00	1	14.14
Sp09	62.00	64.82	62.27	2.42	4	2.37	2.75	2.54	0.20	8	0.18	0.20	0.19	0.01	7	13.37
Su09	51.96	59.82	56.64	4.14	7	2.02	3.22	2.77	0.65	24	0.24	0.26	0.25	0.01	6	11.08
Au09	56.98	58.43	59.30	2.85	5	2.39	3.49	2.83	0.59	21	0.25	0.27	0.26	0.02	7	10.88
Soils under stands of pine trees																
W08	48.25	51.73	49.99	2.47	5	2.07	2.81	2.35	0.40	17	0.14	0.14	0.14	0.00	0	16.79
Sp08	41.73	43.68	42.71	1.37	3	2.07	2.53	2.35	0.26	11	0.14	0.14	0.14	0.00	0	16.79
Su08	66.05	69.00	67.53	2.08	3	2.53	2.67	2.62	0.08	3	0.14	0.15	0.14	0.00	2	18.71
Au08	86.41	88.00	87.20	1.13	1	2.32	2.52	2.41	0.10	4	0.22	0.22	0.22	0.00	0	10.95
W09	64.52	68.00	67.91	3.34	5	2.50	2.56	2.53	0.03	1	0.17	0.20	0.19	0.02	9	13.32
Sp09	48.16	51.71	49.93	2.51	5	0.99	1.60	1.28	0.30	24	0.12	0.30	0.21	0.12	58	6.10
Su09	53.02	55.00	55.48	2.73	5	1.05	2.14	1.77	0.62	35	0.15	0.25	0.20	0.05	24	8.85
Au09	39.54	41.49	40.74	1.04	3	1.25	1.76	1.49	0.26	17	0.16	0.26	0.21	0.05	24	7.10

Min: Minimum; Max: Maximum; \bar{x} : Mean; σ : Standard deviation; CV: Coefficient of variation; W: Winter; Sp: Spring; Su: Summer; Au: Autumn; 08: 2008; 09: 2009.

Moreover C/N ratio was found comparatively lower in pines in 2009 probably to soluble organic carbon loss under higher rainfall.

3.1.2.2.1.3. Soils under pasture

The descriptive statistical analysis of WHC, SOC, and TN for data obtained from soils under pasture is shown in Table 26. The results obtained in Table 26 showed a quite constant SOC and TN content in soils under pasture along the observed period. Likewise WHC values were high, with an annual average of 69% (CV 18%). The seasonal means of SOC content ranged from 3.19% to 4.00% with an average of 3.63% (CV 7%). The TN ranged from 0.41% to 0.50% with a mean amount of 0.44% (CV 7%). The extremely high TN content (on average 185% more than normal values around 0.15%) may have lowered C/N ratio. TN increase in pasture was associated to grazing activity.

Table 26. Descriptive statistical analysis of WHC, SOC, TN, and Carbon/Nitrogen (C/N) ratio from winter 2008 to autumn 2009 for soils under pasture.

Season	WHC (%)					SOC (%)					TN (%)					C/N
	Min	Max	\bar{x}	σ	CV	Min	Max	\bar{x}	σ	CV	Min	Max	\bar{x}	σ	CV	
W08	53.78	57.58	55.68	2.69	5	3.31	4.08	3.80	0.43	11	0.36	0.45	0.41	0.04	10	9.27
Sp08	54.80	55.00	54.90	0.14	0	3.20	4.02	3.54	0.69	19	0.36	0.45	0.44	0.01	2	8.05
Su08	65.11	68.00	66.56	2.04	3	3.05	3.31	3.19	0.13	4	0.40	0.44	0.43	0.02	5	7.42
Au08	88.32	91.00	89.66	1.90	2	3.88	4.08	4.00	0.11	3	0.48	0.50	0.50	0.01	3	8.00
W09	78.03	84.37	80.80	3.25	4	3.71	3.94	3.81	0.12	3	0.45	0.48	0.47	0.02	3	8.11
Sp09	76.09	80.86	78.47	3.37	4	3.19	3.80	3.46	0.31	9	0.34	0.49	0.41	0.11	26	8.44
Su09	60.88	65.68	64.63	3.35	5	3.42	3.86	3.65	0.22	6	0.39	0.47	0.44	0.04	9	8.30
Au09	63.23	63.48	63.53	0.34	1	2.87	4.81	3.57	1.07	30	0.37	0.46	0.43	0.05	13	8.30

Min: Minimum; Max: Maximum; \bar{x} : Mean; σ : Standard deviation; CV: Coefficient of variation; W: Winter; Sp: Spring; Su: Summer; Au: Autumn; 08: 2008; 09: 2009.

3.1.2.2.1.4. Soils under *Cistus* and *Erica* scrub

The descriptive statistical analysis for the seasonal values of water holding capacity (WHC), soil organic carbon (SOC), and total nitrogen (TN) for data obtained from soils under *Cistus* and *Erica* scrubs are shown in Table 27. The seasonal mean of SOC ranged from 2.50% to 3.77%, average of 2.97% in soils under *Cistus* scrub, while in soils under *Erica* scrub the SOC content increased by 15% with range 2.46-4.35%, average of 3.50%. The coefficient of variation among the seasonal means was 7% and 13% for MC and MB soil environments respectively during the observed period. The

seasonal mean of TN content in soils under *Cistus* scrub was 0.25-0.35%, average of 0.22%, CV 10%, while in soils under *Erica* scrub the TN content increased by 29%, range 0.27-0.41%, average of 0.36%. The higher SOC in *Erica* scrub may have contributed to 14% increase in water holding capacity (WHC) with respect to soils under *Cistus* scrub. The highest SOC and TN content were observed in winter 2009 and the lowest content recorded in spring 2009 in agreement with the increasing and the decreasing values of WHC in both soils. By analyzing the results of Table 28, it may be assumed that a given soil environment is able to recover suitable conditions in order to withstand further perturbations. The comparison of SOC values between MC and MB soils is not acceptable considering that the former environment has received 600% more the devastating effect of fire.

Table 27. Descriptive statistical analysis for the seasonal means of WHC, SOC, TN, and Carbon/Nitrogen (C/N) ratio from winter 2008 to autumn 2009 for soils under *Cistus* and *Erica* scrubs.

Season	WHC (%)					SOC (%)					TN (%)					C/N
	Min	Max	\bar{x}	σ	CV	Min	Max	\bar{x}	σ	CV	Min	Max	\bar{x}	σ	CV	
Soils under <i>Cistus</i> scrub																
W08	66.42	70.96	68.69	3.21	5	2.85	2.88	2.87	0.02	1	0.25	0.34	0.28	0.05	17	10.25
Sp08	68.00	69.79	68.90	1.27	2	2.88	2.93	2.89	0.06	2	0.34	0.27	0.26	0.02	6	11.12
Su08	72.77	74.00	73.38	0.87	1	2.93	3.16	3.04	0.11	4	0.27	0.27	0.27	0.00	0	11.26
Au08	76.88	80.00	78.44	2.21	3	2.83	3.17	3.04	0.18	6	0.27	0.27	0.27	0.00	0	11.26
W09	74.91	79.06	74.92	4.14	6	3.29	4.15	3.77	0.44	12	0.34	0.36	0.35	0.02	5	10.77
Sp09	54.12	55.65	54.88	1.08	2	2.25	2.70	2.50	0.23	9	0.24	0.26	0.25	0.01	6	10.00
Su09	56.73	57.98	57.75	0.92	2	2.60	3.10	2.93	0.28	10	0.17	0.34	0.27	0.09	32	10.85
Au09	57.16	58.08	59.25	2.85	5	2.51	3.01	2.74	0.26	9	0.25	0.34	0.30	0.05	16	9.13
Soils under <i>Erica</i> scrub																
W08	73.21	78.15	75.68	3.49	5	3.28	3.93	3.52	0.36	10	0.36	0.36	0.36	0.00	0	9.78
Sp08	78.00	79.67	78.84	1.18	2	2.45	3.93	3.12	1.14	36	0.36	0.40	0.38	0.03	7	8.21
Su08	76.36	77.00	76.68	0.45	1	2.32	2.63	2.46	0.16	6	0.40	0.41	0.41	0.01	2	6.00
Au08	81.00	88.00	86.00	4.36	5	3.74	3.93	3.85	0.10	3	0.39	0.42	0.40	0.02	6	9.63
W09	80.72	86.14	83.43	3.83	5	4.24	4.59	4.35	0.20	5	0.39	0.42	0.41	0.02	4	10.61
Sp09	79.93	81.53	80.23	1.13	1	2.42	4.20	3.25	0.90	28	0.20	0.34	0.27	0.10	37	12.04
Su09	62.80	67.18	66.74	3.74	6	3.45	3.75	3.55	0.17	5	0.27	0.35	0.31	0.04	13	11.45
Au09	74.76	80.33	77.55	3.93	5	3.37	4.18	3.91	0.47	12	0.33	0.40	0.36	0.04	10	10.86

Min: Minimum; Max: Maximum; \bar{x} : Mean; σ : Standard deviation; CV: Coefficient of variation; Winter; Sp: Spring; Su: Summer; Au: Autumn; 08: 2008; 09: 2009.

3.1.2.2.2. Total phosphorus in soil

Given the importance of soil nutrients in plant nutrition processes, soil phosphorus was also measured to add information on soil fertility. The seasonal means during 2009 are showed in Figure 51.

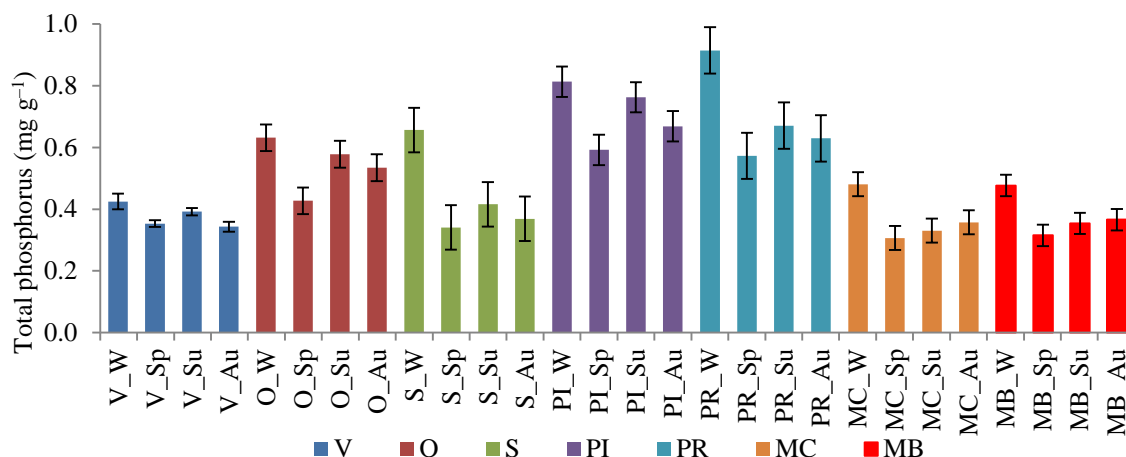


Figure 51. Seasonal means with standard deviation of total phosphorus content for the selected soil environments during 2009.

High interseasonal variability was observed in P concentration, which always increased in winter in all soil environments, associated to chemical weathering that may favor the P removal in this season (Nairn and Mitsch, 2000). The lowest value was found in MC soil (0.31 mg g^{-1}) and the highest in soil PR (0.91 mg g^{-1}) with mean annual averages of 0.38 , 0.54 , 0.45 , 0.71 , 0.70 , 0.37 , and 0.38 mg g^{-1} for V, O, S, PI, PR, MC, and MB soil environments respectively. Total phosphorus values were similar in soils under vines (V), cork trees (S), *Cistus* (MC) and *Erica* (MB) scrubs, whilst higher values were found in olive trees (O), pasture (PR) and pines (PI) soils environments. However, values were considered within the normality in such kind of soils. The variation in TP content among the studied soil environments may be related to the role of soil biological activity at the rhizosphere zones (Nannipieri et al., 2007) of each soil environment that may have varied according to land use and abandonment. It was suggested that manure addition from grazing and deposition of pine needles litter (Kolari and Sarjala, 1995) in PR and PI soil environments respectively may have increased the soil phosphorus content. Following fresh manure addition, ammonium mineralization into nitrate occurs, and in the process H^+ ions are released, contributing to more acidic pH (pH in PR soil below 6.0) thus favoring large amount of the soil

phosphorus bounded to cations, such as iron and aluminum and detected when total phosphorus is analyzed.

3.1.2.3. Nutrient limitations

For assessing nutrient limitation of soil ecosystem processes, the proportions of Carbon/Nitrogen/Phosphorus (C/N/P) ratios were taken into account and therefore calculated on seasonal bases during 2009. It should be noted that, the atomic C/N/P ratio of 106/16/1 in marine water was studied by Redfield (1958) in planktonic biomass, and improved the understanding of the biological processes and nutrient cycling in marine ecosystems. The consistent stoichiometry of C, N, and P in the ocean may enhance the understanding of ocean-atmosphere carbon dioxide (CO₂) exchange, providing valuable insight into the nature and extent of nutrient limitation and ocean C storage, and contributes to the knowledge of biogeochemical cycling of N and P in the world's oceans (Field et al., 1998). Recently, this ratio has been adapted for similar patterns in terrestrial ecosystems relationships, and has even inspired a new discipline "ecological stoichiometry" which seeks to understand the balance of multiple chemical elements in ecological interactions (Sterner and Elser, 2002). The stoichiometric relationships in soils appear to be more variable according to soil nutrient availability and climate (Reich and Oleksyn, 2004). The seasonal means of molar C/N, C/P, and N/P ratios of the studied soils are presented in Figure 52.

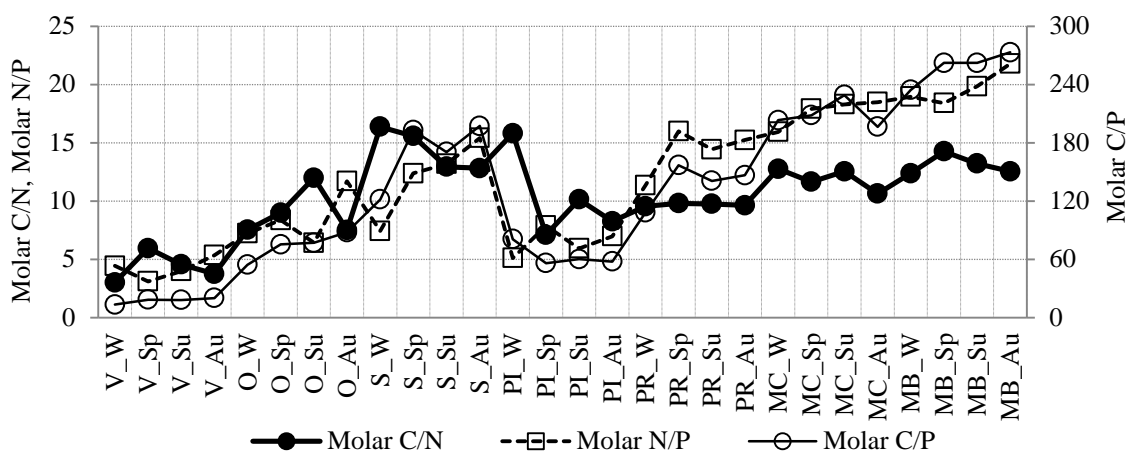


Figure 52. Seasonal patterns of molar C/N, N/P, and C/P ratios for the selected soil environments during 2009.

The graph demonstrates that all soil environments had high seasonal variability along the studied period. Results can help in classifying the studied soils for both nitrogen and phosphorus limitations. Several studies on plant leaves (Aerts and Chapin, 2000; Reich and Oleksyn, 2004) have hypothesized a foliar N/P “breakpoint” between N limitation ($N/P < 14$) and P limitation ($N/P > 16$), indicating that plant N/P ratios may provide a reliable index of nutrient limitation in terrestrial ecosystems. Even though we do not have C, N, and P values for plants, the mean annual averages of N/P ratio for the seven soils were 4.21, 8.42, 12.10, 6.47, 14.25, 17.65, 19.74 for V, O, S, PI, PR, MC, MB soil environments respectively. According to breakpoint N and P limitation it was assumed that V, O, S, PI soil environments may be classified as N limitation and MC and MB soil environments as P limitation. In general, the S, PR, MC, and MB soil environments are presenting higher values for the three ratios. The annual mean average of C/N ratios were 4.31, 9.00, 14.42, 10.32, 9.68, 11.88, 13.09, and C/P ratios were 17.45, 73.34, 170.53, 63.55, 138.06, 209.22, 257.91 for V, O, S, PI, PR, MC, MB soil environments respectively. According to Sterner and Elser (2002), an ecosystem is described as “stoichiometrically balanced” when the C/N/P ratios of autotrophs approximate the Redfield ratio of 106/16/1. When comparing our data with Redfield ratios we noticed that only S and PR soil environments values were closer (162/11/1 for S and 135/14/1 for PR) whilst values were underestimated in soils V, O, and PI and overestimated in soils MC and MB (Table 28). Some considerations about these results may lead to catalogue soils like V, O and PI as with poorer properties and nutrient imbalance whereas soils MC and MB should present better conditions though may have excess in nutrient balance.

Table 28. Average values of soil C, N and P ratios in the studied soils (molar ratios), compared to the Redfield ratio.

	C	N	P
Redfield ratio	106	16	1
ENV			
V	17	4	1
O	73	8	1
S	162	11	1
PI	64	6	1
PR	135	14	1
MC	208	17	1
MB	256	20	1

ENV: Soil environments; V: Soils under vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub.

3.1.2.4. Soil reaction

The seasonal changes in soil moisture, temperature, microbial activity, and plant growth can vary the soil pH values and vice versa. The seasonal effect may be reflected in the loss or formation of salts during the different periods of the year. Thus, the pH values fluctuate as the soil wets and dries. As the soil dries, salt concentration increases, soluble cations replace exchangeable hydronium (i.e., H_3O^+) or aluminum ions, and the soil solution becomes more acid. The presence of the exchangeable acidifying chemical species can be evaluated in soil solution by the measurements of soil pH with a 0.1 N KCl solution. Potassium ions with high ionic strength displace hydronium and aluminum ions. Acid ions, displaced by K^+ from the exchange complex, increases H^+ concentration in the soil solution and the pH decreases. The comparison of $\text{pH}_{\text{H}_2\text{O}}$ to pH_{KCl} provides an assessment of the nature of the net electric charge on the colloidal system (ΔpH). Both the pH in H_2O and KCl were measured on seasonal basis to calculate the ΔpH in the soils under study. A descriptive statistical analysis for the seasonal means of pH in distilled H_2O and 0.1 N KCl solutions with the difference in the pH values (ΔpH) and electrical conductivity is shown in Table 29 by using data obtained from all the studied soils. Soil pH values were lower than neutrality in all soil environments with H_2O and were found even much lower when KCl 0.1 N was used (Table 29). The pH classification allowed to define the soils as weakly acidic, with an average pH of 5.5-6.5. Data were in agreement with the parent material composition. No carbonate content was found in all the studied soils.

The ΔpH was always high ($\Delta\text{pH}\sim 0.5\text{-}1.5$) indicating some imbalance between exchangeable cations and acidification conditions, suggesting lime requirements especially with no tolerant cultivated plants. However, all the plants (spontaneous or cultivated) existing in the area of study are tolerant to acid soil reaction. The lowest pH values were recorded in the soils under pasture (PR). The seasonal fluctuations of soil pH and EC were not strong along the period of study.

The values of electrical conductivity were always found very low (max value 0.17 dS m^{-1} all along the experimental period) confirming no salinity conditions in the studied soils.

Table 29. Descriptive statistical analysis for the seasonal means of pH in H₂O and 0.1 N KCl solution with the difference in the pH values (Δ pH) and electrical conductivity from winter 2008 to autumn 2009.

ENV	Season	pH in H ₂ O solution					pH in 0.1N KCl solution					Δ pH	EC (dS m ⁻¹)				
		Min	Max	Mean	σ	CV	Min	Max	Mean	σ	CV		Min	Max	Mean	σ	CV
V	W08	6.71	6.85	6.78	0.10	1.43	5.88	5.97	5.93	0.06	1.07	0.85	0.05	0.06	0.05	0.00	5.56
	Sp08	6.81	6.93	6.88	0.06	0.91	6.14	6.20	6.17	0.03	0.49	0.71	0.06	0.07	0.07	0.00	7.18
	Su08	6.42	6.71	6.57	0.21	3.12	5.68	5.70	5.69	0.01	0.25	0.88	0.06	0.06	0.06	0.00	0.68
	Au08	7.03	7.07	7.05	0.03	0.40	6.30	6.31	6.31	0.01	0.11	0.75	0.04	0.04	0.04	0.00	2.82
	W09	6.58	6.68	6.63	0.05	0.75	5.41	5.68	5.50	0.16	2.83	1.13	0.06	0.06	0.06	0.00	4.89
	Sp09	6.15	6.43	6.29	0.20	3.15	5.01	5.10	5.06	0.06	1.26	1.24	0.09	0.10	0.10	0.01	5.72
	Su09	5.74	6.21	5.97	0.24	3.95	4.96	5.86	5.29	0.50	9.37	0.68	0.12	0.14	0.13	0.01	7.05
	Au09	6.22	6.61	6.38	0.21	3.23	5.51	5.78	5.62	0.14	2.56	0.76	0.06	0.07	0.07	0.00	7.14
O	W08	6.41	6.60	6.50	0.13	2.01	5.55	5.63	5.59	0.05	0.94	0.91	0.07	0.07	0.07	0.00	5.71
	Sp08	6.40	6.50	6.45	0.07	1.10	5.73	5.80	5.77	0.05	0.86	0.69	0.10	0.11	0.10	0.01	8.31
	Su08	6.10	6.20	6.15	0.07	1.15	5.12	5.15	5.14	0.02	0.41	1.02	0.04	0.04	0.04	0.00	1.51
	Au08	6.80	7.03	6.92	0.16	2.35	6.21	6.22	6.22	0.00	0.08	0.70	0.06	0.06	0.06	0.00	1.40
	W09	6.34	6.65	6.48	0.16	2.43	5.14	5.33	5.26	0.10	1.94	1.22	0.07	0.08	0.08	0.00	2.40
	Sp09	6.26	6.45	6.36	0.13	2.11	5.32	5.32	5.32	0.00	0.00	1.04	0.04	0.05	0.05	0.00	6.23
	Su09	6.40	6.85	6.60	0.23	3.45	5.29	6.21	5.74	0.46	8.01	0.86	0.07	0.08	0.08	0.00	5.00
	Au09	6.31	6.64	6.43	0.18	2.84	5.35	5.73	5.54	0.19	3.43	0.89	0.07	0.08	0.07	0.01	7.45
S	W08	6.11	6.16	6.14	0.04	0.60	5.06	5.15	5.10	0.06	1.25	1.03	0.15	0.16	0.16	0.00	2.83
	Sp08	6.24	6.25	6.25	0.01	0.11	5.47	5.49	5.48	0.01	0.26	0.77	0.08	0.09	0.08	0.00	0.96
	Su08	5.77	5.80	5.79	0.02	0.37	4.11	4.13	4.12	0.01	0.34	1.67	0.25	0.26	0.25	0.01	1.98
	Au08	6.11	6.20	6.16	0.06	1.03	5.30	5.37	5.34	0.05	0.93	0.82	0.20	0.21	0.20	0.01	3.00
	W09	6.32	6.40	6.36	0.06	0.89	5.35	5.60	5.43	0.14	2.66	0.93	0.09	0.09	0.09	0.00	0.91
	Sp09	6.52	6.65	6.59	0.09	1.40	5.75	5.84	5.80	0.06	1.10	0.79	0.09	0.10	0.10	0.00	3.35
	Su09	5.57	6.73	6.32	0.65	10.33	4.48	5.84	5.38	0.78	14.49	0.94	0.12	0.13	0.12	0.01	7.52
	Au09	6.52	6.69	6.61	0.09	1.29	5.46	6.29	5.79	0.44	7.61	0.82	0.11	0.13	0.12	0.01	4.30
PI	W08	5.97	6.00	5.98	0.02	0.41	5.27	5.31	5.29	0.03	0.57	0.69	0.06	0.06	0.06	0.00	3.88
	Sp08	6.00	6.07	6.04	0.05	0.82	5.57	5.60	5.59	0.02	0.38	0.45	0.03	0.04	0.04	0.00	2.86
	Su08	5.72	5.74	5.73	0.01	0.25	4.78	4.87	4.83	0.06	1.32	0.91	0.06	0.06	0.06	0.00	2.28
	Au08	5.88	5.90	5.89	0.01	0.24	5.31	5.34	5.33	0.02	0.40	0.57	0.09	0.09	0.09	0.00	4.01
	W09	6.26	6.29	6.27	0.02	0.28	5.41	5.43	5.42	0.01	0.18	0.85	0.06	0.06	0.06	0.00	1.48

	Sp09	6.70	6.81	6.76	0.08	1.15	4.62	5.83	5.23	0.86	16.38	1.53	0.06	0.07	0.06	0.00	5.60
	Su09	5.78	6.45	6.21	0.37	6.01	4.49	5.41	5.04	0.49	9.67	1.17	0.07	0.08	0.08	0.01	6.80
	Au09	6.01	6.69	6.25	0.38	6.11	4.52	5.63	5.03	0.56	11.12	1.22	0.07	0.07	0.07	0.00	3.66
PR	W08	5.75	5.80	5.77	0.03	0.58	5.04	5.06	5.05	0.02	0.35	0.73	0.11	0.12	0.12	0.00	3.76
	Sp08	5.81	5.84	5.83	0.02	0.36	5.19	5.20	5.20	0.01	0.14	0.63	0.08	0.09	0.08	0.00	4.14
	Su08	5.65	5.75	5.70	0.07	1.24	5.18	5.20	5.19	0.01	0.27	0.51	0.10	0.10	0.10	0.00	2.70
	Au08	5.72	5.75	5.74	0.02	0.37	4.60	4.66	4.63	0.04	0.92	1.11	0.12	0.13	0.12	0.00	3.47
	W09	5.82	5.85	5.84	0.02	0.26	5.17	5.18	5.17	0.01	0.11	0.66	0.16	0.16	0.16	0.00	1.31
	Sp09	5.69	5.91	5.80	0.16	2.68	4.88	5.03	4.96	0.11	2.14	0.85	0.13	0.15	0.14	0.01	7.14
	Su09	5.51	5.96	5.78	0.24	4.15	4.60	5.40	5.03	0.40	8.02	0.75	0.15	0.16	0.15	0.00	2.86
	Au09	5.50	5.63	5.57	0.09	1.65	4.73	5.08	4.85	0.20	4.17	0.72	0.16	0.18	0.17	0.01	8.43
MC	W08	6.08	6.16	6.12	0.05	0.87	5.38	5.39	5.38	0.01	0.10	0.74	0.08	0.08	0.08	0.00	3.95
	Sp08	6.11	6.20	6.16	0.06	1.03	5.28	5.29	5.29	0.01	0.13	0.87	0.06	0.06	0.06	0.00	2.21
	Su08	5.80	5.90	5.85	0.07	1.21	5.37	5.38	5.38	0.01	0.13	0.48	0.09	0.09	0.09	0.00	1.15
	Au08	6.10	6.20	6.15	0.07	1.15	5.37	5.38	5.38	0.01	0.13	0.78	0.07	0.08	0.07	0.01	6.73
	W09	6.32	6.33	6.32	0.01	0.09	5.49	5.49	5.49	0.00	0.00	0.83	0.09	0.09	0.09	0.00	1.70
	Sp09	6.80	6.94	6.87	0.10	1.44	5.17	5.57	5.37	0.28	5.27	1.50	0.08	0.09	0.09	0.01	6.61
	Su09	6.25	6.85	6.51	0.31	4.71	4.78	5.89	5.43	0.58	10.66	1.08	0.10	0.12	0.11	0.01	7.11
	Au09	5.89	6.24	6.11	0.19	3.10	5.07	5.17	5.12	0.05	0.98	0.98	0.08	0.10	0.09	0.01	6.86
MB	W08	5.81	5.89	5.85	0.06	1.06	5.19	5.23	5.21	0.03	0.58	0.64	0.10	0.11	0.11	0.00	3.94
	Sp08	5.90	6.00	5.95	0.07	1.19	5.32	5.34	5.33	0.01	0.27	0.62	0.09	0.09	0.09	0.00	1.52
	Su08	5.56	5.65	5.61	0.06	1.14	5.02	5.10	5.06	0.06	1.12	0.55	0.18	0.19	0.19	0.01	3.84
	Au08	5.70	5.80	5.75	0.07	1.23	5.15	5.20	5.18	0.04	0.68	0.57	0.06	0.07	0.06	0.00	4.04
	W09	6.06	6.12	6.09	0.03	0.49	5.27	5.29	5.28	0.01	0.22	0.81	0.08	0.08	0.08	0.00	1.20
	Sp09	6.10	6.40	6.25	0.21	3.39	4.95	5.87	5.41	0.65	12.02	0.84	0.08	0.09	0.08	0.01	8.60
	Su09	5.87	6.10	6.01	0.12	2.01	5.18	5.37	5.25	0.10	1.94	0.75	0.09	0.10	0.09	0.01	5.50
	Au09	5.75	6.08	5.90	0.17	2.83	5.01	5.34	5.13	0.19	3.61	0.77	0.09	0.10	0.10	0.00	4.29

Min: Minimum; Max: Maximum; σ : Standard deviation; CV: Coefficient of variation; W: Winter; Sp: Spring; Su: Summer; Au: Autumn; 08: 2008; 09: 2009; ENV: Soil environments; V: Soils under cultivated vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub.

3.1.3. General comments on physicochemical characteristics

The mean annual values of the selected soil physical and chemical properties were calculated (\pm standard errors) and reported in Table 30. As above mentioned, all soils showed a sandy loam texture but soils under vines. This evidence has suggested the very high importance exerted by the stable organic compounds which may compensate the minor amount of clay. Effectively, those soil environments increasing in organic compounds show better physical properties like soil moisture, bulk density, and water holding capacity. Despite that, the pH seems to have an independent trend more associated to the acid parent material. The variation in soil organic carbon and total nitrogen content between the soils under the same current soil use has been attributed mainly to the history of post agricultural management, land use change, age of abandonment, plant succession, and periodical perturbations such as wildfire occurrence. The highest organic carbon and nitrogen were found in soils under pasture followed by soils under *Erica* (MB) and *Cistus* (MC) scrubs and soils under cork trees (Table 30). Soils under stands of pine trees may negatively affect the microbial community and cause soil nutrients decline with direct impoverishment on organic carbon content (Iovieno et al., 2006). A 40% decrease in soil organic carbon content (SOC) was recorded in this soil with respect to SOC in soils under cork trees. These findings may be informative on the effectiveness of vegetation cover to soil recovering capacity after abandonment. Similarly, abandoned soil environments often affected by fire occurrence such as *Cistus* scrub may temporarily alter soil parameters like clay, BD, WHC, SOC, and nitrogen content (Pardini et al., 2004b). The SOC content in MC soil environment frequently affected by fire, decreased by 18% with respect to MB soil environment. The relatively low percentage of SOC decrease in the former environment may be attributed to a natural and fast recovering time after the fire, which has been reported by several authors (Pardini et al., 2003; Ferran et al., 2005; Spanos et al., 2005). However, each fire event is a devastating process affecting the relationship between biotic and abiotic soil components and reiterated fires may deeply affect the recover potential of plants and soil. In general, frequent fire occurrence in MC environment, hardly decomposable organic debris in PI environment, and insufficient agricultural management in V and O soil environment decreased their average organic carbon content by 94% with respect to S, PR, and MB soil environments, mainly

affecting water storage capacity and soil structure. Moreover, these soils showed higher erosion rates and nutrient decline (Pardini et al., 2003).

Table 30. Mean annual values (\pm standard deviation) of selected soil physical and chemical parameters determined during the observed period.

ENV	Clay (%)	Silt (%)	Sand (%)	BD (g cm^{-3})	WHC (%)	SM (%)	pH	SOC (%)	TN (%)	C/N
V	4.17 ± 1.44	11.25 ± 2.17	84.59 ± 0.72	1.52 ± 0.07	25.81 ± 3.30	4.20 ± 1.07	6.57 ± 0.35	0.31 ± 0.08	0.06 ± 0.02	5.62 ± 2.79
O	7.92 ± 1.35	29.17 ± 4.81	62.92 ± 4.59	1.34 ± 0.07	46.94 ± 6.54	7.11 ± 1.43	6.49 ± 0.22	1.53 ± 0.23	0.18 ± 0.05	8.80 ± 1.77
S	11.67 ± 2.89	27.50 ± 4.56	60.84 ± 3.95	1.15 ± 0.13	67.16 ± 10.86	10.72 ± 2.45	6.27 ± 0.26	2.94 ± 0.34	0.24 ± 0.02	12.30 ± 1.78
PI	10.21 ± 2.54	21.88 ± 4.97	67.92 ± 3.16	1.20 ± 0.13	57.69 ± 15.62	9.05 ± 2.11	6.14 ± 0.31	2.10 ± 0.51	0.18 ± 0.03	12.27 ± 4.74
PR	15.84 ± 4.08	18.75 ± 5.47	65.42 ± 6.56	1.10 ± 0.12	69.28 ± 12.46	16.99 ± 4.91	5.75 ± 0.11	3.63 ± 0.25	0.44 ± 0.03	8.24 ± 0.51
MC	10.42 ± 0.72	27.92 ± 7.82	61.67 ± 8.53	1.18 ± 0.12	67.02 ± 8.73	12.82 ± 1.94	6.26 ± 0.31	2.97 ± 0.37	0.28 ± 0.03	10.51 ± 0.70
MB	12.09 ± 1.97	25.42 ± 1.45	62.50 ± 2.50	0.98 ± 0.14	78.14 ± 5.77	13.11 ± 3.58	5.93 ± 0.20	3.50 ± 0.57	0.36 ± 0.05	9.78 ± 1.95

BD: Bulk density; WHC: Water holding capacity; SM: Soil moisture; SOC: Soil organic carbon; TN: Total nitrogen; C/N: Carbon/nitrogen ratio; ENV: Soil environments; V: Soils under cultivated vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub.

Within the cultivated soils, soil under vineyards indicated an extremely low organic carbon being total nitrogen added often as chemical fertilizer, thus proportionally higher than organic carbon. This resulted in a very low value of C/N ratio along the observed period with a yearly average of 5.62 for soils under vines and 8.80 for soils under olive groves. The C/N ratio increased to 12.27 in soils under stands of pine trees because of dry needle layer on soil surface which are more resistant to metabolic activity. Manure application following periodical grazing in soils under pasture increased nitrogen content. It is well known that ammonia is trapped at acid pH while CO_2 is lost. The N enrichment enhanced microbial activity consequently producing higher mineralization thus decreasing the C/N ratio to 8.24, despite the relatively acid pH. A more conservative carbon dynamics is likely to occur in S, MC, and MB soil environments with C/N ratios of 12.30, 10.51, and 9.78 respectively.

It must be pointed out that the moisture regime is fundamental in these very shallow soils (0-40 cm depth) with reduced A horizon over a stony C/R horizon, in order to favor humus formation, nutrients dynamics and soil structure improvement. If the capacity to retain water decreases, it would drastically reduce soil moisture content within rainfall events during wetting-drying cycles, reducing soil evolution potential and increasing water loss by runoff and evaporation. Thus, according to the state of the soil, rainfall events may be more or less effective in terms of erosion. Along the period of observation, from winter 2008 to autumn 2009, the mean total amount of rainfall (measured with rain gauges at 1 m height) varied between the soil environments, reaching values of 47, 40, 28, 36, 31, 38, 34 l m⁻² for V, O, S, PI, PR, MC, MB respectively. The rainfall average in V, O, PI, MC soil environments was 40 l m⁻², 1.3 folds the average rainfall recorded in the S, PR, MB soil environments (31 l m⁻²), which may be explained by a cleared plant canopy. Although, higher water storage capacity may be expected in soil receiving higher amount of rainfall, a higher potential for water storage was showed in soils with higher organic carbon content (S, PR and MB), indicating a better water holding capacity. In fact, a proper soil evolution after abandonment occurs when natural plant colonization and succession is not interrupted and may be beneficial to soil itself by contributing to the development of a more organic profile.

3.1.4. General qualification of soil fertility

In all the studied soils, the main three mineral components of slates are feldspars, i.e. Albite, Sanidine, and Plagioclase. Chemical hydrolysis produces Ca²⁺, Na⁺, K⁺, and Mg²⁺ solubilization into the soil solution which is in equilibrium with the adsorbed phase at the liquid/solid interphase. Other products of hydrolysis are clay minerals. The above mentioned cations are named exchangeable bases and their percentage with respect to the soil exchangeable sites function as a buffer to contrast acidification processes. It is well known that acidity in soils comes from H⁺ and Al³⁺ ions in the soil solution and sorbed to soil surfaces. While pH is the measure of H⁺ in solution, Al³⁺ is important in acid soils because between pH 4 and 6, Al³⁺ reacts with water (H₂O) forming AlOH²⁺, and Al(OH)₂⁺, releasing extra H⁺ ions. Many other processes contribute to the formation of acid soils including rainfall, fertilizer use, plant root activity and the weathering of primary and secondary soil minerals (Donald, 2003).

Excess rainfall also leaches cation from the soil, increasing the percentage of Al^{3+} and H^+ with respect to other cations.

Additional sources of soil acidity include oxidation-reduction “redox” reactions, microbial metabolic pathways of organic substrates, addition of soluble salts (fertilizers), and alkaline cations removal during plant uptake may cause acidification of the rhizosphere by releasing H^+ ions or organic acids from the root into the soil and solubilize insoluble micronutrients at neutral pH, such as iron, Cu, and Zn (Thomas and Hargrove, 1984). The exchangeable cations determined in all soils are reported in Table 31, expressed as annual means and standard deviations. Cation exchange capacity, exchangeable Al and base saturation (V) are also presented.

Table 31. Exchangeable cations (Al, Ca, Mg, K, and Na), cation exchange capacity (CEC), and base saturation (V) for the selected soil environments.

ENV	Al	Ca	Mg	K	Na	CEC	V
			cmole (+) kg^{-1}				(%)
V	1.53±0.42	2.05±0.01	7.56 ±0.77	0.50±0.08	5.56±0.23	5.08±4.01	308.50±27.20
O	0.13±0.09	1.70±0.71	7.89 ±0.20	0.72±0.20	5.76±0.07	6.44±3.35	249.50±35.20
S	0.08±0.02	2.78±0.60	10.61 ±0.44	0.84±0.17	5.89±0.16	14.40±5.10	139.70±26.90
PI	0.11±0.01	2.50±0.10	8.56 ±0.34	0.60±0.12	5.77±0.21	10.30±3.24	169.20±23.80
PR	0.23±0.04	1.23±0.20	11.50 ±0.25	0.60±0.34	5.79±0.21	13.60±3.54	140.60±28.20
MC	0.22±0.70	1.20±0.34	11.12 ±0.42	0.46±0.10	6.44±0.80	5.80±2.19	331.40±75.80
MB	0.09±0.60	2.27±0.35	10.97 ±0.33	0.48±0.11	5.95±0.78	18.40±4.12	106.90±38.10

ENV: Soil environments; V: Soils under cultivated vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub.

Exchangeable Al should also be considered an indicator of acidification at the exchange complex. It may be observed that its annual mean concentration decreases strongly from vines to scrubs. Thus, soils under vines indicated a higher susceptibility to acidification. Nevertheless, the low concentration of Ca, which should contrast acidification, is strongly compensated by Mg and K which hinder the soil desaturation but not the acidification process. The Ca/Mg ratio was 0.27, 0.22, 0.26, 0.29, 0.11, 0.11, and 0.21 for V, O, S, PI, PR, MC, MB soil environments, depicting a high Ca deficiency in these soils. Cation exchange capacity (CEC) is used as a measure of fertility and nutrient retention capacity. It varies according to the type of soil. Humus,

the end product of decomposed organic matter, has the highest CEC value because organic colloids have large quantities of negative charges. Clay has a great capacity to attract and hold cations because of its chemical structure. However, CEC varies according to the type of clay. It is very low in soils with weathered kaolinite clay and higher in soils with illite and smectite. As our soils had low clay content and the clay minerals were kaolinite and little illite, their content in organic compounds seems to be the key factor for their stability and fertility. CEC values ordered the soils as MB> S> PR> PI> O> MC> V. Once more results indicate that MB, S, and PR soils may show higher nutrient retention capacity, thus fertility, than V, MC, O and PI soils. A higher CEC with a suited base saturation takes longer time to acidify (as well as to recover from an acidified status) than a site with a low CEC (assuming similar base saturations).

3.1.4.1. Statistical approach to soil fertility

Principal component analysis (PCA) was used to test the relationships between variations in the main soil characteristics considered for the evaluation of soil fertility. PCA provides an orthonormal Eigen-basis for the space of the observed data on the basis that the largest eigenvalues correspond to the principal components associated with most of the variance among a number of observed variables. Soil variables loadings on the first three principal components (PCs) are shown in Table 32. The first PC explained 58.04% of the total variance and included the most relevant variables related to soil fertility (Table 32). Regarding soil acidity the PC I was characterized by high negative loadings on pH and Al and positive loadings on Mg, and Na. Other soil characteristics related to fertility showed high positive loadings on SOC, TN, CEC, SM, and WHC. Negative loadings to the PC II, explaining 16.34% of total variance, were assigned to exchangeable calcium and potassium while positive loading was for base saturation. High positive loading was found for soil phosphorus (TP) at the PC III, with 9.10% of variance explained, probably due to a lower interaction and variability in all the studied soils.

Factor scores are the contribution of each environment to the principal components (Table 33). Negative values may be interpreted as negative contributions to the subject under evaluation. The first PC explained 58.04% of the variance in the analyzed variables and it is reasonable to use it to classify the soil environments

according to their role in the overall fertility level. Soils were then classified as follows: PR> MB> MC> S> PI> O> V, the soils under vines resulted with lower fertility contribution because of the highest negative factor score (−1.73). Conversely, soils under pasture (PR) showed the highest positive score (+1.23) and its fertility potential to the area of study was statistically corroborated.

Table 32. Variables loadings on the three principal components structure for all the soil properties simultaneously. Values below 0.50 omitted.

Variable	PC I	PC II	PC III
BD (g cm ^{−3})	−0.91		
MI (kPa)	0.83		
SS (kPa)	0.89		
SM (%)	0.99		
WHC (%)	0.97		
pH	−0.88		
EC (dS m ^{−1})	0.64		
SOC (mg g ^{−1})	0.98		
TN (mg g ^{−1})	0.97		
TP (mg g ^{−1})			0.86
CEC (cmole kg ^{−1})	0.67		
Ca (cmole kg ^{−1})		−0.86	
Mg (cmole kg ^{−1})	0.93		
Na (cmole kg ^{−1})	0.55		−0.67
K (cmole kg ^{−1})		−0.74	
Al (cmole kg ^{−1})	−0.72		
Base saturation (%)		0.68	
Explained variance (%)			
Total (%)	58.04	16.34	9.10
Cumulative (%)	58.04	74.37	83.47

BD: Bulk density; MI: Mechanic impedance; SS: Shear strength; SM: Soil moisture; WHC: Water holding capacity; EC: Electrical conductivity; SOC: Soil organic carbon; TN: Total nitrogen; Total phosphorus; CEC: Cation exchange capacity.

Table 33. Factor score of the first three principal components.

ENV	Factor I	Factor II	Factor III
V	−1.73	0.54	0.09
O	−0.65	−0.12	0.06
S	0.14	−1.60	−0.82
PI	−0.33	−0.27	1.08
PR	1.23	0.60	1.40
MC	0.52	1.51	−1.42
MB	0.82	−0.66	−0.39

V: Soils under cultivated vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub.

3.1.5. Soil quality evaluation

According to the soil quality evaluation of the USA National Research Council-NARC (1993) and Shukla et al. (2006), soil quality assessment is reported in Table 34. Doran and Parkin (1994) suggested a similar number of soil parameter (minimum data set) to carry out soil quality evaluation based mainly on soil related functions. We have used the main soil quality indicators such as texture, BD, pH, TN, and SOC to assess the fertility and soil quality for each soil environments (Emran, 2011).

Table 34. Soil quality evaluation in the selected environments.

	V	O	S	PI	PR	MC	MB
Indicators	Evaluation						
Texture	Loamy sand	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam
BD (g cm ⁻³)	1.4-1.6	1.2-1.4	0.8-1.2	1.2-1.4	0.8-1.2	0.8-1.2	0.8-1.2
pH	V. compact	Compact	Acceptable	Compact	Acceptable	Acceptable	Acceptable
	6.5-7.5	5.5-6.5	5.5-6.5	5.5-6.5	5.5-6.5	5.5-6.5	5.5-6.5
	N. neutral	W. acidic	W. acidic	W. acidic	W. acidic	W. acidic	W. acidic
TN (%)	0-0.1	0.1-0.25	0.2-0.5	0.1-0.25	0.2-0.5	0.2-0.5	0.2-0.5
	V. depleted	Depleted	Adequate	Depleted	Adequate	Adequate	Adequate
SOC (%)	< 0.5	1.1-2	2.1-4	1.1-2	2.1-4	2.1-4	2.1-4
	V. depleted	Depleted	Adequate	Depleted	Adequate	Adequate	Adequate
Soil assessment							
Fertility level	Low	Moderate	M. High	Moderate	Mod. High	Mod. High	Mod. High
SQI	Very poor	Fair	Good	Fair	Good	Good	Good

SQI: soil quality index; V. compact: Very compact; N. neutral: Nearly neutral; W. acidic: Weakly acidic; V. depleted: Very depleted; M. High: Moderately High.

Also these results corroborated the clear difference between soils under V, O, and PI environments, and S, PR, MC and MB environments suggesting on the one hand that current cultivation practices are inappropriate and that reforestation with pines may delay the achievement of better soil conditions with respect to natural succession represented by S, PR, MC, and MB soil environments (Emran, 2011). When cultivated soils are maintained at minimum agricultural management, soil fertility and soil quality are low and degradation processes may be expected. Likewise, when a forestation is carried out with species not properly contributing to soil health (pines), soil quality is progressively decreasing. This is the first indication on the relationship between soil quality and soil management or abandonment. Soils under meadows or under cork trees and *Erica* scrubs showed higher soil quality level among the studied soils.

3.2. Rainfall/runoff/erosion relationships

Natural rainfall events were recorded during two years (2008-2009) by measuring rainfall gauges installed in each soil environment under study. Three gauges per environment were settled and consisted of plastic bottles of 20 liters capacity, each one inserted in the soil at a depth of 10 cm and equipped with a funnel of 33 cm diameter, to collect water rainfall. The total height of the device was 100 cm. Seven rainfall events were recorded during 2008 whereas eleven events were recorded during 2009 (Table 35). The mean distribution of rainfall events per each DOY (day of the year) and per environments was highly variable and showed a total yearly rainfall precipitation of 214 l m^{-2} during 2008 and 485 l m^{-2} during 2009.

Table 35. The mean rainfall amount (l m^{-2}) recorded for each soil environment at any DOY.

YEAR	DOY	V	O	S	PI	PR	MC	MB
2008	64	14	15	17	8	21	27	21
	101	23	35	28	25	14	26	34
	141	60	40	2	49	37	54	73
	149	55	20	18	16	30	18	17
	158	24	18	21	18	39	24	20
	185	14	66	18	10	14	18	18
	312	115	18	35	54	35	60	60
2009	13	54	121	49	80	35	48	78
	23	1.45	1.45	1.03	1.21	1.09	1.09	1.09
	37	90	88	30	84	90	109	96
	51	0	0	0	0	0	0	0
	65	30	72	72	84	42	36	48
	85	0	0	0	0	0	0	0
	93	25	22	18	22	42	33	27
	118	136	71	64	135	48	75	76
	135	51	37	28	29	35	40	46
	167	36	36	2	24	48	24	36
	247	30	33	28	27	39	36	21
	279	24	8	24	18	40	28	6
	310	60	84	60	66	4	51	1

DOY: Day of the year; V: Soils under cultivated vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub.

A descriptive statistical analysis was tried for all data of rainfall events obtained during the two years of measurements (Table 36), emphasizing the high variability of

rainfall events at each soil environment and between environments as well as the interannual variability for all environments, during the period of measurements.

Table 36. Descriptive statistical analysis of rainfall data ($l\ m^{-2}$) along the whole observed period.

ENV	2008							2009						
	n	Min	Max	Mean	σ	CV	TOT	n	Min	Max	Mean	σ	CV	TOT
V	7	14	115	44	36	84	305	11	1.45	136	49	37	76	540
O	7	15	66	30	18	61	213	11	1.45	121	52	37	72	574
S	7	2	35	20	10	51	140	11	1.03	72	34	24	70	378
PI	7	8	54	26	19	72	181	11	1.21	135	52	41	78	571
PR	7	14	39	27	11	39	190	11	1.09	90	39	24	61	425
MC	7	18	60	32	17	53	227	11	1.09	109	49	28	58	481
MB	7	17	73	35	23	65	244	11	1.00	96	40	33	83	337

ENV: Soil environments; V: Soils under cultivated vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub; n: Number of rainfall events; Min: Minimum rainfall event; Max: Maximum rainfall event; σ : Standard deviation; CV: Coefficient of variation; TOT: Total rainfall for each environment.

3.2.1. Rainfall events and soil environments

The selected soil environments were delimited within approximately $5\ km^2$. The high variability of the rainfall amounts were related to the irregular topography and the speed and direction of the *tramuntana* wind in the area. According to the peculiarity of the environments under study, the recorded rainfall events at each soil environment added further information on the impact of rainfall to soil characteristics. Along the time of observations (winter 2008 to autumn 2009), the mean total amount of rainfall varied considerably between the soil environments, mainly due to plant canopy, with values of 47, 40, 28, 36, 31, 38, $34\ l\ m^{-2}$ for V, O, S, PI, PR, MC, MB respectively, which may be coherent with the total annual mean precipitation in the area ranging from 300 to 450 mm according to dry or wet years. With respect to the interannual variability, the mean rainfall recorded during 2008 were 46, 32, 22, 25, 25, 34, 34, while during 2009 were 48, 49, 33, 48, 37, 42, 34 for V, O, S, PI, PR, MC, MB respectively. This supposed a rainfall increase of 4, 52, 51, 93, 49, and 23% for V, O, S, PI, PR, MC environments whilst in the MB environment a rainfall decrease of 2% was recorded in 2009 with respect to 2008.

3.2.1.1. *Soils under cultivated vines and olive groves*

During 2008, the highest rainfall event (115 l m^{-2}) in soils under vines (V) was recorded in November (DOY 312) and the lowest (14 l m^{-2}) in March (DOY 64) (Figure 53A). Due to higher canopy cover, the rainfall recorded in soils under olive groves was lower in 2008 (maximum 66 l m^{-2} , DOY 185 and minimum 15 l m^{-2} , DOY 64). A 42% decrease of maximum rainfall was therefore recorded in olive groves though the period did not coincide. The minimum rainfall amount in cultivated soils was similar and in the same period (winter). Despite the different rainfall distribution along 2008 between vines and olive groves, the former environment received 305 l m^{-2} against 213 l m^{-2} received from the latter, which represents 30% decrease of rainfall.

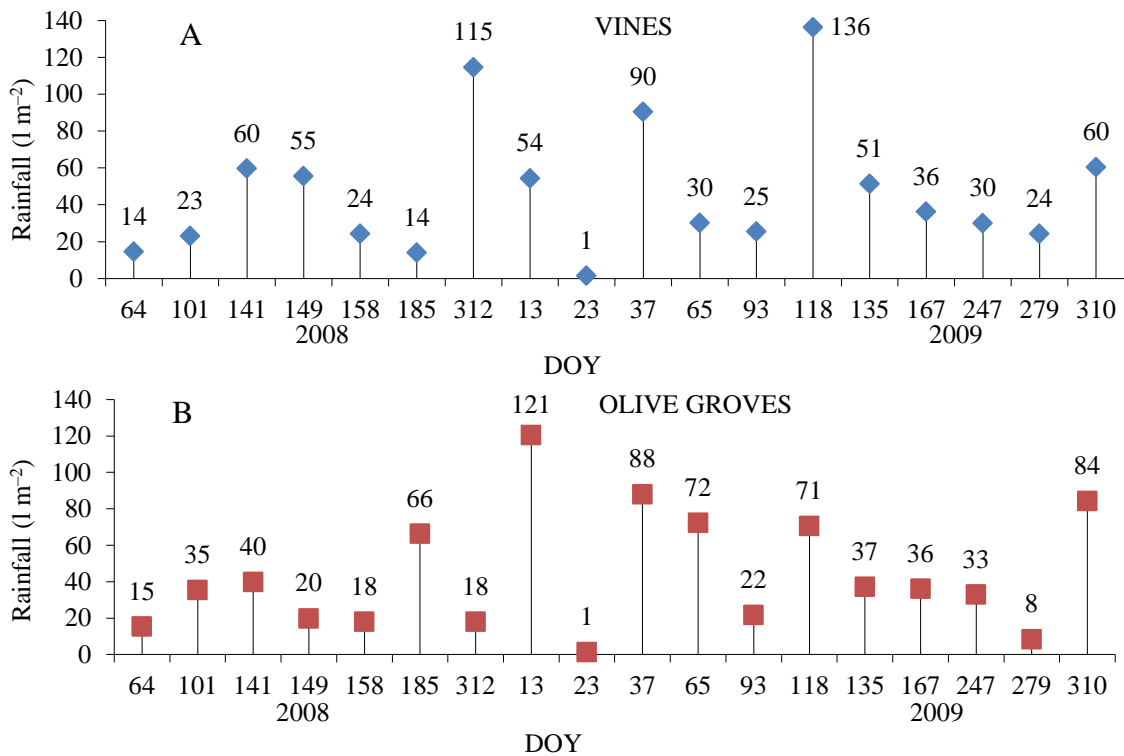


Figure 53. Distribution of rainfall amount recorded in soils under vines (A) and olive groves (B) along the observed period.

During 2009, rainfall distribution was different between vines and olive groves environments. The highest rainfall amount in soils under vines was recorded in April (136 l m^{-2} , DOY 118) and the lowest in January (1 l m^{-2} , DOY 23) (Figure 53A). Conversely, the maximum and minimum amounts of rainfall in soils under olive groves were found in January (121 and 1 l m^{-2} in DOY 13 and 23 respectively). Once more the minimum rainfall amounts in soils under vines and olive groves were recorded in winter

(1 l m⁻²) though in this season the total rainfall in olive groves increased approximately by 60% with respect to vines (Figure 53B). Despite the different rainfall distribution between vines and olive groves the former environment received a total of 540 l m⁻² whereas the latter received 574 l m⁻² which represented 6.3% increase of rainfall.

3.2.1.2. Soils under stands of cork and pine trees

During 2008, the highest rainfall event (35 l m⁻²) in soils under cork trees (S) was recorded in November (DOY 312) and the lowest (2 l m⁻²) was in May (DOY 141) (Figure 54A). The rainfall recorded in soils under pine trees (PI) was higher in 2008 (maximum 54 l m⁻², DOY 312 and minimum 8 l m⁻², DOY 64). In November 2008 (DOY 312), an increase of 54% of rainfall was recorded in soils under pines with respect to soils under cork trees. This pattern may indicate that the interception capacity of pine canopy is lower due to the shape of pine needles. The minimum rainfall records in soils under pine and cork trees forests were not recorded at the same season. During 2008, the soils under cork trees environment received 140 l m⁻² against 181 l m⁻² in pines, which represents an increase of 29% (Figure 54A and B).

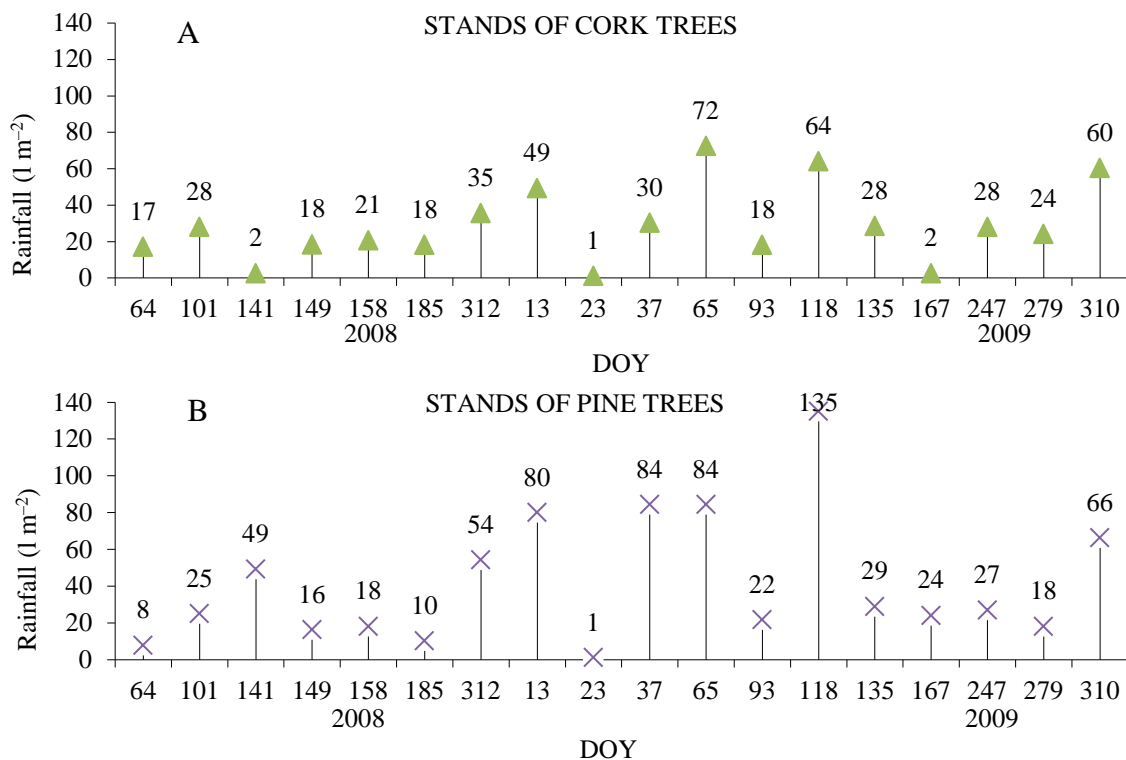


Figure 54. Distribution of rainfall amount recorded in soils under stands of cork (A) and pine trees (B) along the observed period.

In 2009, the highest rainfall amount in soils under cork trees was recorded in March (72 l m^{-2} , DOY 65) and the lowest in January (1 l m^{-2} , DOY 23). The maximum and minimum amounts of rainfall in soils under pine trees were found in April (135 l m^{-2} , DOY 118) and January (1 l m^{-2} , DOY 23) respectively. Therefore, an increase of 88% of maximum rainfall was recorded in pines with respect to cork trees though in different spring periods (Figure 54A and B). In 2009, pines environment received 571 l m^{-2} , 51% more than cork trees environment (378 l m^{-2}).

3.2.1.3. Soils under pasture

In 2008, the highest rainfall amount in soils under pasture (PR) was recorded in June (39 l m^{-2} , DOY 158) and the minimum was in April (14 l m^{-2} , DOY 101). In 2009 the maximum rainfall event was recorded in February (90 l m^{-2} , DOY 37) while the minimum value (1 l m^{-2} , DOY 23) was in January. The total rainfall for pasture during 2009 was 425 l m^{-2} which represent 123% increase with respect to 2008 (190 l m^{-2}) (Figure 55).

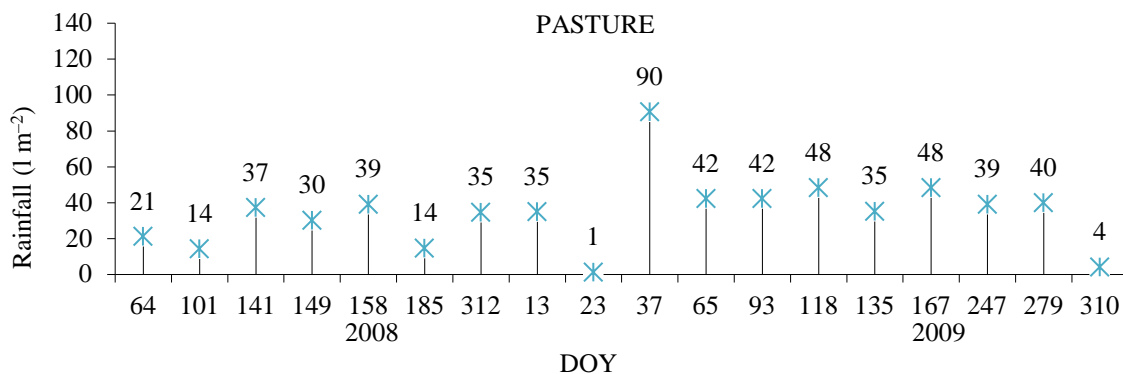


Figure 55. Distribution of rainfall amount recorded in soils under pasture (PR) along the observed period.

3.2.1.4. Soils under *Cistus* and *Erica* scrubs

During 2008, the highest rainfall event in soils under *Cistus* scrub was recorded in November (60 l m^{-2} , DOY 312) and the minimum was in May (18 l m^{-2} , DOY 149). The maximum rainfall recorded in soils under *Erica* scrub was higher (73 l m^{-2} , DOY 141) and recorded in May while the minimum was also in May (17 l m^{-2} , DOY 149). An increase of 22% of maximum rainfall was therefore recorded in soils under *Erica*

scrub though the period did not coincide. Despite the different distribution of rainfall along 2008 between *Cistus* and *Erica* scrub environments, the former environment received 227 l m^{-2} against 244 l m^{-2} received from the latter, which represented 7% decrease of rainfall with respect to *Erica* scrub environment (Figure 56A and B).

During 2009, the rainfall distribution was rather similar between *Cistus* and *Erica* scrub environments. The highest rainfall amount in soils under *Cistus* scrub was recorded in February (109 l m^{-2} , DOY 37) and the lowest was in January (1 l m^{-2} , DOY 23). Similarly, the maximum and minimum rainfall amounts in soils under *Erica* scrub were found in in the same dates (96 l m^{-2} , DOY 37 and 1 l m^{-2} , DOY 23 respectively). Along 2009 the *Cistus* scrub environments received 481 l m^{-2} against 437 l m^{-2} received from *Erica* scrub environment, which represent decrease of 9% of rainfall in soils under *Erica* scrub (Figure 56A and B).

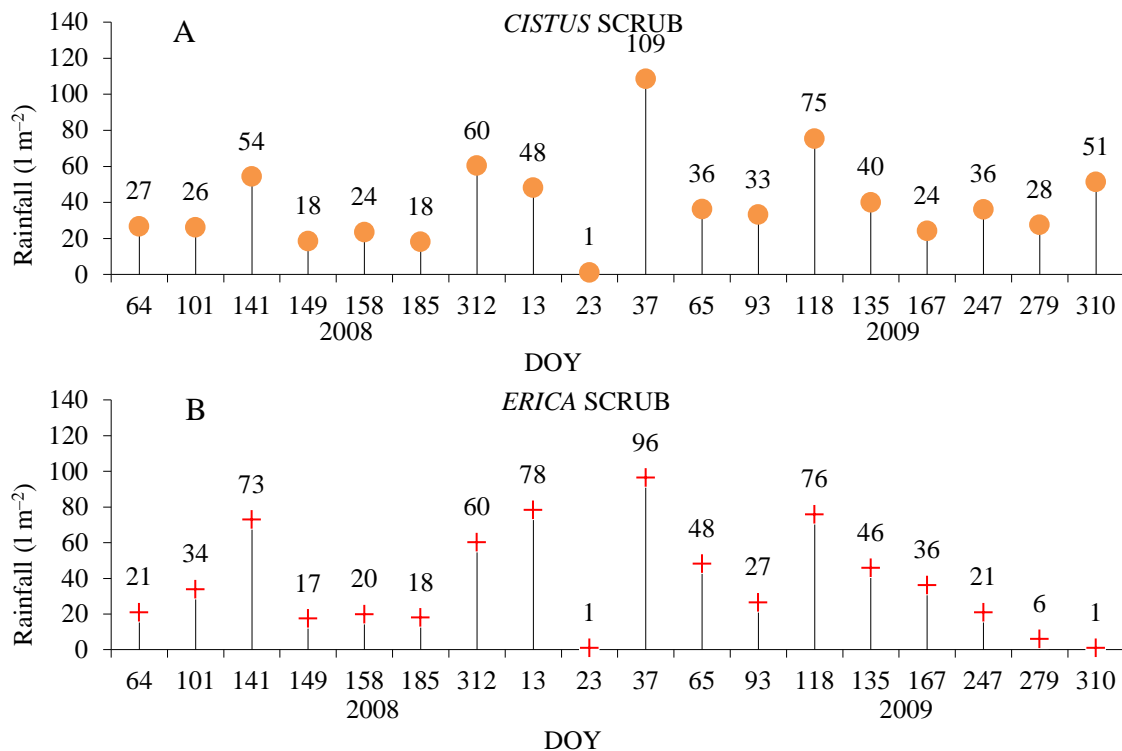


Figure 56. Distribution of rainfall amounts recorded in soils under *Cistus* (A) and *Erica* scrub (B) along the observed period.

3.2.2. Runoff and erosion

The results showed that the majority of water rainfall infiltrated into soil horizons as indicated by the runoff coefficient (RC) values expressed as percent of rainfall (Table 37). Field observations and measurements indicated that runoff generation started after the saturation of soil surface horizons, though patterns showed that runoff values decreased with rainfall increase in all the environments (Figure 57). The overland flow recorded in each environment was thus not considerable but sufficient to activate soil erosion processes able to remove soil particles according to the vulnerability of soil surface to erosion. The mean annual values of water runoff during 2008 were 0.12, 0.20, 0.04, 0.06, 0.17, 0.08, 0.07 l m⁻² producing 5.16, 0.61, 0.35, 0.19, 0.82, 0.26, 0.10 g m⁻² erosion while during 2009 water runoff was 0.19, 0.19, 0.10, 0.14, 0.25, 0.15, 0.17 l m⁻² producing 4.16, 0.59, 0.30, 0.24, 0.75, 0.32, 0.34 g m⁻² erosion for V, O, S, PI, PR, MC, MB soil environments respectively. Soils environments receiving more rainfall amounts (V, O, and PR) produced the highest values of water runoff and eroded particles (values in bold). The highest amount of soil erosion was recorded in the soil under cultivated vines associated to the lowest soil organic carbon (0.31%), lowest clay amount (4.17%), and a highest sand content (85%).

Table 37. The mean annual values for rainfall, runoff, runoff coefficient, and soil erosion.

ENV	2008				2009			
	Rainfall (l m ⁻²)	Runoff (l m ⁻²)	RC (%)	Erosion (g m ⁻²)	Rainfall (l m ⁻²)	Runoff (l m ⁻²)	RC (%)	Erosion (g m ⁻²)
V	43.57	0.12	0.31	5.16	49.08	0.19	0.42	4.15
	±36.45	±0.10	±0.19	±5.79	±37.17	±0.11	±0.29	±1.26
O	30.44	0.20	0.55	0.61	52.18	0.19	0.42	0.59
	±18.46	±0.23	±0.26	±0.45	±37.44	±0.13	±0.25	±0.38
S	19.95	0.04	0.20	0.35	34.37	0.10	0.26	0.30
	±10.21	±0.04	±0.13	±0.09	±24.09	±0.11	±0.19	±0.32
PI	25.87	0.06	0.22	0.19	51.95	0.14	0.29	0.24
	±18.59	±0.06	±0.14	±0.09	±40.71	±0.12	±0.31	±0.10
PR	27.20	0.17	0.64	0.82	38.65	0.25	2.75	0.75
	±10.59	±0.11	±0.43	±0.37	±23.53	±0.26	±5.32	±0.56
MC	32.49	0.08	0.21	0.26	43.76	0.15	0.95	0.32
	±17.36	±0.09	±0.16	±0.17	±28.26	±0.08	±1.85	±0.20
MB	34.81	0.07	0.15	0.10	45.08	0.17	0.99	0.34
	±22.73	±0.08	±0.12	±0.19	±30.67	±0.27	±1.79	±0.65

ENV: Soil environments; RC: Runoff coefficient; V: Soils under vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub.

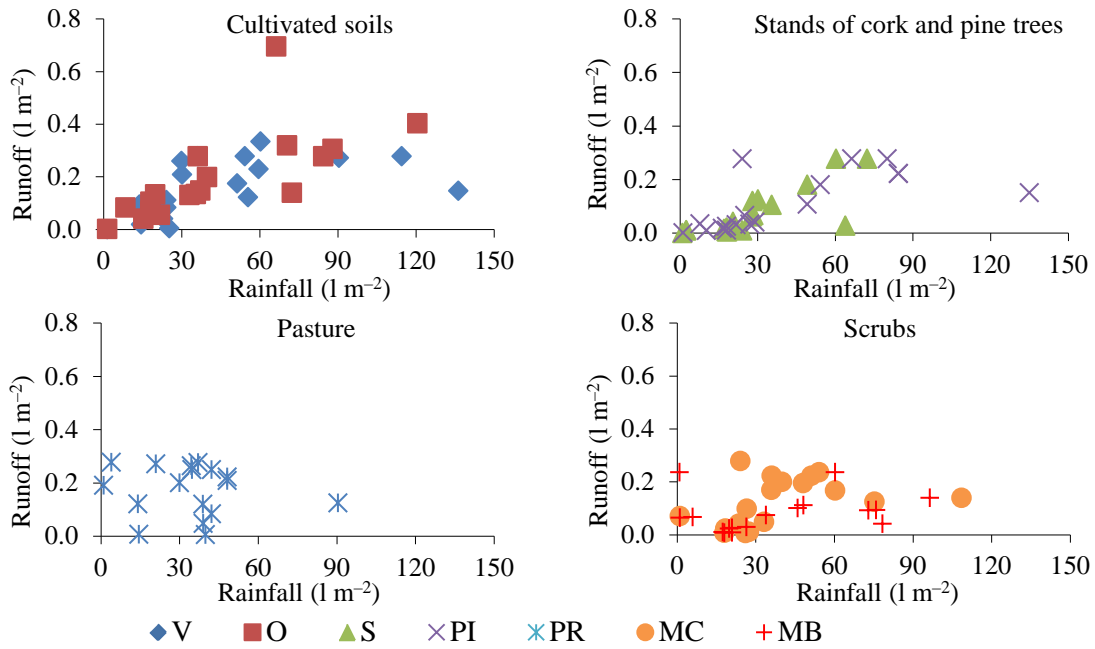


Figure 57. Runoff vs rainfall for the selected soil environments along the observed period.

Linear regressions were performed between cumulative rainfall and runoff along the whole observed period in order to compare patterns in runoff generation within soil environments under the same use and between different environments (Figure 58). The expected positive correlations indicated however a clear trend of runoff generation capacity that can be also seen by the slopes of the equations (Pardini et al., 2012).

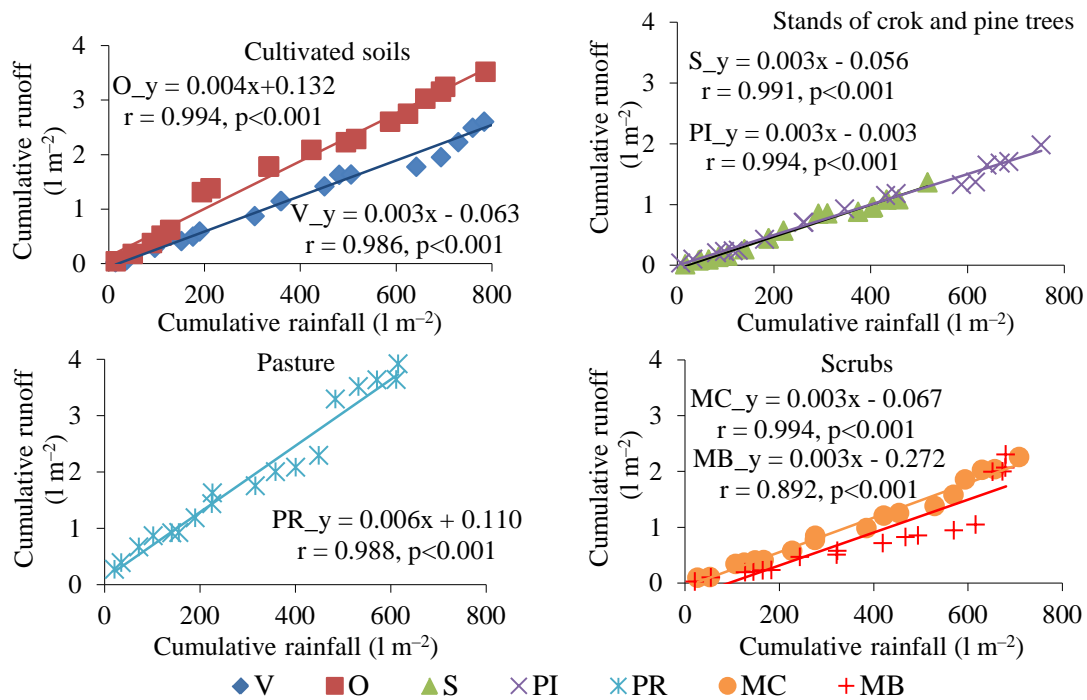


Figure 58. Cumulative runoff vs cumulative rainfall for the selected soil environments separated by land use and abandonment.

Figure 59 and Figure 60 show the overland flow mobilization of soil surface particles both in normal and cumulative arrangement of data.

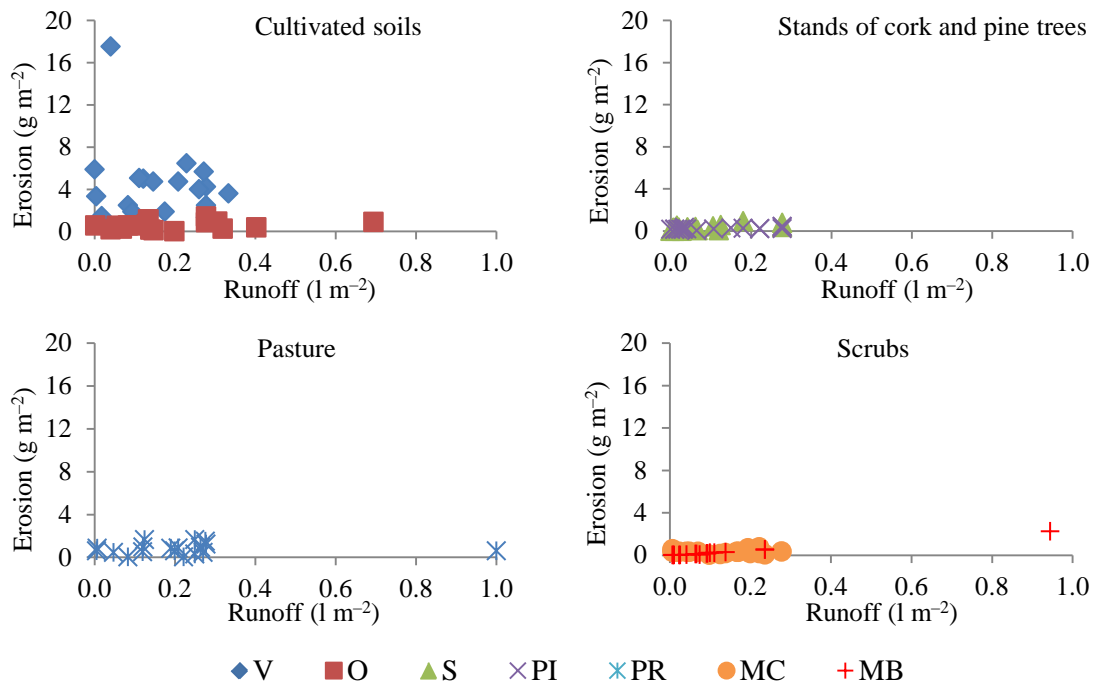


Figure 59. Runoff vs erosion for the selected soil environments separated by land use and abandonment.

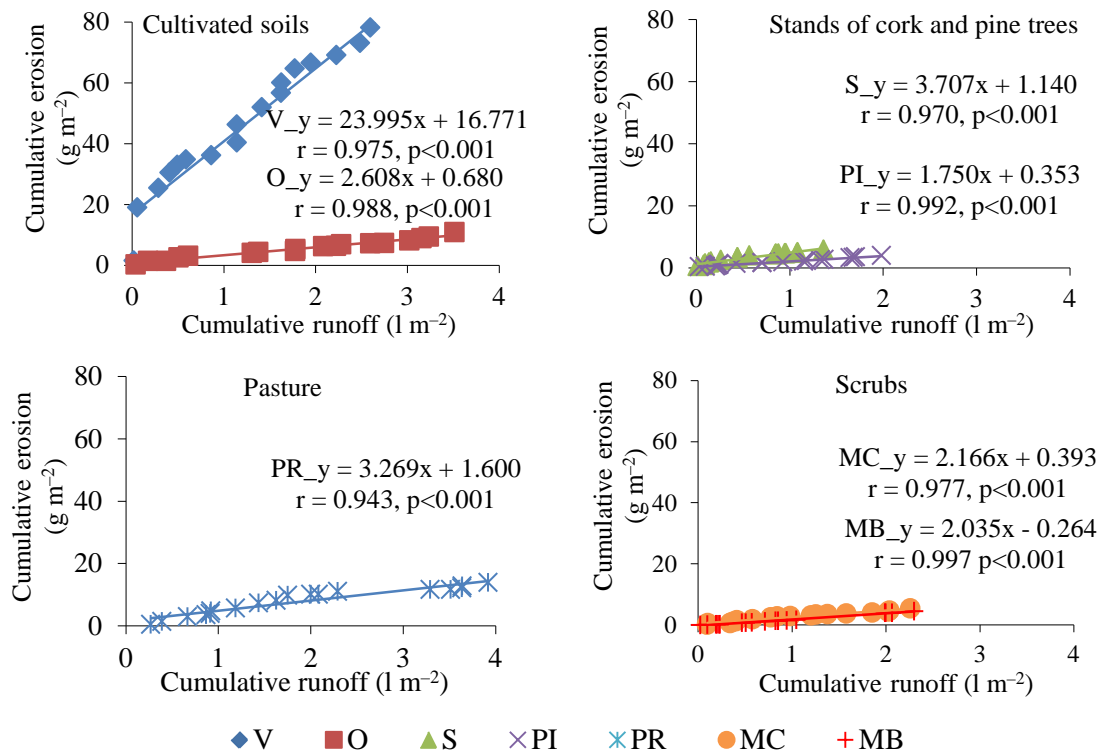


Figure 60. Cumulative erosion vs cumulative runoff for the selected soil environments reported by land use and abandonment.

As expected significant correlations were obtained by processing the values within soil environments under the same land use or abandonment. The amount of eroded soil varied between the soils environments according to the diverse plant cover and soil characteristics (Pardini et al., 2012). All soil environments showed low particle removal by water erosion, with respect to other monitored areas in Spain and in the Mediterranean basin (Grimm et al., 2002). The highest soil erosion per liter runoff and square meter was estimated in soil under cultivated vines as 41 g m^{-2} , corresponding to 0.41 Mg ha^{-1} . Erosion was progressively lower in soil under pasture (4.9 g m^{-2}), soil under stands of cork trees (4.8 g m^{-2}), soil under olive groves (3.3 g m^{-2}), soil under *Cistus* scrub (2.6 g m^{-2}), soil under stands of pine trees (2.1 g m^{-2}), and soil under *Erica* scrub (1.8 g m^{-2}). Despite the low erosion amounts, values may be consistent because of the shallow nature of these soils with very thin A horizons, even though according to the Albaladejo erosion scale built up for Spanish soils (Albaladejo Montoro and Stocking, 1989), the erosion rates of these soils will enter in the very low erosion category ($0\text{-}3 \text{ Mg ha}^{-1} \text{ year}^{-1}$).

In cultivated soils, the runoff production under olive groves was higher than that of soils under vines. For example, for each 100 liters of accumulated rainfall per square meter, the runoff was 0.57 l m^{-2} under cultivated olives, while 0.27 l m^{-2} were collected in soil under cultivated vines. Independently of low runoff coefficient one should expect more erosion with higher runoff production. Conversely, the soil erosion in soils under cultivated vines was estimated in 65 g m^{-2} , with a runoff amount of 2 l m^{-2} (Figure 60) against 6 g m^{-2} in soils under olive groves for the same runoff amount. The shallowness of soil profile, sandy structure, low organic carbon, and low available water content in soils under vines (V) may produce a higher susceptibility of soil surface to wetting/drying cycles and favor surface clods disruption. Findings may alert on the vulnerability of soil surface in soils under vines indicating that considerable erosion rates may be reached with a relatively limited runoff volume. Even though extrapolation of data may be misleading due to the variability of many field and climatic factors, vines could approximately mobilize $350\text{-}400 \text{ kg ha}^{-1} \text{ year}^{-1}$ which may be strongly detrimental for the functionality of soil ecosystem in the current conditions. The extrapolated total erosion rates would decrease substantially in forest soils (25 and $20 \text{ kg ha}^{-1} \text{ year}^{-1}$ for soils S and PI respectively) though soil PI would need 1.4 times runoff than soil S probably due to pine needles accumulated on soil surface hindering

particles removal. In soils under pasture the extrapolated erosion rates will result higher ($100 \text{ kg ha}^{-1} \text{ year}^{-1}$) whereas in soils under scrubs the extrapolated erosion rates will amount to 15 and $13 \text{ kg ha}^{-1} \text{ year}^{-1}$ for MC and MB soil respectively. The soils under MC and MB environments contain the same parent material, same texture (sandy loam), plant cover ($\sim 50\%$), soil depth (30-35 cm), and differ in land use history after abandonment. Even though the MC soil have shown slightly worse soil properties with respect to MB soil (less affected by fire), the surface behavior in term of response to water erosion is low, indicating as elsewhere reported a fast recovery period. However, intense downpours may have an important role on the dynamics of soil surface and the rates of depleted materials, especially if they occur immediately after fire devastation.

3.2.3. Runoff erosion and splash erosion

It is well known that the intensity and duration of rainfall are most likely controlling the rates and magnitudes of soil erosion (Nearing et al., 2005). Splashed soil particles, exacerbated by downpours are usually more abundant during short duration with high intensity rainfall due to highest kinetic energy. Although the erosion caused by long duration and less intensity rainfall is not as spectacular or noticeable as that produced during the intense downpours over shorter time, the amount of soil loss can be of great importance and accumulate over long time. The relationships between runoff erosion and splash erosion along the period of study can be seen in Figure 61.

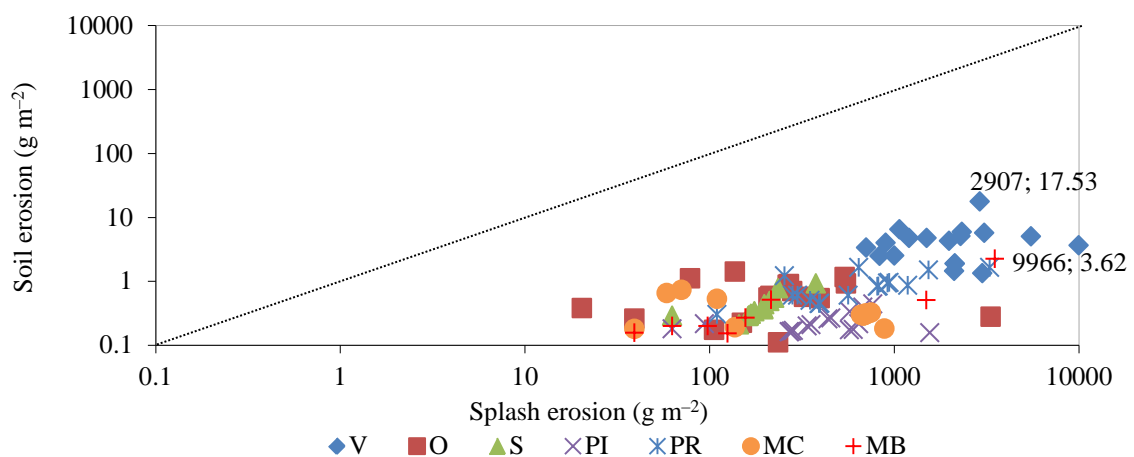


Figure 61. Runoff erosion vs splash erosion for the selected soil environments along the observed period.

A high significant positive correlation ($r=0.500$, $p<0.0001$) was obtained with the following linear regression equation: runoff erosion = 0.0008 splash erosion + 0.4071 when data of runoff and splash erosion were processed for all soil environments. The scatter of point clearly indicates a similar pattern as for erosion rates, and each soil environment is categorized according to the surface aggregate vulnerability to raindrop impact and related kinetic energy. Nevertheless, field conditions did not allow to find significant relationships at each soil environment because of the high variability of data along the period of study. However, the high variability within data recorded in each soil environment improved the understanding of the kinetic energy effect produced by raindrop impact and related to rainfall intensity and duration. Two points at the graph were evaluated, the highest value of soil erosion (17.53 g m^{-2}) and splash erosion (9966 g m^{-2}) respectively recorded in soil under cultivated vines (V).

These data were taken into account for a better understanding the high variability between the data sets in each soil environment. Each value was matched with its couple (x and y values) in the graph (Figure 61) and showed that, at 17.53 g m^{-2} of eroded materials corresponded 2907 g m^{-2} of splashed materials suggesting that a low intensity and large duration rainfall may have occurred, sufficiently able to activate particle removal by surface runoff, because the soil may have been saturated and the laminar flux of water transported particles downslope. At the same time the splashed materials is not so abundant. By contrast, at 3.62 g m^{-2} of eroded materials corresponded 9966 g m^{-2} of the splashed materials suggesting a higher intensity rainfall of short duration, which would have impacted soil surface aggregates with higher kinetic energy strongly splashing particles away. In this case a very high amount of splashed particles is produced even though rainfall did not last enough to activate overland flow with sufficient transport capacity. As indicated elsewhere, soil environments protected by plant canopy may result less affected by splash even under high intensity and long duration rainfall. Therefore, it may be assumed that the dynamics of soil surface aggregation against meteoric elements should be considered under a perspective of soil sustainability, indicating either deterioration or improvement of soil quality, when soil surface aggregates undergo either detachment and dispersion or resilient reaction towards the effect of rainfall (Cerdà, 1998). In fact, soil aggregates composed by very low clay and organic matter content can be easily splashed by raindrop and transported downslope by water runoff, whilst higher clay and organic

matter content are able to form more stable soil aggregates through clay-organic complexes interacting with other mineral and organic soil components and resulting in a soil crumb contrasting raindrop impact and clods disruption, thus limiting erosion (Oades, 1984). Irrespective to the positive significant correlation between runoff and splash erosion (Figure 61), the ratio splashed to eroded particles may vary from tens to thousands in all the studied soil environments which can also be emphasized by the low slope (0.0008) in the equation. Effectively the scattered values plotted down the 1:1 line in the logarithmic graph (Figure 61), are situated under the amount of 20 g m^{-2} of maximum total runoff erosion event (V) and spread until 10000 g m^{-2} of maximum splash erosion event (V) confirming this assumption.

3.2.4. Nutrient depletion

The annual recorded average values of soil erosion during the observation period were 4.54, 0.60, 0.32, 0.22, 0.78, 0.30, 0.25 g m^{-2} for V, O, S, PI, PR, MC, MB soil environment respectively. These values were inversely trended with soil organic carbon and total nitrogen content depicting relevant relationships between soil nutrients and soil erosion. The relationship found between soil erosion and SOC and TN content were statistically significant as can be seen in Figure 62. Graphs show clearly that the organic carbon and nitrogen play a relevant role in contrasting the erosion processes. Evidently the soil itself is able to recover the primitive status before its transformation in agriculture and subsequent abandonment. However, in the abandonment scenario, different factors (grazing, wildfire ...) may delay the natural vegetation succession and cause disturbance to soil evolution. The slight increase in erosion rates recorded in soils under pasture (PR) was probably due to the impact of grazing activity. The nature of soil surface and plant cover colonization have a major role in the assessment of soil surface susceptibility to erosion. That rainwater is mainly infiltrated has been already discussed by runoff coefficient, depicting low runoff generation with consequent relatively low erosion rates. Nevertheless, runoff volume and eroded soil were also analyzed for their content in dissolved organic carbon (DOC) and nitrogen (DN), and eroded organic carbon (EOC) and nitrogen (EN) respectively in order to monitor nutrient dynamics associated to erosion processes along the period of study. Nutrient depletion may be accentuated by land misuse, soil mismanagement and abandonment leading in many cases to soil degradation by decreasing soil structural stability, altering

carbon, nitrogen and other important element's cycles, modifying water regimes and causing adverse impact on biomass productivity, biodiversity and environmental quality. The trend in removal of soil organic carbon and total nitrogen both dissolved and eroded with respect to runoff and erosion was therefore analyzed.

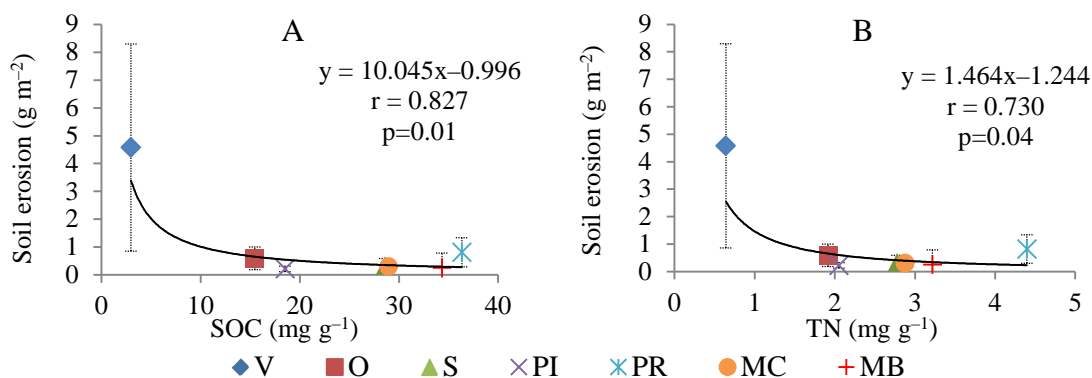


Figure 62. The relationship between the mean annual values of soil erosion SOC (A) and TN (B) for the selected soil environments.

3.2.4.1. Carbon loss as eroded organic carbon (EOC)

The annual average of eroded organic carbon (EOC) during the observation period amounted to 7, 68, 108, 132, 66, 67, and 65 mg for each gram of eroded soil in V, O, S, PI, PR, MC, MB soil environments respectively. The recorded values of EOC were independent of the amount of soil erosion as clearly showed in Figure 63. The largest amount of EOC was washed in most of environments from soil surface with low runoff and mineral particles removal, which may indicate some resilience of soil surface layer aggregates. Nevertheless the same trend may also infer that the percentage of EOC lost with respect to eroded soil is proportionally higher than the percentage of SOC in the soil (De Nobili and Maggioni, 1993). It must be pointed that the annual average of soil organic carbon (SOC) content was 3, 12, 23, 22, 32, 28, 32 mg g⁻¹ for V, O, S, PI, PR, MC, MB soil environments respectively. Thus, it was assumed that the variability in soil carbon loss among the studied soils depended mainly on the variation in organic carbon content and probably quality that may be controlled by different factors such as the type of decaying debris and soil structural stability (S, PR, MB), wildfire occurrence (MC), soil mismanagement (V, O), and a lower contribution of humic substances from hardly decomposable pine needles (PI). The lower carbon loss was recorded in soil under cultivated vines (V), showing the higher erosion rates and the lowest SOC content. Irrespective to this, the organic pool carried by eroded particles was 268%

higher than the SOC in the soil itself corroborating the findings of De Nobili and Maggioni (1993) that reported a steep increase of organic carbon in eroded soil with respect to soil content at 10 cm depth. Similarly the organic pool (EOC) carried by eroded particles in O, S, PI, PR, MC, and MB soil environments was 461%, 418%, 929%, 201%, 258%, 144% respectively higher than the corresponding SOC values of these soils. Despite the noticeable proportional difference, between SOC and EOC, which can be partly explained by the ratio EOC/ES (eroded organic carbon/eroded mineral soil) the values depict a pattern probably related to the stability of surface organic compounds like in soil under PR, MC and MB showing the lowest percent values.

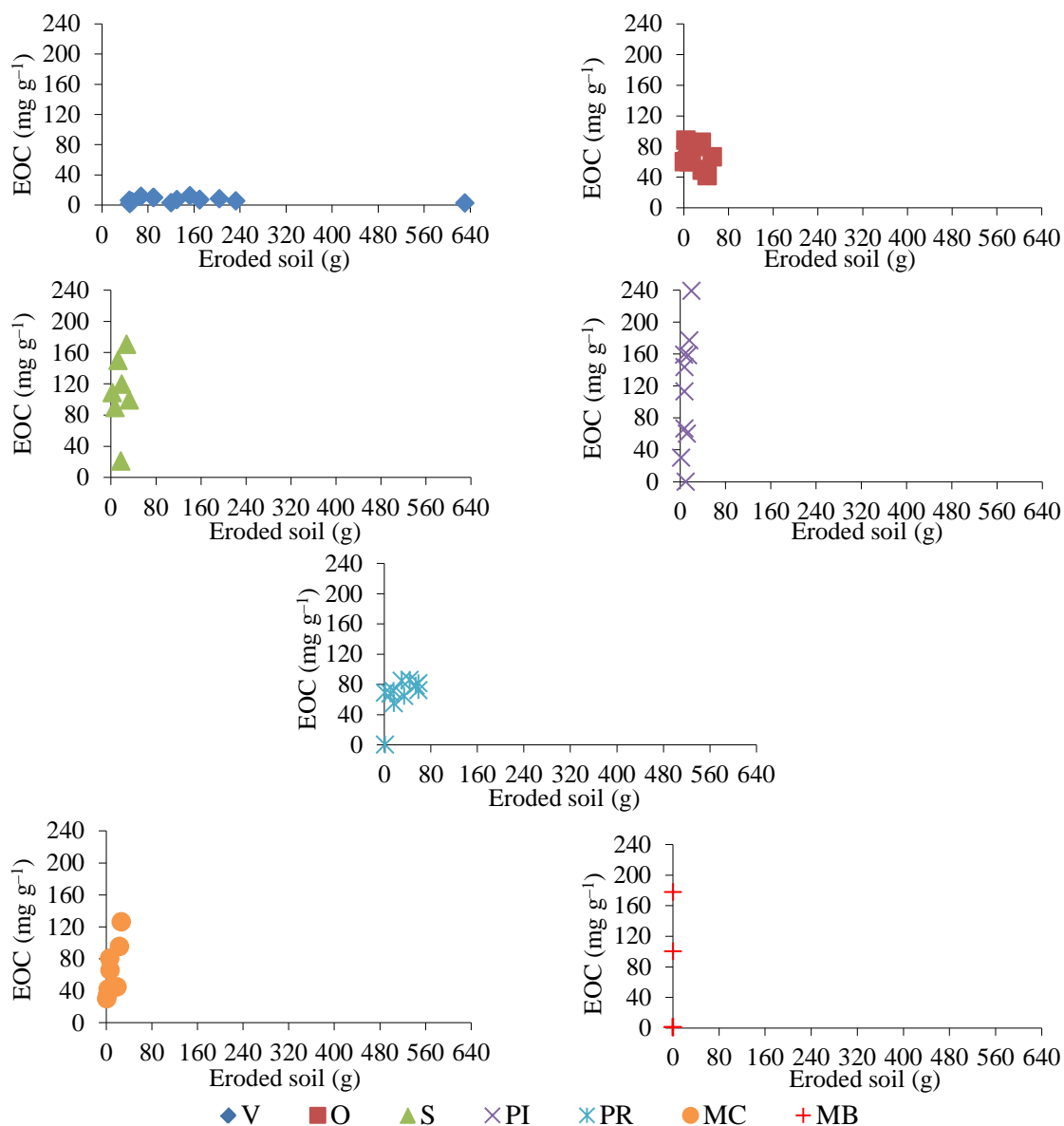


Figure 63. The relationships between eroded organic carbon (EOC) and eroded soil for the selected soil environments along the observed period.

Cumulative relationships between EOC and soil erosion (ES) are shown in Figure 64 to check the trend of this dynamics. As expected, significant correlations were found, though the slope may indicate the susceptibility of carbon loss. The total cumulative values of EOC during the observed period were estimated in 110, 870, 760, 1150, 730, 520, 280 mg g⁻¹ associated to 2206, 332, 122, 103, 325, 89, 39 grams of eroded soil for V, O, S, PI, PR, MC, MB respectively (Figure 64). The higher values were found in soil under stands of pine trees, olive groves, and stands of cork trees respectively.

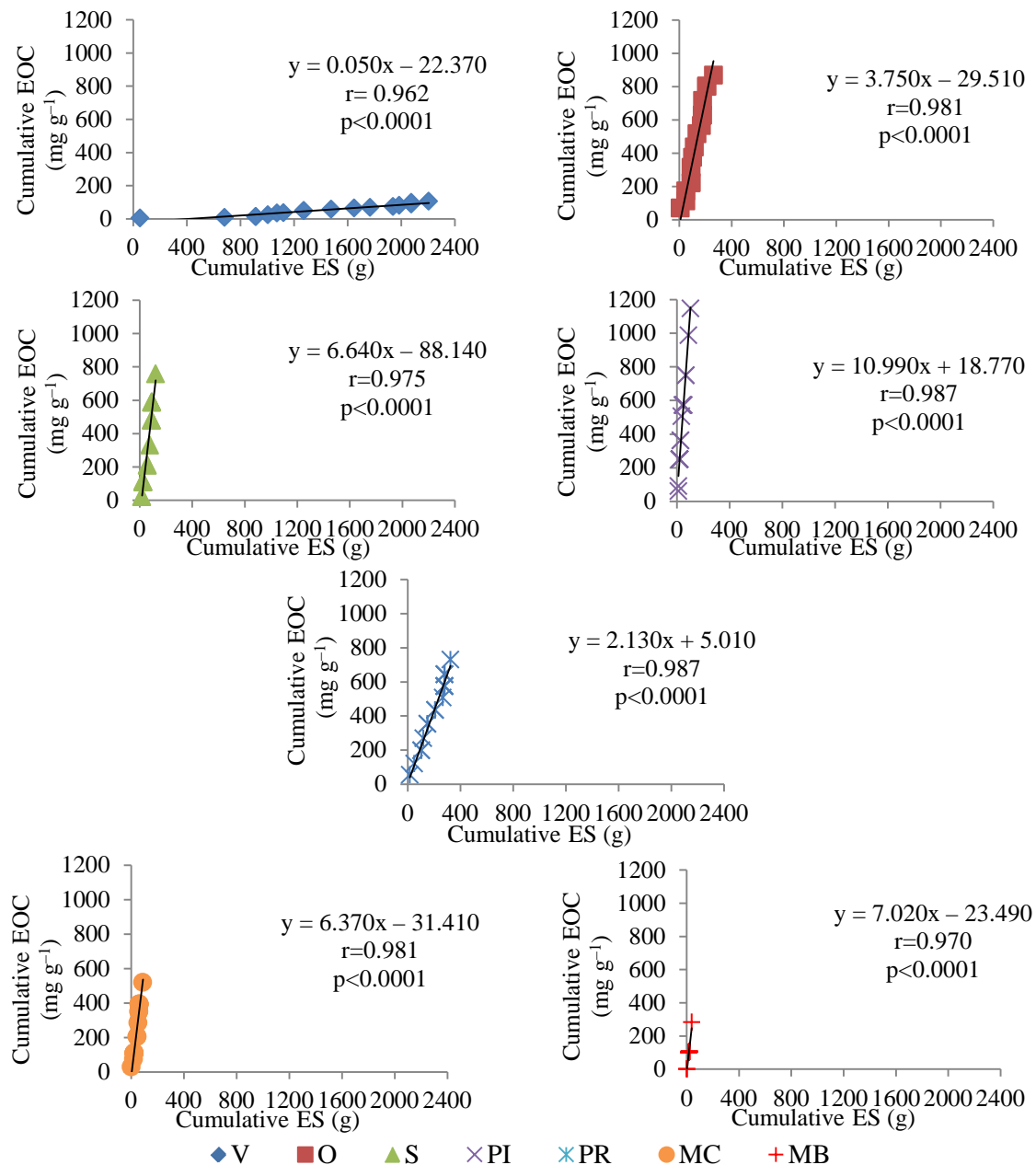


Figure 64. Linear relationships between cumulative eroded organic carbon (EOC) and cumulative erosion (ES) for the selected soil environments along the observed period.

Despite the significant correlations found over all the studied soils, the results showed that the EOC loss is independent of the amount of soil erosion and may be more related with the SOC content and vegetation type. As may be clearly observed by the slope values of the regression equations (Figure 64), the lines may also announce the different type of organic compounds present in the investigated soils: a steeper slope may probably be related to more labile organic compounds except soils under vines for their extremely low carbon content. This trend is evident when the soil loss ratio (that is the ratio of the highest erosion to progressively the lowest): 1.00, 0.13, 0.07, 0.05, 0.18, 0.07, 0.06 is plotted together with the carbon loss ratio (that is the ratio of the highest EOC to progressively the lowest) 0.06, 0.58, 0.94, 1.00, 0.58, 0.57, 0.35 for V, O, S, PI, PR, MC, and MB soils respectively both as function of the percent of plant cover (Figure 65).

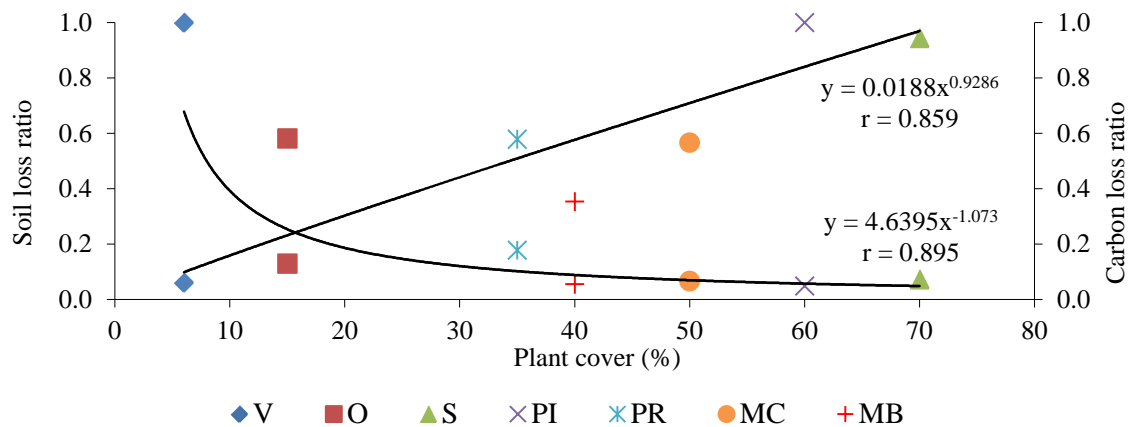


Figure 65. Soil loss ratio (fine black line) and carbon loss ratio (dotted black line) as a function of plant cover for the selected soil environment during the observed period.

From this graph it is observed that the type of plant cover may influence the capability of soil surface to be impoverished of organic compounds. The soils under pasture with the higher soil organic carbon content (3.63%) had erosion rates contrasting with the relatively lower carbon loss ratio with respect to the soils under pines with a lower soil organic carbon content (2.10%), a much higher carbon loss ratio but a lower soil loss ratio. This trend indicates that even with less erosion of mineral particles soils under pines may be easily depleted of particulate forms of carbon.

3.2.4.2. *Nitrogen loss as eroded nitrogen (EN)*

The same trend was found with eroded nitrogen (EN) (Figure 66). The annual average of total eroded nitrogen (EN) during the period of study amounted to 2, 8, 12, 10, 13, 11, 5 mg for each gram of eroded soil in V, O, S, PI, PR, MC, MB soil environments respectively. The eroded values of EN were independent of the amount of soil erosion.

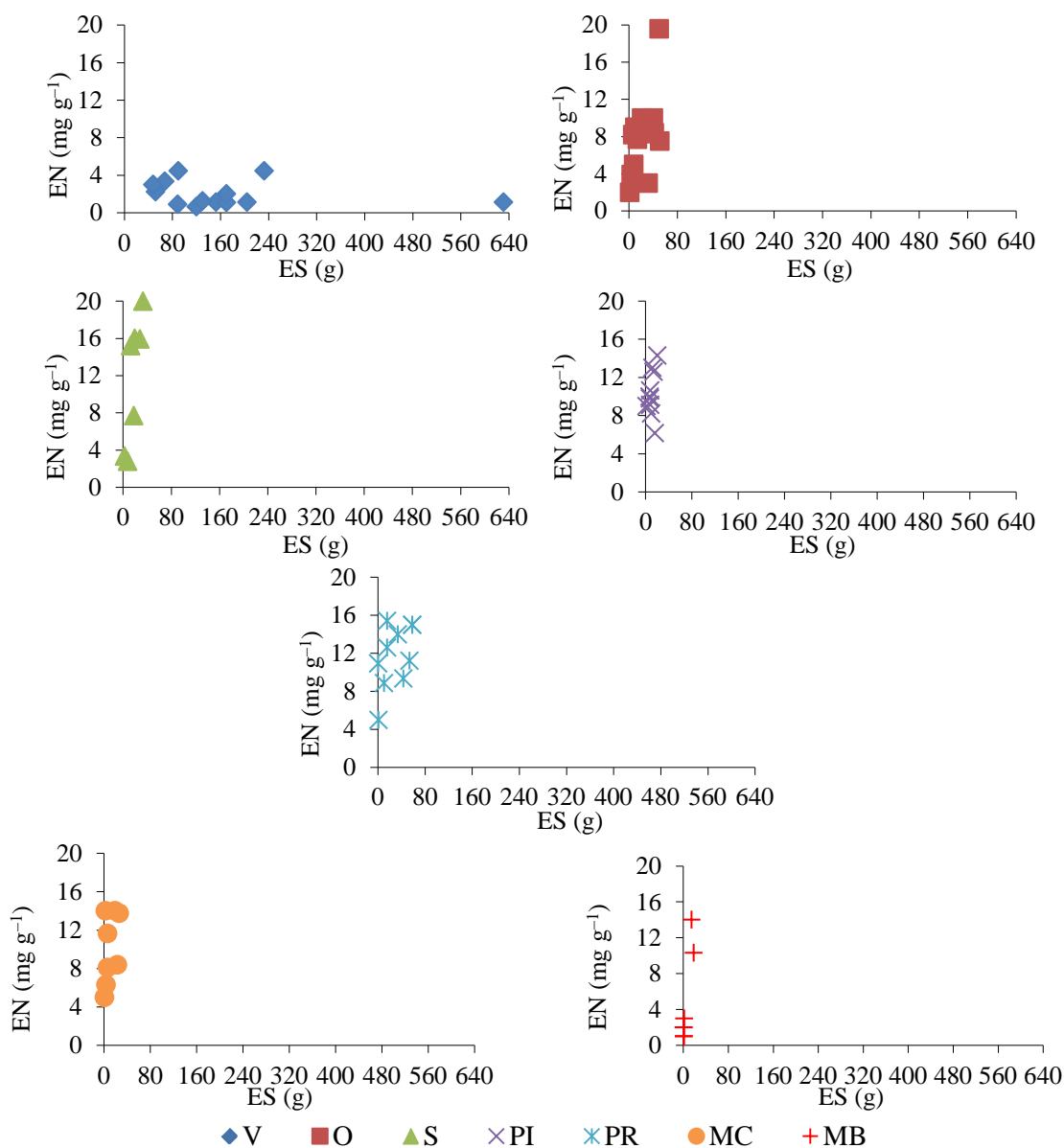


Figure 66. Relationship between eroded nitrogen (EN) and eroded soil (ES) for the selected soil environments along the observed period.

As for the EOC values, EN values may indicate that the percentage lost with respect to eroded soil is proportionally higher than the percentage of total nitrogen for

the soil itself. It must be pointed that the mean annual average of total nitrogen was 0.06, 0.18, 0.26, 0.18, 0.44, 0.28, 0.34% for V, O, S, PI, PR, MC, MB soil environments respectively. The lower EN loss was recorded in soil under cultivated vines (V) with the higher erosion rates. According to data, the nitrogen eroded during erosion processes was 398% higher than the total nitrogen in soil itself under vines in agreement of what found from De Nobili and Maggioni (1993). Similarly the nitrogen pool exported from O, S, PI, PR, MC, and MB soil environments corresponded to 488%, 444%, 636%, 295%, 348%, 134% respectively, over the total nitrogen content of these soils.

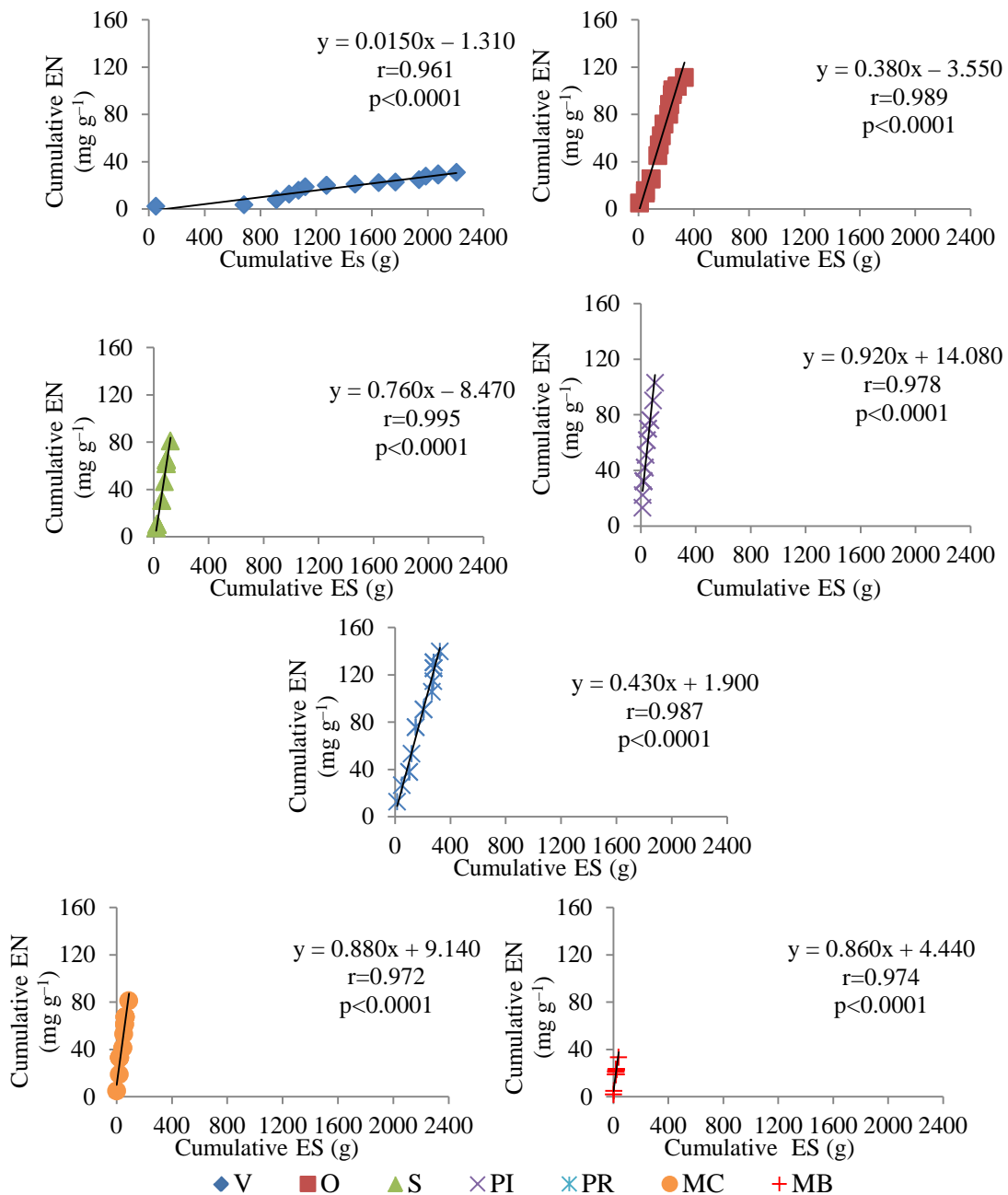


Figure 67. Linear regression equations between the cumulative eroded nitrogen (EN) and cumulative eroded soil (ES) for the selected soil environments along the observed period.

The cumulative EN loss in soils under V, O, S, PI environments was found to be 1.3 fold of the EN depleted from soils under soil PR, MC, and MB environments. Cumulative relationships between EN and soil erosion (ES) were drawn (Figure 67) in order to check the trend of the process of nitrogen removal. Expected significant correlations were found between the cumulative values of EN and the cumulative values of eroded soil (ES). The total cumulative values during the observed period was estimated in 30, 110, 810, 100, 140, 810, 330 mg g⁻¹ associated to 2206, 332, 122, 103, 325, 89, 39 grams of eroded soil for V, O, S, PI, PR, MC, MB respectively. EN is thus independent of the amounts of soil erosion as carbon and may be related on the TN content of soil. The organic nitrogen lost by soils under pasture may be susceptible to runoff erosion both in the form of NH₄⁺ and NO₃⁻. Moreover, the slope of the equation in Figure 68 may directly indicate those environments more susceptible to nitrogen loss. This trend is also clear when the soil loss ratio is plotted together with nitrogen loss ratio both against the plant cover (%) in each soil environment (Figure 68). In this case the highest nitrogen loss was found in the soils under pasture for the frequent manuring during grazing.

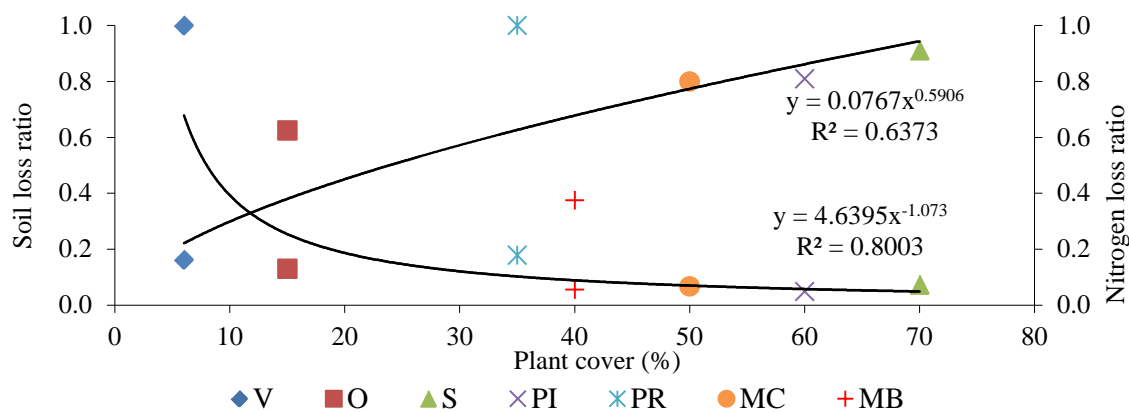


Figure 68. Soil loss ratio (fine black line) and nitrogen loss ratio (dotted black line) as a function of plant cover for the selected soil environment during the observed period.

3.2.4.3. Carbon loss as dissolved organic carbon (DOC)

Organic carbon dissolved by runoff water and transported downslope represents also a significant part of the carbon pool removal from soils. The annual average of dissolved organic carbon (DOC) during the observation period was recorded as 0.99, 1.06, 1.70, 1.70, 1.14, 0.73, 0.46 g l⁻¹ for V, O, S, PI, PR, MC, MB soil environments respectively. Despite the low runoff coefficient recorded in these soils along the

observed period, runoff water was able to solubilize and transport the decomposable fractions of soluble organic compounds as indicated by the values observed in Figure 69, where it is also indicated that DOC was uptaken in the first phases of runoff generation and later on promptly decreased.

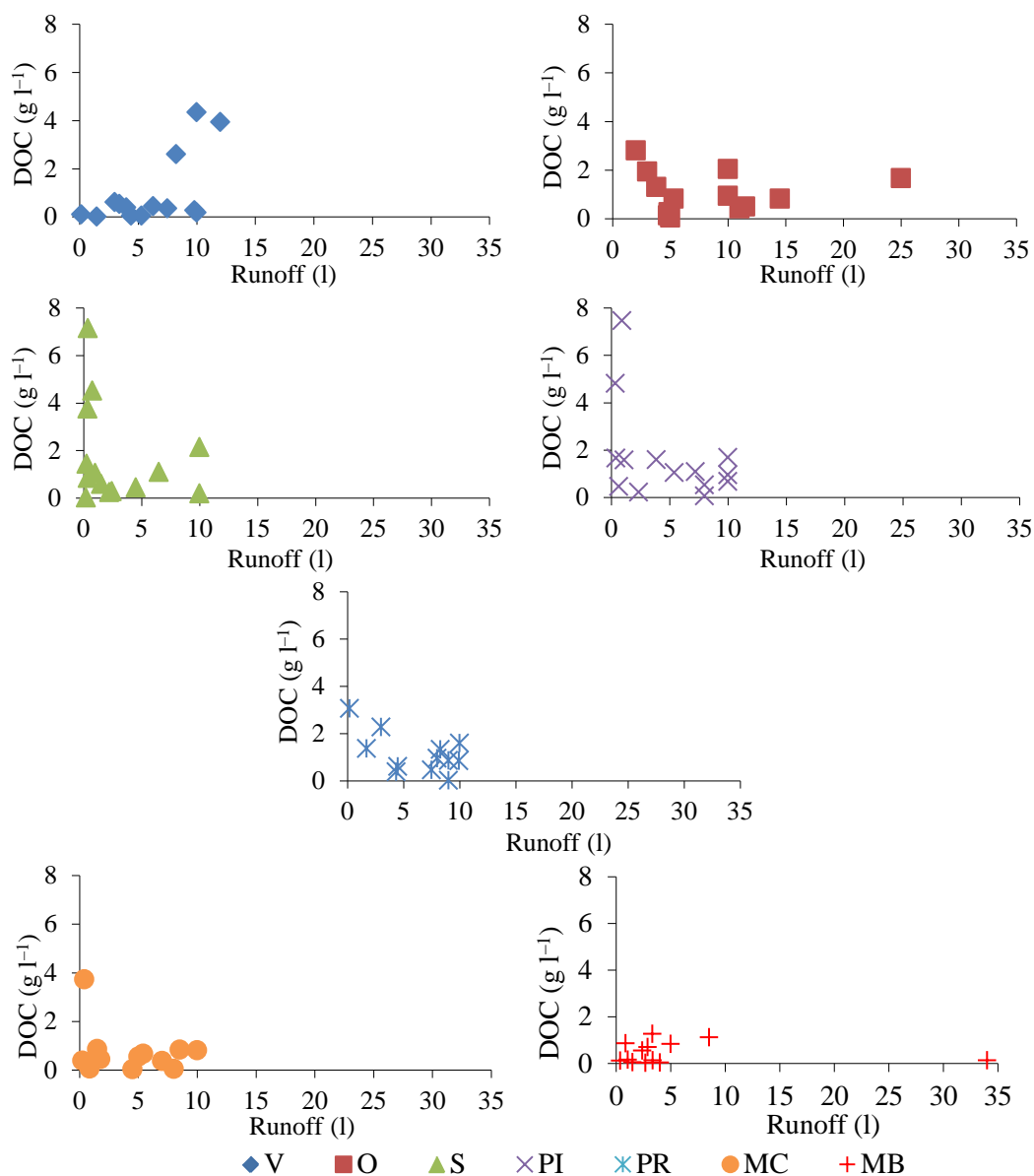


Figure 69. Relationship between the dissolved organic carbon (DOC) and water runoff for the selected soil environments along the observed period.

This concept differed in soils under vines which, besides the low SOC content is continuously depleted of organic carbon under eroded or dissolved form. As soil structure and SOC content may be considered the key factors able to contrast degradation processes and maintain nutrient cycling, the soils under vines appear as very unstable from a point of view of soil quality. DOC was 42% higher than the SOC

content in soils under vines. Similarly DOC in O, S, PI, PR, MC, and MB soil environments was 8%, 7%, 11%, 3%, 2%, 1% respectively higher than the correspondent SOC values of these soils. This pattern was probably related to the stability of surface organic compounds. As above mentioned the soils under stands of cork and pine trees showed a higher susceptibility to organic carbon loss. The total cumulative values during the observed period were estimated in 14, 14, 24, 24, 14, 9, 6 g l⁻¹ contained in 96, 122, 45, 70, 134, 81, 83 liters of runoff for V, O, S, PI, PR, MC, MB respectively.

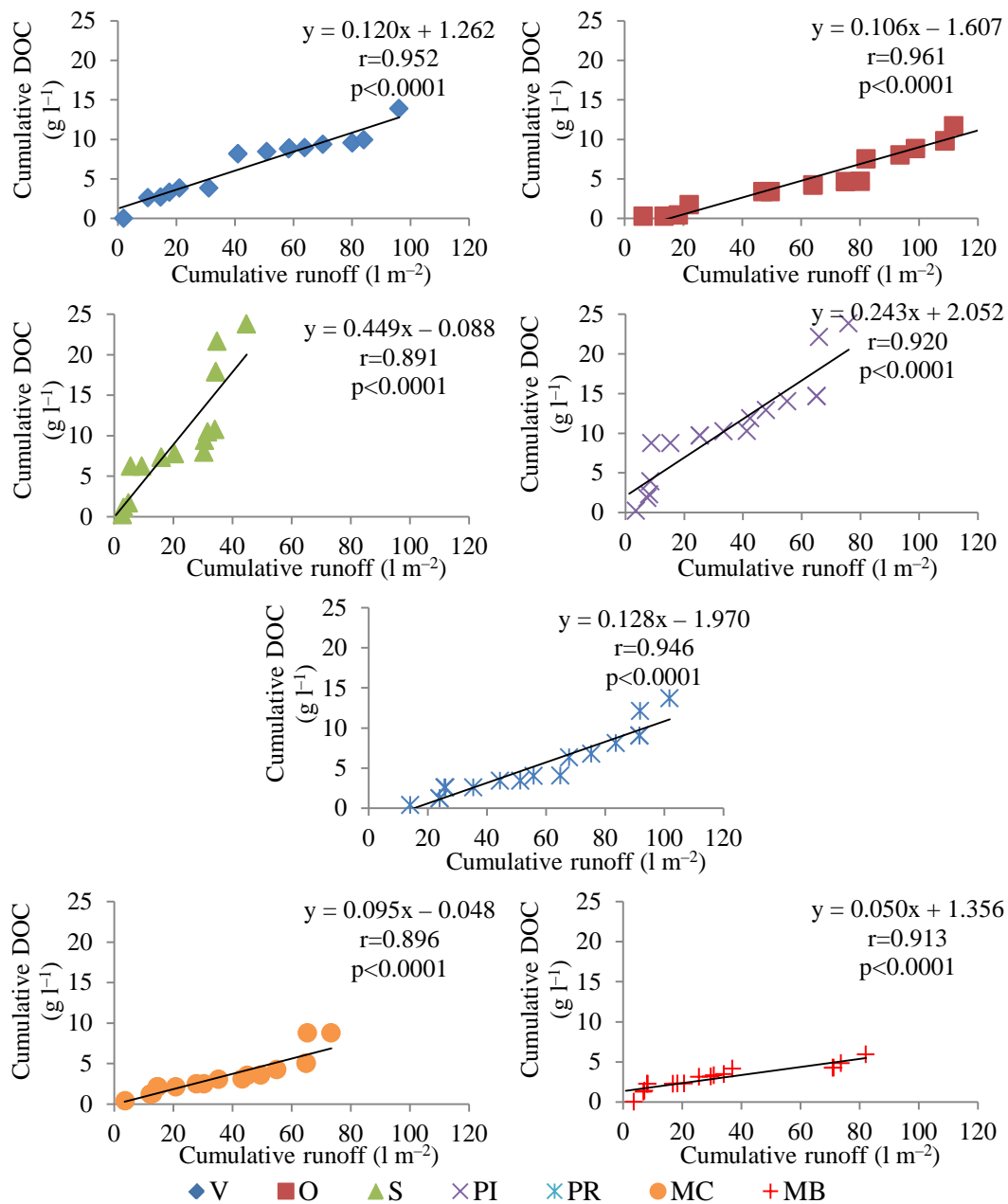


Figure 70. Cumulative dissolved organic carbon (DOC) vs cumulative runoff for the selected soil environments along the observed period.

The cumulative DOC was plotted against the cumulative runoff in order to have a better view of the potential of water runoff to DOC depletion (Figure 70). As expected, significant correlations were found. As may be observed by the slope values of the regression equations (Figure 70), the lines may also announce the different type of organic compounds present in the investigated soils: a steeper slope may be related to more labile organic compounds except soils under vines for their extremely low carbon content.

3.2.4.4. *Nitrogen loss as dissolved nitrogen (DN)*

Dissolved nitrogen (DN) showed approximately the same trend of DOC (Figure 71). The annual recorded average of DN was 6, 18, 46, 47, 82, 9, and 6 mg l⁻¹ for V, O, S, PI, PR, MC, and MB soil environments respectively. As for DOC, it was noticed that, when runoff water increases the DN concentration decreases. The highest DN concentration was found in soils under stands of pine (PI) and cork (S) trees and pasture (PR) respectively may be related to the abundance of soluble nitrogenous compounds. Whilst the dynamics of soil erosion is explained by runoff transport capacity, nutrient losses are more difficult to explain because controlled by abiotic and biotic processes (Cole, 1995; Pardini et al., 2003).

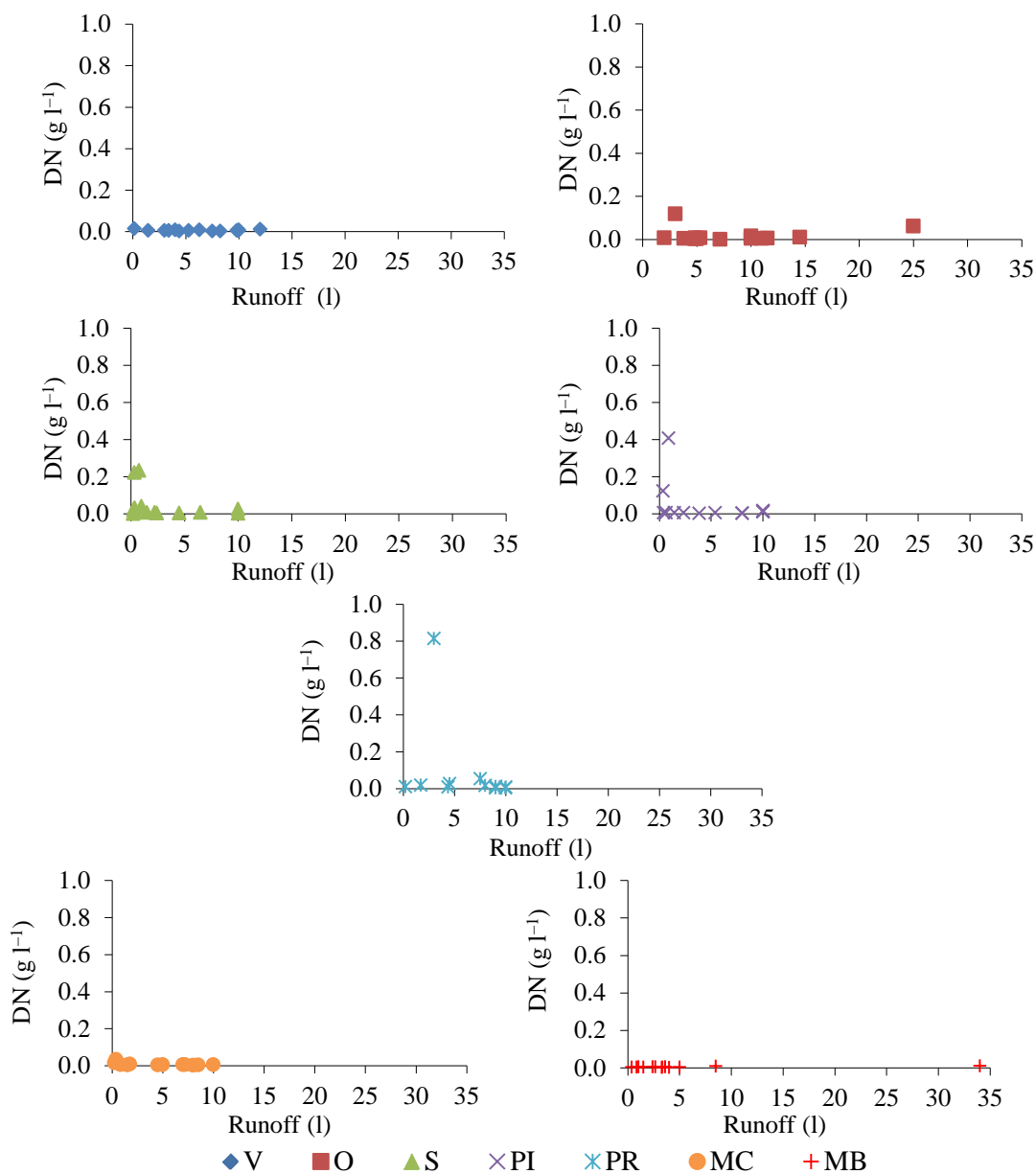


Figure 71. Relationship between the dissolved nitrogen (DN) and water runoff for the selected soil environments along the observed period.

Cumulative assessments were obtained in order to check the potential of water runoff to DN depletion (Figure 72).

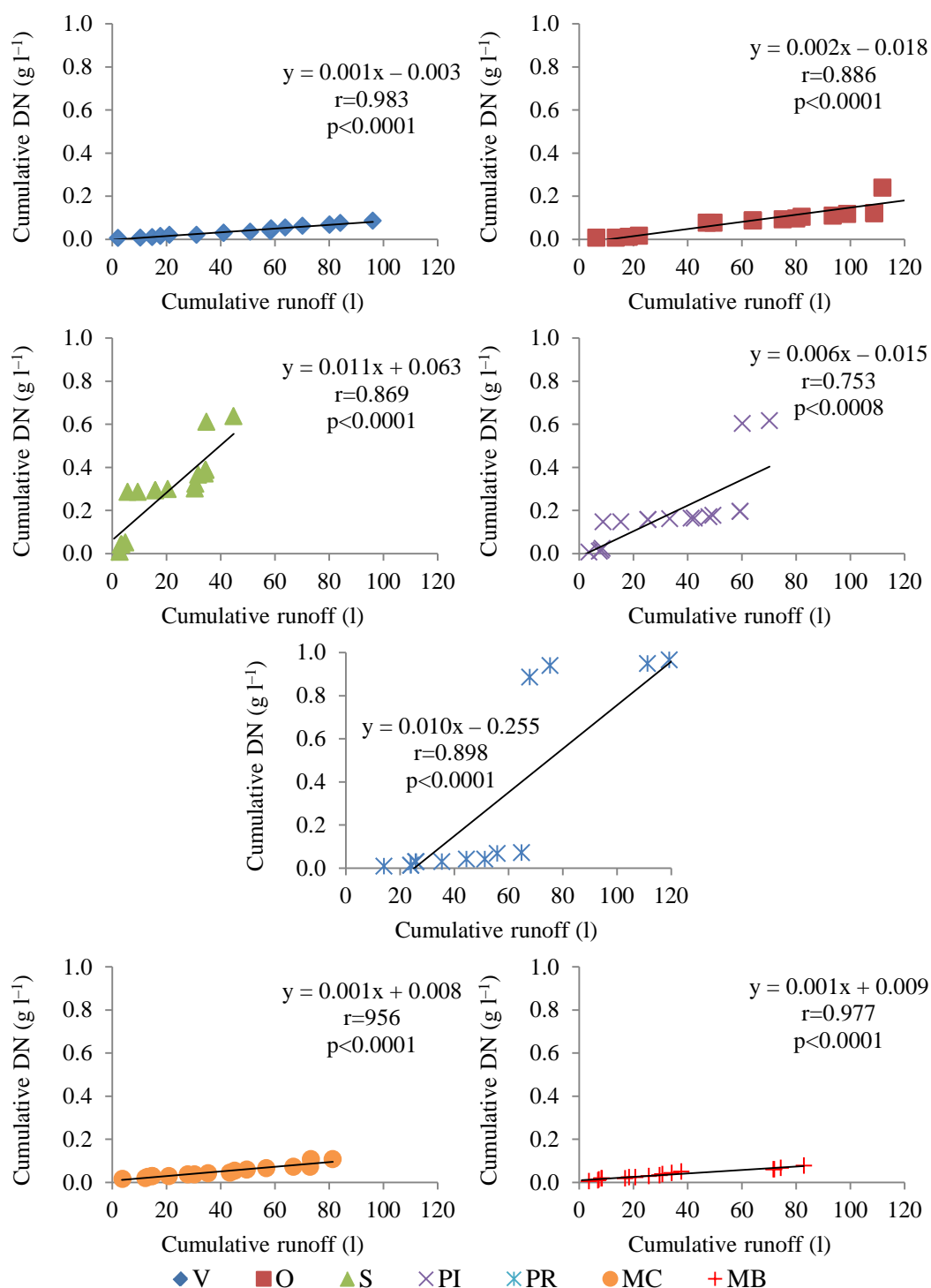


Figure 72. Cumulative dissolved nitrogen (DN) vs cumulative runoff for the selected soil environments along the observed period.

The total cumulative values during the observed period were estimated in 86, 255, 638, 617, 985, 167, 78 mg l^{-1} contained in 96, 122, 45, 70, 134, 81, 83 liters of the generated runoff for V, O, S, PI, PR, MC, MB respectively.

A summarized view of the total amounts of sediment yields on related nutrient depletion is shown in Table 38. Values expressed as kg ha^{-1} or l ha^{-1} indicate the different response of the studied soil to erosion in the two rainfall scenarios.

Table 38. Total estimation of soil erosion, runoff, and corresponding depleted nutrients from each soil environment during the two observed periods.

Year	ENV	n	Erosion (kg ha^{-1})	EOC (kg ha^{-1})	EN (kg ha^{-1})	Runoff (l ha^{-1})	DOC (kg ha^{-1})	DN (kg ha^{-1})
2008	V	7	311.57	11.26	5.82	8,647	33.03	0.18
	O	7	28.08	10.47	1.52	13,750	46.48	1.06
	S	7	7.02	0.77	0.07	2,622	16.28	0.75
	PI	7	5.75	1.43	0.18	4,264	37.27	0.63
	PR	7	41.74	14.76	3.16	9,861	25.44	0.30
	MC	7	6.33	0.69	0.21	5,797	12.29	0.17
	MB	7	4.78	0.49	0.09	4,692	10.56	0.09
2009	V	13	301.15	326.49	3.68	18,064	181.90	1.17
	O	13	44.66	7.18	2.55	20,103	208.67	3.60
	S	13	26.79	2.58	1.89	9,822	172.67	3.45
	PI	13	22.96	1.90	1.63	16,839	254.10	7.91
	PR	13	48.43	8.44	3.10	18,428	205.10	17.60
	MC	13	18.44	1.22	0.89	14,603	97.11	1.12
	MB	13	5.94	0.13	0.09	18,131	66.72	1.07

ENV: Soil environments; n: Number of observations; EOC: Eroded organic carbon; EN: Eroded nitrogen; DOC: Dissolved organic carbon; DN: Dissolved nitrogen; V: Soils under cultivated vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub.

3.3. Soil infiltration and permeability

As showed elsewhere the low runoff coefficient allowed the mobilization of few but important amounts of sediments in all the studied soil environments during the observed period. Infiltration rates were therefore worth to be studied to record the potential of soil infiltration capacity. It is well known that soil infiltration rate is the water entry into the soil caused by rainfall or irrigation. Conversely, hydraulic conductivity represents the water flux through the soil pore system to ensure sufficient moist conditions for physical, chemical, and biological reactions. Accordingly, measurements of soil infiltration rates and soil hydraulic conductivity were carried out during the experimental period. The values of soil infiltration rates (IR) and soil hydraulic conductivity (k) per each DOY can be seen in Table 39 and Figure 73 and Figure 74.

Table 39. Values of soil infiltration rates IR (mm h^{-1}) and soil hydraulic conductivity k (cm h^{-1}) for each soil environment per each DOY during 2008 and 2009.

DOY	V		O		S		PI		PR		MC		MB	
	IR	k	IR	k	IR	k	IR	k	IR	k	IR	k	IR	k
64	828	0.68	1698	0.42	382	0.07	790	1.11	65	0.35	1343	0.14	142	2.56
101	818	7.39	2001	1.73	450	3.25	1525	0.90	154	5.05	1330	2.22	765	2.49
141	1546	4.10	1446	4.71	159	1.32	1040	1.52	38	1.32	1070	0.14	489	3.46
149	1205	1.80	1087	0.35	309	4.85	777	1.45	34	1.50	472	1.87	516	4.92
158	1540	5.46	1779	0.35	309	1.18	716	0.97	41	5.12	350	1.04	245	0.07
185	1235	2.73	1606	2.42	296	4.71	1088	0.97	61	2.28	195	2.22	303	4.02
312	1193	3.15	1582	1.94	305	3.72	989	1.22	58	3.16	260	1.46	266	3.27
13	1120	8.88	790	0.14	483	20.08	186	0.35	64	0.28	273	1.87	558	2.56
23	825	5.41	515	0.87	336	13.15	324	0.82	88	0.44	177	5.12	312	1.90
37	911	8.01	347	1.38	485	27.55	139	1.18	151	1.11	111	1.18	451	2.28
51	811	7.14	325	0.14	372	4.15	653	0.00	143	1.04	84	2.84	149	2.70
65	1067	9.25	214	10.38	353	11.63	342	0.62	75	0.83	74	19.94	101	6.44
85	741	11.67	436	3.39	306	5.40	518	0.69	41	1.04	111	1.32	214	1.32
93	573	4.59	193	0.48	419	3.74	340	0.62	77	0.62	53	1.32	127	1.87
118	829	3.10	440	1.25	322	3.18	139	0.35	69	0.69	210	0.21	171	0.28
135	799	5.00	500	1.54	368	4.21	151	2.10	78	1.23	88	0.99	162	1.85
167	769	11.98	693	1.94	434	6.85	190	3.32	145	3.67	49	1.52	174	1.59
247	690	9.11	388	3.32	346	4.10	210	1.40	86	1.07	126	3.70	218	2.06
279	532	15.70	564	5.75	524	4.22	387	1.18	204	4.02	64	0.83	89	0.83
310	1168	13.16	837	5.61	373	1.04	207	1.45	1331	0.21	280	1.45	193	3.53

DOY: Day of the year; V: Soils under cultivated vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub.

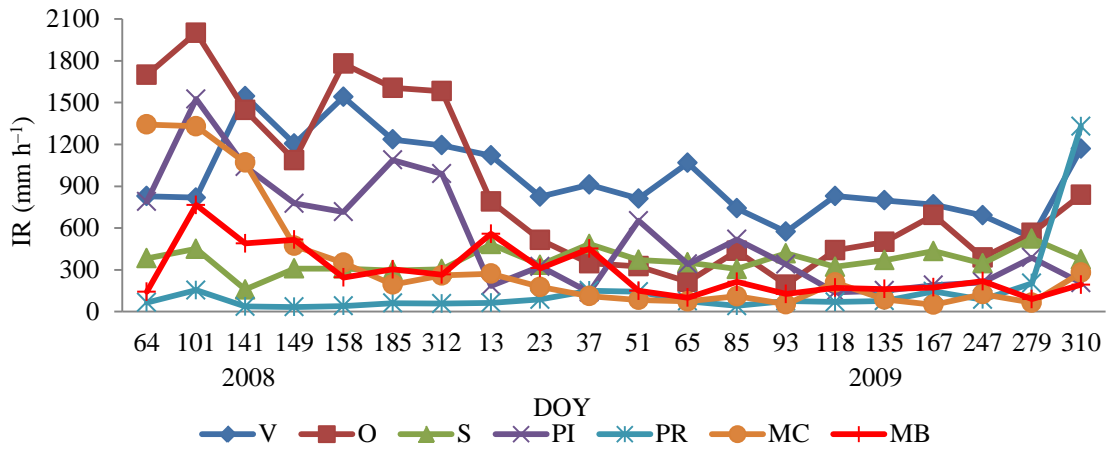


Figure 73. Trends of soil infiltration rates (IR) during the two years of measurements.

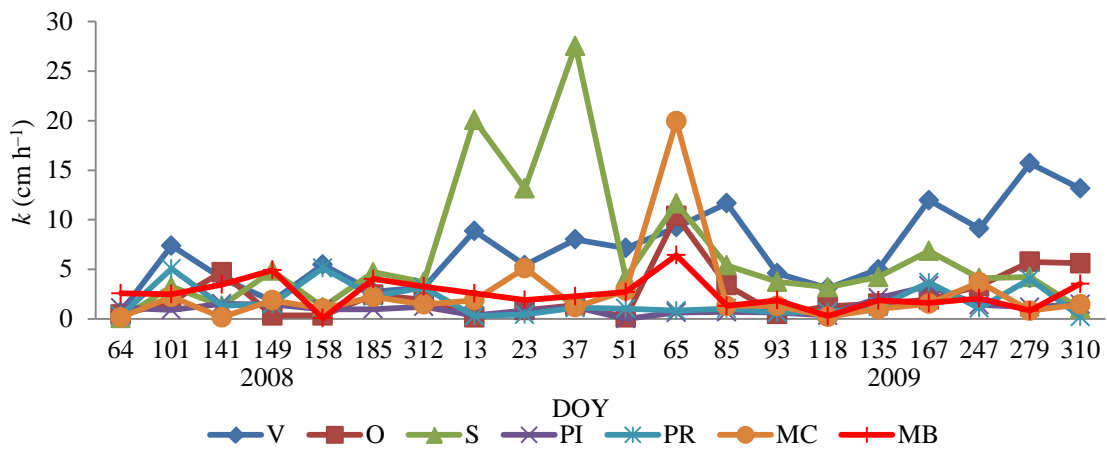


Figure 74. Trends of soil hydraulic conductivity (k) during the two years of measurements.

Results plotted in Figure 73 showed higher soil IR values during 2008 than 2009, which is in agreement with the recorded rainfall amounts in these two years. Effectively, in a first broad observation, the interannual variability is considerable in all the studied soil environments. The values of soil IR during 2008 varied largely and different trends were observed among the studied soil environments. It is reasonable to assume that each soil response to variable amount of rainfall depended on several factors or soil properties such as the time within rainfall event affecting soil moisture, the texture, the soil structural arrangement at surface, and the organic matter content, all of them influencing the IR. This resulted in the higher variability per each DOY at each soil environment (Table 39 and Figure 73). Conversely, when rainfall amounts are abundant, like in 2009, some soil properties may reduce their ability in retaining or losing water from the profile. At the same time, a shorter within rainfall time and relatively higher moisture content may maintain the soil over field capacity conditions, lowering the IR variability between the soil environments.

The measurements of soil hydraulic conductivity (k) also showed higher interannual variability between the two years of measurements and, as expected, the higher values were recorded during 2009 (Figure 74). This year showed also a much higher variability of k between the selected soil environments, probably related to the moisture content and hydraulic properties of the investigated soils at any measurement period. It may be pointed out that the mini disk infiltrometer used, is able to measure the hydraulic conductivity of the soil when it is placed upon at field conditions. Hydraulic conductivity is defined as “the meters per day of water seeping into the soil under the pull of gravity or under a unit hydraulic gradient” which is different than infiltration rate, which is defined as “the meters per unit time of water entering into the soil, regardless of the types or values of forces or gradients”. Because the minidisk infiltrometer has an adjustable suction ($0.5 < 2.0 < 6.0$ cm) one can get additional information about the soil surface pore range involved in the measurement having a water tension higher than the suction of the infiltrometer. When the water is under higher tension or suction, it will not enter pores such as cracks or wormholes, but will only move into and through the soil pore system as determined by the hydraulic forces in the soil. As macropore flow is extremely variable from place to place and therefore difficult to quantify, infiltrating water under a higher tension prevents the filling of the macropores and gives a hydraulic conductivity characteristic of the soil matrix, and is less spatially variable. Therefore soil hydraulic conductivity is a function of water potential and water content of the soil. The decrease in conductivity as the soil dries is due primarily to the movement of air into the soil to replace the water. As the air moves in, the pathways for water flow between soil particles becomes smaller and more tortuous, and flow becomes more difficult. Although we had no porosity information, this trend was likely to occur in all soil environments during 2008 which was a less rainy year than 2009, and where a higher “antagonism” between water and air may have probably occurred in the soil pore system, thus producing a general delay in water permeability and reduction of k values (Figure 74). In order to partly clarify the different k values recorded along the experiments the following assumptions were proposed:

If we view the soil matrix as an idealized mixture of solids and pores which take the form of capillary tubes, equilibrium between the upward forces of surface tension and the downward force of gravity will be found at:

$$h = \frac{2\sigma \cos \alpha}{\rho g r}$$

where: h is the height of rise of water in the capillary pores; σ is the surface tension of water against air (0.073 kg s^{-2} at 20°C); α is the contact angle of water with the soil pore ($\cos\alpha \approx 1$); ρ is the water density (998 kg m^{-3} at 20°C); g is the acceleration due to gravity (9.81 m s^{-2}); r is the equivalent pore radius (m). According to Kabat and Beekma (1994), if we assume that the soil matrix is a bundle of uniform tubes, and neglect other forces such as osmotic potential, we can set $hpg = -\Psi_m$ (Pa) and solve for the radius at any given matric potential. The result, for the properties of water at 20°C ,

$$\text{should then be: } r = \frac{0.000146}{-\Psi_m}$$

where Ψ_m is measured in kPa and r in meters. The assumptions stated above make it clear that this result is a simplified version of reality. Nonetheless, it illustrates the effect increase/decrease in moisture may have on the function of supposed air/water filled pores. By using the 2 cm suction minidisk infiltrometer on the soil surface at given natural soil moisture conditions, all soil surface pores forcing water suction larger than 2 cm, that is, pores showing -0.197 kPa matric potential ($pF \approx 1$) or higher, will be susceptible to be water filled whereas pores with lower matric potential will be susceptible to be air filled. Accordingly the threshold soil pore radius ($r = \mu\text{m}$) sensitive to favor hydraulic conductivity may be calculated by:

$$r_{(m)} = \frac{0.000146}{0.197} = 0.000741 = 741_{(\mu\text{m})}$$

The critical water filled pore radius ($741 \mu\text{m}$) activated by working with the 2 cm suction infiltrometer should be related with any given soil moisture content. Pores larger than this critical dimension will remain largely air filled probably delaying hydraulic conductivity, while smaller pores will show higher hydrodynamic properties. Despite this theoretical assumption, the reality is much more complex and many factors such as clay, organic matter, physical surface compaction, sealing, among others, may play simultaneously in the soil surface system. For instance if we look at the soil moisture content in Table 40 (each value is the mean of three replications), and rainfall amounts recorded in the investigated soil environments during 2008 and 2009 (Table 35), we observe that many hydraulic conductivity values at the same DOY are difficult to explain whilst others seem to follow some pattern. Let analyze the rainfall interval between DOY 13 and 85 of 2009 (Table 35): In DOY 13 soil environments V, O, S, PI,

PR, MC, and MB received 54, 121, 49, 80, 35, 48, and 78 l m⁻² respectively and the soil moisture in the first 10 cm depth amounted to 6.41, 14.30, 19.71, 21.34, 40.59, 19.96, and 19.01% in the same soils. In DOY 23 (2009) the recorded rainfall amount was considerably lower: 1.45, 1.45, 1.03, 1.21, 1.09, 1.09, and 1.09 l m⁻² respectively (the same order from soil V to MB) and the soil moisture content decreased by 49.77, 48.25, 48.19, 40.02, 32.47, 34.11, and 26.24% after ten days with respect to the previous measurements. This pattern may suggest once more that these very shallow soils may undergo frequent wetting-drying processes and are prone to easy water loss. Soil surface moisture decline may therefore favor air entry in the pore system hindering soil response to hydraulic conductivity pulse, giving k values difficult to explain, and justifying some of the above mentioned statements. A similar and clearer pattern may be observed in k values from DOY 37 to 65 (2009). In DOY 37 (after 14 days during which the soils may have been subjected to further drying), V, O, S, PI, PR, MC, and MB soil environments received 90, 88, 30, 84, 90, 109, and 96 l m⁻² rainfall respectively, and moisture content increased by 177.02%, 78.65%, 87.26%, 53.67%, 78.69%, 87.83%, and 85.59% respectively. Nevertheless, the majority of recorded k values were relatively low except the soil under stands of cork trees, may be due to antecedent soil dryness conditions which may interfere with soil surface hydraulic properties. In DOY 51, no rain was recorded and measurements of hydraulic conductivity showed further low values of k , may be related to a considerable moisture decline. Effectively, moisture decrease by 61.66%, 59.53%, 46.23%, 33.63%, 75.72%, 33.64%, and 23.82% was recorded for V, O, S, PI, PR, MC, and MB soil environments respectively. It seems reasonable to assume that a hysteresis effect caused by the continuous wetting-drying processes in these peculiar soils may strongly affect the surface matric potential. However, a more regular trend of k values was observed in DOY 65 when soil surface was able to recover 22.22%, 184.49%, 74.61%, 34.86%, 72.49%, 23.55%, 6.67% moistures respectively when the same environments received 30, 72, 72, 84, 42, 36, 48 l m⁻² of rainfall respectively. Moreover, higher k values were recorded in those environments showing a lower moisture increase, that is, soils that have maintained a comparatively higher water retention capacity which would be able to activate the water suction at 0.197 kPa. More difficult was to explain the behavior of soils under vines and pasture. The first, with a sandy texture and low organic matter content is often drip-irrigated and this agricultural practice may have influenced the k values. The latter resulted generally in very low k values all along the period of study

which may be attributed to a very hard surface due to frequent grazing. Despite that, a significant variability was found for soil moisture values in each environment both for 2008 and 2009 (Table 40).

Table 40. The mean values of soil moisture contents for the studied soils at each DOY. ANOVA results (F, p) indicated the data significance.

DOY	V	O	S	PI	PR	MC	MB
64	4.02±1.11	6.11±1.39	17.56±5.03	4.95±1.49	8.52±1.75	13.81±2.39	13.71±4.17
101	4.13±1.15	1.58±0.50	4.92±0.55	2.62±1.02	5.37±0.41	11.29±1.93	4.22±0.89
141	7.92±2.62	1.60±0.51	3.68±0.11	3.23±1.14	30.68±11.16	4.95±0.78	2.75±0.38
149	7.75±2.55	13.82±2.90	10.92±2.67	9.10±2.32	20.00±6.62	17.70±3.09	11.54±3.42
158	3.73±1.00	8.05±1.77	16.35±4.60	8.08±2.12	9.49±2.16	6.92±1.14	4.77±1.08
185	5.10±1.53	1.23±0.43	1.96±0.50	1.60±0.81	2.72±0.72	1.78±0.21	2.21±0.19
312	5.88±1.83	9.42±2.04	15.60±4.33	14.99±3.51	26.96±9.58	18.33±3.21	19.17±6.05
F	2.852	26.483	12.479	17.029	9.508	27.353	13.154
p	0.050	0.000	0.000	0.000	0.000	0.000	0.000
13	6.41±2.04	14.30±2.99	19.71±5.79	21.34±4.79	40.59±15.37	19.96±3.50	19.01±5.99
23	3.22±0.80	7.40±1.64	10.21±2.42	12.80±3.07	27.41±9.77	13.15±2.27	14.02±4.27
37	8.92±2.67	13.22±5.60	19.12±9.77	19.67±4.24	48.98±17.85	24.70±2.75	26.02±5.06
51	3.42±0.50	5.35±1.23	10.28±1.37	13.05±1.35	11.89±4.76	16.39±0.65	19.82±5.02
65	4.18±0.58	15.22±0.58	17.95±1.87	17.60±2.76	20.51±2.04	20.25±1.28	21.14±3.12
85	2.32±0.42	3.54±1.44	5.49±0.65	5.16±2.37	2.98±0.26	6.00±0.85	7.63±1.46
93	3.86±0.22	9.75±0.52	12.82±2.07	7.11±0.57	22.48±2.07	21.05±0.27	26.34±13.96
118	4.12±0.27	8.87±1.60	13.52±1.47	12.02±4.40	19.19±2.61	16.01±1.93	20.46±0.51
135	2.22±0.41	6.00±1.37	10.00±2.35	8.00±2.10	15.00±4.50	12.00±2.06	14.00±4.26
167	1.06±0.12	1.82±0.13	4.41±0.82	3.69±1.60	2.75±0.52	4.66±0.38	5.72±1.13
247	0.76±0.16	1.32±0.07	2.36±0.59	1.74±0.15	2.32±0.44	2.90±0.59	3.49±0.38
279	0.80±0.27	1.18±0.36	2.15±0.34	1.58±0.66	2.44±0.34	2.49±0.68	3.73±1.89
310	4.27±1.18	12.45±1.53	15.30±1.68	12.69±1.82	19.58±5.27	22.14±8.89	22.48±8.34
F	13.992	46.093	27.663	20.461	14.357	18.934	12.727
p	0.000	0.000	0.004	0.000	0.000	0.000	0.001

DOY: Day of the year; V: Soils under cultivated vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub.

A descriptive statistical analysis was tried using all data of IR and k parameters obtained during the two years of measurements (Table 41). Analysis was carried out per each DOY all environments, and per environment all DOY. The statistical data showed an interannual variability of soil IR and k both for DOY and Environments. The mean annual values of soil IR per each DOY over all the studied soils were always higher during 2008 than 2009 because of the higher Max and Min values recorded during 2008, a lower rainy year. Conversely the data of soil k showed a different tendency with higher mean value in 2009 than 2008. The higher variability of k during 2009 may be referred to the different response of soils between rainfall events generally higher during

2009, and different soil hydraulic properties, which have reacted according to the wetting-drying cycles occurred at soil surface. When environment vs all DOY is analyzed the trend of IR and k is very similar though with different mean values corroborating what stated before.

Table 41. Descriptive statistical analysis of soil infiltration rate (IR) and soil hydraulic conductivity (k) from March 2008 to November 2009 for all data obtained per each DOY and per environments.

Year	Parameters	DOY		Environment	
		IR (mm h ⁻¹)	k (cm h ⁻¹)	IR (mm h ⁻¹)	k (cm h ⁻¹)
2008	n	7	7	7	7
	Minimum	4400.56	5.32	65.58	1.15
	Maximum	7043.63	23.03	1602.75	3.69
	Mean	5271.16	16.16	767.74	2.27
	σ	901.03	5.98	540.61	0.94
	CV (%)	17.09	37.00	70.42	41.32
2009	n	13	13	7	7
	Minimum	1780.11	9.06	130.69	1.08
	Maximum	4388.29	59.09	833.37	8.69
	Mean	2550.17	27.72	364.31	3.96
	σ	679.92	13.13	238.76	3.23
	CV (%)	26.66	47.39	65.54	81.59

DOY: Day of the year; n: Number of observations; σ : Standard deviation; CV: Coefficient of variation.

The initial infiltration rate is normally higher at each soil site because of the combination of capillary and gravity forces that is gradually decreasing until the infiltration point is approached when the water infiltration rates are reaching the primary saturated permeability point (basic infiltration rate). Thus the soil infiltration capacity has been instantly considered as the maximum infiltration rate at which the power equation curve started to be constant because of the saturation of soil surface (Table 42). Depending on rainfall intensity and duration, and soil moisture content, the initial infiltration rates may vary upon the soil surface (Lado et al., 2004). In some environments, sensitive to moisture change, soil hydraulic conductivity and infiltration capacity may be less stable over time. For example, soils with low organic matter and high bulk density (like soils under vines) showed higher water loss under drier conditions though with high soil infiltration capacity.

3.3.1. Soil infiltration rates and dynamic physical soil parameters

3.3.1.1. Soils under cultivated vines

Descriptive statistical analysis of the soil infiltration rates (IR) is shown per each DOY (Table 42) together with power equations.

Table 42. Descriptive statistical analysis of the soil infiltration rate values (mm min^{-1}) for soil under cultivated vines along the experimental period.

DOY	I	Fi	\bar{x}	σ	CV	I/Fi	Equation	r	p-level
64	85.71	13.80	32.00	17.61	46	6.21	$y=45.71x^{-0.29}$	0.998	0.0001
101	200.00	13.63	50.37	38.56	77	14.67	$y=56.79x^{-0.35}$	0.991	0.0001
141	200.00	25.77	79.67	38.66	49	7.76	$y=80.10x^{-0.28}$	0.995	0.0001
149	85.71	20.09	47.72	17.72	37	4.27	$y=54.51x^{-0.24}$	0.992	0.0001
158	75.00	25.67	51.59	14.73	29	2.92	$y=57.38x^{-0.20}$	0.986	0.0001
185	120.00	20.58	48.36	26.16	54	5.83	$y=63.12x^{-0.27}$	0.999	0.0001
312	103.46	19.88	46.66	20.24	43	5.20	$y=36.86x^{-0.26}$	0.989	0.0001
Mean	124 \pm 54	20 \pm 5	50.91	24.81	48	6.69	$y=56.35x^{-0.27}$	0.993	0.0001
2008	CV=43	CV=25							
13	53.43	18.67	31.49	10.68	34	2.86	$y=42.31x^{-0.20}$	0.995	0.0001
23	43.78	13.75	23.84	8.41	35	3.18	$y=33.35x^{-0.21}$	0.992	0.0001
37	54.55	15.18	26.06	9.93	38	3.59	$y=36.55x^{-0.22}$	0.987	0.0001
51	54.55	13.51	25.50	10.99	43	4.04	$y=37.32x^{-0.25}$	0.998	0.0001
65	40.00	17.78	27.41	7.60	28	2.25	$y=36.56x^{-0.18}$	0.984	0.0001
85	42.86	12.34	22.36	8.99	40	3.47	$y=33.36x^{-0.24}$	0.997	0.0001
93	40.00	9.55	17.47	8.25	47	4.19	$y=27.72x^{-0.26}$	0.992	0.0001
118	40.00	13.82	23.64	8.21	35	2.89	$y=34.14x^{-0.22}$	0.989	0.0001
135	42.71	13.32	23.20	8.18	35	3.21	$y=32.59x^{-0.21}$	0.999	0.0001
167	30.00	12.82	19.64	5.85	30	2.34	$y=27.70x^{-0.19}$	0.971	0.0001
247	38.22	11.50	20.52	7.20	35	3.32	$y=29.40x^{-0.21}$	0.994	0.0001
279	23.08	8.86	13.74	4.16	30	2.60	$y=21.46x^{-0.22}$	0.987	0.0001
310	57.14	19.46	33.23	11.75	35	2.94	$y=44.28x^{-0.20}$	0.992	0.0001
Mean	43 \pm 10	14 \pm 3	23.70	8.48	36	3.15	$y=33.59x^{-0.22}$	0.991	0.0001
2009	CV=23	CV=23							

DOY: Day of the year; I: Initial IR value; Fi: Final IR value; \bar{x} : Mean; σ : Standard deviation; CV: Coefficient of variation (%); I/Fi: Ratio of initial and final IR values.

All soil infiltration measurements per each DOY lasted one hour in order to reach a constant IR value that is: basic infiltration rate (Fi), the mean of this value was 20 mm min^{-1} and 14 mm min^{-1} for 2008 and 2009 respectively being the former 43% higher. Likewise, a 48% of mean variability (CV) was found within the IR measurements carried out during 2008 with respect to 36% (CV) of IR measurements within 2009 (Table 42). As elsewhere mentioned this was attributed to the soil

conditions at any rainfall events. This trend was also indicated by the higher I/Fi ratio values. Power law equations fitted very well the infiltration rates data (Table 42). Highly significant negative correlations were found at any DOY, indicating a quite normal process of water entry into the soil. By using the power equations of 2008 a mean annual theoretical value of soil infiltration rate was calculated ($56 \pm 14 \text{ mm min}^{-1}$, $CV=24\%$), which differ by 10% with respect to the real mean ($51 \pm 14 \text{ mm min}^{-1}$) due to the fact that the power equations do not fit perfectly the experimental data. During 2009 highly significant correlations were also found. By using the same procedure, the mean annual value of soil infiltration rate was $34 \pm 6 \text{ mm min}^{-1}$ ($CV=18\%$), against a real mean of $24 \pm 5 \text{ mm min}^{-1}$. The higher negative slopes obtained in power equations for 2008 are also indicating that higher IR values were recorded with respect to 2009 and stress once more the driest soil conditions during 2008. Another significant statistical evidence of the different trend in infiltration rates is provided by the mean standard deviation and coefficient of variation (CV) of initial and final IR values for each DOY in soils under vines (Table 42). It may be observed that in the drier year (2008) the mean CV value is 48% against 36% of 2009 (rainy year) suggesting that the soil was subjected to higher variations of water entry into the soil due to drier soil conditions. In addition, relationships were tried between the coefficient of variation (CV) and the initial/final ratio of IR values from each DOY during 2008 and 2009 (Figure 75). The related power equations demonstrated that a smoother trend was shown by the points of 2008 (lower slope) with respect to 2009 (higher slope) indicating that a lower I/Fi ratio in the infiltration line against a CV of the same magnitude may correspond to the lower infiltration rates.

This trend may also be seen in the graphs of Figure 76. That water regimes in soil are subjected to rainfall events is well known, though it seems that in these soils it get particular importance for their singular conditions. If rainfall events, runoff coefficient, and soil moisture contents must be considered relevant parameters to help in the comprehension of the dynamics of soil infiltration rates and soil hydraulic conductivity, (Figure 76) other field and laboratory soil parameters must also be evaluated to better understand the soil dynamics.

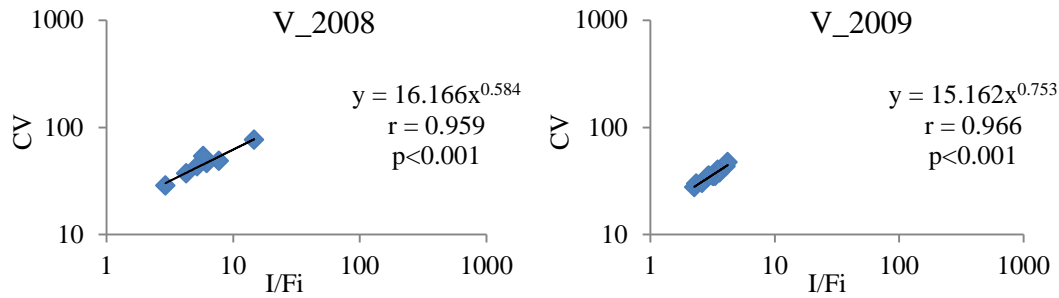


Figure 75. Relationships between the coefficient of variation (CV) and (I/Fi) ratio of soil infiltration rates in soil under cultivated vines along 2008 and 2009.

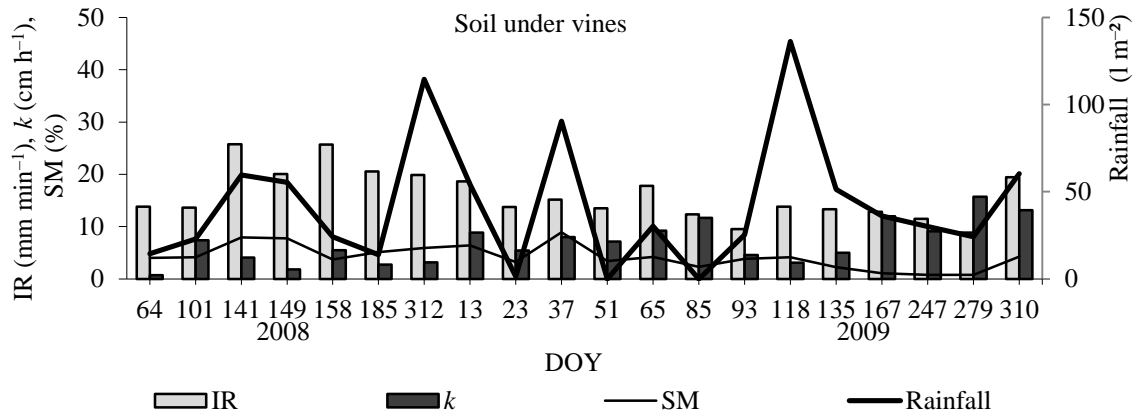


Figure 76. Values of soil infiltration rate (IR) (clear grey bar) and hydraulic conductivity (k) (dark grey bar) for soils under vines along the period of study. Trending of soil moisture (SM) (fine line) and rainfall (coarse line) is also represented.

The trend of soil IR and k data show the direct dependence of IR values with drier soil conditions and an inverse relationship of k values with wetter soil conditions along 2008 and 2009 (Figure 76). Different concepts have to be taken into account to improve the understanding of soil infiltration dynamics at each period of the year and under the effects of different land use and abandonment. Soils under cultivated vines periodically receive agricultural practices such as tillage, disrupting the soil structure and consequently increasing soil porosity. This process is able to increase the soil infiltration rates and the soil moisture is also increased despite the loamy sand texture. It may be postulated that the soils under vines, even in moist conditions may form a thin crust layer (Isikwue and Onyilo, 2010) which is suddenly broken by rainfall kinetic energy but is able to respond to suction pulse giving also relatively high k values (Figure 76). This trend was also detected by the MI and SS values showed in Table 20. When the recorded rainfall amount decreases by 7% (ex: from 59.58 l m^{-2} at DOY 141, 2008, to 55.48 l m^{-2} at DOY 149, 2008) a relatively low decrease of surface mechanic impedance (MI) by 24% and shear strength (SS) by 29% was recorded, indicating the continuity of still moist conditions at the soil surface. Conversely a decrease of rainfall

amount by 56% (DOY 149, 2008, 55.48 l m⁻² to DOY 158, 2008, 24.12 l m⁻²) caused a consistent increase in MI and SS values by 189% and 83% respectively indicating a hard but fragile soil surface structural arrangement.

A correlation matrix was tried between the data obtained in Table 20 (SM, BD, MI, and SS) together with rainfall, runoff, infiltration rates (IR), and hydraulic conductivity (k) in order to detect the relationships of the parameters depicting the soil surface dynamics (Table 43).

Table 43. Correlation matrix between the dynamic physical soil parameters in soils under cultivated vines (Data for 2008 and 2009).

Soil parameters	Rainfall (l m ⁻²)	Runoff (l m ⁻²)	SM (%)	BD (g cm ⁻³)	MI (kPa)	SS (kPa)	IR (mm h ⁻¹)
Runoff (l m ⁻²)	0.540 <i>p=0.014</i>						
SM (%)	0.450 <i>p=0.047</i>	0.197 p=0.405					
BD (g cm ⁻³)	-0.436 p=0.055	-0.108 p=0.649	-0.730 p=0.000				
MI (kPa)	-0.348 p=0.133	-0.247 p=0.293	-0.530 <i>p=0.016</i>	0.416 p=0.068			
SS (kPa)	0.244 p=0.299	-0.275 p=0.241	-0.039 p=0.870	-0.241 p=0.306	0.248 p=0.292		
IR (mm h ⁻¹)	0.211 p=0.371	0.276 p=0.238	0.615 p=0.004	-0.433 p=0.057	-0.125 p=0.599	-0.235 p=0.318	
k (cm h ⁻¹)	-0.200 p=0.399	0.388 p=0.091	-0.470 <i>p=0.036</i>	0.465 <i>p=0.039</i>	0.009 p=0.969	-0.316 p=0.175	-0.348 p=0.132

SM: Soil moisture; BD: Bulk density; MI: Mechanic impedance; SS: Shear strength; IR: Infiltration rate; k : Hydraulic conductivity; Bold: $p < 0.01$; Italic: $p < 0.05$.

The correlation matrix is emphasizing significantly the effects of rainfall regimes on runoff and soil moisture content (Table 43). A significant effect of SM content on the soil bulk density and mechanic impedance confirmed the response of soil surface structure to the changes in soil water regimes. A significant negative correlation was also observed between SM and k values obtained during 2008 and 2009. The positive correlation found between SM and IR may be explained by rapid loss of water by drainage in this sandy soil. Similarly, the positive correlation between soil k and BD was attributed to a most favorable water distribution from surface when soil particles are more sealed increasing soil pulse, as recorded during k measurements with minidisk infiltrometer.

3.3.1.2. *Soils under olive groves*

Descriptive statistical analysis of the soil infiltration rates (IR) is shown per each DOY during the two years of measurements for soil under cultivated olives (Table 44). The power equations, correlation coefficient, and significance level are also presented.

Table 44. Descriptive statistical analysis of the soil infiltration rates (mm min^{-1}) for soils under olive groves along the experimental period.

DOY	I	Fi	\bar{x}	σ	CV	I/Fi	Equation	r	p-level
64	57.14	28.30	40	9.17	23	2.02	$y=48.00x^{-0.13}$	0.960	0.0001
101	171.43	33.35	83	43.48	53	5.14	$y=95.64x^{-0.26}$	0.974	0.0001
141	200.00	24.09	79	54.02	69	8.30	$y=90.45x^{-0.32}$	0.995	0.0001
149	200.00	18.11	59	42.98	73	11.04	$y=72.94x^{-0.34}$	0.996	0.0001
158	85.71	29.65	53	19.39	37	2.89	$y=64.74x^{-0.19}$	0.980	0.0001
185	120.00	26.76	61	30.23	50	4.48	$y=74.87x^{-0.25}$	0.996	0.0001
312	130.00	21.37	57	35.66	62	6.08	$y=80.66x^{-0.28}$	0.947	0.0001
Mean 2008	138 \pm 56 CV=40	26 \pm 5 CV=20	61.48	33.56	52	5.71	$y=75.33x^{-0.25}$	0.978	0.0001
13	37.50	13.17	22	7.40	34	2.85	$y=31.78x^{-0.22}$	0.990	0.0001
23	29.86	8.58	13	6.12	47	3.48	$y=35.36x^{-0.22}$	0.947	0.0001
37	28.57	5.78	11	5.03	48	4.94	$y=20.04x^{-0.30}$	0.997	0.0001
51	15.38	5.42	9	2.93	34	2.84	$y=15.50x^{-0.26}$	0.992	0.0001
65	5.00	3.56	4	0.43	11	1.40	$y=5.53x^{-0.11}$	0.936	0.0001
85	33.33	7.27	14	6.70	48	4.59	$y=24.98x^{-0.30}$	0.998	0.0001
93	15.00	3.21	6	2.98	54	4.67	$y=12.23x^{-0.33}$	0.992	0.0001
118	30.00	7.33	15	7.11	48	4.09	$y=26.13x^{-0.31}$	0.990	0.0001
135	28.00	8.33	15	6.00	41	3.36	$y=22.36x^{-0.35}$	0.994	0.0001
167	21.82	11.55	15	3.27	22	1.89	$y=19.93x^{-0.13}$	0.937	0.0001
247	27.99	6.47	19	8.60	46	4.33	$y=21.90x^{-0.16}$	0.992	0.0001
279	42.86	9.41	20	10.26	52	4.56	$y=32.28x^{-0.30}$	0.995	0.0001
310	52.63	13.95	28.97	14.46	50	3.77	$y=49.04x^{-0.02}$	0.495	0.0001
Mean 2009	28 \pm 12 CV=44	8 \pm 3 CV=42	14.57	6.25	41	3.60	$y=24.39x^{-0.23}$	0.943	0.0001

DOY: Day of the year; I: Initial IR value; Fi: Final IR value; \bar{x} : Mean; σ : Standard deviation; CV: Coefficient of variation (%); I/Fi: Ratio of initial and final IR values.

All soil infiltration measurements lasted one hour in order to reach a constant IR value, that is: basic infiltration rate. The mean annual variability of IR values was 52% within 2008 IR measurements while during 2009 was 41% (Table 44). Nevertheless in the 2009 values of the basic infiltration rate showed a decrease by 69% with respect to 2008, recorded as a drier year and accepting more infiltrated water. This trend was also showed by the CV values plotted against I/Fi ratio (Figure 77), indicating higher IR values in 2008 by the slope values of the fitting equations. As stated before, the

difference in IR values found in 2008 with respect to 2009 is clearly indicated by the slope (0.669 for 2008 and 1.159 for 2009) of equations in Figure 98. A steeper trend of CV to I/Fi ratio will depict lower infiltration rate. Power law equations fitted very well the IR data per each DOY, giving significant correlations (Table 44). The theoretical mean IR value of 2008 was calculated by using the mean power equation ($r=0.978$, $p<0.0001$), resulting in $75\pm16 \text{ mm min}^{-1}$ ($CV=21\%$) and differing by 18% with respect to the experimental point of the IR line. Similarly during 2009 the mean power equation (Table 44) showed significant correlations ($r=0.943$, $p<0.0001$), and the theoretical IR mean value was $14\pm11 \text{ mm min}^{-1}$ ($CV=46\%$) against a real mean value of $14.57 \text{ mm min}^{-1}$.

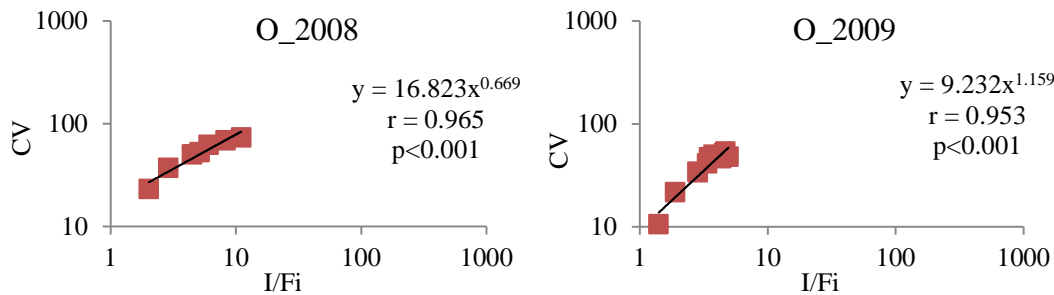


Figure 77. Relationships between the coefficient of variation (CV) and I/Fi ratio of soil infiltration rates in soil under cultivated olives along 2008 and 2009.

The dynamics of water regimes in soils under olive and its effect on IR and k values is reported in Figure 78 for the two years of investigations. From this figure it may be observed how rainfall trend may affect these two parameters. Rainfall regimes and soil moisture contents showed a direct impact on the dynamics of soil infiltration rates and soil hydraulic conductivity.

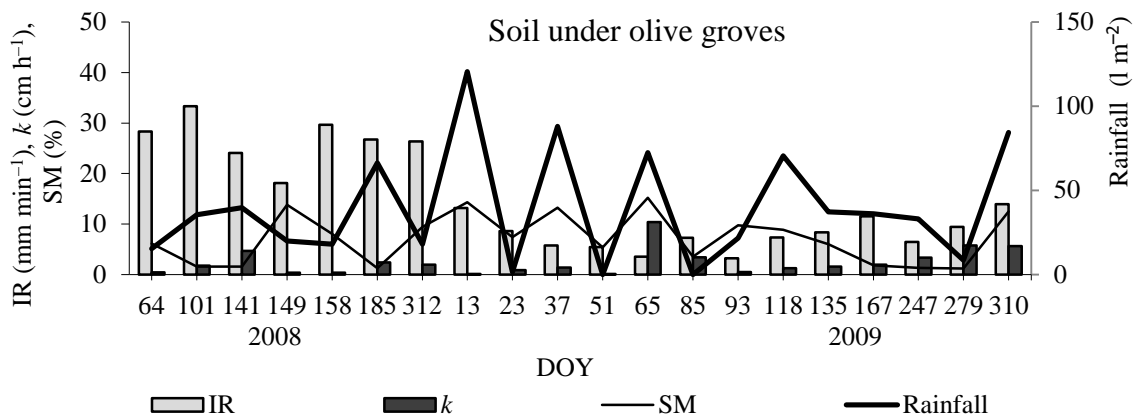


Figure 78. Values of soil infiltration rate (IR) (clear grey bar) and hydraulic conductivity (k) (dark grey bar) for soils under olive groves along the period of study. Trending of soil moisture (SM) (fine line) and rainfall (coarse line) is also presented.

The trend of soil IR and k data showed the direct dependence of IR values with drier soil condition and an inverse relationship of k values with wetter soil conditions along 2008 and 2009 (Figure 78). Therefore other surface parameters such as trends detected by MI and SS values in Table 20 may help to improve the understanding of soil infiltration dynamics at each period of the year observed and under the effects of different land use and abandonment. Soil infiltration capacity and water permeability at soil surface may not depend totally on underneath structure. Low clay and organic matter content may favor sealing mechanisms at surface, and even it may be higher with higher compacted surface crust. This was proved by the correlation matrix between the data obtained (Table 45). Effectively, a significant positive correlation was found between IR and MI ($r=0.546$, $p=0.013$) during 2008 and 2009. Moreover, the correlation matrix emphasized the effect of rainfall on runoff and soil moisture content with positive significant correlations. SM showed also negative significant correlations with bulk density and mechanic impedance, indicating its importance in soil surface dynamics.

Table 45. Correlation matrix between the dynamic physical soil parameters in soils under olive groves (Data for 2008 and 2009).

Soil parameters	Rainfall (l m ⁻²)	Runoff (l m ⁻²)	SM (%)	BD (g cm ⁻³)	MI (kPa)	SS (kPa)	IR (mm h ⁻¹)
Runoff (l m ⁻²)	0.711 p=0.000						
SM (%)	0.478 p=0.033	-0.017 p=0.945					
BD (g cm ⁻³)	-0.591 p=0.006	-0.109 p=0.647	-0.855 p=0.000				
MI (kPa)	-0.304 p=0.192	-0.013 p=0.955	-0.527 p=0.017	0.346 p=0.135			
SS (kPa)	-0.169 p=0.477	0.032 p=0.894	-0.384 p=0.094	0.418 p=0.066	0.546 p=0.013		
IR (mm h ⁻¹)	-0.155 p=0.514	0.113 p=0.636	-0.256 p=0.277	0.085 p=0.722	0.546 p=0.013	0.111 p=0.641	
k (cm h ⁻¹)	0.190 p=0.423	0.008 p=0.975	0.028 p=0.907	-0.011 p=0.965	-0.080 p=0.737	0.127 p=0.593	-0.217 p=0.359

SM: Soil moisture; BD: Bulk density; MI: Mechanic impedance; SS: Shear strength; IR: Infiltration rate; k : Hydraulic conductivity; Bold: $p<0.01$; Italic: $p<0.05$.

3.3.1.3. *Soils under stands of cork trees*

Descriptive statistical analysis of the soil infiltration rate (IR) is shown per each DOY during the two years of measurements for soil under stands of cork trees (Table 46). The power equations, correlation coefficient, and significance level are also presented. The measurements of soil infiltration rates per each DOY lasted one hour until the basic infiltration rate. In 2009 the values of basic infiltration rate showed a mean increase by 40% with respect to 2008. Even though this was a drier year, the final infiltration rate values were rather similar to those of 2009. It may be observed that the mean annual variability of IR values was 117% in 2008, while during 2009 was 47% (Table 46).

Table 46. Descriptive statistical analysis of the soil infiltration rate values for soil under stands of cork trees along the experimental period.

DOY	I	Fi	\bar{x}	σ	CV	I/Fi	Equation	r	p-level
64	12.77	6.37	8.73	1.96	22	2.00	$y=13.79x^{-0.19}$	0.983	0.0001
101	100.00	7.50	22.06	19.23	87	13.33	$y=38.12x^{-0.40}$	0.999	0.0001
141	300.00	2.65	17.95	58.88	328	113.38	$y=28.18x^{-0.58}$	0.972	0.0001
149	260.00	5.16	38.91	60.96	157	50.43	$y=51.29x^{-0.56}$	0.996	0.0001
158	15.38	5.16	9.15	2.73	30	2.98	$y=15.57x^{-0.24}$	0.966	0.0001
185	46.15	4.93	12.60	10.08	80	9.37	$y=24.07x^{-0.39}$	0.999	0.0001
312	100.00	5.08	15.99	18.56	116	19.67	$y=31.23x^{-0.41}$	0.999	0.0001
Mean	119±116	5±1.5	17.91	24.63	117	30.17	$y=34.00x^{-0.44}$	0.988	0.0001
2008	CV=97	CV=28							
13	16.67	8.06	11.41	2.86	25	2.07	$y=17.02x^{-0.18}$	0.956	0.0001
23	17.00	5.60	12.21	3.21	26	3.04	$y=18.12x^{-0.21}$	0.992	0.0001
37	18.95	8.09	12.71	4.14	33	2.34	$y=19.69x^{-0.22}$	0.928	0.0001
51	11.04	6.19	8.17	1.77	22	1.78	$y=12.10x^{-0.16}$	0.964	0.0001
65	16.67	5.88	8.94	2.91	33	2.83	$y=14.92x^{-0.23}$	0.998	0.0001
85	20.00	5.10	8.97	4.02	45	3.92	$y=16.84x^{-0.29}$	0.998	0.0001
93	30.00	6.98	13.15	6.58	50	4.30	$y=22.07x^{-0.28}$	0.999	0.0001
118	24.00	5.36	10.38	5.17	50	4.48	$y=20.58x^{-0.33}$	0.993	0.0001
135	35.00	6.14	12.65	6.50	51	5.70	$y=25.66x^{-0.33}$	0.948	0.0001
167	66.67	7.24	22.47	17.34	77	9.21	$y=36.22x^{-0.39}$	0.998	0.0001
247	71.00	5.77	20.12	16.98	84	12.31	$y=32.26x^{-0.39}$	0.993	0.0001
279	70.59	8.74	25.12	17.45	69	8.08	$y=39.97x^{-0.37}$	0.992	0.0001
310	22.22	6.22	11.01	4.72	43	3.57	$y=19.37x^{-0.28}$	0.997	0.0001
Mean	32±22	7±1.2	13.64	7.20	47	4.90	$y=22.68x^{-0.28}$	0.981	0.0001
2009	CV=68	CV=18							

DOY: Day of the year; I: Initial IR value; Fi: Final IR value; \bar{x} : Mean; σ : Standard deviation; CV: Coefficient of variation (%); I/Fi: Ratio of initial and final IR values.

This trend was also showed by the CV values plotted against I/Fi ratio (Figure 79), indicating higher IR values in 2008 by the slope values of the fitting equations. Power law equations fitted very well the IR data per each DOY, giving significant correlations (Table 46). The theoretical mean of IR value of 2008 was calculated when $t=1$ by using the mean of power equations ($r=0.988$, $p<0.0001$), resulting in 34 ± 14 mm min^{-1} (CV=41) and differing by 47% with respect to the experimental point of IR line. Similarly during 2009 the mean power equation (Table 46) showed significant correlations ($r=0.981$, $p<0.0001$), and the theoretical IR mean value when $t=1$ was 23 ± 9 mm min^{-1} (CV=37%) against a real mean value of 13.64 mm min^{-1} . As stated before, the difference in IR values found in 2008 with respect to 2009 is clearly indicated by the slope (0.637 for 2008 and 0.712 for 2009) of equations in Figure 79. A steeper trend of CV to I/Fi ratio will depict a lower infiltration rate dynamics. In Figure 80 it is noticed that infiltration rates and hydraulic conductivity dynamics may be associated to physical parameters presented for 2008 and 2009 in Table 21.

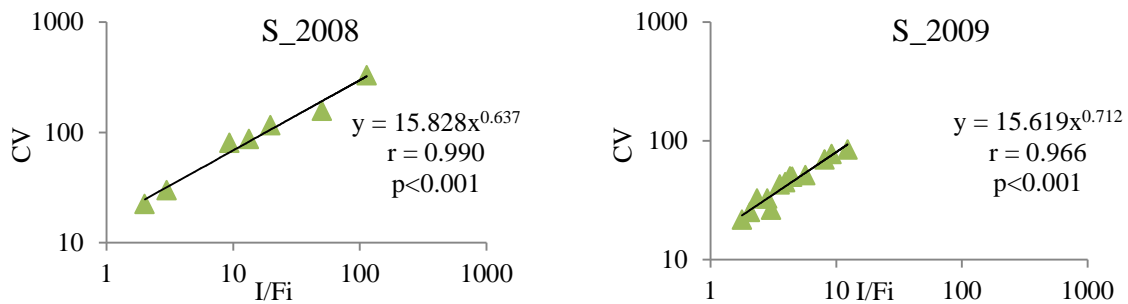


Figure 79. Relationships between the coefficient of variation (CV) and I/Fi ratio of soil infiltration rates in soil under stands of cork trees along 2008 and 2009.

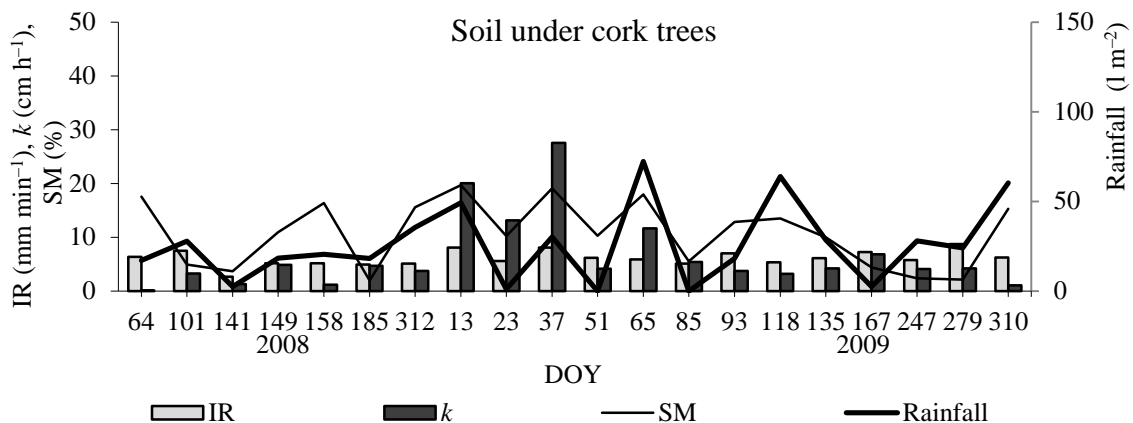


Figure 80. Values of soil infiltration rate (IR) (clear grey bar) and hydraulic conductivity (k) (dark grey bar) for soil under stands of cork trees along the period of study. Trending of soil moisture (SM) (fine line) and rainfall (coarse line) is also presented.

IR values showed an inverse trend with soil moisture content along 2009 and 2008. The same trend was found with the k values despite the increasing of k values during winter 2009 (DOY 13, 23, and 37) with wetter soil conditions (Figure 80). The dynamics of soil infiltration rate and permeability at soil surface was thought to be more related to the soil structure which may play an important role. At this regard a correlation matrix was tried between the physical data sets of SM, BD, MI, and SS presented in Table 21 in order to find crossed relationships (Table 47).

Table 47. Correlation matrix between the dynamic physical soil parameters in soils under stands of cork trees (Data for 2008 and 2009).

Soil parameters	Rainfall (l m ⁻²)	Runoff (l m ⁻²)	SM (%)	BD (g cm ⁻³)	MI (kPa)	SS (kPa)	IR (mm h ⁻¹)
Runoff (l m ⁻²)	0.798 p=0.000						
SM (%)	<i>0.523</i> <i>p=0.018</i>	<i>0.500</i> <i>p=0.025</i>					
BD (g cm ⁻³)	-0.354 p=0.126	-0.208 p=0.378	-0.710 p=0.000				
MI (kPa)	-0.133 p=0.576	-0.332 p=0.153	<i>-0.533</i> <i>p=0.016</i>	0.398 p=0.083			
SS (kPa)	<i>-0.451</i> <i>p=0.046</i>	-0.291 p=0.213	-0.321 p=0.168	0.426 p=0.061	0.405 p=0.077		
IR (mm h ⁻¹)	0.193 p=0.414	0.182 p=0.442	0.181 p=0.445	-0.206 p=0.385	0.151 p=0.524	0.173 p=0.466	
k (cm h ⁻¹)	0.165 p=0.488	0.316 p=0.175	0.404 p=0.077	0.020 p=0.932	-0.192 p=0.418	0.076 p=0.751	<i>0.455</i> <i>p=0.044</i>

SM: Soil moisture; BD: Bulk density; MI: Mechanic impedance; SS: Shear strength; IR: Infiltration rate; k : Hydraulic conductivity; Bold: $p < 0.01$; Italic: $p < 0.05$.

The most relevant positive significant correlation was found between IR and K . This was interpreted as the positive role of soil structure receiving water from rain and controlling its distribution along the profile. Similarly, the matrix showed significant correlation between rainfall, runoff and soil moisture. Soil bulk density and mechanic impedance were significantly and negatively correlated with SM. The same was found between rainfall and shear strength (Table 47). These data may confirm the behavior of soil surface structure to changes in water regimes.

3.3.1.4. *Soils under stands of pine trees*

Descriptive statistical analysis of the soil infiltration rate (IR) is shown per each DOY during the two years of measurements for soil under stands of pine trees (Table 48). The power equations, correlation coefficients and significance level are also presented.

Table 48. Descriptive statistical analysis of the soil infiltration rates (mm min^{-1}) for soil under stands of pine trees along the experimental period.

DOY	I	Fi	\bar{x}	σ	CV	I/Fi	Equation	r	p-level
64	150.00	13.17	44.15	34.15	77	11.39	$y=60.58x^{-0.373}$	0.983	0.0001
101	150.00	25.42	71.19	41.53	58	5.90	$y=85.30x^{-0.296}$	0.999	0.0001
141	85.71	17.34	43.01	23.48	55	4.94	$y=58.41x^{-0.297}$	0.972	0.0001
149	120.00	12.95	51.29	40.07	78	9.27	$y=66.11x^{-0.398}$	0.996	0.0001
158	150.00	11.93	39.78	32.22	81	12.57	$y=55.76x^{-0.377}$	0.966	0.0001
185	85.71	18.13	39.29	19.16	49	4.73	$y=53.50x^{-0.264}$	0.999	0.0001
312	90.00	16.48	37.00	18.69	51	5.46	$y=54.98x^{-0.29}$	0.999	0.0001
Mean	119 \pm 31	16 \pm 5	46.53	29.90	64.09	7.75	$y=62.09x^{-0.33}$	0.987	0.0001
2008	CV=26	CV=28							
13	30.00	3.10	6.99	6.19	89	9.69	$y=17.05x^{-0.42}$	0.956	0.0001
23	20.00	5.40	5.32	3.23	61	3.70	$y=11.54x^{-0.36}$	0.992	0.0001
37	8.33	2.32	3.62	1.60	44	3.59	$y=8.60x^{-0.32}$	0.928	0.0001
51	66.67	10.88	26.76	16.07	60	6.13	$y=41.43x^{-0.33}$	0.964	0.0001
65	8.57	5.70	6.87	0.97	14	1.50	$y=9.42x^{-0.12}$	0.998	0.0001
85	20.69	8.64	12.88	3.60	28	2.39	$y=19.78x^{-0.20}$	0.998	0.0001
93	10.00	5.66	7.14	1.50	21	1.77	$y=10.53x^{-0.15}$	0.999	0.0001
118	3.75	2.32	2.85	0.45	16	1.61	$y=4.14x^{-0.14}$	0.993	0.0001
135	9.00	2.51	3.60	1.25	35	3.58	$y=20.89x^{-0.26}$	0.948	0.0001
167	300.00	3.16	21.43	60.90	284	94.88	$y=31.42x^{-0.56}$	0.998	0.0001
247	70.00	3.50	23.00	30.00	130	20.00	$y=40.98x^{-0.33}$	0.993	0.0001
279	20.00	6.45	10.54	3.87	37	3.10	$y=18.24x^{-0.25}$	0.992	0.0001
310	26.09	3.44	8.25	6.40	78	7.58	$y=18.86x^{-0.42}$	0.997	0.0001
Mean	46 \pm 79	4.85 \pm 2.6	10.71	10.46	68.92	12.27	$y=19.45x^{-0.30}$	0.986	0.0001
2009	CV=174	CV=54							

DOY: Day of the year; I: Initial IR value; Fi: Final IR value; \bar{x} : Mean; σ : Standard deviation; CV: Coefficient of variation (%); I/Fi: Ratio of initial and final IR values.

The measurements of soil infiltration rates were conducted until basic infiltration rate was achieved in all DOYs. In 2009 values of basic infiltration rate showed a decrease by 70% with respect to 2008. Nevertheless the mean annual variability of IR values was 64% within the IR measurements of 2008 while during 2009 was 68% (Table 48). This trend was also showed by the CV values plotted against I/Fi ratio (Figure 81), indicating higher IR values in 2008 by the slope values of the fitting

equations. Power law equations fitted very well the IR data per each DOY, giving significant negative correlation (Table 48). The theoretical mean of IR value of 2008 was calculated when $t=1$ min by using the mean power equation ($r=0.987$, $p<0.0001$), resulting in 62 ± 11 mm min⁻¹ (CV=18) and differing by 24% with respect to the experimental point of IR line. Similarly during 2009 the mean power equation (Table 48) showed significant correlations ($r=0.986$, $p<0.0001$), and the theoretical IR mean value when $t=1$ min was 18 ± 12 mm min⁻¹ (CV=61%) against a real mean value of 11 mm min⁻¹. As stated before, the difference in IR values found in 2008 with respect to 2009 is clearly indicated by the slope (0.517 for 2008 and 0.710 for 2009 respectively) of equations in Figure 81. A steeper trend of CV to I/Fi ratio will depict a lower infiltration rate dynamics. Figure 82 shows the values of rainfall, IR, k , and SM plotted against the DOYs. The values pictured in this figure clearly indicate the response of soil surface in the PI environment. It may be observed that IR are high in 2008 (drier year) and low in 2009 (more rainfall). Soil moisture also follows the rainfall patterns. Nevertheless, hydraulic conductivity (k) shows low variability during 2008 and 2009.

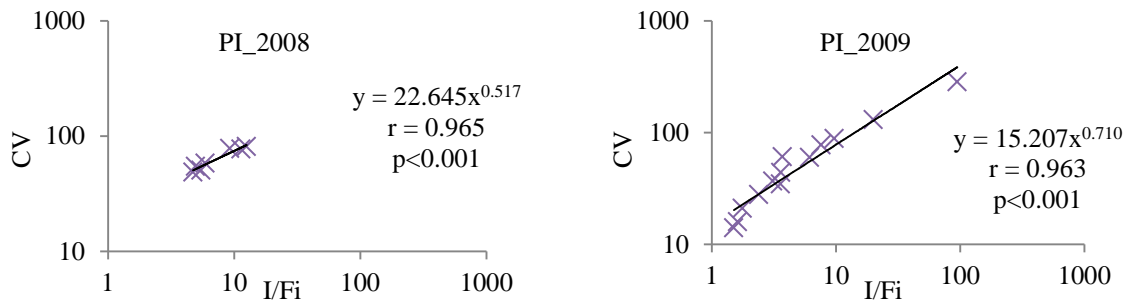


Figure 81. Relationships between the coefficient of variation (CV) and I/Fi ratio of soil infiltration rates in soil under stands of pine trees along 2008 and 2009.

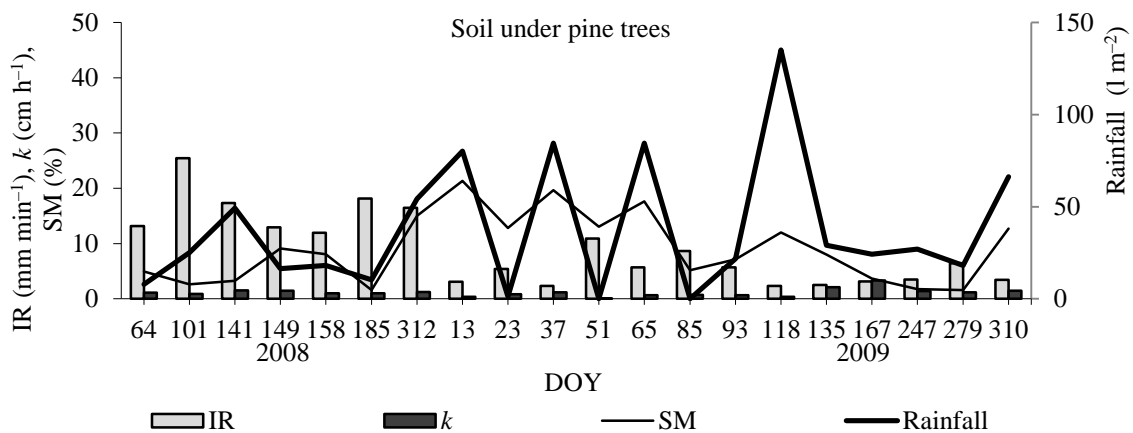


Figure 82. Values of soil infiltration rate (IR) (clear grey bar) and hydraulic conductivity (k) (dark grey bar) for soil under stands of pine trees along the period of study. Trending of soil moisture (SM) (fine line) and rainfall (coarse line) is also represented.

The correlation matrix tried between the data of Table 21 (SM, BD, MI, and SS) together with rainfall, runoff, IR, and k values allowed to check the relationships between soil dynamic physical parameters (Table 49).

Table 49. Correlation matrix between the dynamic physical soil parameters in soils under stands of pine trees (Data for 2008 and 2009).

Soil parameters	Rainfall (l m ⁻²)	Runoff (l m ⁻²)	SM (%)	BD (g cm ⁻³)	MI (kPa)	SS (kPa)	IR (mm h ⁻¹)
Runoff (l m ⁻²)	0.711						
	p=0.000						
SM (%)	0.580	<i>0.558</i>					
	p=0.007	<i>p=0.011</i>					
BD (g cm ⁻³)	<i>-0.543</i>	<i>-0.520</i>	<i>-0.541</i>				
	<i>p=0.013</i>	<i>p=0.019</i>	<i>p=0.014</i>				
MI (kPa)	-0.072	-0.311	-0.393	0.016			
	p=0.764	p=0.182	p=0.086	p=0.947			
SS (kPa)	0.155	-0.017	-0.184	0.033	0.388		
	p=0.513	p=0.942	p=0.439	p=0.889	p=0.091		
IR (mm h ⁻¹)	-0.367	-0.367	-0.399	0.244	0.271	-0.339	
	p=0.112	p=0.112	p=0.082	p=0.299	p=0.248	p=0.143	
k (cm h ⁻¹)	-0.154	0.265	-0.376	0.058	0.002	0.043	-0.124
	p=0.516	p=0.259	p=0.102	p=0.809	p=0.994	p=0.858	p=0.602

SM: Soil moisture; BD: Bulk density; MI: Mechanic impedance; SS: soil moisture; IR: Infiltration rate; k : Hydraulic conductivity; Bold: $p < 0.01$; Italic: $p < 0.05$.

The correlation matrix is emphasizing significantly the effects of rainfall regimes on the soil moisture content ($r=0.580$, $p=0.007$). A significant negative correlation was found between SM content and the soil bulk density ($r=-0.541$, $p=0.014$). No significant correlations were found between data of mechanic impedance and shear strength with other parameters. Similarly IR and k values did not show any significant correlations.

3.3.1.5. Soils under pasture

Descriptive statistical analysis of the soil infiltration rates (IR) is shown per each DOY during the two years of measurements for soils under pasture (Table 50). The power equations, correlation coefficients and significance level are also presented. The basic infiltration rate per each DOY was reached after one hour of water infiltration. In 2009 the values of basic infiltration rate increased by 206% with respect to 2008. These results were rather surprising even though the soil under pasture is known to have a very

high water retention capacity. Moreover, the mean annual variability of IR values was 211% higher within the IR measurements of 2008 (Table 50) than 2009 (34%). These values may partly explain the occurred process. In 2008 the driest conditions of soil surface caused a high variability of initial water entry into the soil. Conversely, during 2009 the soil response to initial water entry was more regular giving less variability within rainfall events.

Table 50. Descriptive statistical analysis of the soil infiltration rate values for soils under pasture along the experimental period.

DOY	I	Fi	\bar{x}	σ	CV	I/Fi	Equation	r	p-level
64	40.00	1.09	5.89	10.73	182	36.71	$y=16.95x^{-0.67}$	0.995	0.0001
101	120.00	2.57	11.93	25.13	211	46.68	$y=24.68x^{-0.55}$	0.994	0.0001
141	150.00	0.63	11.59	38.31	331	239.39	$y=16.74x^{-0.80}$	0.999	0.0001
149	149.00	0.57	12.00	39.00	325	262.94	$y=16.00x^{-0.75}$	0.947	0.0001
158	150.00	0.68	13.41	39.63	296	220.00	$y=17.99x^{-0.80}$	0.999	0.0001
185	15.35	1.02	2.25	1.78	79	15.00	$y=9.13x^{-0.54}$	0.998	0.0001
312	12.00	0.97	3.90	2.00	51	12.41	$y=11.23x^{-0.21}$	0.999	0.0001
Mean 2008	91±65 CV=72	1.07±0.7 CV=64	8.71	22.37	211	119.02	$y=16.10x^{-0.62}$	0.990	0.0001
13	2.34	1.06	1.47	0.39	27	2.21	$y=3.84x^{-0.31}$	0.991	0.0001
23	4.00	1.47	2.00	0.60	30	2.73	$y=5.00x^{-0.27}$	0.993	0.0001
37	5.66	2.52	3.39	0.83	25	2.25	$y=6.49x^{-0.23}$	0.999	0.0001
51	6.00	2.38	3.30	0.93	28	2.53	$y=6.74x^{-0.26}$	0.999	0.0001
65	2.75	1.25	3.00	0.50	20	2.20	$y=4.36x^{-0.20}$	0.998	0.0001
85	1.00	0.68	0.79	0.09	11	1.46	$y=1.26x^{-0.15}$	0.997	0.0001
93	20.00	1.28	4.12	4.67	113	15.67	$y=12.64x^{-0.56}$	0.998	0.0001
118	2.61	1.15	1.53	0.39	26	2.27	$y=2.97x^{-0.23}$	0.997	0.0001
135	2.60	1.29	1.60	0.40	25	2.01	$y=2.70x^{-0.27}$	0.998	0.0001
167	14.46	2.41	1.84	0.60	50	6.00	$y=4.04x^{-0.29}$	0.948	0.0001
247	4.45	1.43	6.58	2.57	39	3.11	$y=12.36x^{-0.26}$	0.999	0.0001
279	5.22	3.40	4.02	0.52	13	1.54	$y=5.88x^{-0.13}$	0.991	0.0001
310	66.67	22.18	38.26	14.40	38	3.01	$y=49.87x^{-0.20}$	0.995	0.0001
Mean 2009	11±18 CV=167	3.27±5.7 CV=175	5.53	2.07	34	3.61	$y=9.09x^{-0.26}$	0.993	0.0001

DOY: Day of the year; I: Initial IR value; Fi: Final IR value; \bar{x} : Mean; σ : Standard deviation; CV: Coefficient of variation (%); I/Fi: Ratio of initial and final IR values.

This trend was also showed by the CV values plotted against I/Fi ratio (Figure 83), indicating higher IR values in 2008 by the slope values of the fitting equations. Power law equations fitted very well the IR data per each DOY, giving significant correlation (Table 50). The theoretical mean of IR value of 2008 was calculated when $t=1$ min by using the mean power equation ($r=0.990$, $p<0.001$), resulting in 16 ± 5 mm min^{-1} (CV=31) and differing by 46% with respect to the experimental point of IR line. Similarly during 2009 the mean power equation (Table 50) showed significant

correlation ($r=0.993$, $p<0.001$), and the theoretical IR mean value when $t=1$ min was 9 ± 13 mm min⁻¹ (CV=140%) against a real mean value of 5.53 mm min⁻¹. As stated before, the difference in IR values found in 2008 with respect to 2009 is clearly indicated by the slope (0.520 for 2008 and 0.883 for 2009) of equations in Figure 83. A steeper trend of CV to I/Fi ratio will depict a lower infiltration rate dynamics. Figure 84 depicts the trend of rainfall, IR, k , and SM along the DOYs.

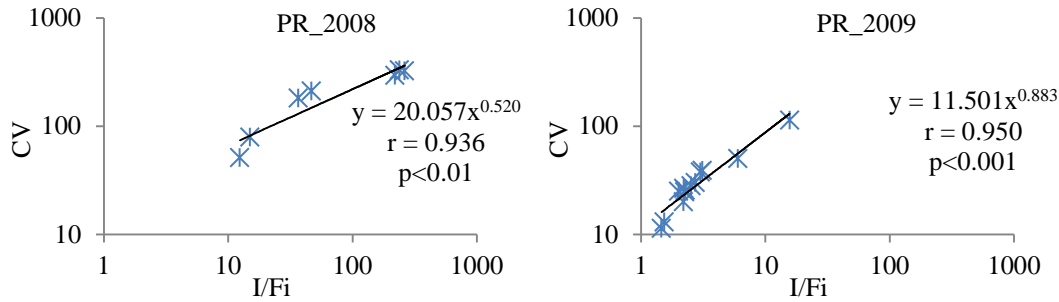


Figure 83. Relationships between the coefficient of variation (CV) and I/Fi ratio of soil infiltration rates in soils under pasture along 2008 and 2009.

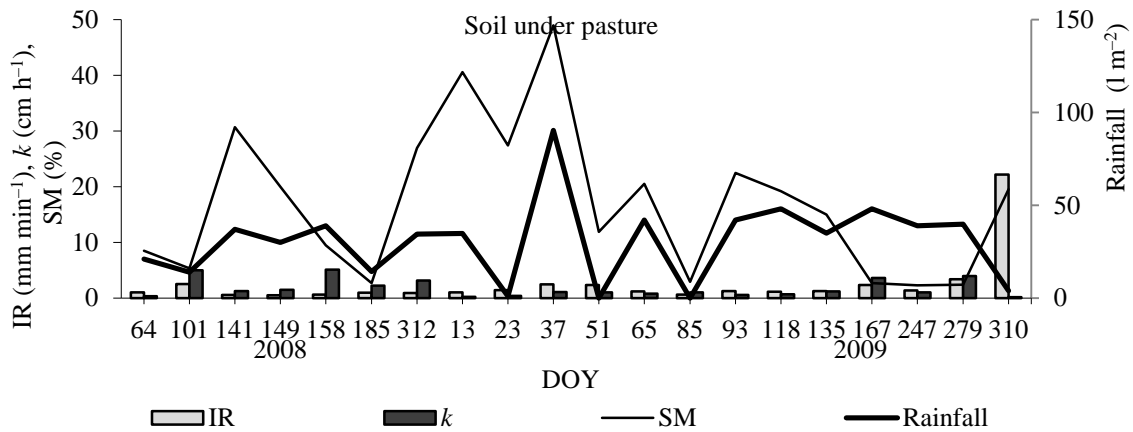


Figure 84. Values of soil infiltration rate (IR) (clear grey bar) and hydraulic conductivity (k) (dark grey bar) for soils under pasture along the period of study. Trending of soil moisture (SM) (fine line) and rainfall (coarse line) is also presented.

It may be observed that SM in soils under pasture is strongly influenced by rainfall events during the experimental period and may affect the IR and k parameters. Although IR and k are expressed with different units for graphical constraints we may notice a rather similar trend which is given by the soil capability for water storage. When the rainfall decreases as in DOY 310, IR values are suddenly increased. The same soil physical parameters (SM, BD, MI, and SS in Table 22) together with data of rainfall, infiltration capacity (IR), and hydraulic conductivity (k) were evaluated to better understand the physical dynamics. The correlation matrix in Table 51 showed significant negative correlation between IR values and both MI and SS values. As

elsewhere reported, soils under pasture have the disadvantage to be subjected to periodical grazing, starting from winter into late spring. The surface compaction by grazing is partially compensated by the capability of soils to water storage. MI and SS were also significantly positively correlated.

Table 51. Correlation matrix between the water regimes and dynamic physical soil parameters in soils under pasture (Data for 2008 and 2009).

Soil parameters	Rainfall (l m ⁻²)	Runoff (l m ⁻²)	SM (%)	BD (g cm ⁻³)	MI (kPa)	SS (kPa)	IR (mm h ⁻¹)
Runoff (l m ⁻²)	0.116 p=0.628						
SM (%)	0.442 p=0.051	0.189 p=0.425					
BD (g cm ⁻³)	0.106 p=0.658	-0.094 p=0.693	-0.583 p=0.007				
MI (kPa)	0.028 p=0.906	0.142 p=0.550	-0.420 p=0.065	0.088 p=0.714			
SS (kPa)	-0.061 p=0.797	-0.143 p=0.547	-0.362 p=0.117	0.299 p=0.200	<i>0.444</i> <i>p=0.050</i>		
IR (mm h ⁻¹)	-0.257 p=0.274	0.057 p=0.813	0.020 p=0.932	-0.050 p=0.835	-0.608 p=0.004	-0.505 <i>p=0.023</i>	
<i>k</i> (cm h ⁻¹)	0.100 p=0.675	-0.226 p=0.338	-0.433 p=0.057	0.255 p=0.279	0.429 p=0.059	0.124 p=0.601	-0.170 p=0.474

SM: Soil moisture; BD: Bulk density; MI: Mechanic impedance; SS: Shear strength; IR: Infiltration rate; *k*: Hydraulic conductivity; Bold: $p < 0.01$; Italic: $p < 0.05$.

3.3.1.6. Soils under *Cistus scrub*

A descriptive statistical analysis of the soil infiltration rates (IR) is shown per each DOY during the two years of measurements for soils under *Cistus* (Table 52). The equations correlation coefficients and significance level are also presented. In 2009, the values of basic infiltration rate (F_i values) (Table 52) showed a decrease by 82% with respect to 2008, recorded as drier year, favoring more water entry from surface. The mean annual variability of IR values was 71% within the IR measurements of 2008 while during 2009 was 57% (Table 52). This trend was also showed by the CV values plotted against I/F_i ratio (Figure 85), indicating higher IR values in 2008 by the slope values of the fitting equations. Power law equations fitted very well the IR data per each DOY, giving significant correlation (Table 52). The theoretical mean of IR value of 2008 was calculated when $t=1$ min by using the mean power equations, resulting in 46 ± 13 mm min⁻¹ (CV=49) and differing by 34% with respect to the experimental point of IR line. Similarly during 2009 the mean power equation (Table 52) showed

significant correlations ($r=0.993$, $p<0.0001$), and the theoretical IR mean value when $t=1$ min was 8 ± 3 mm min⁻¹ (CV=36%) against a real mean value of 3.21 mm min⁻¹. The difference in IR values found in 2008 with respect to 2009 is then indicated by the slope (0.593 for 2008 and 0.901 for 2009) of equations in Figure 85. A steeper trend of CV to I/Fi ratio will depict a lower infiltration rate dynamics.

Table 52. Descriptive statistical analysis of the soil infiltration rate (IR) (mm min⁻¹) values for soils under *Cistus* scrub along the experimental period.

DOY	I	Fi	\bar{x}	σ	CV	I/Fi	Equation	r	p-level
64	60.00	22.39	37.47	12.44	33	2.68	$y=48.52x^{-0.19}$	0.991	0.0001
101	53.33	22.17	35.46	10.97	31	2.41	$y=45.60x^{-0.18}$	0.971	0.0001
141	94.74	17.83	46.35	26.43	57	5.31	$y=61.85x^{-0.30}$	0.986	0.0001
149	120.00	7.86	31.76	28.84	91	15.26	$y=48.77x^{-0.45}$	0.993	0.0001
158	89.00	5.83	32.68	30.00	92	15.26	$y=60.98x^{-0.40}$	0.938	0.0001
185	60.00	3.25	12.41	14.21	115	18.48	$y=27.16x^{-0.52}$	0.998	0.0001
312	50.00	4.33	15.70	12.00	76	11.54	$y=31.66x^{-0.46}$	0.998	0.0001
Mean	75±26	12±9	30.26	19.27	71	10.13	$y=46.36x^{-0.36}$	0.982	0.0001
2008	CV=35	CV=71							
13	7.59	4.55	5.39	0.92	17	1.67	$y=7.84x^{-0.13}$	0.999	0.0001
23	8.00	2.95	4.65	1.20	26	2.71	$y=8.98x^{-0.35}$	0.994	0.0001
37	8.33	1.85	3.48	1.98	57	4.50	$y=10.30x^{-0.42}$	0.993	0.0001
51	2.61	1.40	1.75	0.34	19	1.87	$y=3.49x^{-0.22}$	0.998	0.0001
65	2.44	1.23	1.60	0.35	22	1.99	$y=3.52x^{-0.26}$	0.999	0.0001
85	4.00	1.84	2.77	0.68	25	2.17	$y=6.66x^{-0.314}$	0.999	0.0001
93	20.00	0.88	3.45	4.89	142	22.65	$y=12.32x^{-0.64}$	0.999	0.0001
118	5.00	3.50	4.05	0.42	10	1.43	$y=5.13x^{-0.094}$	0.989	0.0001
135	11.00	1.47	1.00	0.80	80	7.50	$y=7.45x^{-0.32}$	0.994	0.0001
167	12.00	0.82	2.27	2.71	120	14.62	$y=10.71x^{-0.63}$	0.999	0.0001
247	25.00	2.10	2.65	2.60	98	11.90	$y=9.64x^{-0.53}$	0.947	0.0001
279	15.00	1.07	2.98	3.31	111	13.99	$y=11.76x^{-0.59}$	0.999	0.0001
310	7.50	4.66	5.66	0.85	15	1.61	$y=7.30x^{-0.11}$	0.998	0.0001
Mean	10±7	2.18±1.3	3.21	1.62	57	6.82	$y=8.08x^{-0.35}$	0.993	0.0001
2009	CV=68	CV=61							

DOY: Day of the year; I: Initial IR value; Fi: Final IR value; \bar{x} : Mean; σ : Standard deviation; CV: Coefficient of variation (%); I/Fi: Ratio of initial and final IR values.

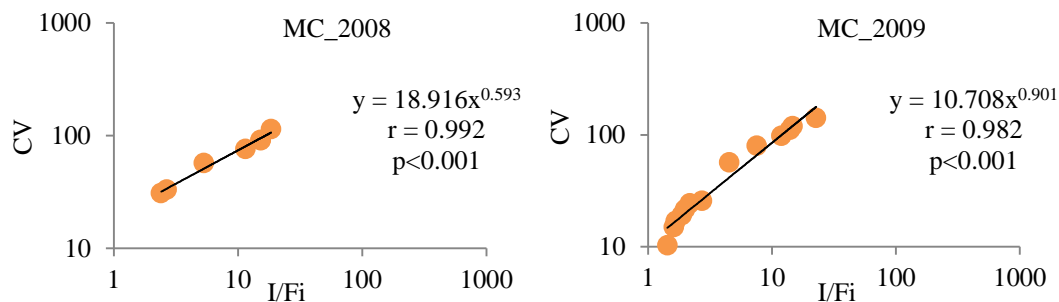


Figure 85. Relationships between the coefficient of variation (CV) and I/Fi ratio of soil infiltration rates in soils under *Cistus* scrub along 2008 and 2009.

Along the experimental period the trend of soil IR values showed an inverse trend with soil moisture content (Figure 86). A mean increase of SM by 24% in 2009 showed a decrease of 449% in soil infiltration rates whilst soil hydraulic conductivity increased 60% with respect to 2008.

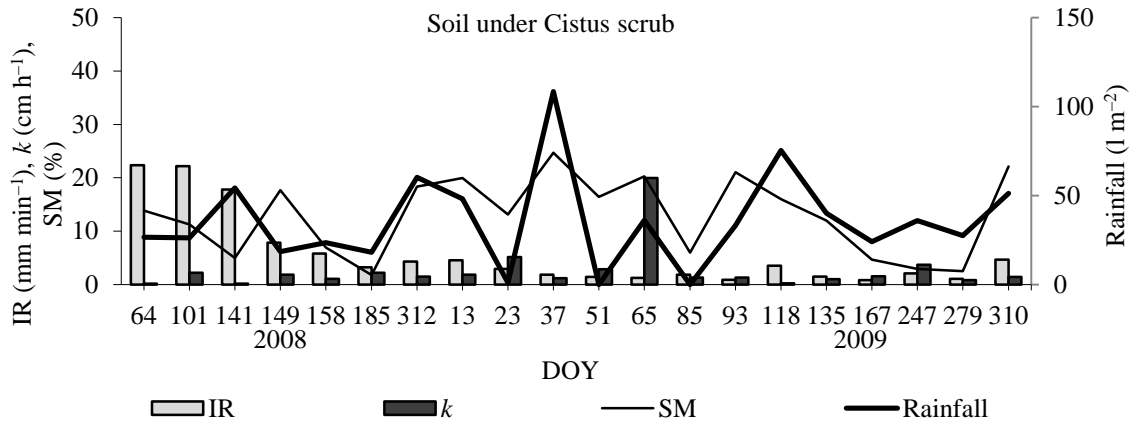


Figure 86. Values of soil infiltration rate (IR) (clear grey bar) and hydraulic conductivity (k) (dark grey bar) for soils under *Cistus* scrub along the period of study. Trending of soil moisture (SM) (fine line) and rainfall (coarse line) is also represented.

The correlation matrix tried with physical parameters of Table 23 (SM, BD, MI, and SS) together with rainfall, runoff, infiltration rates (IR), and hydraulic conductivity (k) data allowed to relate soil surface dynamics (Table 53).

Table 53. Correlation matrix between the dynamic physical soil parameters in soils under *Cistus* scrub (Data for 2008 and 2009).

Soil parameters	Rainfall ($l\ m^{-2}$)	Runoff ($l\ m^{-2}$)	SM (%)	BD ($g\ cm^{-3}$)	MI (kPa)	SS (kPa)	IR ($mm\ h^{-1}$)
Runoff ($l\ m^{-2}$)	0.498 <i>$p=0.025$</i>						
SM (%)	0.454 <i>$p=0.044$</i>	0.176 $p=0.459$					
BD ($g\ cm^{-3}$)	-0.350 $p=0.131$	-0.157 $p=0.508$	-0.644 $p=0.002$				
MI (kPa)	-0.101 $p=0.672$	-0.133 $p=0.576$	-0.599 $p=0.005$	0.755 $p=0.000$			
SS (kPa)	0.007 $p=0.977$	-0.192 $p=0.417$	-0.377 $p=0.102$	0.545 <i>$p=0.013$</i>	0.687 $p=0.001$		
IR ($mm\ h^{-1}$)	-0.012 $p=0.961$	-0.074 $p=0.756$	-0.085 $p=0.720$	0.319 $p=0.170$	0.272 $p=0.245$	0.047 $p=0.845$	
k ($cm\ h^{-1}$)	-0.119 $p=0.618$	0.211 $p=0.372$	0.214 $p=0.365$	-0.111 $p=0.641$	-0.197 $p=0.406$	0.076 $p=0.751$	-0.220 $p=0.351$

SM: Soil moisture; BD: Bulk density; MI: Mechanic impedance; SS: Shear strength; IR: Infiltration rate; k : Hydraulic conductivity; Bold: $p<0.01$; Italic: $p<0.05$.

Relevant positive significant correlations were found between BD and both MI and SS respectively. This soil may be more vulnerable for recent and repeated wildfire occurrence, which may explain these direct relationships. Rainfall, runoff and SM were significantly correlated whilst significant negative correlation emphasized the positive effect that moisture increase may have on soil surface compaction (BD) and mechanic impedance. A significant positive correlation was found with data of MI and SS.

3.3.1.7. Soils under *Erica scrub*

Descriptive statistical analysis of the soil infiltration rates (IR) is shown per each DOY during the two years of measurements for soils under *Erica scrub* (Table 54).

Table 54. Descriptive statistical analysis of the soil infiltration rate (IR) (mm min^{-1}) values for soils under *Erica scrub* along the experimental period.

DOY	I	Fi	\bar{x}	σ	CV	I/Fi	Equation	r	p-level
64	150.00	2.37	16.33	35.22	216	63.17	$y=27.17x^{-0.60}$	0.999	0.0001
101	50.00	12.75	25.62	11.42	45	3.92	$y=38.52x^{-0.27}$	0.978	0.0001
141	75.00	8.15	24.13	17.98	74	9.20	$y=40.75x^{-0.39}$	0.996	0.0001
149	109.09	8.60	39.70	35.44	89	12.68	$y=55.86x^{-0.46}$	0.987	0.0001
158	120.00	4.08	26.06	34.13	131	29.40	$y=38.87x^{-0.55}$	0.996	0.0001
185	85.71	5.05	17.38	19.40	112	16.96	$y=31.54x^{-0.45}$	0.995	0.0001
312	90.56	4.43	19.99	21.33	107	20.43	$y=35.66x^{-0.43}$	0.989	0.0001
Mean	97 \pm 33	6 \pm 4	24.17	24.99	110	22.25	$y=38.34x^{-0.45}$	0.992	0.0001
2008	CV=33	CV=55							
13	171.43	9.30	38.00	52.32	138	18.42	$y=51.46x^{-0.42}$	0.999	0.0001
23	89.47	5.20	6.00	6.60	110	17.21	$y=30.70x^{-0.27}$	0.994	0.0001
37	13.33	7.52	9.69	2.19	23	1.77	$y=13.80x^{-0.15}$	0.969	0.0001
51	6.25	2.49	3.54	1.06	30	2.51	$y=7.32x^{-0.26}$	0.996	0.0001
65	5.00	1.68	2.74	1.10	40	2.98	$y=5.85x^{-0.31}$	0.939	0.0001
85	5.54	3.57	4.37	0.63	14	1.55	$y=7.60x^{-0.18}$	0.999	0.0001
93	16.22	2.11	5.00	3.81	76	7.68	$y=12.46x^{-0.43}$	0.999	0.0001
118	3.75	2.86	3.18	0.20	6	1.31	$y=3.39x^{-0.02}$	0.889	0.0001
135	9.66	2.71	4.00	1.20	30	3.57	$y=5.36x^{-0.24}$	0.943	0.0001
167	10.00	2.90	4.59	1.77	39	3.45	$y=10.45x^{-0.31}$	0.993	0.0001
247	6.53	3.63	5.00	1.20	24	1.80	$y=6.12x^{-0.29}$	0.957	0.0001
279	3.33	1.49	2.05	0.48	23	2.24	$y=4.35x^{-0.26}$	0.997	0.0001
310	7.14	3.22	4.36	1.08	25	2.22	$y=7.83x^{-0.22}$	0.991	0.0001
Mean	27 \pm 49	4 \pm 2	7.12	5.66	44	5.13	$y=12.82x^{-0.26}$	0.994	0.0001
2009	CV=184	CV=62							

DOY: Day of the year; I: Initial IR value; Fi: Final IR value; \bar{x} : Mean; σ : Standard deviation; CV: Coefficient of variation (%); I/Fi: Ratio of initial and final IR values.

The power equations, correlation coefficients and significance level are also presented. Basic infiltration rate was considered the constant IR value which did not change with time. In 2009, the values of basic infiltration rate showed a decrease by 33% as in this year higher rainfall was recorded, with respect to 2008. The mean annual variability of IR values was 110% within the IR measurements of 2008 while during 2009 was 44% (Table 54). When plotting CV values against I/Fi ratio higher IR values in 2008 were indicated with respect to 2009 by the slope values of the fitting equations (Figure 87). Power law equations fitted very well the IR data per each DOY (Table 54), giving significant correlation. The theoretical mean of IR value of 2008 was calculated, resulting in $38 \pm 9 \text{ mm min}^{-1}$ (CV=24) and differing by 37% with respect to the experimental point of IR line. Similarly during 2009 the mean power equation (Table 54) showed significant correlations ($r=0.9915$, $p<0.0001$), and the theoretical IR mean value was $13 \pm 14 \text{ mm min}^{-1}$ (CV=106%) against a real mean value of 7.12 mm min^{-1} .

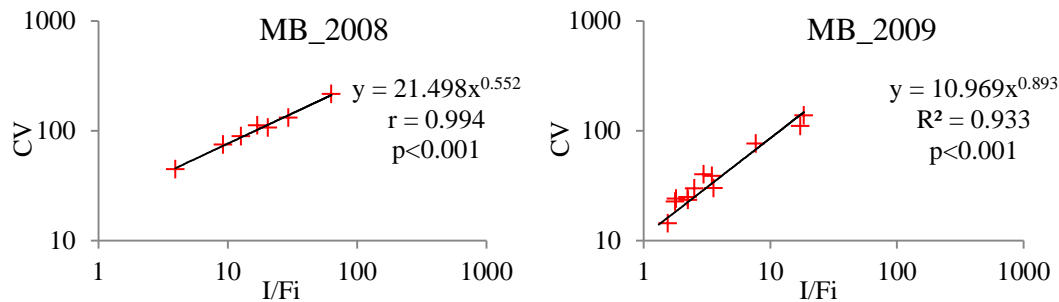


Figure 87. Relationships between the coefficient of variation (CV) and I/Fi ratio of soil infiltration rates in soils under *Erica* scrub along 2008 and 2009.

The dynamics of water rainfall, SM, IR, and k values is reported in Figure 88 during the experimental period. As in other soil environments the soil moisture resulted closely related to rainfall events. This influenced clearly the trend of soil IR and k (Figure 88). An increase by 60% in the soil moisture average during 2009 resulted in decreasing by 42% and 24% of the annual average of IR and k values respectively, with respect to 2008, recorded as drier year. The above parameters were integrated with other field and laboratory parameters to evaluate the physical dynamics of soil surface.

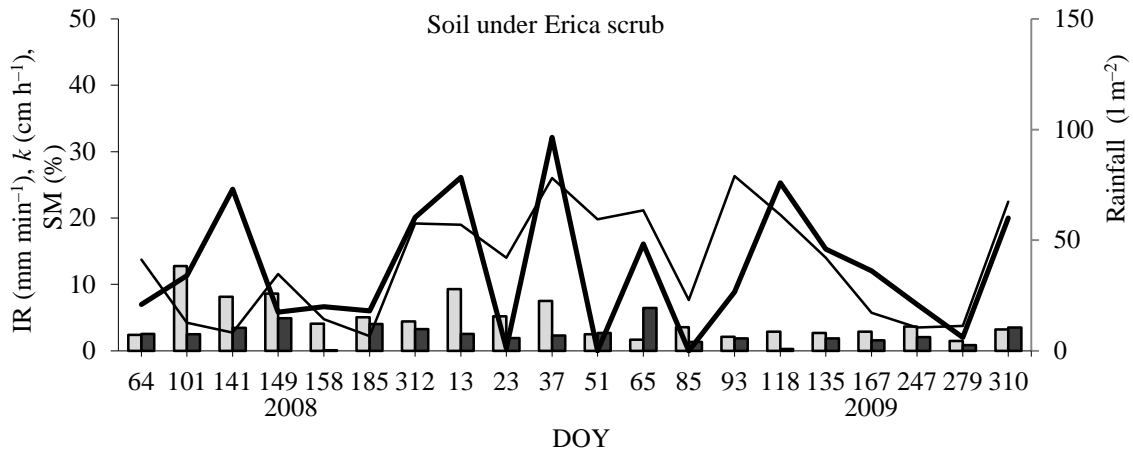


Figure 88. Values of soil infiltration rate (IR) (clear grey bar) and hydraulic conductivity (k) (dark grey bar) for soils under *Erica* scrub along the period of study. Trending of soil moisture (SM) (fine line) and rainfall (coarse line) is also presented.

A correlation matrix was tried between the data of Table 23 (SM, BD, MI, and SS) together with rainfall, runoff, infiltration rates (IR), and hydraulic conductivity (k) trying to find better explanation on their relationships (Table 55). Soil moisture content is revealed once more as the soil dynamic property contributing to beneficial soil conditions as it may attenuate compaction. Significant negative correlations were recorded between SM and BD, MI, and SS (Table 55).

Table 55. Correlation matrix between the dynamic physical soil parameters in soils under *Erica* scrub.

Soil parameters	Rainfall ($l\ m^{-2}$)	Runoff ($l\ m^{-2}$)	SM (%)	BD ($g\ cm^{-3}$)	MI (kPa)	SS (kPa)	IR ($mm\ h^{-1}$)
Runoff ($l\ m^{-2}$)	0.202 $p=0.393$						
SM (%)	0.286 $p=0.020$	-0.054 $p=0.820$					
BD ($g\ cm^{-3}$)	-0.051 $p=0.831$	-0.190 $p=0.423$	-0.634 $p=0.003$				
MI (kPa)	-0.203 $p=0.392$	-0.134 $p=0.573$	-0.663 $p=0.001$	0.508 $p=0.022$			
SS (kPa)	-0.169 $p=0.475$	-0.098 $p=0.681$	-0.518 $p=0.019$	0.409 $p=0.073$	0.751 $p=0.000$		
IR ($mm\ h^{-1}$)	0.306 $p=0.190$	-0.137 $p=0.564$	-0.197 $p=0.406$	0.372 $p=0.106$	0.066 $p=0.783$	0.104 $p=0.663$	
k ($cm\ h^{-1}$)	0.128 $p=0.591$	-0.067 $p=0.780$	0.177 $p=0.455$	-0.027 $p=0.911$	-0.176 $p=0.457$	-0.077 $p=0.749$	0.181 $p=0.446$

SM: Soil moisture; BD: Bulk density; MI: Mechanic impedance; SS: Shear strength; IR: Infiltration rate; k : Hydraulic conductivity; Bold: $p<0.01$; Italic: $p<0.05$.

3.3.2. Seasonal variability of soil infiltration rates (IR)

Soil infiltration rates may vary in time due to the influence of the antecedent soil moisture conditions. The Mediterranean environments are subjected to climatic fluctuations, which modify the soil conditions and hydrological processes, such as runoff and water regimes within the soil profile along the year (Cerdà, 1996). The study of the seasonal variability of infiltration rates can supply information about these fluctuations due to soil conditions. Soil infiltration rates were substantially higher in dry (2008) than in wet (2009) soil conditions due to the rainfall events and the time within them. Significant variability of seasonal IR values was checked with ANOVA within and between soil environments (Table 56).

Table 56. ANOVA for seasonal IR values during 2008 and 2009.

Groups	2008		2009	
	F	p	F	p
Within soil environments				
V	0.537	0.670	0.488	0.700
O	0.810	0.523	2.393	0.144
S	0.208	0.888	1.042	0.425
PI	0.212	0.885	0.162	0.717
PR	0.127	0.942	10.467	0.004
MC	1.896	0.209	0.707	0.574
MB	0.152	0.925	1.158	0.384
Between soil environments				
Winter	1.777	0.177	<i>2.977</i>	<i>0.043</i>
Spring	1.822	0.166	6.524	0.002
Summer	1.408	0.279	<i>3.543</i>	<i>0.024</i>
Autumn	<i>3.746</i>	<i>0.020</i>	<i>4.096</i>	<i>0.014</i>

V: Soils under cultivated vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub; Bold: $p < 0.01$; Italic: $p < 0.05$.

Despite the higher rainfall variability in 2008 significance in IR values was found neither within each environment nor between environments but autumn 2008, where IR values showed high significant values between soil environments ($F=3.746$, $p=0.020$). Only the soils under pasture showed significant IR variability within environments in 2009 ($F=10.467$, $p=0.004$) probably due to longer variations in surface configuration during season (grazing, drying-wetting cycles, sealing ...). High significant variability was found between soil environment's IR in each season of 2009 resulting $F=2.977$, $p=0.043$ in winter, $F=6.524$, $p=0.002$ in spring, $F=3.543$, $p=0.024$ in summer, and $F=4.096$, $p=0.014$ in autumn, indicating that soil conditions may have

been seasonally different at soil surface. Despite the lack of significant differences in IR variability in 2008 and 2009 within each environment but pasture in 2009 and between environments in 2008 except autumn, the infiltration curves showed in Figure 89 show clear differences in experimental surface water entry in 2008 and 2009. The mean seasonal values of the final infiltration rates (expressed in mm h^{-1}) are shown in Figure 89 for 2008 and 2009.

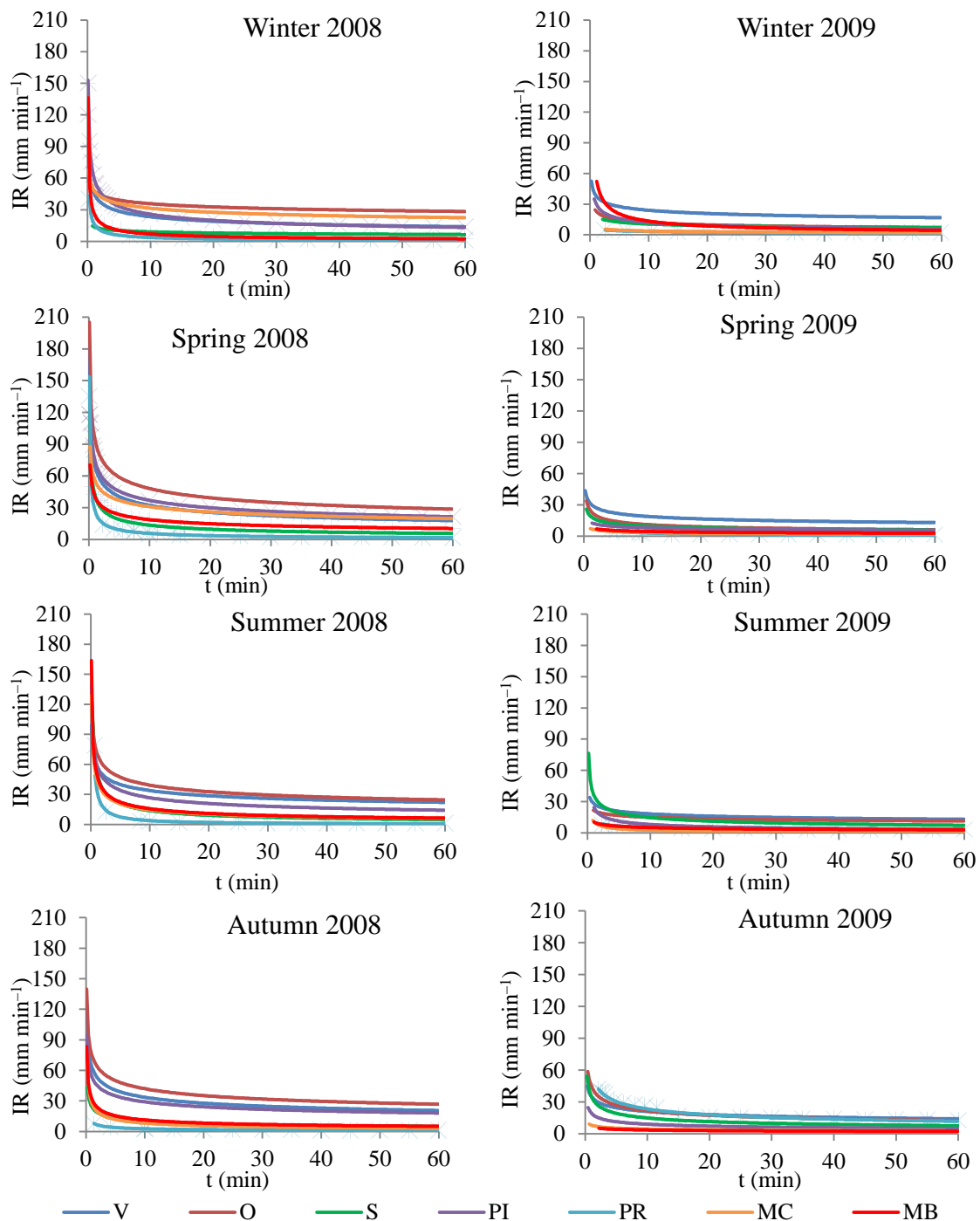


Figure 89. Seasonal trends of soil infiltration rate (IR) vs time during the two years of observations.

3.4. Statistical evaluation for the studied soil parameters

3.4.1. Correlation matrix for both data of 2008 and 2009

All field and laboratory data were subjected to statistical analysis to obtain correlation matrix for both 2008 and 2009 including all soil environments. The correlation matrix containing correlation coefficients and the significant p-level for 2008 is showed in Table 57. Highly significant positive correlations were found between the seasonal means of soil erosion and sand fraction ($r=0.591$, $p=0.001$), bulk density ($r=0.459$, $p=0.01$), soil hydraulic conductivity ($r=0.565$, $p=0.002$), and soil pH ($r=0.457$, $p=0.01$). Moreover, erosion was highly negatively correlated with clay ($r=-0.418$, $p=0.02$), silt ($r=-0.493$, $p=0.008$), mechanic impedance ($r=-0.480$, $p=0.01$), shear strength ($r=-0.406$, $p=0.03$), water holding capacity ($r=-0.513$, $p=0.005$), soil organic carbon ($r=-0.499$, $p=0.007$), and total nitrogen ($r=-0.379$, $p=0.04$). These correlations may indicate the effect of erosion on the soil environments. That is: more erosion, even though with absolute low values, may produce bulk density increase for the collapse of surface particles after overland flow; sand fraction may increase due to fine particles removal; this in turn may favor hydraulic conductivity increase, whilst soil pH may also increase due to soluble components mobilized by erosion processes. Conversely, the significant negative correlations may be in agreement with the configuration of these soil environments, with low content of clay and silt (easily exhausted with erosion), MI and SS decrease when erosion increases, as well as a decrease of WHC, SOC and TN. The mean seasonal values of eroded organic carbon (EOC) were positively correlated with dissolved organic carbon (DOC) ($r=0.814$, $p=0.000$) (Figure 90A) and eroded nitrogen (EN) with dissolved nitrogen (DN) ($r=0.393$, $p=0.039$) (Figure 90B). This proved the susceptibility of nutrients depletion due to removal of soil organic decaying debris in the upper soil profiles by runoff water. This assumption may be supported by the highly significant positive correlations between eroded nitrogen and soil organic carbon ($r=0.517$, $p=0.005$) and total nitrogen ($r=0.445$, $p<0.02$). Dissolved organic carbon was also negatively correlated with mechanic impedance ($r=-0.409$, $p=0.03$) and shear strength ($r=-0.376$, $p=0.05$).

Table 57. Correlation matrix between erosion and physical and chemical soil parameters obtained during 2008 for all soil environments.

Variables	Rainfall	Runoff	Erosion	EOC	EN	DOC	DN	Clay	Silt	Sand	BD	MI	SS	SM	WHC	IR	<i>k</i>	pH	EC	SOC
Runoff	0.614																			
Erosion	0.124	0.118																		
EOC	0.099	0.147	-0.209																	
EN	0.011	0.314	-0.256	0.141																
DOC	0.153	0.170	0.023	0.814	0.171															
DN	0.096	0.199	0.017	0.086	<i>0.393</i>	0.346														
Clay	-0.140	-0.028	<i>-0.418</i>	0.028	0.354	0.015	0.041													
Silt	-0.2737	-0.185	<i>-0.493</i>	0.136	0.185	-0.060	0.063	0.210												
Sand	0.329	0.150	0.591	-0.155	-0.354	0.005	-0.120	<i>-0.492</i>	<i>-0.922</i>											
BD	0.015	-0.053	<i>0.459</i>	-0.230	<i>-0.486</i>	-0.236	-0.304	<i>-0.642</i>	-0.266	0.562										
MI	-0.295	0.035	<i>-0.480</i>	-0.240	0.258	<i>-0.409</i>	-0.242	0.607	<i>0.406</i>	<i>-0.583</i>	<i>-0.377</i>									
SS	-0.047	0.241	<i>-0.406</i>	-0.248	0.304	<i>-0.376</i>	-0.139	0.646	0.304	<i>-0.515</i>	<i>-0.499</i>	0.887								
SM	0.103	0.333	-0.130	0.204	0.333	0.282	0.515	0.495	-0.026	-0.198	<i>-0.631</i>	0.169	0.310							
WHC	-0.018	-0.005	<i>-0.513</i>	<i>0.391</i>	<i>0.423</i>	<i>0.420</i>	<i>0.377</i>	0.685	<i>0.422</i>	<i>-0.648</i>	<i>-0.837</i>	<i>0.344</i>	<i>0.406</i>	0.619						
IR	0.127	0.038	0.183	-0.003	-0.314	-0.129	-0.248	<i>-0.5407</i>	0.217	0.127	0.730	-0.212	-0.332	<i>-0.533</i>	<i>-0.556</i>					
<i>k</i>	0.306	0.143	0.565	-0.127	0.032	-0.001	0.155	-0.2270	-0.271	0.245	0.004	-0.222	-0.140	0.041	-0.039	-0.175				
pH	0.294	0.050	<i>0.457</i>	-0.301	<i>-0.472</i>	<i>-0.374</i>	-0.115	<i>-0.620</i>	-0.174	<i>0.462</i>	0.793	<i>-0.419</i>	<i>-0.380</i>	<i>-0.384</i>	<i>-0.732</i>	0.654	0.111			
EC	-0.265	-0.258	-0.165	0.046	0.143	0.123	0.193	0.303	0.227	<i>-0.403</i>	<i>-0.501</i>	0.050	0.076	0.275	0.524	<i>-0.506</i>	0.255	<i>-0.415</i>		
SOC	-0.169	0.042	<i>-0.499</i>	0.272	0.517	0.292	0.335	0.784	0.265	<i>-0.591</i>	<i>-0.897</i>	0.508	0.572	0.620	0.856	<i>-0.711</i>	-0.097	<i>-0.828</i>	<i>0.433</i>	
TN	-0.111	0.141	<i>-0.379</i>	0.090	<i>0.445</i>	0.048	0.109	0.706	0.115	<i>-0.455</i>	<i>-0.848</i>	0.661	0.745	0.599	0.722	<i>-0.736</i>	0.064	<i>-0.742</i>	<i>0.408</i>	0.855

EOC: Eroded organic carbon; DOC: Dissolved organic carbon; EN: Eroded nitrogen; DN: Dissolved nitrogen; BD: Bulk density; MI: Mechanic impedance; SS: Shear strength; SM: Soil moisture; WHC: Water holding capacity; IR: Infiltration rate; *k*: Hydraulic conductivity; EC: Electrical conductivity; SOC: Soil organic carbon; TN: Total nitrogen. Bold: $p < 0.01$; Italic: $p < 0.05$.

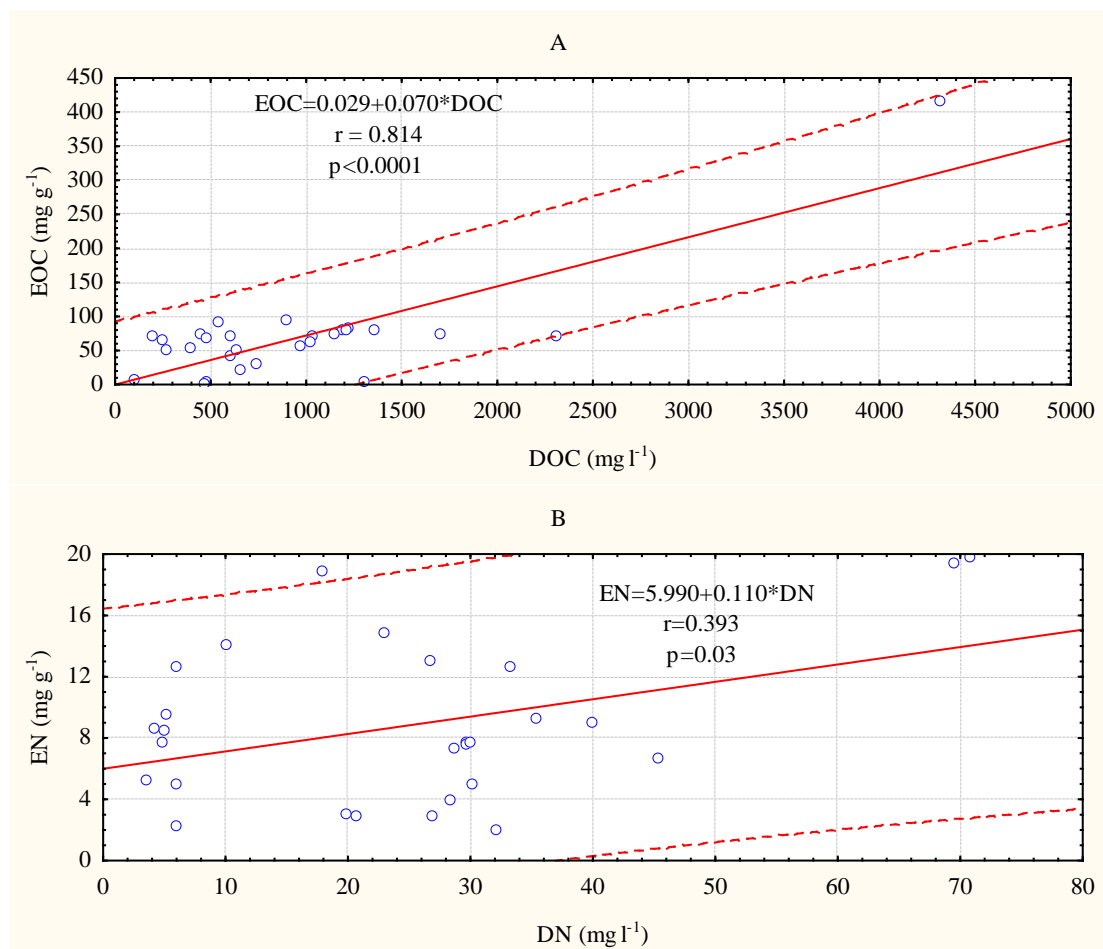


Figure 90. Relationships between the seasonal means of A): Eroded organic carbon (EOC) versus dissolved organic carbon (DOC); B): Eroded nitrogen (EN) versus dissolved nitrogen (DN) during 2008 for the studied soil environments.

The correlation matrix of Table 57 also showed significant correlations among the soil physical and chemical parameters from field and laboratory determinations. It was interesting to observe significant positive correlations between clay, MI, SS, WHC, SOC, and TN. All of them may be explained by the role that clay may exert to these parameters which may be either positive (WHC, SM, SOC, and TN) or negative (MI and SS). Despite that, clay showed also negative correlations with sand, BD, and IR, which can support the effect of clay in increasing BD, decreasing IR and contrasting the sand amount. Between the physical parameters it was outlined the negative correlation between BD and WHC ($r=0.837$, $p=0.000$), and the positive correlation between MI and SS ($r=0.887$, $p=0.000$). BD was also negatively correlated with SOC and TN. The positive correlation between MI and SS with SOC and TN may be explained by the peculiar conditions of soil surface after wetting-drying cycles which may create a hard but fragile sealed surface even in more organic soils. SOC and TN were obviously

positively correlated. Those soils with good structure and high organic carbon content are able to storage more water was showed by the positive correlations between WHC and SOC and TN. This usually allows water availability for plant and soil microorganisms favoring better steady state soil conditions able to resist soil degradation and erosion processes. Soil infiltration rates showed negative correlation with soil moisture ($r=-0.533$, $p=0.003$) and water holding capacity ($r=-0.556$, $p=0.002$) validating that seasonal dynamics of soil infiltration rates depend on water regimes in soils under different soil use and land abandonment. Highly significant negative correlation was found between soil pH and electrical conductivity ($r=-0.415$, $p=0.02$). Moreover soil pH was negatively correlated with clay ($r=-0.620$, $p<0.001$), organic matter ($r=-0.828$, $p<0.001$), and total nitrogen ($r=-0.742$, $p<0.001$).

The correlation matrix with data of 2009 is reported in Table 58. Negative correlation between rainfall and EOC ($r=-0.399$, $p=0.035$) were found indicating that the organic compounds contained in the eroded soils tend to decrease with increasing rainfall amounts. Effectively, erosion was found negatively correlated with EOC and EN indicating that higher erosion rates may rapidly decrease the amount of removable nutrients in eroded particles. As for 2008 erosion, clay, silt, MI, SS, SM, WHC, and SOC were negatively correlated whilst erosion was positively correlated with sand, BD, IR, and k . EOC, EN, DOC, and DN showed positive correlation among them. EOC and EN were further negatively correlated with sand and BD. Yet, DOC and DN showed positive correlation with pH and EC in runoff water indicating that soluble organic components may mutually interact with soil reaction and salts concentration in soil.

Table 58. Correlation matrix between erosion and physical and chemical soil parameters obtained during 2009 for all soil environments.

Variables	Rainfall	Runoff	Erosion	EOC	EN	DOC	DN	Clay	Silt	Sand	BD	MI	SS	SM	WHC	IR	<i>k</i>	pH	EC	SOC
Runoff	-0.026																			
Erosion	0.164	0.146																		
EOC	<i>-0.399</i>	-0.082	-0.476																	
EN	-0.122	-0.151	-0.545	0.599																
DOC	-0.353	-0.164	-0.037	<i>0.436</i>	0.265															
DN	-0.185	-0.184	-0.185	<i>0.375</i>	0.707	<i>0.386</i>														
Clay	-0.289	0.140	-0.651	0.251	0.475	0.033	0.152													
Silt	-0.187	-0.213	-0.798	<i>0.411</i>	0.509	0.065	0.253	0.310												
Sand	0.275	0.096	0.903	<i>-0.427</i>	-0.604	-0.064	-0.261	-0.696	-0.899											
BD	0.193	0.124	0.741	<i>-0.421</i>	-0.561	0.007	-0.189	-0.774	-0.607	0.816										
MI	-0.343	0.314	-0.587	0.157	0.218	0.008	-0.007	0.609	0.490	-0.651	-0.363									
SS	-0.334	0.094	-0.660	0.093	0.312	-0.062	0.011	0.712	0.577	-0.765	-0.538	0.903								
SM	0.171	-0.169	<i>-0.419</i>	0.041	<i>0.467</i>	-0.291	0.087	0.571	0.362	-0.537	-0.670	0.201	<i>0.459</i>							
WHC	-0.131	-0.055	-0.708	0.234	0.488	-0.228	0.141	0.800	0.559	-0.792	-0.910	<i>0.428</i>	0.625	0.798						
IR	0.087	-0.059	0.765	-0.322	<i>-0.449</i>	0.155	-0.052	-0.634	<i>-0.638</i>	0.774	0.696	-0.620	-0.707	-0.524	-0.744					
<i>k</i>	-0.058	0.015	0.587	-0.213	-0.206	-0.042	0.269	-0.553	-0.347	0.518	0.527	-0.491	-0.525	-0.270	<i>-0.407</i>	0.586				
pH	<i>0.380</i>	-0.052	0.018	-0.053	-0.078	-0.141	0.080	<i>-0.450</i>	0.145	0.099	0.221	-0.139	-0.162	-0.084	-0.174	-0.216	0.108			
EC	<i>-0.440</i>	0.239	-0.119	-0.013	0.160	0.024	0.152	0.594	-0.031	-0.251	-0.263	0.502	<i>0.445</i>	0.226	0.366	-0.173	-0.137	-0.549		
SOC	0.011	0.061	-0.506	0.221	<i>0.416</i>	-0.259	0.016	0.629	<i>0.445</i>	-0.626	-0.757	0.281	<i>0.444</i>	0.865	0.840	-0.632	-0.281	-0.149	0.284	
TN	-0.253	0.213	-0.578	0.165	<i>0.379</i>	-0.006	0.089	0.885	<i>0.428</i>	-0.732	-0.763	0.619	0.718	0.641	0.823	-0.599	<i>-0.460</i>	<i>-0.419</i>	0.599	0.748

EOC: Eroded organic carbon; DOC: Dissolved organic carbon; EN: Eroded nitrogen; DN: Dissolved nitrogen; BD: Bulk density; MI: Mechanic impedance; SS: Shear strength; SM: Soil moisture; WHC: Water holding capacity; IR: Infiltration rate; *k*: Hydraulic conductivity; EC: Electrical conductivity; SOC: Soil organic carbon; TN: Total nitrogen. Bold: $p < 0.01$; Italic: $p < 0.05$.

Similarly, the regression equations between the seasonal means of eroded organic carbon (EOC) versus dissolved organic carbon (DOC) and eroded nitrogen (EN) versus dissolved nitrogen (DN) may be seen in Figure 91A and B. Highly significant positive correlations were found between eroded nitrogen and soil moisture ($r=0.467$, $p=0.012$) and water holding capacity ($r=0.488$, $p=0.008$). As for 2008 clay was negatively correlated with sand, BD, IR, and k whereas positive correlations were found between clay and MI, SS, SM, WHC, SOC and TN.

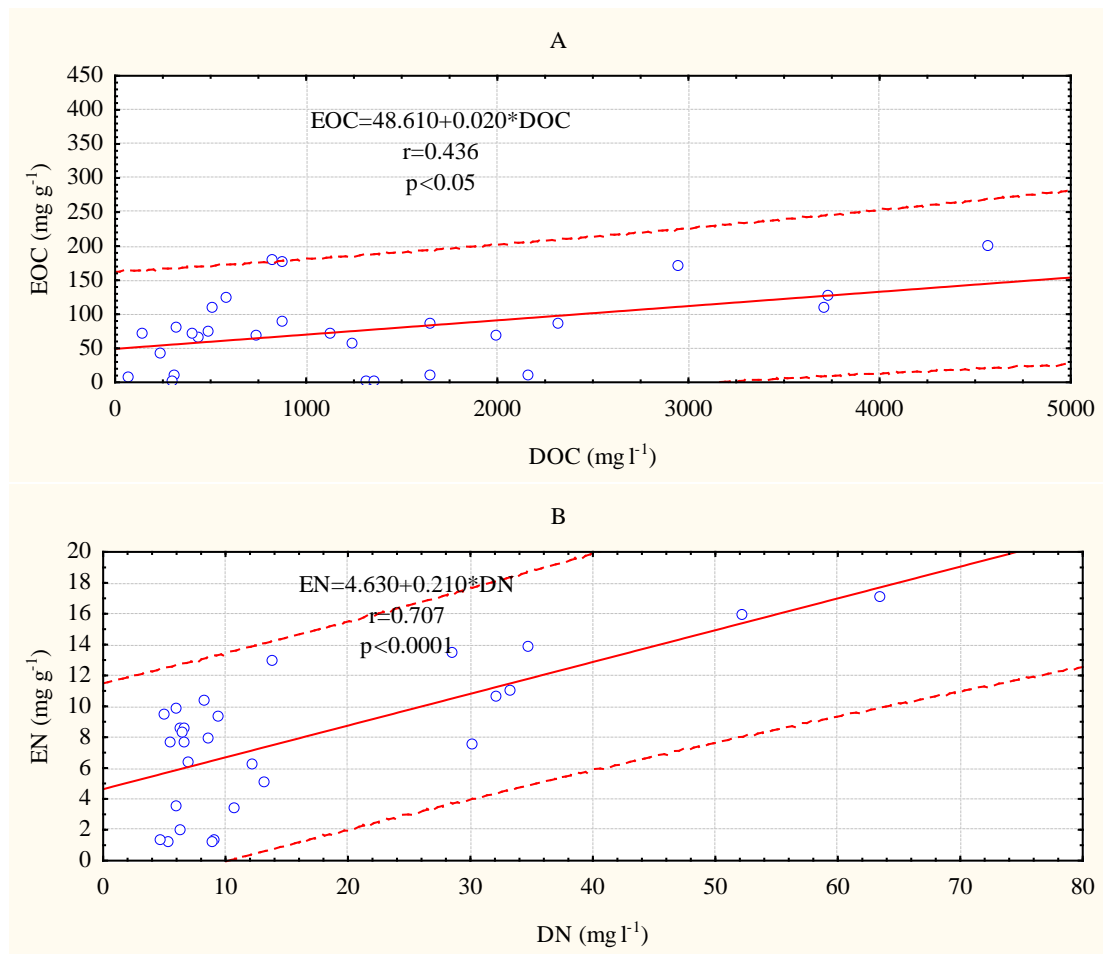


Figure 91. Relationships between the seasonal means of A): Eroded organic carbon (EOC) versus dissolved organic carbon (DOC); B) Eroded nitrogen (EN) versus dissolved nitrogen (DN) during 2009 for the studied soil environments.

The lack of correlation between runoff water with any of the analyzed parameters in 2008 and 2009 suggested that this erosion component did not have a relevant role in the overall erosion dynamics and its relevance was attenuated by the high sandy texture of the majority of investigated soils. Positive correlation was found between MI and SS ($r=0.903$, $p=0.000$) indicating that in all soils there is a close

relationship between vertical and horizontal surface strength. IR was also positively correlated with k suggesting that the water entry in all soils has continuity along the profile through permeability. SOC and TN were both negatively correlated with sand, BD, and pH. The negative correlation of both SOC and TN with IR may be explained, as for 2008, by the shallow nature of these soils that may often show crusting and sealing mechanisms in drier periods independently of the organic matter content. SOC and TN were positively correlated ($r=0.748$, $p=0.000$).

3.4.2. Principal component and factor analysis for 2008 and 2009 data

3.4.2.1. *Factor analysis and communality*

The factor analysis was carried out by the statistical program STATISTICA 7.1 of StatSoft Inc. (2005) by using the analyzed physical and chemical soil parameters as well as soil erosion parameters of all the environments simultaneously. These types of factor structures were tried in order to find statistical evidence of the natural dynamics occurred in the area of study during the two observed years separately. The factor analysis was carried out to determine whether any correlation among the measured variables existed using the minimum possible number of factors to describe them (Norušis, 1993). The first three factors explained the most significant part of the variance within the variables. Factors were explained as indices of overall soil dynamics. A conceptual name was given to each factor as to identify the relevance of the variables included (Paniagua et al., 1999; Emran, 2011). The factor analysis was used to rank the soil attributes and calculate the related communality values. The sum of the squared factor loadings for all factors for a given variable (row) is the variance in that variable accounted for by all the factors, and this is called the communality. The communality measures the percent of variance in a given variable explained by all the factors jointly and may be interpreted as the reliability of the indicator (Paniagua et al., 1999; Emran, 2011). The loadings of varimax rotated factor analysis and percentages of the explained absolute and cumulative variance by the three factors of 2008 data are reported in Table 59.

The first three factors explained the most significant variance (66.67%) within the variables. Factor 1 explained 35.14% of the total variance and was named the

“organic reserve and water storage”. High positive loadings were found for TN (+0.94), SOC (+0.92), WHC (+0.81), clay (+0.78), SM, (+0.73), SS (+0.71), MI (+0.58), and EN (+0.57) whereas negative loadings were found for bulk density (BD) (−0.89) and soil infiltration rate (IR) (−0.76). The positive loadings outline the presence of environments that contribute to organic reserve, even in peculiar condition of soil depth. Factor 2 explained 16.97% of the total variance and showed high positive loadings for sand (+0.75), soil erosion (+0.66), hydraulic conductivity (+0.63), and rainfall (+0.55) and negative loadings for silt (−0.81). This factor was named “erosion and hydraulics” and may be related to the erosion dynamics and soil permeability. The third factor named “nutrient depletion” explained 14.56% of the total variance with positive loadings for MI (+0.58) and negative loadings for DOC (−0.90), EOC (−0.81), and DN (−0.50).

Table 59. Variable’s loadings in the factor analysis (FA) using the studied soil parameters obtained during 2008 for the selected soil environments. Values below 0.50 omitted.

Variable	Organic reserve and water storage	Erosion and hydraulics	Nutrient depletion	Communality
Rainfall		0.55		0.34
Runoff				0.31
Erosion		0.66		0.60
EOC			−0.81	0.70
EN	0.57			0.36
DOC			−0.90	0.84
DN			−0.50	0.40
Clay	0.78			0.69
Silt		−0.81		0.69
Sand		0.76		0.82
BD	−0.89			0.85
MI	0.58		0.58	0.85
SS	0.71			0.84
SM	0.73			0.66
WHC	0.81			0.88
IR	−0.76			0.62
<i>k</i>		0.63		0.40
SOC	0.92			0.93
TN	0.94			0.90
Explained variance				
Total (%)	35.14	16.97	14.56	66.67
Cumulative (%)	35.14	52.11	66.67	66.67

EOC: Eroded organic carbon; EN: Eroded nitrogen; DOC: Dissolved organic carbon; DN: Dissolved nitrogen; BD: Bulk density; MI: Mechanic impedance; SS: Shear strength; SM: Soil moisture; WHC: Water holding capacity; IR: Infiltration rate; *k*: Hydraulic conductivity; SOC: Soil organic carbon; TN: Total nitrogen.

The communality for a given variable can be interpreted as the proportion of variation in that variable explained by the three factors jointly. Communalities are computed by taking the sum of the squared loadings for that variable (Table 59). The soil organic carbon is the most important variable in this factor structure of the data obtained during 2008 as it shows a communality of 0.9248 obtained from $(0.9200)^2 + (-0.2176)^2 + (-0.1760)^2$. The sum of all the communalities values divided by the number of variables gives the proportion of total variance explained by the three factors ($14.67/19=0.6667=66.67\%$).

The loadings of varimax rotated factor analysis and percentages of the explained absolute and cumulative variance by the three factors of 2009 are reported in Table 60.

Table 60. Variable's loadings in the factor analysis using the studied soil parameters obtained during 2009 for the selected soil environments. Values below 0.50 emitted.

Variable	Organic reserve and water storage	Nutrient depletion	Soil compaction	Communality
Rainfall		-0.50		0.50
Runoff				0.34
Erosion	-0.74			0.77
EOC		0.75		0.62
EN		0.66		0.77
DOC		0.73		0.62
DN		0.74		0.64
Clay	0.75			0.74
Silt	0.61			0.60
Sand	-0.81			0.91
BD	-0.88			0.84
MI			0.82	0.85
SS	0.63		0.65	0.83
SM	0.88			0.86
WHC	0.95			0.91
IR	-0.78			0.73
<i>k</i>				0.45
SOC	0.89			0.81
TN	0.79			0.77
Explained variance				
Total (%)	41.52	15.96	13.90	71.38
Cumulative (%)	41.52	57.48	71.38	71.38

EOC: Eroded organic carbon; EN: Eroded nitrogen; DOC: Dissolved organic carbon; DN: Dissolved nitrogen; BD: Bulk density; MI: Mechanic impedance; SS: Shear strength; SM: Soil moisture; WHC: Water holding capacity; IR: Infiltration rate; *k*: Hydraulic conductivity; SOC: Soil organic carbon; TN: Total nitrogen.

The three factor structure explained 71.38% of the total variance into the analyzed variables during 2009. Factor 1 named “organic reserve and water storage” explained the 41.52% of the total variance with positive loadings for WHC (+0.95), SOC (+0.89), SM (+0.88), TN (+0.88), clay content (+0.75), SS (+0.63), and silt content (+0.61) and negative loadings for BD (−0.88), sand content (−0.81), infiltration rate (−0.78), and erosion (−0.73). The second factor named “nutrient depletion” explained 15.96% of total variances with positive loadings for EOC (+0.75), dissolved nitrogen (+0.74) and dissolved organic carbon (+0.73), and eroded nitrogen (+0.66), and negative loadings (−0.50) related with the effect of rainfall amount on soil surface. The third factor named “soil compaction” explained the 13.90% of the total variance with positive loadings for mechanic impedance (+0.82) and shear strength (+0.65).

The proportion of the total variance explained by the three factors is $13.56/19 \text{ variables} = 0.7138$; this means that a 71.38% of variance explained through these factors as total communalities. Soil organic carbon lowered its weight in the factor 1 during 2009. A lower communality of 0.81 with respect to 0.93 of 2008 may be probably attributed to different overall soil conditions in the area caused by 130% increase of rainfall during 2009. This assumption was also supported by higher loadings in WHC and new loads for sand and erosion. In fact, the first factor in 2009 increased 18% of the total variance with respect to the first factor in 2008 probably due to the negative loadings of sand and soil erosion.

3.4.2.2. *Factor scores*

Factor analysis provided information about the relationships between the variables correlated with a factor on seasonal basis and each soil environment to obtain factor scores set to deepen into the overall dynamics of the area of study. Factor scores are theoretically the scores of each group of variables on each factor, corresponding to environments and seasons. To compute the factor score one takes the standardized score on each variable, multiplies by the corresponding factor loading of the variable for the given factor, and sums these products. Positive or negative charges (scores) in each factor are the contribution of the variable loadings in the factor, associating the variables loadings in that factor to each environment and season, thus indicating those environments and seasons that better contribute to soil dynamics. The values of factor

scores for the first three factor structure obtained during 2008 are presented in Figure 92.

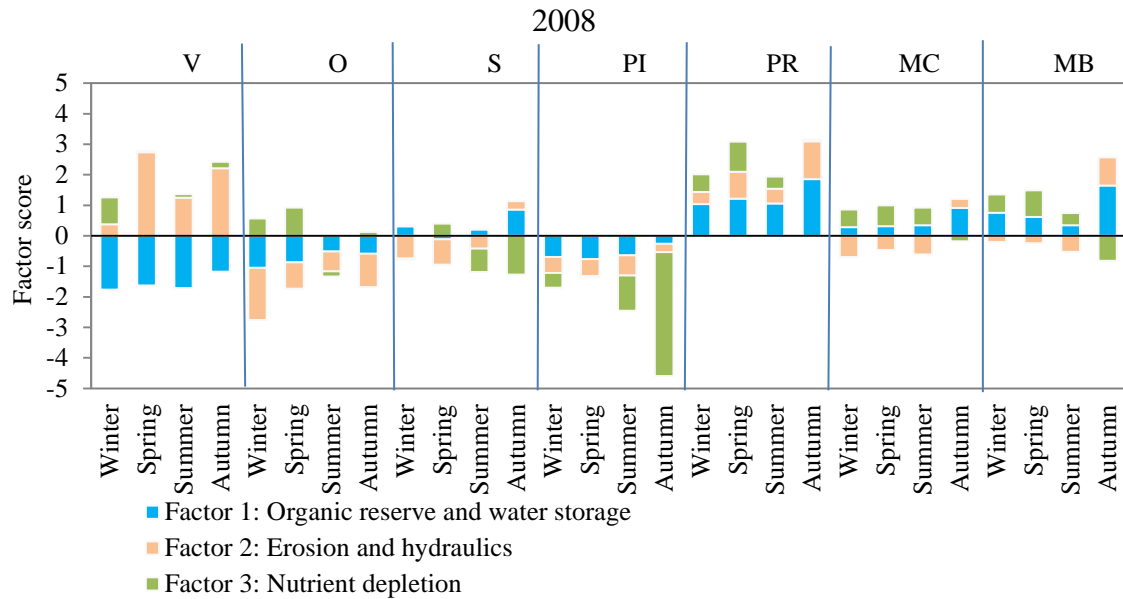


Figure 92. Factor score values of the first three factors structure obtained during 2008.

During 2008, factor score refers to soil under cultivated vines during the four seasons as the environment with the highest contribution to soil infiltration capacity supported by the high negative loadings in BD, IR, sand, and silt contents respectively in the first factor. It is also pointed out that soil under PR, MB, MC, and S showed the highest contribution to organic reserve and water storage by the positive loadings in the first factor. Nevertheless, the soils under pasture showed also a relatively high sensitivity to erosion along seasons (Figure 92). A stronger resistance to erosion was recorded in O, S, PI, MC, and MB soil environments because of the same higher positive loadings in clay, MI, SS, WHC, SOC, and TN variables. The second factor score refers to the soil under cultivated vines as the highest contributor to soil erosion with highest positive loadings in sand content, soil erosion, and soil hydraulic conductivity. The third factor score related to nutrient depletion explained the environments and seasons as major contributors of nutrients removal. The highest nutrient removal was recorded in autumn in PI followed by PR, MB and MC (winter, spring and autumn). Soils under olive groves (O), vines (V), and corks (S) showed comparatively less ability to nutrients loss. It was noticed that soil nutrients depletion seemed to be more consistent in rainy seasons (2009) than in dry seasons mainly depending on the soil condition and organic matter content.

The factor score values related to the factor structure obtained with data of 2009 can be seen in Figure 93.

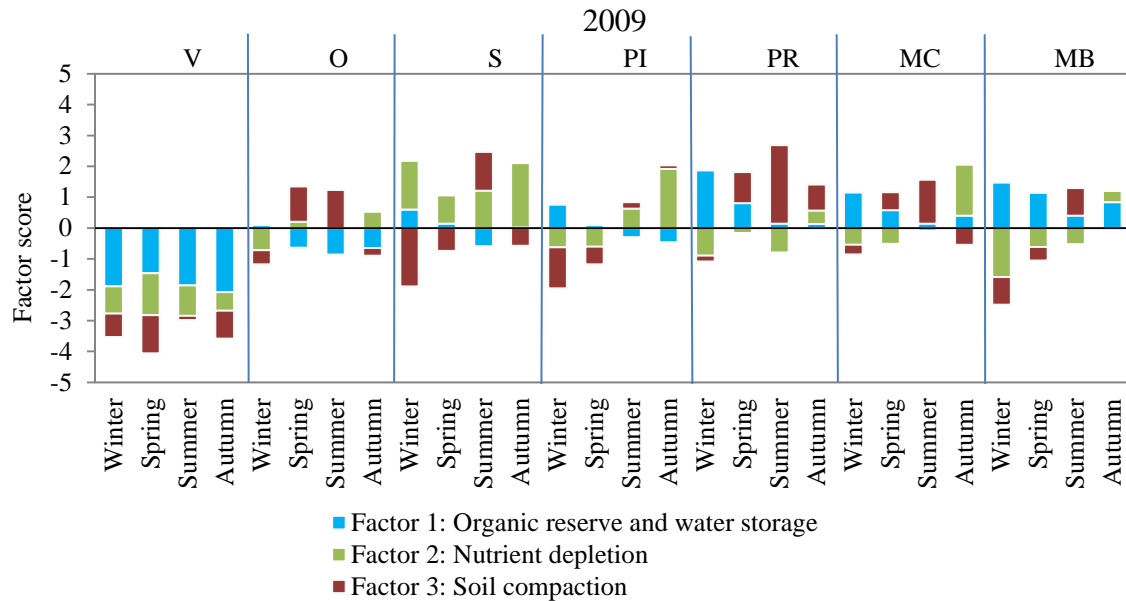


Figure 93. Factor score values of the first three factors structure obtained during 2009.

The factor scores indicating the degree of association of each environment and season to each factor and related variables underline that soil under cultivated vines showed a different trend during 2009 (Figure 93). As may be observed, this environment contributed negatively to the three factors. The first factor was associated to texture, water dynamics, organic matter and nitrogen that may be better reflected in the PR, MB, MC, PI and S environments. Likewise, these environments have some role in nutrients depletion, especially soils under cork trees due to a recent fire. High positive scores were showed in the second factor “nutrient depletion” in soils under stands of cork and pine trees. Regarding factor 3 “soil compaction”, the soils under pasture (PR) seems to be the main contributor independently of its high organic carbon content. This soil is often wetted-dried forming a hardened surface. High positive contribution to the third factor was showed by MC, O, S, MB and PI soil environments.

3.4.2.3. Principal component analysis (PCA) plot

The PCA plot was generated to combine the outcome of two orthogonal diagrams of variables and cases for 2008 and 2009 (Figure 94). The position of both variables and soil environments along seasons and their related contribution to the overall dynamics of soil properties in the area of study may be observed for the two

years. In the diagram related to 2008 data, it is worth to outline the gradient formed by the soil environments along the factor one. From the extreme left (indicating less contribution of soil environments to the overall soil dynamics) to extreme right indicating a greater contribution. As elsewhere indicated statistics confirmed the sequence $V < O < PI < S < MC < MB < PR$. However, it is also informative to outline the second distribution of each soil environment in the orthogonal diagram. The soils under pasture during the four seasons of 2008 indicated that it may regularly favor the formation of suited physical and chemical parameters though may be depleted from nutrients (especially EN) because of fresh manure addition by grazing activity. Effectively in autumn animal arrive from the high mountains (Pyrenees) to this area where some pasture is still possible for suited climate. It may be observed that PR (autumn) is very close to EN to indicate loss of nitrogen in eroded sediments. Soil under pasture is positioned together with the most relevant soil properties and practically contributes to them all over the year. Similarly soils under S, MC and MB are grouped together as relevant contributors to soil properties along seasons. Looking at factor 3 (Table 59), it may be stated that soil under PI (autumn) is the major contributor of depleted nutrients, already observed in Figure 92. The negative part of factor 1 (low contribution to soil properties) outlined soils under cultivated vines, olive groves, and stands of pine trees, being vines more strictly related to soil erosion, high bulk density and soil infiltration rates.

The PCA plot diagram for data of 2009 (Figure 94) depicts a different picture. The trends of soil characteristics and nutrient depletion changed during this year with respect to the data obtained in 2008 due to the higher rainfall amounts received during 2009. This may have caused the changes observed in seasonal distribution of V, O, and P soil environments in the diagram. However, the highest contribution to soil physical and chemical properties is still given by soils under pasture, *Cistus* and *Erica* scrub though these soils moved to the center of the diagram, losing some relevance. Once more the soils under vines show a very particular dynamics along the season with high susceptibility to erosion. The parameters related to nutrient depletion are opposed to rainfall in factor 2 indicating that they may be controlled by rainfall-runoff-erosion dynamics. That is: the soil surface availability of solid or soluble decaying debris. Rainfall events may be able to differentiate the soil response along the sequence of study especially according to the age of abandonment. Low rainy year may preserve the

2008

PC 2: 16.81%

PC 1: 37.87%

2009

PC 2: 13.55%

PC 1: 47.71%

251

3.5. Carbon loss as soil respiration

The change in the previous mosaic of land exploitation, the erosion processes and the periodical occurrence of wildfire have changed the soil properties as well (Pardini et al, 2003; Pardini et al., 2004b; Dunjó et al., 2004) arising questions on how these changes may have affected the organic reserve and the C flux from the soil. Furthermore, it is well known that the consumption of organic matter in soil by heterotrophic microorganisms and the respiration of plant roots are known to produce carbon dioxide, which is finally released to the atmosphere (Jensen et al., 1996). Thus mineralization and humification processes may affect atmospheric CO₂ concentration as much as mineralization exceeds humification. The humification process is based on the relative susceptibility of the type of fresh organic field wastes and decaying debris to biological decomposition, mainly depending on C/N ratio, soil moisture and temperature. This process implies also consequent biochemical transformations of labile (carbohydrates) and more stable (lignin and phenolic compounds) organic fractions into humus. In case of carbohydrates as starting point the carbon percentage that makes it to humus is less than 20%, so that mineralizable of labile organic compounds are most easily lost as CO₂ through mineralization (Zech et al., 1997). If the starting point is lignin, tannins, or other phenolic groups (mostly found in wood and leaves) the humus percentage may reach 75%. In this case, low-mineralizable humic substances may increase in the soil and be preserved in the form of stable organic compounds for decades or even centuries if the soil is accurately managed (Emran et al., 2012a).

Soil structure and porosity as well as bulk density are tightly related with mineralization and humification processes. When intensive soil tillage or mismanagement cause low structure and poorly distributed porosity, metabolization of labile or even stable organic fractions into CO₂ is likely to increase, favoring mineralization, decline in structural stability and compaction. Conversely, a stable structure with positively sorted porosity tends to maintain better soil conditions through stable organic compounds persistence and structural stability improvement. To minimize the carbon dioxide emission by mineralization of organic matter fractions, any agricultural practice or spontaneous plant succession preserving humic compounds in the soil system, may favor this system to act as an organic carbon sink, playing an

important role in balancing carbon dioxide concentrations in the global carbon cycle (Emran et al., 2012a).

There are contrasting opinions on how land use and cover change and land abandonment may influence soil organic carbon (SOC) content and consumption or its redistribution in time and space, entering in a new dynamics with biological activity, porosity, soil temperature and water regimes (Bajracharya et al., 2000; Levy et al., 2004). As elsewhere mentioned eroded soils contain relevant amounts of SOC which may be redistributed and preserved in depositional areas (Bajracharya et al., 1998). Also, farmland abandonment may lead to natural vegetation succession increasing plant species colonization and the recover of suitable organic horizons. Because of the varied effects on SOC and microbial activity, land use and cover change and land abandonment have important influence upon soil respiration, carbon (C) flux to the atmosphere and C sequestration in soil. The effectiveness of soil microbial activity on the organic substrate depends primarily on moisture, temperature, infiltration capacity, clay content, and soil aeration. Moreover, soil respiration rates may be very sensitive to seasonal changes in soil temperature and water content because of their influence in microbial activity and root growth (Davidson et al., 1998; Emran et al., 2012a).

The potential for soil respiration is indicating the soil biological activity especially in the upper soil layer that is often influenced by land use change and abandonment. Measurements of carbon dioxide fluxed from the soil are therefore important in order to add information on the carbon dynamics. Nevertheless, there is a considerable uncertainty in the CO₂ measurements from soil surface, mainly due to differences in the methods used (Raich and Schlesinger, 1992).

3.5.1. Measurements of soil respiration

Two different methods for determining CO₂ concentration were performed on monthly basis in the field in each environment from winter 2009 to autumn 2009: the soda lime method at surface (SLF) and the IRGA method at surface and 5 cm depth. Three replicates of each measurement were performed. The CO₂ measurements were also performed in the laboratory by using the soda lime method (SLJ) at the same time interval of field measurements. Then four different data sets were obtained on seasonal

basis to evaluate the CO₂ respired from soil, two from the soda lime method (field and laboratory) and two with the IRGA method (surface and 5 cm depth). Data of corresponding soil moisture and temperature were also recorded (Table 61).

3.5.1.1. *Soda lime methods as a static method*

The static (soda lime) method used in the field and laboratory was tried to check the sensitivity of the measurements under limited and natural conditions. From Table 61 the seasonal effect on CO₂ emission from soil environments recorded with SLF method is shown. Testing data variability with one-way ANOVA, no significance was found within each environment along seasons ($F=0.126$, $p=0.991$) whilst data were highly significant ($F=27.73$, $p=0.000$) between environments each season. The same trend was observed in the SLJ data variability with no significance within environments ($F=0.837$, $p=0.561$) and with high significance between environments ($F=4.619$, $p=0.037$). By calculating the annual mean of CO₂ measurements by soda lime method in the field (SLF) during the time of observation (from winter to autumn 2009) results showed CO₂ concentrations of 5.19, 5.20, 5.47, 5.57, 5.74, 5.80, and 6.72 $\mu\text{mole CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ corresponding to the environments PI, V, MB, O, S, MC, and PR respectively. Generally the highest production of carbon dioxide was recorded in soils with higher amount of SOC, though the C-CO₂ concentration is proportionally low indicating a higher suitability to preserve soil organic carbon. This can be seen clearly in soils under pasture with the highest SOC content (36.3 mg g^{-1}) between the studied soils and a C-CO₂ loss representing less than 1%.

The highest CO₂ emission recorded by soda lime in the field is found in summer season with respect to the other seasons for all soil environments (Table 61). An inverse trend was found with the soda lime method in the laboratory, which showed the lowest value of CO₂ in summer, may be related to the incubation of a finite amount of dry soil; soil microorganisms may have been more sensitive to moisture loss in these conditions. The highest values of CO₂ in laboratory were recorded in winter which may be associated to the maintenance of 5%, 11%, 15%, 17%, 30%, 19%, 20% of moisture content for V, O, S, PI, PR, MC, MB respectively at constant laboratory temperature (25 °C), positively affecting soil microbial activity at each particular soil.

Table 61. Mean seasonal values (\pm standard error) of CO₂ concentrations by soda lime method at field (SLF) and laboratory (SLJ), infrared gas analyzer (IRGA) method at soil surface and at 5 cm depth, soil temperature (ST), and soil moisture (SM) for the selected soil environments.

Season	ENV	SLJ (mg g ⁻¹ day ⁻¹)	SLF (μ mole m ⁻² s ⁻¹)	IRGA surface (μ mole m ⁻² s ⁻¹)	IRGA 5 cm (μ mole m ⁻² s ⁻¹)	ST (°C)	SM (%)
Winter	V	0.41 \pm 0.16	3.05 \pm 0.58	2.44 \pm 0.53	3.04 \pm 0.63	11.58 \pm 1.38	5.23 \pm 1.32
	O	0.60 \pm 0.24	3.37 \pm 0.78	3.61 \pm 0.66	4.40 \pm 1.10	20.30 \pm 1.11	11.10 \pm 2.41
	S	0.85 \pm 0.32	4.09 \pm 0.36	2.87 \pm 0.63	4.24 \pm 0.75	16.50 \pm 1.04	15.45 \pm 4.24
	PI	0.85 \pm 0.46	3.68 \pm 0.59	4.05 \pm 0.94	5.37 \pm 0.75	14.20 \pm 1.09	16.89 \pm 3.24
	PR	1.23 \pm 0.55	5.32 \pm 1.34	2.65 \pm 0.72	5.03 \pm 0.85	9.00 \pm 0.97	29.88 \pm 9.96
	MC	1.17 \pm 0.45	3.19 \pm 1.12	2.67 \pm 0.55	4.54 \pm 0.66	8.50 \pm 0.88	18.89 \pm 2.09
	MB	0.82 \pm 0.36	2.98 \pm 0.37	2.85 \pm 0.55	4.57 \pm 0.78	9.06 \pm 0.97	20.00 \pm 4.69
Spring	V	0.54 \pm 0.18	5.75 \pm 0.50	3.84 \pm 0.98	5.83 \pm 1.03	13.67 \pm 0.75	3.43 \pm 0.30
	O	0.63 \pm 0.24	7.01 \pm 1.45	6.01 \pm 0.95	7.26 \pm 0.94	18.50 \pm 0.97	7.38 \pm 1.18
	S	0.95 \pm 0.14	6.76 \pm 0.64	5.08 \pm 0.78	6.77 \pm 0.74	15.50 \pm 0.93	10.61 \pm 1.40
	PI	0.67 \pm 0.29	5.11 \pm 0.44	4.17 \pm 0.62	5.46 \pm 0.78	14.00 \pm 0.79	8.09 \pm 2.45
	PR	0.95 \pm 0.36	9.04 \pm 1.19	2.54 \pm 0.39	5.02 \pm 0.48	10.67 \pm 0.53	14.89 \pm 1.65
	MC	1.06 \pm 0.32	7.10 \pm 0.85	4.00 \pm 0.61	6.22 \pm 0.70	11.00 \pm 1.13	14.35 \pm 1.02
	MB	1.31 \pm 0.39	6.65 \pm 0.54	4.40 \pm 0.52	5.82 \pm 1.55	11.83 \pm 0.49	18.15 \pm 5.31
Summer	V	0.21 \pm 0.17	8.99 \pm 0.48	5.04 \pm 0.89	7.37 \pm 0.55	30.75 \pm 1.28	0.91 \pm 0.14
	O	0.34 \pm 0.06	8.39 \pm 1.06	5.74 \pm 1.07	8.84 \pm 0.90	34.75 \pm 0.93	1.57 \pm 0.10
	S	0.27 \pm 0.24	8.26 \pm 0.68	5.78 \pm 0.98	6.36 \pm 1.12	31.00 \pm 1.01	3.38 \pm 0.71
	PI	0.29 \pm 0.22	8.09 \pm 0.38	5.06 \pm 0.53	6.01 \pm 0.87	29.75 \pm 2.68	2.71 \pm 0.88
	PR	0.36 \pm 0.30	8.24 \pm 1.01	2.28 \pm 0.56	3.60 \pm 0.77	30.75 \pm 0.53	2.54 \pm 0.48
	MC	0.36 \pm 0.35	9.47 \pm 0.89	5.07 \pm 0.89	5.58 \pm 1.08	29.50 \pm 1.44	3.78 \pm 0.49
	MB	0.39 \pm 0.23	8.89 \pm 0.84	4.99 \pm 0.89	7.06 \pm 0.68	28.25 \pm 1.53	4.60 \pm 0.75
Autumn	V	0.68 \pm 0.11	3.02 \pm 0.27	3.37 \pm 0.60	6.52 \pm 1.27	39.00 \pm 1.79	2.54 \pm 0.72
	O	0.67 \pm 0.19	3.50 \pm 0.79	5.53 \pm 0.82	8.93 \pm 1.55	35.50 \pm 1.91	6.81 \pm 0.94
	S	0.81 \pm 0.29	3.87 \pm 0.25	3.89 \pm 0.88	5.23 \pm 1.01	26.00 \pm 1.92	8.72 \pm 1.01
	PI	0.76 \pm 0.17	3.88 \pm 0.67	4.13 \pm 1.31	5.23 \pm 1.23	23.50 \pm 1.53	7.13 \pm 1.24
	PR	0.70 \pm 0.30	4.28 \pm 0.70	1.79 \pm 0.39	3.33 \pm 0.71	15.75 \pm 0.64	11.01 \pm 2.81
	MC	0.85 \pm 0.27	3.45 \pm 0.42	3.12 \pm 0.46	5.49 \pm 0.99	17.50 \pm 1.01	12.31 \pm 4.79
	MB	1.00 \pm 0.39	3.36 \pm 0.27	2.32 \pm 0.56	5.14 \pm 1.07	16.75 \pm 0.67	13.11 \pm 5.11

ENV: Soil environments; V: Soils under vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub.

3.5.1.2. IRGA method as a dynamic method

The infrared gas analyzer (IRGA) was used as a dynamic method in the field in order to determine faster measurements of CO₂ concentrations emitted from the soil surface and at 5 cm depth, and to compare the results with the static soda lime methods. As may be observed in Table 61, the values of CO₂ measured by IRGA are generally lower than those obtained by the soda lime when measurements at soil surface are

carried out. It must be pointed out that the IRGA detects the concentration of CO₂ (ppm) until an equilibrium value is obtained which may be considered constant. Generally this value is stable after 5 minutes of continuous air pumping from the soil to the CO₂ detector. Further measurements in longer time have given approximately the same values indicating that the equilibrium value reached after 5 minutes is reliable.

The mean seasonal values of CO₂ concentration measured by the IRGA method in the two sets of data, (surface and 5 cm depth) along the observed period are showed in Table 61. The higher values of CO₂ were found at 5 cm depth than at soil surface. Probably, the fresh soil portion exposed allowed the IRGA CO₂ detector to measure the immediate emission of carbon dioxide by microbial activity from the just opened soil hole. A highly significant positive correlation ($r=0.830$, $p<0.0001$) was found between the two sets of measurements (Figure 95). In summer the CO₂ concentration increased both at surface and 5 cm depth with respect to other seasons probably due to higher temperature (a mean of 31 °C was recorded during the season) and suited moisture conditions, enhancing the metabolic activities of soil surface microbial habitats. Irrespective to this, data variability was not significant within each environment ($F=2.395$, $p=0.084$) and between environments ($F=1.749$, $p=0.234$) at surface measurements and at 5 cm depth ($F=1.463$, $p=0.263$; $F=1.277$, $p=0.346$).

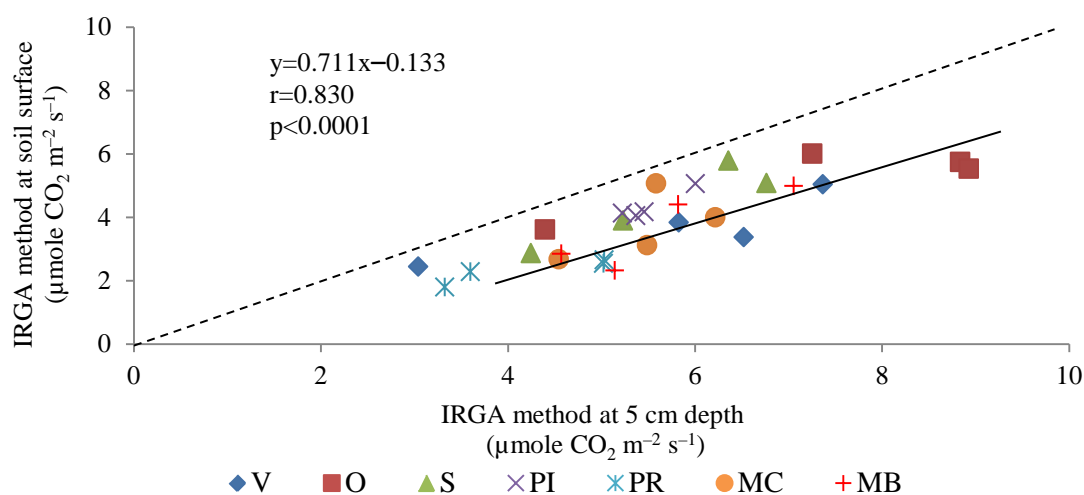


Figure 95. Linear regression and significant positive correlation between the seasonal means of CO₂ measurements at soil surface and at 5 cm soil depth for the selected soil environments.

On annual basis the CO₂ produced at 5 cm depth was higher by 155%, 141%, 128%, 127%, 183%, 147%, and 155% than the CO₂ produced from soil surface for V,

O, S, PI, PR, MC, MB soil environments respectively, indicating a higher biological activity of soil microbial biomass. A clear example is given in soils under pasture with the higher soil microbial activity at 5 cm depth, +183% of CO₂ emitted with respect to the measurements at soil surface. This pattern may suggest a twofold behavior in few centimeters of the soil profile. The soil shallowness and the climatic conditions affect directly the soil moisture content at surface through frequent drying and hardening processes. Carbon dioxide measurements may be therefore reduced in such particular conditions. However, when deeping 5 cm the CO₂ concentration measured increases consistently with respect to surface due to more favorable conditions for microbial activity.

3.5.2. Variability of CO₂ production at different soil use and abandonment

The measurements of CO₂ corroborate the close relationships between the IRGA and SLF under the same field conditions. The variations in the carbon dioxide production from the selected soil environments and within a same environment explain the complexity of CO₂ production under different land use and age of abandonment. Soils with a better soil structure and more stable organic compounds, achieved along the sequence of abandonment through the colonization of spontaneous vegetation, are assumed to preserve organic carbon in a stable form, and only labile organic carbon may be mineralized by the microbial activity, and released as CO₂ from the soil surface to the atmosphere.

The increase of organic compounds in soils changes with soil use, land management, and abandonment, and this sequence may control the efflux of CO₂ from soil surface. Soil use and management must be accurately planned if imbalance of CO₂ emission with respect to SOC content is to be avoided. Land abandonment without any management is not a suggestible practice because of the risk of fire occurrence in a disorganized scrubland. Nevertheless it is proved that progressive recolonization of ancient agricultural land by plant may have a primary role in recovering stable organic compounds and a good soil structure against degradation processes. This would suggest that also land abandonment is worth of special management in order to recover a mosaic of land use and prevent environmental damages.

A correlation matrix was tried by using the data of CO₂ measurements in the field and laboratory together with soil moisture and soil temperature (Table 62). Highly significant correlations between the values of the two applied techniques along the period of observation were obtained (Table 62).

Table 62. Correlation matrix between the mean seasonal values of soil moisture (SM), soil surface temperature (ST), and CO₂ concentration measured by soda lime method at laboratory (SLJ) and field (SLF) condition and infrared gas analyzer (IRGA) method at soil surface and at 5 cm depth.

Variables	SM	ST	SLJ	SLF	IRGA soil surface
ST	-0.760 p=0.000				
SLJ	0.861 p=0.000	-0.683 p=0.000			
SLF	<i>-0.389</i> <i>p=0.041</i>	0.313 p=0.105	<i>-0.434</i> <i>p=0.021</i>		
IRGA soil surface	-0.477 p=0.010	0.514 p=0.005	<i>-0.416</i> <i>p=0.028</i>	0.501 p=0.007	
IRGA 5 cm depth	-0.358 p=0.061	0.537 p=0.003	-0.219 p=0.262	<i>0.379</i> <i>p=0.047</i>	0.830 p=0.000

Bold: $p < 0.01$; Italic: $p < 0.05$.

When the seasonal mean values of the SLJ method were plotted against the values of SLF method a highly significant negative correlation was found ($r = -0.434$, $p < 0.05$) (Figure 96A). Likewise, SLJ values were negatively correlated with the CO₂ values of IRGA method ($r = -0.416$, $p < 0.05$) (Figure 96B). This trend was attributed to summer field values of CO₂ concentration which increased considerably in natural conditions by both methods.

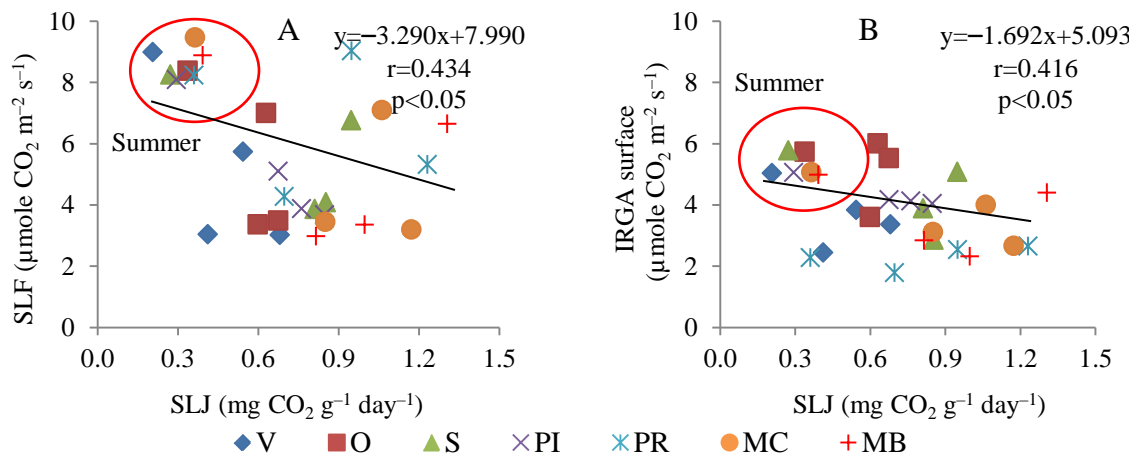


Figure 96. Linear regression and significant negative correlation between SLF and IRGA measurements against SLJ CO₂ values for the selected soil environments.

3.5.3. Role of soil temperature and soil moisture on soil respiration capacity

Data of CO₂ measurements with soda lime method under laboratory conditions (SLJ) indicated that CO₂ production was related to soil temperature and moisture content as highly significant negative correlation ($r=-0.683$, $p<0.0001$) was found with soil temperature, whereas a significant positive correlation ($r=0.861$, $p<0.001$) was found with soil moisture (Figure 97A, B). It was noticed that, if soils under laboratory conditions are able to maintain certain amount of moisture, the CO₂ production increased as a result of the increasing soil microbial activities, enhanced by the amount of soil water content that each environment is able to hold. However, loss of water determines a rapid decrease in CO₂ production. Laboratory values showed that soil temperature may influence the CO₂ measurements in an inverse trend with respect to the other field techniques. The content of soil organic matter and its ability to preserve moisture within the pore space system may be therefore fundamental.

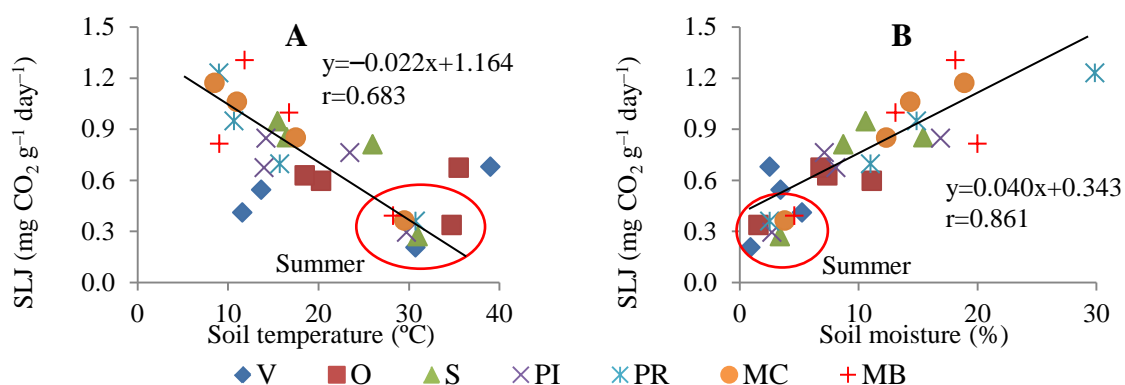


Figure 97. Linear regressions equations between CO₂ values of soda lime methods at laboratory conditions, soil surface temperature (A) and soil moisture (B) for the selected soil environments.

The soil response to SLF measurements of CO₂ may emphasize the role of surface temperature and moisture towards the CO₂ emission at field conditions. Patterns of CO₂ concentrations estimated by SLF method are shown (Figure 98) as a function of soil surface temperature, both measured at each DOY (year 2009). Data presented per DOY had the objective to display the natural soil surface temperature at the time of CO₂ measurements. Independently of the scatter of points for each soil environment the regression equation showed a significant p level and indicated those environments more sensitive to CO₂ emission despite the organic carbon content.

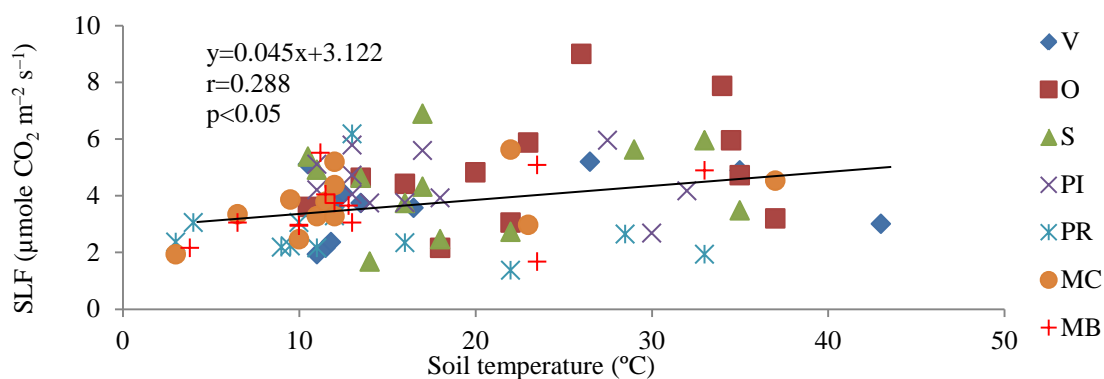


Figure 98. The relationship between the values of CO₂ by SLF method and soil temperature per each DOY.

By contrast, CO₂ values by SLF method showed a negative correlation with soil moisture (Figure 99). The highest CO₂ estimation was recorded in summer 2009 (8.62 μmole m⁻² s⁻¹) with the highest soil surface temperature (30.68 °C) and the lowest water content (2.79%), whilst the lowest CO₂ value was recorded during autumn 2009 (3.62 μmole m⁻² s⁻¹) with a soil temperature of 24.86 °C and a water content of 8.81%.

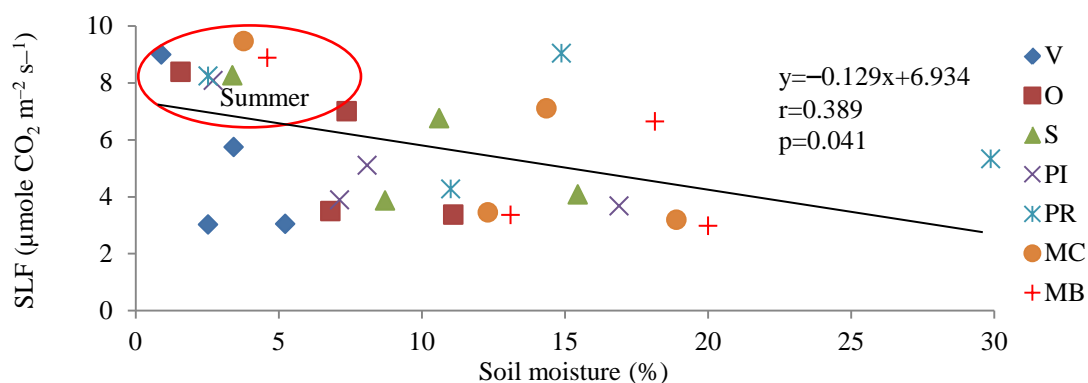


Figure 99. The relationship between the CO₂ values by soda lime (SLF) method and soil moisture.

The same trend was recorded by the IRGA method. CO₂ concentration at soil surface was positively correlated with soil temperature and negatively correlated with soil moisture (Figure 100A and B).

Similarly, the CO₂ values measured with IRGA method at 5 cm depth were positively correlated with soil temperature (Figure 101A) whilst no significant correlation was found between CO₂ values and soil moisture at 5 cm depth (Figure 101B).

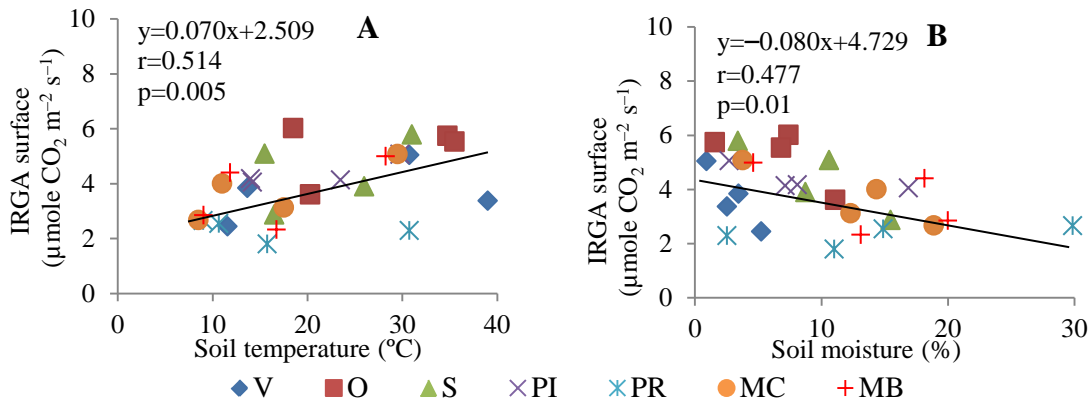


Figure 100. The relationship between CO₂ values by IRGA method at soil surface versus soil temperature (A) and soil moisture (B).

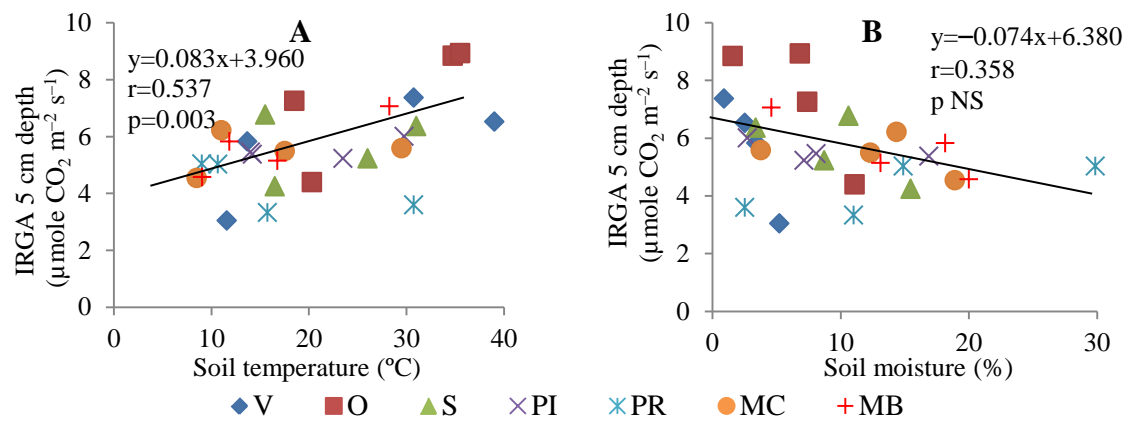


Figure 101. The relationship between the seasonal means of CO₂ values by IRGA method at 5 cm depth versus soil temperature (A) and soil moisture (B).

3.5.4. ANOVA for comparing the reliability of CO₂ measurement methods

Analysis of variance (ANOVA) was used in order to emphasize the reliability of these methods for the evaluation of CO₂ emission. ANOVA was run for each seasonal data set of CO₂ measurements separately as a dependent variable versus the other three data sets as independent variables (Table 63). Results showed in Table 63 indicate that both soda lime and IRGA methods are significantly different one another. The highest p levels of the IRGA method may suggest a higher reliability of the measurements for the evaluation of carbon dioxide production from soil.

Table 63. Analysis of variance (ANOVA) used to test statistical significance ($p<0.05$).

	SLJ	SLF	IRGA surface	IRGA 5 cm depth
F-test	3.173	3.631	25.444	19.471
p level	0.042	0.027	<0.0001	<0.0001

df: Degree of freedom; SLJ: Soda lime method at laboratory conditions; SLF: Soda lime method at field conditions; IRGA: Infrared gas analyzer method.

3.5.5. Comparison of CO₂ concentrations at field conditions by SLF and IRGA methods

Measurements of CO₂ concentration fluxed from soil surface (three replications each method) were performed in the field in each soil environment from autumn 2008 to autumn 2009 by using both the soda lime (SLF) and infrared gas analyzer (IRGA) methods. Then two different data sets were obtained to evaluate the CO₂ emitted from soil surface in different days of the year (DOY). Though the two methods may be conceptually different the degree of similarity in the emitted CO₂ values was noticeable. The fluxed CO₂ expressed as $\mu\text{mole CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ recorded by both methods in each DOY (from DOY 312 corresponding to autumn 2008 to DOY 310 corresponding to autumn 2009) show a rather similar trend along the temporal sequence of measurements (Figure 102). The highest values of fluxed CO₂ were recorded by the SLF method. As elsewhere said after 24 h a higher absorption of CO₂ by soda lime occurred in the closed chamber, with respect to the transient IRGA measurements made in a shorter time. IRGA method seems to measure the immediate CO₂ concentration in five minutes constraints. Irrespective to this, the values from both methods were considered at a steady state, because: i) the soda lime collected after 24 h did ever show indications of CO₂ saturation (light pink color), ii) the CO₂ values from IR apparatus was always constant after 5 minutes.

3.5.5.1. Statistical analysis for comparing CO₂ concentrations

A descriptive statistics was carried out in order to check the standard deviation (SD) and the coefficient of variability (CV) either for all the analyzed soil environments/per each DOY (day of the year), or for each soil environment/per all DOY (Table 64).

Table 64. Descriptive statistical analysis of CO₂ values expressed as $\mu\text{mole CO}_2 \text{ m}^{-2} \text{ s}^{-1}$.

Statistical parameters	SLF method		IRGA method	
	Environments/ each DOY	Environment/ all DOY	Environments/ each DOY	Environment/ all DOY
x	5.36	5.36	3.81	3.81
SD	0.96	3.36	1.10	1.31
CV (%)	20.24	63.17	28.33	34.42

DOY; Day of the year; SLF: Soda lime method at field; IRGA: Infrared gas analyzer method; x: Mean; SD: Standard deviation; CV: Coefficient of variation.

The variation of CO₂ data is higher along the days of measurements, with respect to each day of measurement for all the soil environments. This indication was already clear reading in Figure 102.

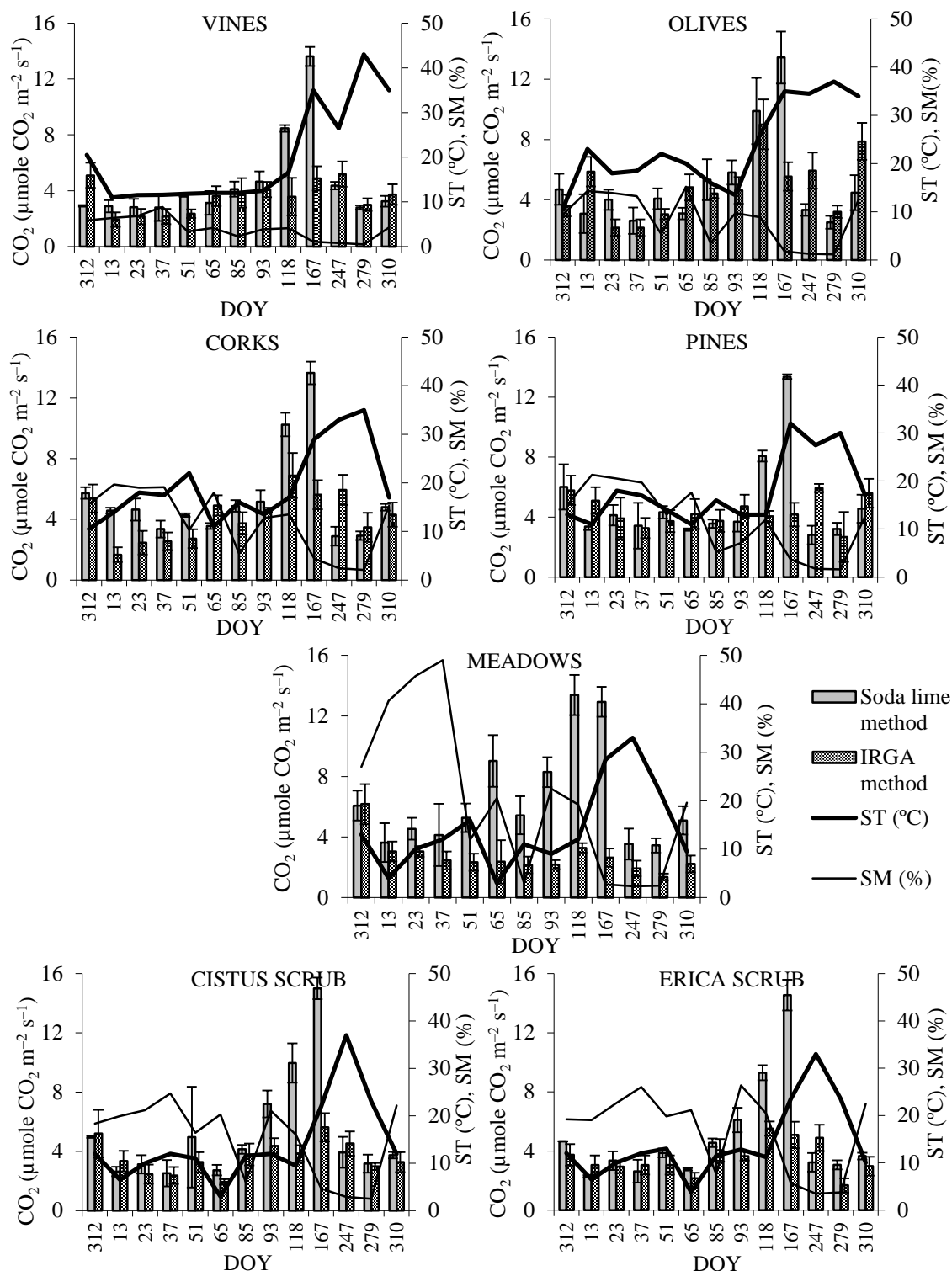


Figure 102. Temporal mean values of CO₂ flux from soil ($\mu\text{mole CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) obtained by SLF (clear grey bar) and IRGA (dotted grey bar) methods along the period of study indicated by the day of the year (DOY) for the studied soil environments. Trending of soil moisture (SM) (fine line) and soil temperature (ST) (coarse line) are also represented.

Linear relationships were then tried between all sets of SLF and IRGA surface data though the best linear fits were found from winter period (January, DOY 13) to the beginning of summer (June, DOY 167). By using these data a total of 53 positive significant correlations were obtained (Table 65).

Table 65. Correlation matrix between CO₂ values from the SLF and IRGA methods for the selected soil environments, from DOY 13 (January 2009) to DOY 167 (June 2009).

ENV	V	O	S	PI	PR	MC	MB
	IRGA SLF	IRGA SLF	IRGA SLF	IRGA SLF	IRGA SLF	IRGA SLF	IRGA SLF
V	IRGA SLF 0.736						
O	IRGA0.446 0.535 SLF <i>0.752</i> 0.983 <i>0.581</i>						
S	IRGA 0.806 <i>0.709</i> <i>0.711</i> <i>0.751</i> SLF 0.658 0.983 0.605 0.985 <i>0.697</i>						
PI	IRGA0.120 0.058 0.455 0.068 0.020 0.102 SLF 0.616 0.982 0.451 0.953 0.615 0.976 0.001						
PR	IRGA0.318 0.200 0.462 0.233 0.084 0.361 0.227 0.292 SLF <i>0.786</i> 0.837 0.720 0.853 0.963 0.829 0.104 <i>0.774</i> 0.198						
MC	IRGA 0.698 0.825 0.448 0.846 0.470 0.802 0.295 <i>0.758</i> 0.026 0.569 SLF <i>0.751</i> 0.977 0.531 0.981 <i>0.730</i> 0.960 0.103 0.944 0.152 0.846 0.873						
MB	IRGA0.587 0.827 0.674 0.890 <i>0.688</i> 0.869 0.011 <i>0.774</i> 0.321 <i>0.720</i> <i>0.788</i> 0.841 SLF <i>0.767</i> 0.991 0.516 0.992 <i>0.731</i> 0.974 0.057 0.961 0.164 0.843 0.856 0.992 0.849						

IRGA; Infrared gas analyzer method; SLF; Soda lime method at field conditions; ENV; Soil environments; V: Soils under cultivated vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub; Bold: $p < 0.01$; Italic: $p < 0.05$.

By relating the CO₂ values of both SLF and IRGA method for each environment separately, 5 positive significant correlations were found (Table 65). Linear regression equations showed $r=0.873$, $p < 0.01$, $r=0.849$, $p < 0.01$, $r=0.736$, $p < 0.05$, $r=0.581$, $p < 0.05$, $r=0.697$, $p < 0.05$ for MC, MB, V, O, S soils respectively. An example of the linear fitting on yearly basis may be observed in Figure 103, where CO₂ mean values from IRGA measurements are plotted against CO₂ mean values from SLF measurements in all soil environments.

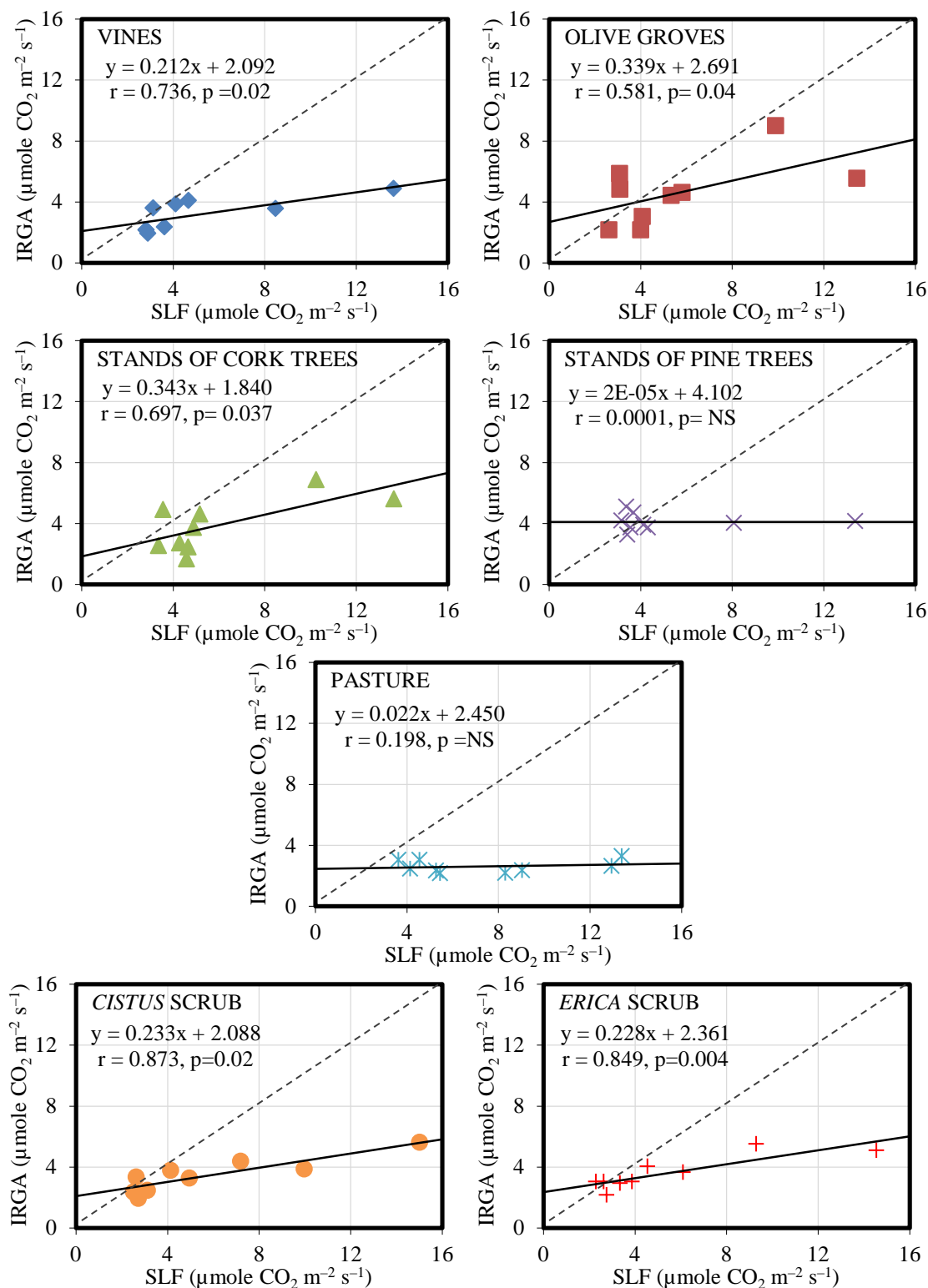
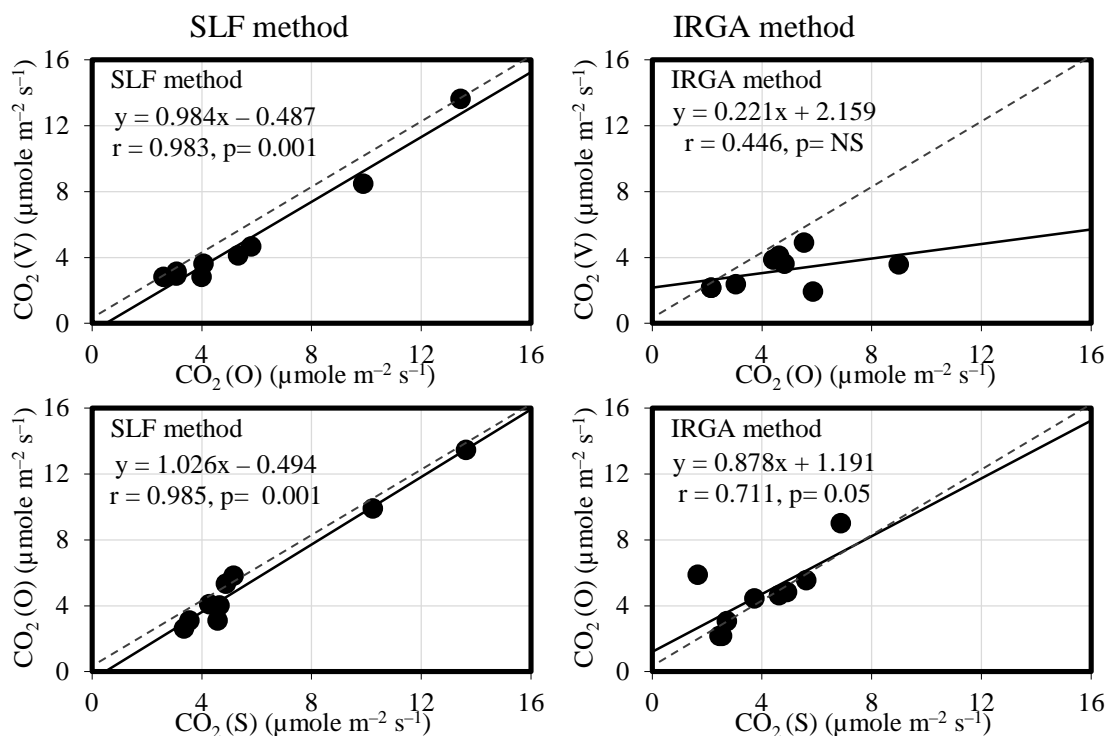


Figure 103. Examples of fitting linear regression equations obtained by using IRGA vs SLF data of all soil environments from DOY 13 to DOY 167.

The slope of the equations depicts the proportionality of CO_2 data between the two methods with respect to the 1:1 line (Keith and Wong, 2006). Soils under pines (PI) or pasture (PR) did not show any relationships when CO_2 flux data of IRGA vs SLF

method were tested for these two environments separately. Probably, fresh deposition of manure following grazing activity in PR soil environment during some periods of the year may produce stronger variability in CO₂ flux, altering the comparison with one another technique. Similarly, the particular composition of hardly decomposable pine tree leaves in PI soil environment may have hindered the temporal and spatial comparison of the two methods. Moreover, 21 positive significant correlations were obtained by relating values of fluxed CO₂ when using SLF method between one another soil environments, i.e. V vs O, O vs S, S vs PI... (Table 65). Accordingly, as each environment was correlated with all the others, we may postulate that the SLF method is detecting approximately the same amount of CO₂ emitted from each soil environment at any measurement date independently of SOC content. By using the IRGA method with the same purpose, 6 positive significant correlations were obtained (Table 65) through the same soil environments, accounting for some diversity in CO₂ detection by this method, which may be probably due to the immediate response of soil to CO₂ measurements by IRGA apparatus.

Linear fitting of CO₂ flux measured from different soil environments is shown in Figure 104.



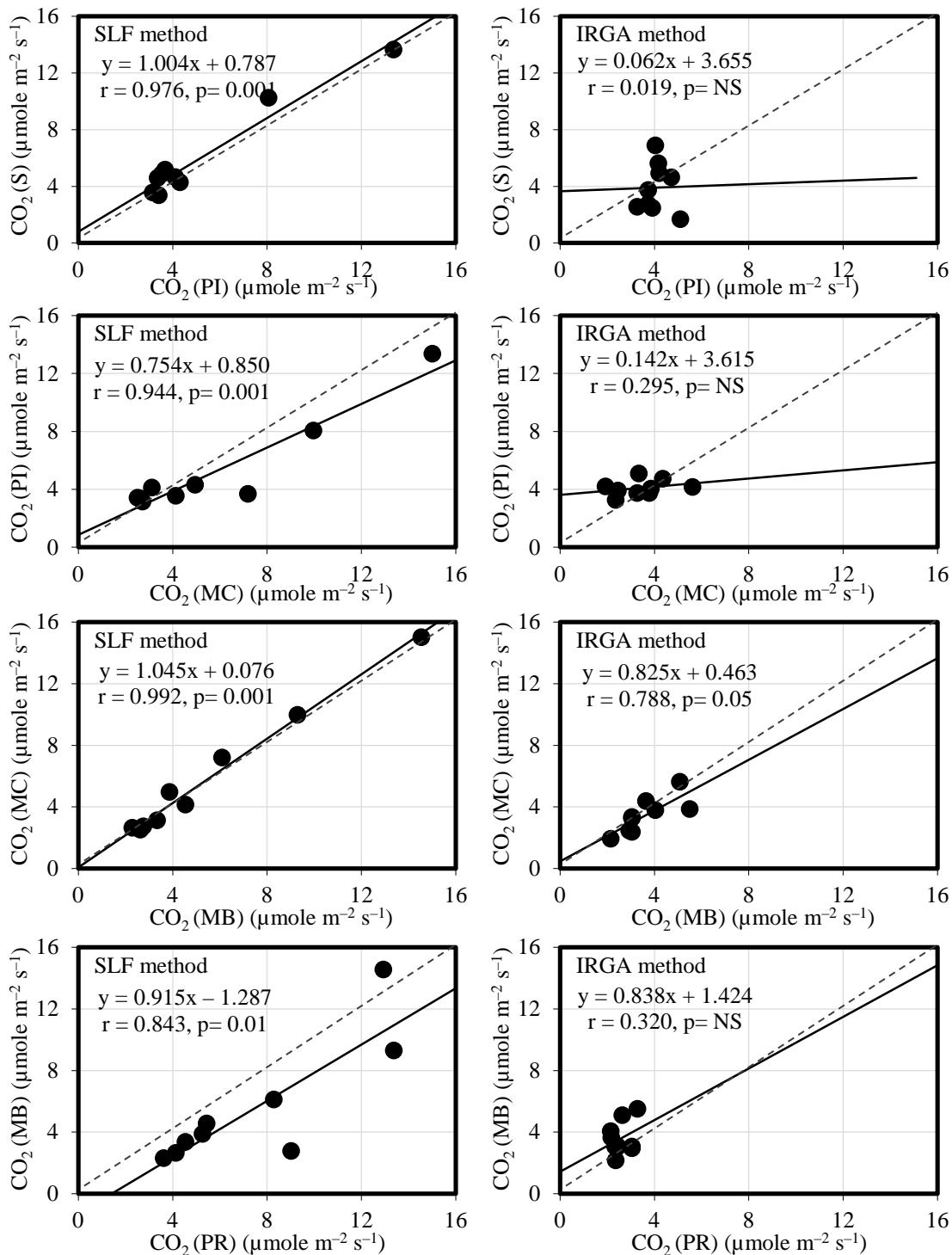


Figure 104. Examples of fitting linear regression equations by using CO₂ concentrations to check each method (IRGA and SLF) with two consecutive soil environments (V vs O; O vs S; S vs PI; PI vs MC; MC vs MB; MB vs PR) from DOY 13 to DOY 167.

When the CO₂ data from soils under vines (V) are plotted against CO₂ data from soils under olive groves (O), SLF method showed much more linearity with respect to the 1:1 line and higher significant and positive correlation coefficient was obtained (Figure 104). A similar pattern was obtained by comparing other environments, i.e. O vs S, S vs PI, PI vs MC, MC vs MB, MB vs PR (Figure 104). According to these results,

we may postulate that the SLF method is detecting approximately the same amount of CO₂ respired from each environment at any measurement date. Effectively the slope deviation with respect to the 1:1 line was 0.020, 0.030, 0.004, 0.293, 0.259, and 0.040 (mean 0.108) for the above mentioned couples of analyzed environments, corroborating this assumption. Conversely lower or no correlations were found by trying to relate the CO₂ data from IRGA method through the same soil environments (Figure 104), where only olive groves (O) vs Cork trees (S) and *Cistus* scrub (MC) vs *Erica* scrub (MB) showed a significant correlation. On average, the mean slope deviation from the 1:1 line in this set of regressions was 1.045, accounting for the diversity in CO₂ detection by this method among the analyzed soil environments.

Soil moisture showed a significant negative correlation with soil temperature in each soil environment and the period of study as showed by the following mean correlations (V, $r=-0.618$, $p=0.024$; O, $r=-0.626$, $p=0.022$; S, $r=-0.801$, $p=0.001$; PI, $r=-0.700$, $p=0.008$; PR, $r=-0.618$, $p=0.024$; MC, $r=-0.748$, $p=0.003$; MB, $r=-0.766$, $p=0.002$). Data confirmed that soil temperature was found to be more related to carbon dioxide emission than moisture which did not show any correlation neither with SLF nor with IRGA method. Positive significant correlation coefficients were found between the SLF method and soil temperature. Values of CO₂ recorded with the SLF method in V, O, S, PI, PR, MC, MB soil environments gave the following statistical data respectively: V $r=0.954$, $p=0.000$; O $r=0.750$, $p=0.002$; S $r=0.694$, $p=0.03$; PI $r=0.819$, $p=0.007$; PR $r=0.437$, $p=0.204$; MC $r=0.766$, $p=0.01$; MB $r=0.826$, $p=0.006$. As indicated, only the soil under meadows (PR) did not show a significant correlation, which may be related to its particular dynamics: is the shallowest soil (25 cm depth) though is the more organic but subjected to rapid wetting-drying cycles because very exposed to wind and is periodically amended by grazing activity; the process of natural decaying vegetal debris comes mostly from the roots of herbaceous plants, though manure is another important source of organics. The values of CO₂ flux increased almost two or threefold either with SLF or IRGA measurements at the beginning of summer season (a mean of 33 °C was recorded), compared with other periods of the year, which is in agreement with Rochette et al. (1991) stating that higher temperature and sufficient moisture content may enhance microbial activity in the soil system.

As reported elsewhere, SLF and IRGA methods were used in the field to check the sensitivity of the measurements under natural conditions. For the SLF method, the annual mean values of the thirteen averaged measurements of fluxed CO₂ in V, O, S, PI, PR, MC, MB soils, from autumn 2008 to autumn 2009 were the following: 4.57(±3.12), 5.10(±3.16), 5.44(±3.09), 4.90(±2.92), 6.53(±3.40), 5.24(±3.61), 4.92(±3.44) $\mu\text{mole CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. A quantitative extrapolation of fluxed CO₂ values was obtained by using the molecular weight of carbon dioxide, resulting in the following annual means: 43.30(±30.24), 49.42(±30.64), 52.68(±29.93), 47.45(±28.26), 63.21(±32.93), 50.74(±34.95), 47.69(±33.28) $\text{Mg ha}^{-1} \text{ year}^{-1}$ corresponding to the soils under vines (V), olive groves (O), stands of cork trees (S), stands of pine trees (PI), pasture (PR), *Cistus monspeliensis* scrubs (MC), and *Erica arborea* scrubs (MB) respectively. The same procedure was applied when using the IRGA method. First, the annual mean values of CO₂ fluxed in V, O, S, PI, PR, MC, MB soils, from autumn 2008 to autumn 2009 were: 3.51(±1.14), 4.79(±2.07), 4.18(±1.57), 4.38(±2.99), 2.71(±1.16), 3.61(±1.10), 3.52(±1.12) $\mu\text{mole CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, and the same quantitative extrapolation gave these results: 33.27(±10.78), 45.36(±19.57), 39.57(±14.83), 41.48(±9.43), 25.66(±10.99), 34.22(±10.41), 33.37(±10.66) $\text{Mg ha}^{-1} \text{ year}^{-1}$, corresponding to the soils under vines (V), olive groves (O), stands of cork trees (S), stands of pine trees (PI), pasture (PR), *Cistus monspeliensis* scrubs (MC), and *Erica arborea* scrubs (MB) respectively. Subsequently, amounts of C-CO₂ ($\text{Mg ha}^{-1} \text{ year}^{-1}$) were calculated for both SLF and IRGA methods by multiplying the CO₂ values by the 12/44 ratio being 12 the molecular weight of carbon and 44 the molecular weight of CO₂.

3.5.5.2. Carbon loss and mineralization index

An indication of carbon loss potential (%) was obtained by relating the C-CO₂ amounts ($\text{Mg ha}^{-1} \text{ year}^{-1}$) for each soil environment to the average SOC stock ($\text{Mg ha}^{-1} \text{ year}^{-1}$) of the same environment, calculated by using the average means of soil bulk density (Mg m^{-3}) and organic carbon (%) measured along the thirteen CO₂ experiments (Table 66).

Table 66. Indicative percentage of carbon loss calculated on the basis of CO₂ fluxes from the studied environments along the studied period.

ENV	SOC (Mg ha ⁻¹ year ⁻¹)	SLF method		IRGA method	
		C-CO ₂ (Mg ha ⁻¹ year ⁻¹)	C loss (%)	C-CO ₂ (Mg ha ⁻¹ year ⁻¹)	C loss (%)
V	6.49 ±1.59	12.07±8.07	185±148	9.27±2.94	143±41
O	30.89±5.56	13.47±8.17	43±34	12.65±5.34	41±22
S	49.49±5.82	14.37±7.98	29±25	11.04±4.05	23±11
PI	32.39±8.30	12.94±7.54	39±53	11.57±2.67	36±16
PR	61.00±2.63	17.25±8.78	28±14	7.15±3.00	12±5
MC	51.83±8.89	13.84±9.32	26±21	9.54±2.84	18±7
MB	53.37±10.4	12.99±8.88	24±21	7.70±2.91	14±8

ENV: Soil environments; SLF method: Soda lime method at field conditions; IRGA method: Infrared gas analyzer method at soil surface; SOC: Soil organic carbon; C-CO₂: Carbon-carbon dioxide; C loss: Carbon loss; V: Soils under vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub.

The dramatic differences in C loss (%) showed in Table 66 are produced by the differences in bulk density and organic carbon in the soil environments and may be informative, despite the high SD values, on the carbon dynamics occurring in the sequence of these peculiar studied soils. Effectively, an indication of the high variability in CO₂ field measurements by both methods was first observed by plotting the mineralization index, calculated in each soil environment by dividing the mean value of C-CO₂ (Mg ha⁻¹ year⁻¹) to the mean of SOC value (Mg ha⁻¹ year⁻¹) from the initial three measurements at each DOY (Figure 105).

In order to have a better reading of mineralization index values, a logarithmic y-axis has been used and the x-axis crossed y scale at 1 mineralization index. Despite the scatter of points for each soil environment, which defines the complexity of such type of measurements, stressed also by the SD values reported in Figure 105, the findings may allow to compare the soil response to different land use and abandonment and the potential for carbon loss or storage (Emran et al., 2012a). Though mineralization index values for soils under vines may appear meaningful, they may be justified by the fact that these soils are also often irrigated being the major CO₂ producers probably because of an extraordinary microbial activity with respect to the real SOC content. Results demonstrated also the reliability of the methods used to evaluate the soil carbon loss as C-CO₂ and allowed to classify those environments with increasing potential for carbon

sequestration. The classification was rather similar by using both methods indicating a higher susceptibility to carbon loss (Table 66).

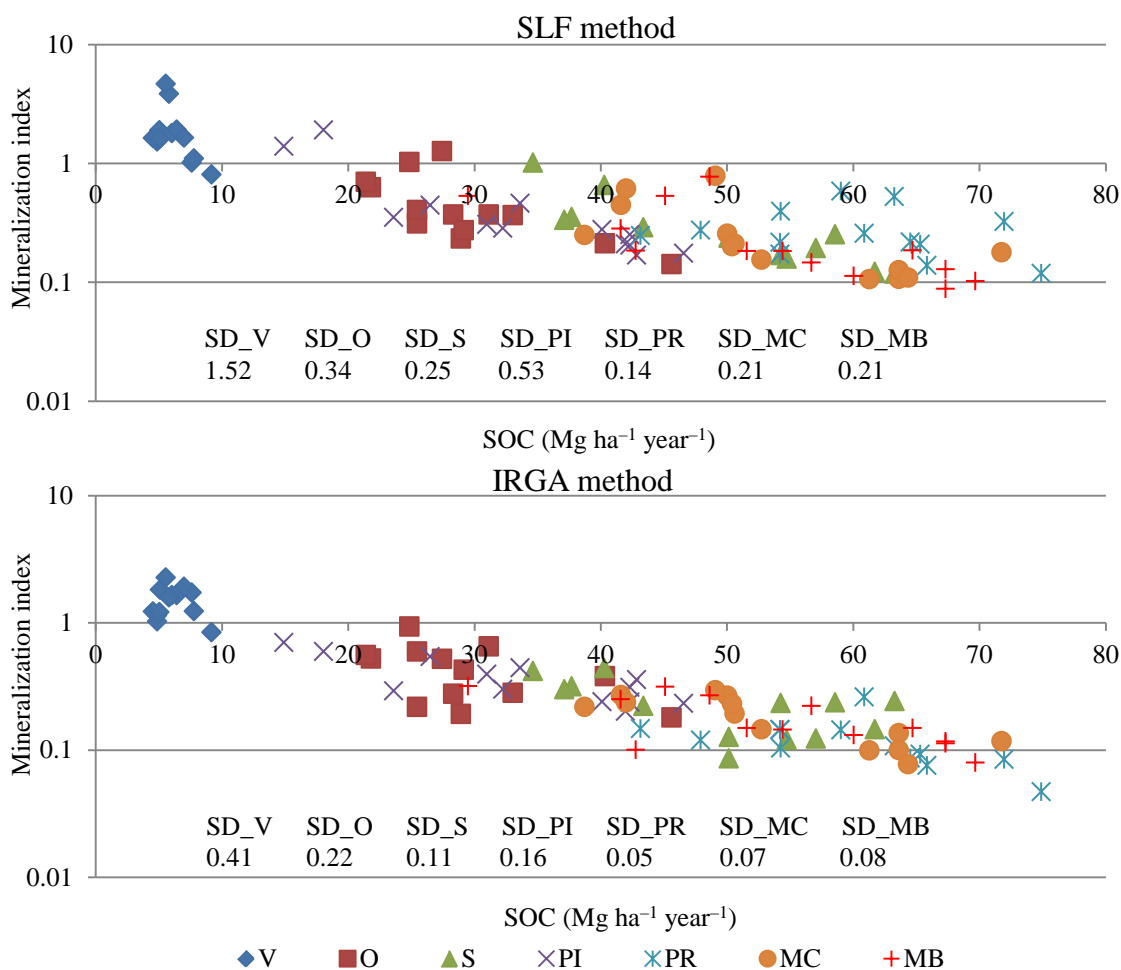


Figure 105. Mineralization index as a function of soil carbon loss estimated by SLF method and IRGA method vs soil organic carbon (SOC) for the studied soil environments.

According to the presented results the sequence of susceptibility to carbon loss from the studied environments should then be the following: V > O > PI > S > PR > MC > MB by using the SLF method and: V > O > PI > S > MC > MB > PR by using the IRGA method. Soil environments can be divided into soils showing a clear ability to preserve SOC, such as soils under pasture (PR), *Erica* scrub (MB) and cork trees (S), and soils with intermediate CO₂ emission because of historical fire perturbations (MC and PI environments) which may have affected their carbon storage capacity. Generally it is reported that environments with lower production of carbon dioxide are potentially carbon sequestering systems, though our data show that not always it may correspond with the highly variable field dynamics.

3.6. Role of microbiota in soil processes**3.6.1. Bacterial and fungal populations in soils under study**

The variability in the dynamics of soil organic carbon is mainly related to the variation in the productivity and activity of soil microbial biomass. Microbial count of bacterial and fungal populations is estimated in a soil sample. The activity of soil microbiota may respond much more rapidly than soil organic matter as a whole to changes in soil management that alter the annual input of organic material into a soil as carbon pool (Powelson et al., 1987). The metabolic activities of soil microorganisms may favor the humification, increasing the carbon pools along with the mineralization, with a continuous loss of soil carbon to be transformed into inorganic forms released as the end-product of their metabolic activities. The dynamic susceptibility of soil organic matter to be metabolized by soil microorganisms is thus controlling both humification and mineralization processes. Litter deposition and their susceptibility in the transformation and decomposition by soil microbiota have to be taken into account in the formation of labile and stable organic matter fractions. The soil organic matter components with relatively short residence time are mainly consisting of microorganisms and metabolites, produced through the metabolization processes. These components are referred to as the active or labile organic fractions and considered at the same time as the main substrate for soil microbiota and plant nutrition. Fungi and bacteria are involved in the transformations of carbon within soil particles. Principally, fungi and bacteria govern most of the transformations and long-term storage of organic carbon in soils (Bailey et al., 2002). Ananyeva et al. (2006) reported that fungi dominate in soil for the most part as well as bacteria. The pathways of the microbial transformations favored by the two major groups of soil microorganisms differ. Fungi tend to be predominant in drier soils than bacteria as they have hyphae that allow them to move, colonize, and degrade surface litters and have the ability to extend through soil pores and obtain water and nutrients, but bacteria do not share the same advantage (Sylvia et al., 2005). Nevertheless, bacteria may have much more populations forcing other transformation processes. The chemical composition of the fungal hyphae is more complex than bacteria. For example, two components of fungal cell wall are the polymers of melanin and chitin, while the main components of bacterial membranes are phospholipids. The polymers are much more resistant to degradation, while the

phospholipids are energy-rich, readily-decomposable substrates, available to a wide range of soil microorganisms. Fungi tend to have higher carbon assimilation efficiency than bacteria and thus store more carbon during the metabolization processes (Bailey et al., 2002). Thus the carbon stored by fungi is expected to be more persistent when mediated by fungal biomass. Changes in soil microbiota measured over relatively short periods can indicate the dynamic transformations trends of soil organic carbon. Soil bacteria and fungi are generally estimated and expressed in colony forming unit per grams of oven dry soil (cfu g⁻¹). The total are summed and presented as total soil microbiota as referring to the major two groups of soil microorganisms in soil. The ratio of fungi to bacteria (F/B) is used to establish the dominating group of soil microorganisms in soil (Table 67).

Our results demonstrate that bacteria population dominate the studied soils more than fungi population, especially in soils under cork trees and pasture in winter and spring. The annual average of fungal productivity was 0.68×10^5 , 1.70×10^5 , 3.89×10^5 , 3.54×10^5 , 8.93×10^5 , 3.29×10^5 , and 2.72×10^5 cfu g⁻¹ while the bacteria average was 1.42×10^6 , 5.40×10^6 , 9.05×10^6 , 5.93×10^6 , 17.20×10^6 , 5.99×10^6 , 6.34×10^6 cfu g⁻¹ giving fungi to bacteria ratios of 4.79:100, 3.15:100, 4.30:100, 5.97:100, 5.19:100, 5.49:100, and 4.29:100 for V, O, S, PI, PR, MC, and MB soil environments respectively. The higher fungi and bacteria populations were found in soils under pasture. Periodical manuring increases both fungi and bacteria, thereby increasing the humification and mineralization rates leading to organic carbon storage, though efflux of carbon dioxide due to higher metabolic activity in this active fraction of organic matter may be expected. Between the agricultural soils fungi increased by 15% from V to O soils whilst bacteria increased by 280%. This produced a decrease of F/B ratio by 35% in soils under olive groves. Fungi and bacteria decreased respectively by 9% and 35% from soils under stands of cork trees to soils under stands of pine trees. This trend produced a F/B ratio higher in soils under stands of pine trees than under cork trees, probably due to the abundance of dried pine needles under decomposition. In soils under scrubs, bacteria increased by 6% in soils under *Erica* scrub, but the fungi decreased by 21% with respect to soils under *Cistus* scrub. The F/B ratio was then 22% lower in soil MB.

Table 67. Seasonal means (\pm standard error) of the soil bacteria and fungi, total soil microbiota, and fungi to bacteria ratio (F/B).

Season	ENV	Fungi (cfu g ⁻¹)	CV (%)	Bacteria (cfu g ⁻¹)	CV (%)	Soil microbiota (cfu g ⁻¹)	F/B ratio
Winter	V	$0.67 \times 10^5 \pm 2.08 \times 10^4$	31	$2.41 \times 10^6 \pm 0.31 \times 10^6$	13	2.48×10^6	0.03
	O	$1.14 \times 10^5 \pm 1.53 \times 10^4$	13	$5.56 \times 10^6 \pm 1.06 \times 10^6$	19	5.68×10^6	0.02
	S	$2.90 \times 10^5 \pm 4.04 \times 10^4$	14	$12.00 \times 10^6 \pm 1.73 \times 10^6$	14	12.30×10^6	0.02
	PI	$3.62 \times 10^5 \pm 2.08 \times 10^4$	6	$5.79 \times 10^6 \pm 1.15 \times 10^6$	20	6.15×10^6	0.06
	PR	$10.60 \times 10^5 \pm 2.83 \times 10^4$	3	$20.10 \times 10^6 \pm 1.41 \times 10^6$	7	21.10×10^6	0.05
	MC	$3.49 \times 10^5 \pm 9.24 \times 10^4$	26	$6.43 \times 10^6 \pm 3.54 \times 10^6$	55	6.78×10^6	0.05
	MB	$2.20 \times 10^5 \pm 7.07 \times 10^4$	3	$8.49 \times 10^6 \pm 0.71 \times 10^6$	8	8.72×10^6	0.03
Spring	V	$1.00 \times 10^5 \pm 7.21 \times 10^4$	72	$1.10 \times 10^6 \pm 0.61 \times 10^6$	56	1.20×10^6	0.09
	O	$1.11 \times 10^5 \pm 2.52 \times 10^4$	23	$5.77 \times 10^6 \pm 1.00 \times 10^6$	17	5.88×10^6	0.02
	S	$3.85 \times 10^5 \pm 7.57 \times 10^4$	20	$11.20 \times 10^6 \pm 2.08 \times 10^6$	19	11.5×10^6	0.03
	PI	$3.68 \times 10^5 \pm 4.62 \times 10^4$	13	$6.26 \times 10^6 \pm 1.13 \times 10^6$	18	6.63×10^6	0.06
	PR	$11.60 \times 10^5 \pm 5.69 \times 10^4$	5	$24.30 \times 10^6 \pm 2.83 \times 10^6$	12	25.5×10^6	0.05
	MC	$3.73 \times 10^5 \pm 7.07 \times 10^4$	2	$5.97 \times 10^6 \pm 2.12 \times 10^6$	36	6.34×10^6	0.06
	MB	$3.53 \times 10^5 \pm 5.03 \times 10^4$	14	$3.88 \times 10^6 \pm 0.57 \times 10^6$	15	4.23×10^6	0.09
Summer	V	$0.46 \times 10^5 \pm 1.53 \times 10^4$	33	$1.05 \times 10^6 \pm 0.61 \times 10^6$	57	1.10×10^6	0.04
	O	$2.10 \times 10^5 \pm 3.21 \times 10^4$	15	$4.89 \times 10^6 \pm 0.99 \times 10^6$	20	5.10×10^6	0.04
	S	$5.61 \times 10^5 \pm 1.59 \times 10^4$	3	$5.61 \times 10^6 \pm 1.13 \times 10^6$	20	6.17×10^6	0.10
	PI	$3.97 \times 10^5 \pm 7.00 \times 10^4$	18	$5.16 \times 10^6 \pm 1.36 \times 10^6$	26	5.56×10^6	0.08
	PR	$7.00 \times 10^5 \pm 7.81 \times 10^4$	11	$11.90 \times 10^6 \pm 9.54 \times 10^6$	80	12.60×10^6	0.06
	MC	$3.26 \times 10^5 \pm 1.50 \times 10^4$	5	$5.54 \times 10^6 \pm 0.57 \times 10^6$	10	5.87×10^6	0.06
	MB	$1.41 \times 10^5 \pm 3.21 \times 10^4$	23	$3.98 \times 10^6 \pm 3.21 \times 10^6$	81	4.12×10^6	0.04
Autumn	V	$0.58 \times 10^5 \pm 3.37 \times 10^4$	58	$1.11 \times 10^6 \pm 0.61 \times 10^6$	55	1.17×10^6	0.05
	O	$2.46 \times 10^5 \pm 7.96 \times 10^4$	32	$5.39 \times 10^6 \pm 1.04 \times 10^6$	19	5.63×10^6	0.05
	S	$3.21 \times 10^5 \pm 9.80 \times 10^4$	31	$7.45 \times 10^6 \pm 1.25 \times 10^6$	17	7.77×10^6	0.04
	PI	$2.88 \times 10^5 \pm 8.99 \times 10^4$	31	$6.51 \times 10^6 \pm 1.15 \times 10^6$	18	6.80×10^6	0.04
	PR	$6.56 \times 10^5 \pm 1.60 \times 10^4$	24	$12.50 \times 10^6 \pm 1.75 \times 10^6$	14	13.10×10^6	0.05
	MC	$2.69 \times 10^5 \pm 8.53 \times 10^4$	32	$6.00 \times 10^6 \pm 1.10 \times 10^6$	18	6.27×10^6	0.04
	MB	$3.73 \times 10^5 \pm 1.11 \times 10^4$	30	$9.02 \times 10^6 \pm 1.41 \times 10^6$	16	9.39×10^6	0.04

ENV: Soil environments; CV: Coefficient of variation; V: Soils under cultivated vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub.

As can be observed in Table 67, high seasonal variability is shown among the studied soils. For all soil environments, the mean seasonal average of fungi was 3.51×10^5 , 4.07×10^5 , 3.40×10^5 , and 3.16×10^5 cfu g⁻¹ and bacteria was 8.68×10^6 , 8.35×10^6 , 5.45×10^6 , and 6.85×10^6 cfu g⁻¹ revealing Fungi: Bacteria ratio of 4.04:100, 4.87:100, 6.23:100, and 4.61:100, all data referring progressively to winter, spring, summer, and autumn respectively. The higher fungi to bacteria ratio was found in summer probably because of higher sensitivity of soil bacteria to climatic changes than fungi, indicating that when the frequency of soil wetting/drying cycles increases, soil

microbiota community may be affected (Landesman and Dighton, 2011). The higher fungi and bacteria along all seasons were found in soils under pasture and cork trees respectively, and were especially higher in spring and winter. Thus, seasonal changes in soil moisture and soil surface temperature may coherently affect the number of these populations as well as activities increasing either bacteria or fungi populations under their suitable environmental conditions. Accordingly, soil microbiota seemed to proliferate more in soils able to maintain water content (15-30%) during the seasons with moderate soil surface temperature (10-20 °C).

3.6.2. Impact of changes in environmental and soil conditions on the productivity and activity of soil microbiota

As soil microbiota increases, microbial activity increases. This concept has been generally observed in soil ecosystems under optimal environmental conditions with a moisture range of 20-60% and a soil temperature range of 20-45 °C (Allende et al., 2004) and referred to the mesophilic microorganisms as the predominant group in soil ecosystems. Soil temperature greatly influences the rates of biological, physical, and chemical processes in the soil. Within a limited range, the rates of chemical reactions and biological processes may double for every 10 degree of temperature increasing. While the lack of adequate moisture in the soils under study may dehydrate the microbial cell walls, consequently producing the death of soil microbes indicated by the decreasing number of soil microbiota in summer season (Table 67), this dead biomass may be considered a part of active organic matter fractions (Ekschmitt et al., 2005). Tolerance to restrictive soil moisture levels varies within major microbial groups as well as within individual microbial species. Generally, bacteria are more sensitive than fungi. Hence, the latter organisms tend to better survive (latent survival) in drier soils. This observation results at least in part from their capacity to form “resting” structures, which activity is re-stimulated when favorable conditions occur. Biological activity may increase under natural field conditions with soil moisture content less than 5%, soil pH ranges from 5.5-7.5, and soil surface temperature range from 20 to 45 °C. These conditions are normally found in the studied soils during summer season.

Independently of fungi and bacteria content, the higher carbon dioxide flux obtained by SLF, was recorded in summer season for all soil environments (Figure

106). The soil surface temperature range from 28 °C to 35 °C and water content around 3-5% may have been sufficiently feasible for microorganism's activity. These parameters combined with soil carbon pool may help in understanding the role of environmental conditions in organic decaying debris dynamics. Results seem to indicate that when water content increases (15-20%) and low to moderate soil surface temperature (10-20 °C) is recorded (winter and spring) environmental conditions favor the increasing of soil microbial growth (Figure 106). A slight increase in CO₂ production was also recorded from winter to spring even not as much as in summer. Effectively, bacteria and fungi in winter increased by 125%, 11%, 99%, 11%, 68%, 16%, and 112% and soil moisture content also increased by 475%, 607%, 357%, 523%, 1076%, 400%, and 335% in V, O, S, PI, PR, MC, and MB soil environments respectively, compared to summer season. In winter, when the soil temperature decreased by 62%, 42%, 47%, 52%, 71%, 71%, and 68% for the same soil sequence, the production of carbon dioxide also decreased by 66%, 60%, 50%, 55%, 35%, 66%, and 66% respectively with respect to summer season. Moreover, the proportional increase in soil moisture recorded in spring was 277%, 370%, 214%, 199%, 486%, 280%, and 295% higher when compared with summer values of the same environments. This produced also a slight constraint in CO₂ production (Figure 106). Soil microbiota in autumn was higher by 179%, 334%, 158%, 163%, 333%, 226%, and 185% than in summer, for V, O, S, PI, PR, MC, and MB soil environments respectively. Soil moisture increased as well by 6%, 10%, 26%, 22%, 4%, 7%, and 128%. During this season, the S, PI, PR, MC, and MB soil environments showed a decrease by 53%, 52%, 48%, 64%, and 62% in the carbon dioxide production with respect to summer associated to 16%, 21%, 49%, 41%, and 41% decrease of soil surface temperature.

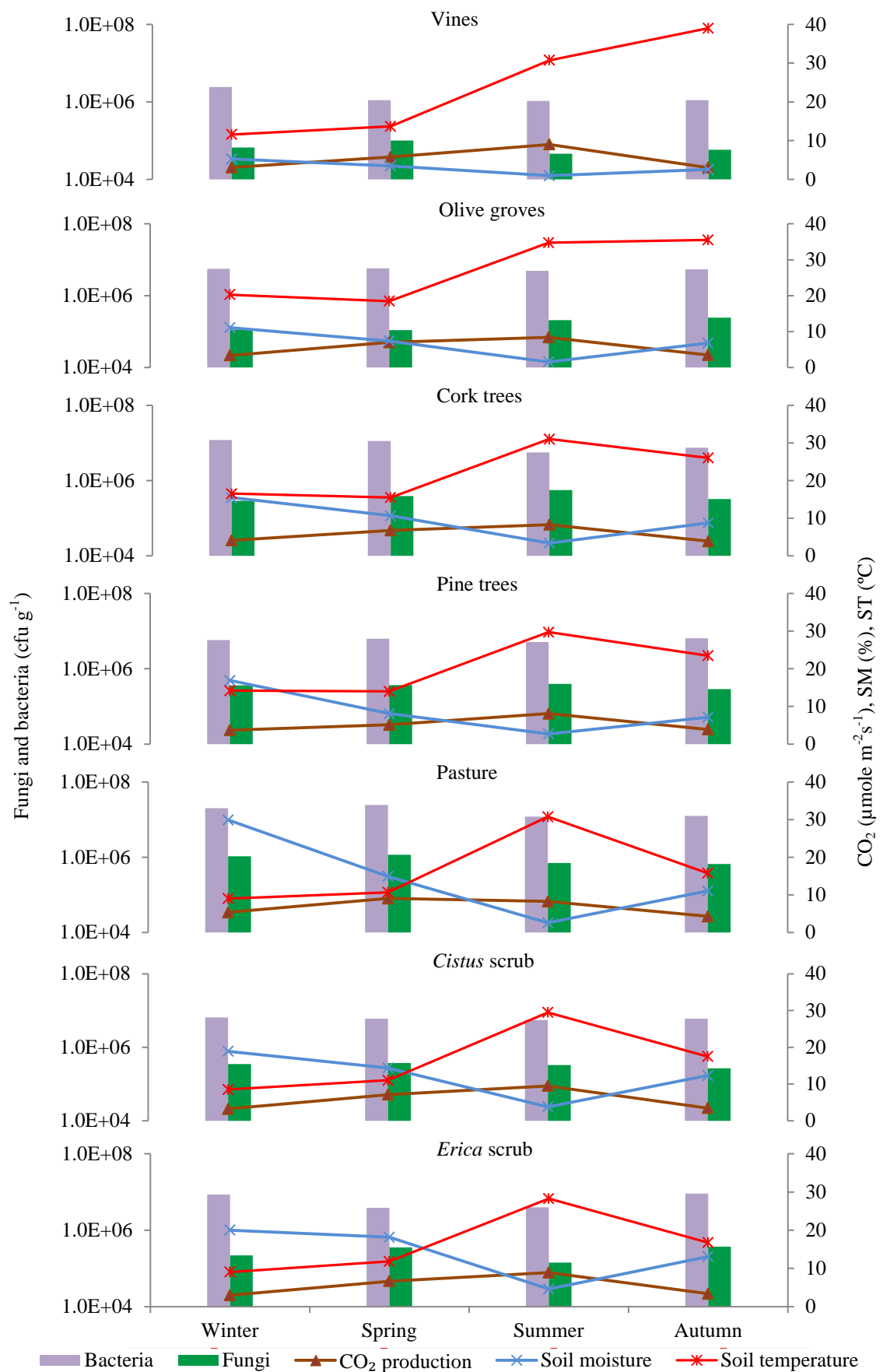


Figure 106. Seasonal means of bacteria and fungi yield, trend in carbon dioxide (CO₂) production, soil moisture, and soil temperature for all soil environments.

3.6.2.1. *Correlation matrix with soil physicochemical characteristics*

The response of soil bacteria and fungi populations to changes in soil and environmental conditions was tested by checking their significant correlations with the most relevant soil physical and chemical characteristics by using all seasonal data obtained from all soil environments (Table 68).

Table 68. Correlation matrix between the seasonal means of relevant soil physical and chemical characteristics against bacteria, fungi and soil microbiota.

Variables	Bacteria (cfu g ⁻¹)	Fungi (cfu g ⁻¹)	Soil microbiota (cfu g ⁻¹)
Clay (%)	0.800 p=0.000	0.839 p=0.000	0.806 p=0.000
BD (g cm ⁻³)	-0.480 p=0.010	<i>-0.460</i> <i>p=0.014</i>	-0.481 p=0.010
MI (kPa)	0.505 p=0.006	0.575 p=0.001	0.511 p=0.005
SS (kPa)	0.658 p=0.000	0.690 p=0.000	0.662 p=0.000
ST (°C)	<i>-0.389</i> <i>p=0.041</i>	<i>-0.289</i> <i>p=0.135</i>	<i>-0.386</i> <i>p=0.042</i>
SM (%)	0.564 p=0.002	0.470 p=0.012	0.563 p=0.002
WHC (%)	0.635 p=0.000	0.607 p=0.001	0.637 p=0.000
IR (mm h ⁻¹)	<i>-0.456</i> <i>p=0.015</i>	-0.502 p=0.007	<i>-0.460</i> <i>p=0.014</i>
<i>k</i> (cm h ⁻¹)	<i>-0.272</i> <i>p=0.162</i>	<i>-0.397</i> <i>p=0.037</i>	<i>-0.279</i> <i>p=0.150</i>
pH	-0.478 p=0.010	-0.497 p=0.007	-0.482 p=0.009
EC (dS m ⁻¹)	0.604 p=0.001	0.703 p=0.000	0.612 p=0.001
SOC (mg g ⁻¹)	0.609 p=0.001	0.588 p=0.001	0.611 p=0.001
TN (mg g ⁻¹)	0.724 p=0.000	0.728 p=0.000	0.727 p=0.000
TP (mg g ⁻¹)	0.472 p=0.010	0.476 p=0.010	0.474 p=0.010

BD: Bulk density; MI: Mechanic impedance; SS: Shear strength; ST: soil temperature; SM: Soil moisture; WHC: Water holding capacity; IR: Infiltration rate; *k*: Hydraulic conductivity; EC: Electrical conductivity; SOC: Soil organic carbon; TN: Total nitrogen; TP: Total phosphorus. Bold: $p < 0.01$; Italic: $p < 0.05$.

Bacteria, fungi and soil microbiota were particularly strongly correlated with soil physical and chemical characteristics using data from all soil environments. Soil bulk

density, soil surface temperature, infiltration rate and permeability, and soil pH were negatively correlated with all microbial groups. Conversely, no significant correlation was found between soil surface temperature (ST) and fungi indicating that fungi may be not sensitive to seasonal changes in soil temperature. Significant negative correlation was found between soil fungi and hydraulic conductivity. Clay content was positively correlated with microbial groups, inferring the role of clay for protecting the soil microbes in a soil micro crumb. MI, SS, SM, and WHC were positively correlated with soil microbial groups. Soil organic carbon, total nitrogen, and phosphorus also showed positive correlations with soil microbial groups indicating their successful implication in biological activity to build a better soil structure through the accumulation of organic compounds.

3.6.2.2. *Carbon dioxide and its relation to microbial groups in soils*

The seasonal means of carbon dioxide were plotted against the seasonal means of both fungi and bacteria for the selected soil environments (Figure 107). Significant linear correlations were found between CO₂ and both fungi and bacteria by excluding the summer measurements because of the highest soil carbon dioxide emission reported in summer (highest biological activity in this season may correspond to lower soil microbiota) followed by spring and autumn while the lowest was recorded in winter.

3.6.2.2.1. *Multiple regression analysis*

Multiple regression analysis was run for the data of CO₂ production as a dependent variable versus the data sets of fungi, bacteria, soil moisture, soil organic carbon, total nitrogen, soil surface temperature, clay, bulk density, and soil pH as independent variables (Table 69).

Table 69. Multiple regression analysis used to test the significant differences of the CO₂ production as dependent variable against data sets of fungi, bacteria, and soil parameters as independent variables.

	Sum of squares	Degree of freedom	Mean squares	F-test	p level
Regression	75.120	5	15.024	4.903	0.004
Residuals	67.416	22	3.064		
Total	142.535				

Even though results indicated highly significant effects of the independent variables on the carbon dioxide emission ($p=0.004$), regression equation was as follows; CO_2 production $= +0.00001(\text{Fungi}) - 0.257(\text{SM}) + 1.395(\text{SOC})$.

Thus, as already documented elsewhere the soil moisture seems to be a contrasting parameter during the metabolization processes of carbon dioxide.

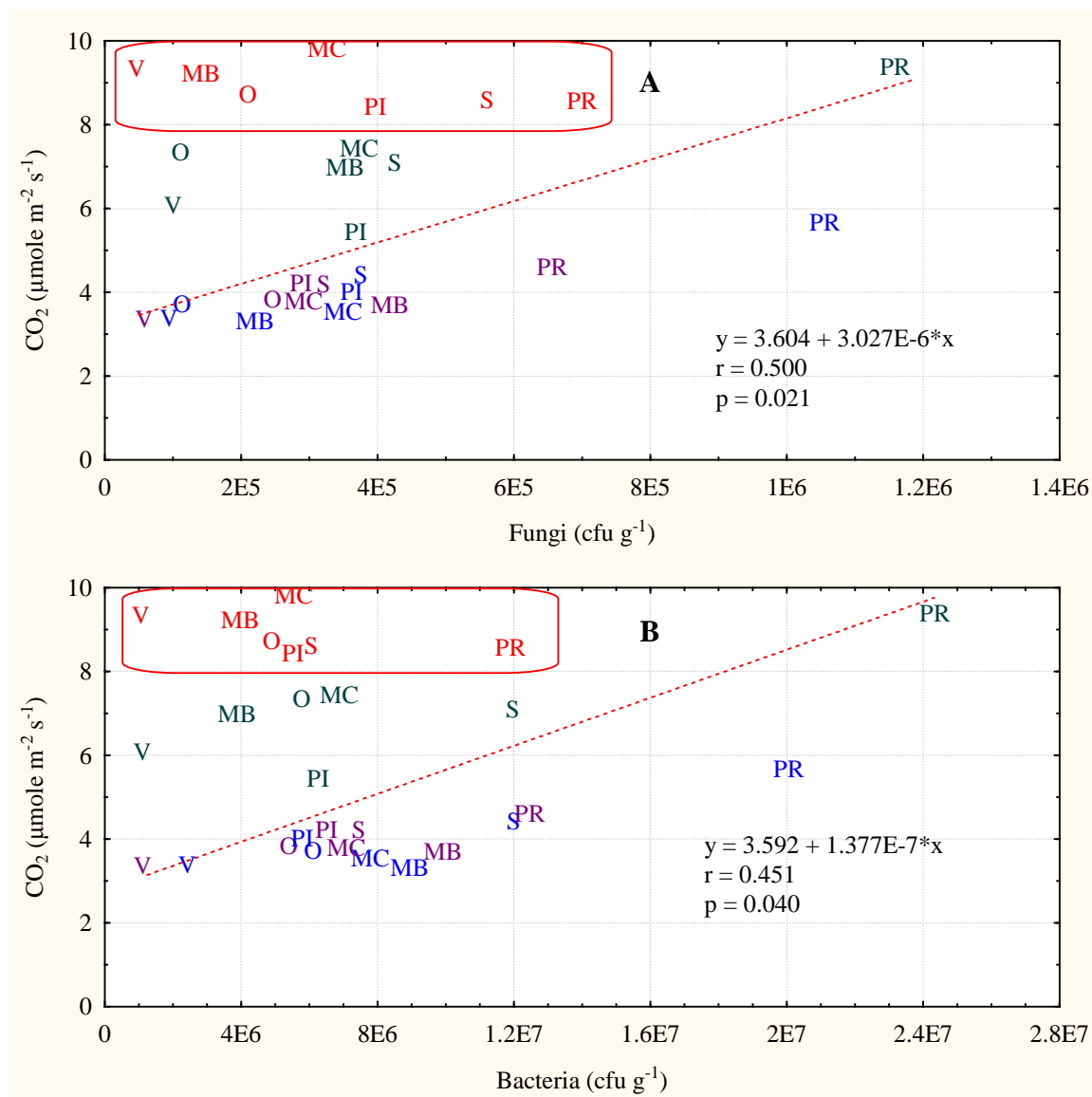


Figure 107. Relationships between the seasonal means of carbon dioxide (CO_2) production and fungi (A) and bacteria (B) for all soil environments. Blue color: Winter; Dark green color: Spring; Red color: Summer; Violet color: Autumn.

3.7. Glomalin production

Glomalin is a specific component of fungal activity and has a relevant role in increasing soil carbon pool. Glomalin is a glycoprotein produced by Arbuscular Mycorrhizal Fungi (AMF), sloughs from hyphae (Wright et al., 1996; Wright and Upadhyaya, 1996; Wright et al., 1998), and glues soil surface particles together as a binding agent for soil aggregates formation (Rillig, 2004; Wright et al., 2007). As it is a glycoprotein, still chemically unknown (Rillig and Steinberg 2002; Gillespie et al., 2011), carbon is stored in both its protein and carbohydrate (glucose or sugar) subunits. This process is considered a key question in the dynamics of soil carbon storage capacity against carbon loss, especially in abandoned soils that may favor higher accumulation of organic matter in the ecosystem.

It should to be pointed that glomalin concentrations are assessed by using a general assay for protein content, the Bradford protein analysis, therefore using the term Bradford reactive soil protein (BRSP) for total glomalin fraction and easily extractable Bradford reactive soil protein (EE-BRSP) for that fraction obtained after only one extraction (Wright and Upadhyaya, 1996). The EE-BRSP has been generally defined as a more labile fraction of the glycoprotein ephemerally joined to soil particles than other unquantified fraction within the total BRSP content (Nichols and Wright, 2005). These operationally defined glomalin pools have a different behavior and their dynamics should be accurately studied in order to establish the persistence of soil carbon within the two fractions.

As elsewhere reported, Rillig (2004) proposed four terms for glomalin measurements based on the extraction and quantification procedures: Bradford reactive soil protein (BRSP), easily extractable Bradford reactive soil protein (EE-BRSP), immunoreactive (MAb32B11) soil protein (IRSP), and easily extractable immunoreactive (MAb32B11) soil protein (EE-IRSP). All show interferences with other substances such as humic acids, tannins, polyphenols (Halvorson and González, 2008; Janos et al., 2008), with the antibody measurement with MAb32B11 being more specific for glomalin than the Bradford assay. However, Hontoria et al., (2009) have assessed the role of glomalin estimated by the first two methods on the formation of soil aggregates (1-2 mm) and carbon pools (total and particulate SOC) in a Haplic Calcisol

in central Spain, where abandoned and active cultivation of olive groves exist. The results indicated that abandoned areas were associated with the increasing of soil organic carbon, aggregate stability, EE-BRSP, and BRSP. Nevertheless, no significant differences were noticed in the role of EE-BRSP on soil aggregates. Therefore, Bradford reactive soil protein seemed to have a positive influence on aggregate stability, even in soils rich in calcium carbonate, under semi-arid climatic conditions.

3.7.1. Global survey of glomalin concentrations

The estimation of glomalin by Bradford reactive soil protein in the studied soils is likely in agreement with the global glomalin survey (Table 70). The global patterns of glomalin abundance varied mainly due to the differences in the dynamic mechanisms of soil ecosystems and the environmental factors such as the climatic conditions, soil parent materials composition, colonization of plant species, and the abundance of organic nutrients in soils. Few studies have been recorded during the short period of glomalin discover, in natural soil ecosystems at the upper soil horizons where the abundance of arbuscular mycorrhizal fungi occurs e.g. agricultural fields (Rillig et al., 2003b; Bedini et al., 2007; Hontoria et al., 2009), forests (Steinberg and Rillig, 2003; Rillig et al., 2003b; Lovelock et al., 2004; Nichols and Wright, 2005; Bedini et al., 2007), pastures and grasslands (Lutgen et al., 2003; Nichols and Wright 2005), and scrublands (Rillig et al., 2003a; Caravaca et al., 2005; Bedini et al., 2007). The glomalin concentrations detected by Bradford method and identified as BRSP and EE-BRSP glomalin fractions in these ecosystems are reported in Table 70. Yearly mean values of easily extractable glomalin (EE-BRSP) and total glomalin (BRSP) recorded in this study in the 0.00-2.00 mm soil fraction are also listed for each group of soil use.

The data in Table 70 are showing a higher variation of glomalin concentration at different locations, soil types, and uses. Many concentrations are coherent with our survey in soils of the Cap de Creus Peninsula (NE Spain). Plant cover seemed to be a coarse indicator of available photosynthate to glomalin producers and both glomalin fractions were greater in scrubland, pasture, and grassland than in soils under forests and agricultural areas.

Table 70. Survey of annual mean glomalin concentrations (\pm standard error) from different studies.

Study	Location	Soil type	Textural class	EE-BRSP (mg g ⁻¹)	BRSP (mg g ⁻¹)
Cultivated soils					
Rillig et al., 2003b	Ohio, USA	Mesic Typic Hapludalf	Morley silt loam	0.35	2.60
Bedini et al., 2007	Tuscany, Italy	Entisol	...	0.25	1.90
Hontoria et al., 2009	Central Spain	Haplic Calcisol	...	0.27 \pm 0.03	0.77 \pm 0.09
This study (V)	NE Spain	Lithic Xerorthent	Loamy sand	0.48\pm0.12	1.04\pm0.32
This study (O)	NE Spain	Lithic Xerorthent	Sandy loam	0.92\pm0.08	2.85\pm1.85
Forest soils					
Lovelock et al., 2004	NE Costa Rica, USA	[Ultisol Inceptisol	...	2.00 \pm 0.50	4.10 \pm 0.60
Steinberg and Rillig 2003	Missoula	Typic Eutroboralf	Clayey skeletal	1.90 \pm 0.50	6.50 \pm 2.50
Rillig et al., 2003b	Ohio, USA	Mesic Typic Hapludalf	Morley silt loam	0.30	2.50
Bedini et al., 2007	Tuscany, Italy	Entisol	Fluvent soil	0.50	6.00
Nichols and Wright 2005	Mary land, USA	Mollic Hapludalf	Loam	...	2.10 \pm 0.70
Nichols and Wright 2005	Georgia, USA	Typic Kanhapludult	Sandy loam	...	3.14
This study (S)	NE Spain	Lithic Xerorthent	Sandy loam	1.06\pm0.13	3.49\pm1.44
This study (PI)	NE Spain	Lithic Xerorthent	Sandy loam	0.80\pm0.06	2.45\pm1.50
Pasture soils					
Nichols and Wright 2005	Colorado, USA	Pachic Argiustolls	Loam	...	1.97
Nichols and Wright 2005	Georgia, USA	Typic Kanhapludult	Sandy clay loam	...	4.50
Lutgen et al., 2003	Missoula, USA	...	Sandy loam	1.70 \pm 0.33	8.00 \pm 0.90
This study (PR)	NE Spain	Lithic Xerorthent	Sandy loam	1.28\pm0.22	4.85\pm1.90
Scrublands					
Caravaca et al., 2005	SE Spain	Typic Petrocalcic	Silt loam	...	3.60 \pm 0.10
Bedini et al., 2007	Tuscany, Italy	Entisol	Fluvent soil	1.50	9.00
Rillig et al., 2003a	SE Spain	Lithic Calciorthid	...	0.40 \pm 0.02	1.40 \pm 0.10
This study (MC)	NE Spain	Lithic Xerorthent	Sandy loam	0.94\pm0.09	3.67\pm1.54
This study (MB)	NE Spain	Lithic Xerorthent	Sandy loam	0.94\pm0.08	3.88\pm1.30

Our soils under vines and olive groves, and under cork and pine trees forests showed similar BRSP concentrations to Morley silt loam soils in Ohio, USA, under agricultural fields of corn (*Zea mays*) and soybean (*Glycine max*) and under forest dominated by maple (*Acer* spp.), elm (*Ulmus* spp.), and walnut (*Juglans* spp) (Rillig et al., 2003b) but showed higher EE-BRSP concentrations (Table 70). The Entisols investigated by Bedini et al. (2007) with low organic matter contents (1.5%) in soil under agricultural maize in Tuscany, Italy, contained higher BRSP concentrations than

our soils under cultivated vines (V), and lower BRSP than our soils under olive groves (O); however, their EE-BRSP content was lower than both V and O soils. In the same study, Bedini et al. (2007) reported higher BRSP concentrations in soils dominated by poplar (*Populus alba*) and grassland with respect to our soils under forest (S and PI) and scrubs (MC and MB), may be due to the high SOC content (3.8% and 5.2% respectively). The EE-BRSP content was lower in soil under poplar grove than in our S and PI environments, while higher values were found in grassland compared with our MC and MB environments. Glomalin concentrations were generally lower in soils rich in calcium carbonate content. Hontoria et al. (2009) have reported lower concentrations of both glomalin fractions in agricultural soils under olive groves (*Olea europaea*) in Haplic Calcisol ($\text{CaCO}_3 \sim 71\%$) than in our V and O environments. Similarly, Rillig et al. (2003a) recorded low BRSP and EE-BRSP content in Lithic Calciorthids under vegetated patches dominated by *Stipa tenacissima* steppes and the sprouting grass *Brachypodium retusum* in SE Spain under semi-arid Mediterranean climate. The low glomalin production in those soils with high calcium carbonate content may be related to the detrimental effect of Ca on AMF biomass (Roldán et al., 1997). A clear pattern was also reported by Lovelock et al. (2004), indicating the increasing of EE-BRSP and BRSP in Ultisols with low Ca content than in Inceptisols with higher Ca content.

The clayey soils investigated by Steinberg and Rillig (2003) under *Pinus ponderosa*/*Festuca idahoensis* community were richer in glomalin stocks than our S and PI soil environments. However, higher organic matter content found in loam and sandy loam soils under two forest areas investigated by Nichols and Wright (2005) showed lower glomalin stocks than our soils under cork (S) and pine (PI) trees. This difference may be related to the lower CEC (4.6 and 4.2 $\text{cmole}_{(+) } \text{kg}^{-1}$), acid soil pH (4.4 and 5.1), and low phosphorus content (0.02 mg g^{-1}) in a Mollic Hapludalf and a Typic Kanhapludult respectively (Nichols and Wright, 2005). The same authors reported that increasing of phosphorus to 0.2 mg g^{-1} associated with a slow increasing in soil exchangeable cations (17 $\text{cmole}_{(+) } \text{kg}^{-1}$) in a Pachic Argiustolls of Colorado under tall and mid-grass prairie decreased the glomalin content by 25% with respect to the study under forest and our S and PI soil environments (Table 70). The Typic Kanhapludult (3.8% organic matter) under pasture investigated by Nichols and Wright (2005) showed a glomalin content similar to our soils under pasture (PR) independently of low P content (0.03 mg g^{-1}).

Plant colonization in adequate climate conditions may have favored the formation of higher concentrations of both glomalin fractions studied by Lutgen et al. (2003) in sandy loam soils under the dense Idaho fescue (*Festuca idahoensis*) and blue bunch (*Agropyron spicatum*) wheat grass community, with more than 3% of SOC. The review of glomalin production in other studies with different soil use and high calcium carbonate content (Rillig et al., 2003a; Caravaca et al., 2005; Hontoria et al., 2009) and different phosphorus levels (Nichols and Wright, 2005), is emphasizing some much higher levels of glomalin in our soils under study, classified as Lithic Xerorthents with lack of CaCO_3 . Soil use and management may also be defined as key parameters useful in further clarifying global glomalin concentrations in soils as outlined Rillig et al. (2003b), Lutgen et al. (2003), Lovelock et al. (2004), and Bedini et al. (2007), and have also caused noticeable differences among the soils under this study with the same parent material composition. Accordingly, climate, soil mineralogy and properties, vegetation type and productivity, and fungal species and diversity, may all have interactive roles in determining the differences in glomalin concentrations across sites (Rillig et al., 2001b; Lovelock et al., 2004).

3.7.2. Glomalin concentrations in the studied soils

The production of glomalin was estimated as total glomalin (BRSP) extracted from soils. In addition, the easily extractable glomalin (EE-BRSP) was determined because it was said to contain freshly produced glomalin (Wright and Upadhyaya, 1998). The seasonal mean values of EE-BRSP and BRSP determined from winter to autumn 2009 in the 0.00-2.00 mm soil fraction of the selected soil environments are shown in Table 71.

3.7.2.1. Seasonal variability in glomalin concentrations

ANOVA was tried to explore the seasonal variations of data in Table 71 within a soil environment and between soil environments. Accordingly, 3 values per season were run when comparing variability within an environment and 21 values per season (3 values x 7 environments) were run to compare the variability between environments (Table 72).

Table 71. Descriptive statistical analysis of the mean seasonal values for easily extractable glomalin (EE-BRSP) and total glomalin (BRSP) in all soil environments.

Season	ENV	EE-BRSP (mg g ⁻¹)					BRSP (mg g ⁻¹)					EE- BRSP /BRSP
		Min	Max	Mean	SD	CV	Min	Max	Mean	SD	CV	
Winter	V	0.34	0.38	0.36	0.02	6.68	0.83	1.32	1.05	0.25	23.90	0.35
	O	0.87	0.96	0.93	0.05	5.90	0.95	1.23	1.12	0.15	13.44	0.83
	S	1.21	1.31	1.25	0.05	4.31	1.90	2.14	2.00	0.12	6.07	0.62
	PI	0.77	0.83	0.80	0.03	3.72	0.50	1.40	0.87	0.47	53.50	0.92
	PR	1.42	1.65	1.51	0.13	8.38	2.54	3.63	3.02	0.56	18.55	0.50
	MC	0.53	1.15	0.91	0.33	36.77	2.11	2.30	2.20	0.10	4.32	0.41
	MB	0.98	1.05	1.01	0.04	3.98	1.92	2.42	2.18	0.25	11.40	0.46
Spring	V	0.38	0.39	0.38	0.01	1.49	0.49	1.66	0.91	0.48	53.25	0.42
	O	0.71	0.89	0.80	0.09	11.17	0.61	2.89	2.07	0.96	46.53	0.39
	S	0.99	1.06	1.03	0.03	3.20	2.13	4.81	3.37	1.06	31.39	0.31
	PI	0.67	0.77	0.73	0.05	6.88	1.00	3.35	2.04	1.14	55.81	0.36
	PR	1.35	1.51	1.41	0.08	5.73	2.65	6.12	4.61	1.56	33.81	0.31
	MC	1.01	1.15	1.07	0.07	6.66	2.36	4.74	3.50	0.92	26.41	0.31
	MB	0.93	1.09	0.98	0.09	9.15	1.58	7.28	4.48	2.03	45.32	0.22
Summer	V	0.58	0.60	0.59	0.01	2.32	0.95	2.22	1.48	0.66	44.47	0.40
	O	0.89	1.06	1.00	0.10	10.09	2.72	9.15	5.42	3.34	61.62	0.18
	S	0.86	1.33	1.02	0.26	25.78	3.11	8.83	5.46	3.00	54.86	0.19
	PI	0.79	1.01	0.87	0.13	14.58	1.96	7.79	4.47	3.00	67.09	0.19
	PR	1.04	1.15	1.08	0.05	5.07	4.76	11.90	7.50	3.85	51.35	0.14
	MC	0.87	0.91	0.89	0.02	2.01	3.48	9.24	5.82	3.03	51.98	0.15
	MB	0.77	0.96	0.84	0.11	12.99	3.16	8.42	5.21	2.82	54.06	0.16
Autumn	V	0.56	0.60	0.58	0.02	3.66	0.68	0.85	0.74	0.10	13.01	0.79
	O	0.92	0.94	0.93	0.01	0.84	2.56	2.90	2.76	0.17	6.31	0.34
	S	0.89	1.02	0.95	0.06	6.60	3.06	3.26	3.12	0.12	3.75	0.30
	PI	0.79	0.81	0.80	0.01	1.70	2.32	2.48	2.41	0.09	3.53	0.33
	PR	1.09	1.13	1.11	0.02	1.86	4.20	4.28	4.26	0.05	1.10	0.26
	MC	0.86	0.89	0.88	0.02	2.01	3.06	3.30	3.17	0.12	3.95	0.28
	MB	0.90	0.95	0.92	0.02	2.66	3.49	3.79	3.64	0.15	4.12	0.25

ENV: Soil environments; V: Soils under vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub.

ANOVA showed that the seasonal pattern of BRSP concentration within each environment was not significant. However data may have been skewed by the lack of tendency for seasonal values to be significantly distributed, as very high BRSP values were found in summer with respect to other seasons. Statistically, the intraclass correlation coefficient (ICC) is known to describe how strongly units in the same group resemble each other. The ICC values were given by using the between-groups mean square, the within-groups mean square, and the number of subjects in each group through the following equation (Kosmelj et al., 2001):

$$ICC = \frac{MS_{Between} - MS_{Within}}{MS_{Between} + MS_{Within}(n - 1)}$$

where $MS_{Between}$ is the between-groups mean square, MS_{Within} is the within-groups mean square, and n is the number of subjects in each group. Small ICC values define non-significantly distributed data along a dataset, whereas large ICC values identify a significant arrangement in a set of data. Effectively small ICC values were found for V (0.136), O (0.372), S (0.360), PI (0.281), PR (0.266), MC (0.312), and MB (0.143) environments corroborating the above mentioned assumptions.

The significance in data variability was also checked between soil environments per each season with ANOVA (Table 72). High significant variability ($p < 0.01$) were found for EE-BRSP values accounting for the diversity in soil response to land use and age of abandonment. Conversely BRSP data showed a high significant variability along winter, spring and autumn 2009 whilst no significance was found in summer, because of the low values recorded in vines with respect to other environments (Emran et al., 2012b). The variation in glomalin contents was mainly attributed to i) the changes in soil use and age of abandonment, and ii) the changes in environmental climatic conditions.

Table 72. ANOVA to check the significant variation of easily extractable glomalin (EE-BRSP) and total glomalin (BRSP) data within and between soil environments.

	EE-BRSP (mg g ⁻¹)		BRSP (mg g ⁻¹)	
	F	p	F	p
Within soil environments				
V	63.097	0.000	1.628	0.258
O	6.876	<i>0.013</i>	3.369	0.075
S	7.393	<i>0.011</i>	2.463	0.137
PI	17.009	0.001	2.563	0.128
PR	17.899	0.001	2.453	0.138
MC	23.493	0.000	2.810	0.108
MB	4.800	<i>0.050</i>	1.670	0.250
Between soil environments				
Winter	58.002	0.000	18.426	0.000
Spring	70.019	0.000	5.611	0.002
Summer	5.029	0.006	1.138	0.391
Autumn	102.593	0.000	258.267	0.000

V: Soils under cultivated vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub. Bold: $p < 0.01$; Italic: $p < 0.05$.

The variability of EE-BRSP data was highly significant within and between environments. The highest EE-BRSP values were always detected in winter but vines followed by a downward trend in other seasons. The seasonal fluctuation as a percent change was calculated as follow;

$$= \frac{\text{highest mean seasonal value} - \text{lowest mean seasonal value}}{\text{lowest mean seasonal value}} \times 100$$

The concentration of EE-BRSP fluctuated significantly through seasons with 64%, 25%, 32%, 19%, 40%, 22%, and 20% change between the lowest and highest average concentrations in V, O, S, PI, PR, MC, and MB environment respectively (Figure 108). Rillig et al. (2003b) documented losses of 11-57% of EE-BRSP and BRSP from Ohio soils (USA) during 400 days incubations whilst Steinberg and Rillig (2003) reported a 25% decline in BRSP from temperate forest soil during five months in laboratory incubations at dark. At field conditions other authors referred to 25% fluctuation of BRSP in grassland sandy loam soils in Missoula, USA from May to November (Lutgen et al., 2003). We found the lowest BRSP production in winter and the highest in summer. Thus, the fluctuation in each environment was 101%, 386%, 173%, 413%, 149%, 165%, and 139% from January to August for V, O, S, PI, PR, MC, and MB soil environments (Figure 108). Seasonal fluctuations of both EE-BRSP and BRSP resulted higher by 21% and 48% in less stable soils (V, O, PI) suggesting deficiency in carbon storage with respect to other more stable soils (S, PR, MC, MB) (Emran et al., 2012b).

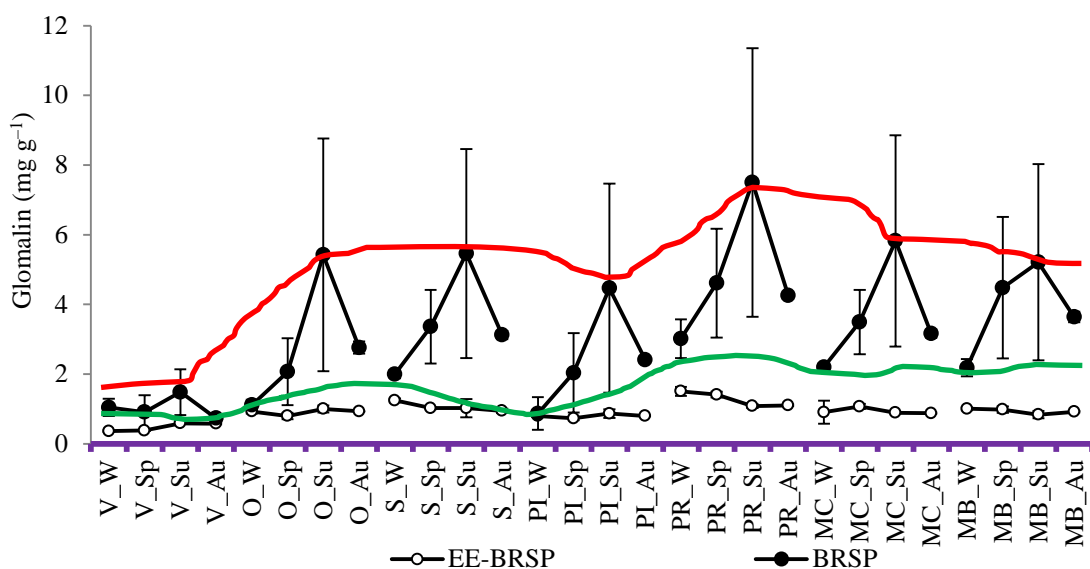


Figure 108. Seasonal patterns and standard errors of easily extractable Bradford reactive soil protein (EE-BRSP) and Bradford reactive soil protein (BRSP) in the seven studied soil environments.

The seasonal trend of EE-BRSP and BRSP showed in Figure 108 may even support that EE-BRSP is probably transformed into recalcitrant carbon along the sequence of land use and abandonment. In this study we have proved that both EE-BRSP and BRSP are subjected to a significant seasonal turnover among the studied soil ecosystems, in agreement with other findings reported in the literature. In spite of the high seasonal fluctuations (between the red and green lines), relatively constant glomalin pools may be expected in the dormant season (under green line) where lower soil biological and root activity occur. However, additional glomalin pools may be captured at the beginning of the growing season. According to soil conditions glomalin pools will increase as following: $V < O < PI < S < MC < MB < PR$. Results in our soils may also suggest a much faster glomalin turnover because of higher EE-BRSP/BRSP ratio with respect to the values recorded in Lutgen et al. (2003). Their EE-BRSP/BRSP ratios were in a range of 0.20-0.30, whilst we found ranges of 0.34-0.79 in V, 0.18-0.83 in O, 0.19-0.62 in S, 0.19-0.92 in PI, 0.14-0.50 in PR, 0.15-0.41 in MC, 0.16-0.46 in MB soils from January to August 2009 (Table 71). The mean annual fluctuation ratios were 0.49 ± 0.20 , 0.44 ± 0.28 , 0.36 ± 0.19 , 0.45 ± 0.32 , 0.30 ± 0.15 , 0.29 ± 0.11 , 0.27 ± 0.13 for the same soil order (Emran et al., 2012b). Lovelock et al. (2004) hypothesized that glomalin turnover may be enhanced in more fertile than less fertile soils according to what they found in a tropical rain forest landscape in Costa Rica. Our results showed that soil environments like V, O, and PI combined less fertility conditions with a much higher turnover with respect to other more fertile environments (S, PR, MC, MB) showing more conservative carbon dynamics, probably suggesting that fluctuation rates may be faster when the labile fraction of organic carbon is more abundant with respect to more stable organic compounds. Field investigations on soil carbon losses showed that soils with less fertility (V, O, PI) emitted a comparatively higher amount of CO_2 with respect to more fertile soils (S, PR, MC, MB) in the same period (Emran et al., 2012a).

Independently of data our results showed that EE-BRSP and BRSP concentrations were higher in those soil environments with better structure as also reported by Rillig et al. (2003a) and Rillig (2004). We found that EE-BRSP and BRSP were highly positively correlated ($r=0.489$, $p<0.01$) corroborating that EE-BRSP may tend to be highly transformed into recalcitrant glomalin forms, mainly depending on soil and environmental conditions (Figure 109). The EE-BRSP/BRSP ratio was always higher in winter than autumn and spring respectively while the lowest ratios were found

in summer in all soil environments but vines, higher in autumn. This ratio increased 3.52, 2.35, 3.71, 2.48, 1.71, and 1.87 folds from summer to winter in O, S, PI, PR, MC, and MB soils respectively and was 0.98 fold higher in autumn than summer in soils under vines. The previous results for environments and seasons suggested that the formation of recalcitrant glomalin fractions may be faster in those soils with high glomalin levels, and this process may probably occur from winter to summer. For example, when observing Figure 109 we may assume that the slope of the regression line indicate that a part of EE-BRSP is transformed to BRSP along seasons.

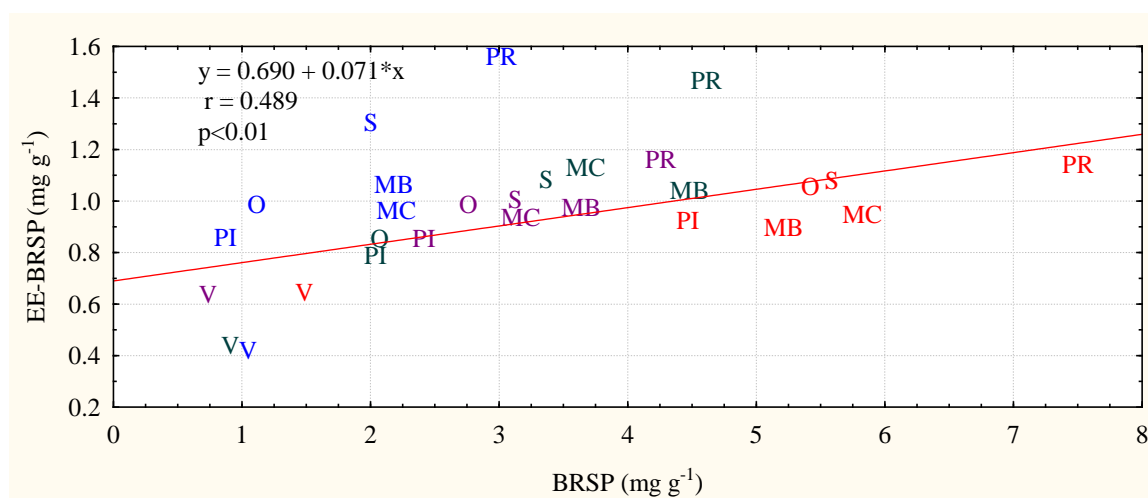


Figure 109. Linear regression equation, correlation, and significant level between the seasonal means of EE-BRSP and BRSP for the selected soil environments. Blue color: Winter; Dark green color: Spring; Red color: Summer; Violet color: Autumn.

3.7.2.1.1. Impact of land use change on glomalin concentrations

The BRSP content varied among the studied soils and the mean annual average was in this ascending order 1.04, 2.45, 2.84, 3.49, 3.67, 3.88, 4.85 mg g⁻¹ for V, PI, O, S, MC, MB, PR soil environments. Low agricultural management as in soils under cultivated vines and olive groves is able to decrease the production of glomalin probably because of i) the low fungal biomass productivity, ii) the low organic substrates available for AMF, iii) the higher oxidation of organic matter content includes the labile fraction of glomalin. In addition, plant species such as pine trees, may be able to minimize the microorganisms metabolic activity due to worse soil conditions. Moreover, AMF have low ability to colonize the roots of pine trees (Wagg et al., 2011). However, different plant species can supply the AMF colonization and therefore the production of glomalin may increase according to the higher active organic

matter available for these groups of fungi. The highest glomalin content was found in soils under pasture. The predominant herbaceous species such as *Brachipodium retusum*, *Lavandula stoechas*, *Trifolium stellatum*, *Dactylis glomerata*, and *Ulex parviflorus* together with Cistaceae associations such as *Cistus monspeliensis* and *Calicotome espinosa* and Ericaceae associations such as *Erica arborea* may support the colonization around the roots of AMF groups in soils under PR, MC, and MB environments. However, further investigations are needed to clearly identify the plant species forming AMF associations.

3.7.2.1.2. Impact of environmental changes on glomalin concentrations

The environmental changes have negative/low or positive/high impact on the production of glomalin (BRSP). This picture can be displayed in Figure 110. The increasing (positive bars) and the decreasing (negative bars) proportion of glomalin production was calculated on the basis of the mean annual average of glomalin produced during the investigated year (2009) for each soil environment. The mean annual averages of BRSP were 1.04, 2.84, 3.49, 2.45, 4.85, 3.67, and 3.88 mg g⁻¹ for V, O, S, PI, PR, MC, and MB soil environments respectively. These values are representing the zero values on the graph for each soil environment (Figure 110). The changes in climate conditions at all environments may have stimulated the soil biological ecosystems as in summer season, positively favoring the production of glomalin with respect to other seasons. As reported elsewhere, it can be observed that the highest proportional differences were found in V, O, and PI probably indicating less stability of the soil ecosystems.



Figure 110. The proportional seasonal variations in glomalin production (BRSP) with respect to its mean annual average for all soil environments.

3.7.3. Glomalin production and fungal populations

Another interesting point is the relationship between the EE-BRSP fractions and the total fungal populations along the seasonal changes under environmental soil conditions (Figure 111).

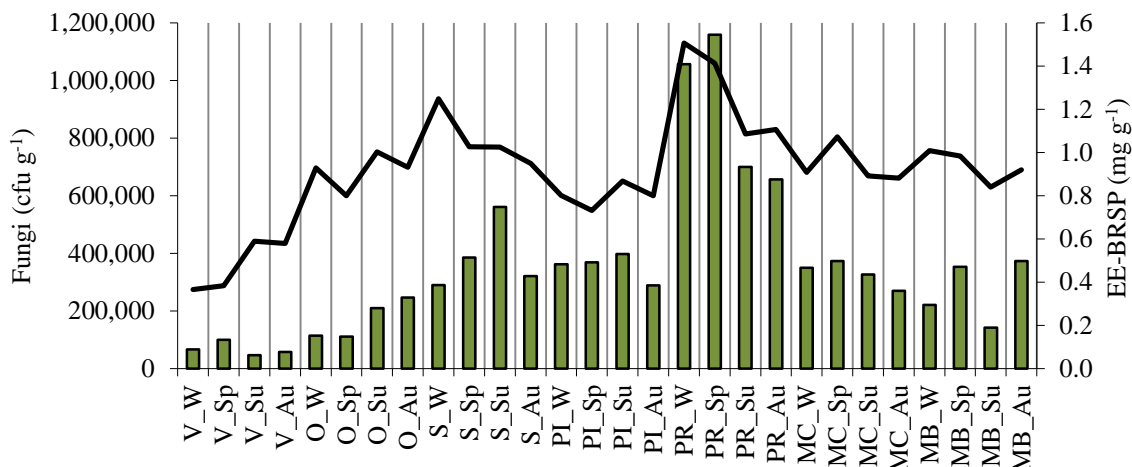


Figure 111. The seasonal trend of EE-BRSP (black line) and the fungi (dark olive color bars) for all soil environments.

Even though the fungi-producing glomalin (AMF) were not identified, this picture closely indicate the predomination of the AMF groups in the studied soils, especially in soils under forest ($S > PI$), under scrubs ($MC = MB$), and under pasture (PR), where the highest amount of freshly produced glomalin (EE-BRSP) was recorded. As reported in the literature AMF appear to be the most important fungal mediators of soil aggregation because they represent a dominant biological soil component accounting for 30% of total soil microbial biomass (Rillig et al., 1999; Borie et al., 2008). Positive significant correlations were found when relating both glomalin fractions to fungi (Figure 112A and B) corroborating the above mentioned mechanisms of soil arbuscular mycorrhizal fungi in the studied soil environments. It is also observed that the labile glomalin fraction (EE-BRSP), ephemeral deposited in soil, is highly correlated to soil fungal populations than the other glomalin fraction (BRSP) with longer resident time. Moreover the trend in increasing both EE-BRSP and BRSP concentration is respecting the sequence of the soils under study.

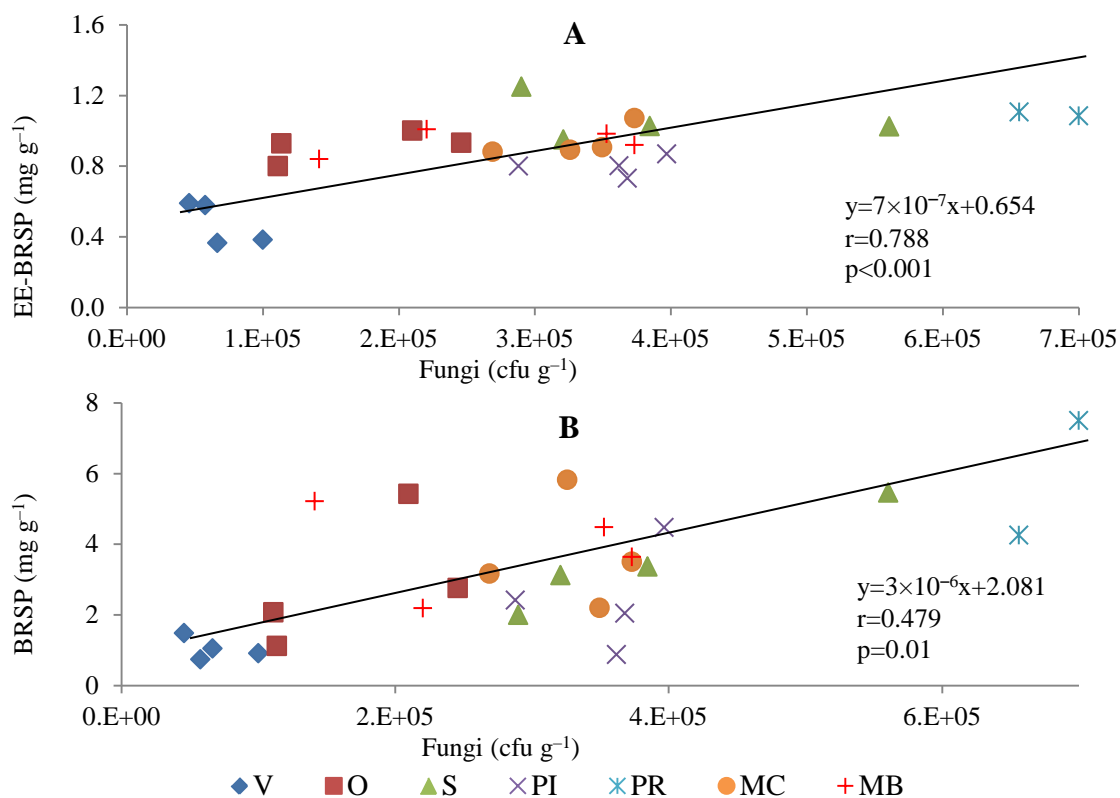


Figure 112. Linear regression equation between easily extractable glomalin (EE-BRSP) (A) and total glomalin (BRSP) (B) versus fungal populations for all soil environments.

3.7.4. Deposition of both glomalin fractions in the studied soils

As the freshly produced glomalin is significantly correlated with the fungal propagules, the dynamics of EE-BRSP and BRSP (recalcitrant glomalin) in soil should be distinguished. In the literature, the BRSP was initially identified as the total amount of glomalin extractable from the soil containing older fractions of glomalin, whereas the EE-BRSP contained freshly produced glomalin (Wright and Upadhyaya, 1998). More recent researches have demonstrated that these theories were not correct (Nichols and Wright, 2005; Steinberg and Rillig, 2003). A more recalcitrant fraction of glomalin was removed from soil by first extracting the soil humic acids with 0.1 N sodium hydroxide and successively extracting glomalin with 50 mM sodium citrate at pH 8.0 (Nichols and Wright, 2005). Other authors said that glomalin may move in and out of the BRSP and EE-BRSP pools regardless of incubation time (Steinberg and Rillig, 2003). This may indicate that these procedures may not be able to separate the oldest glomalin fraction by age but it is just operationally defining glomalin pools.

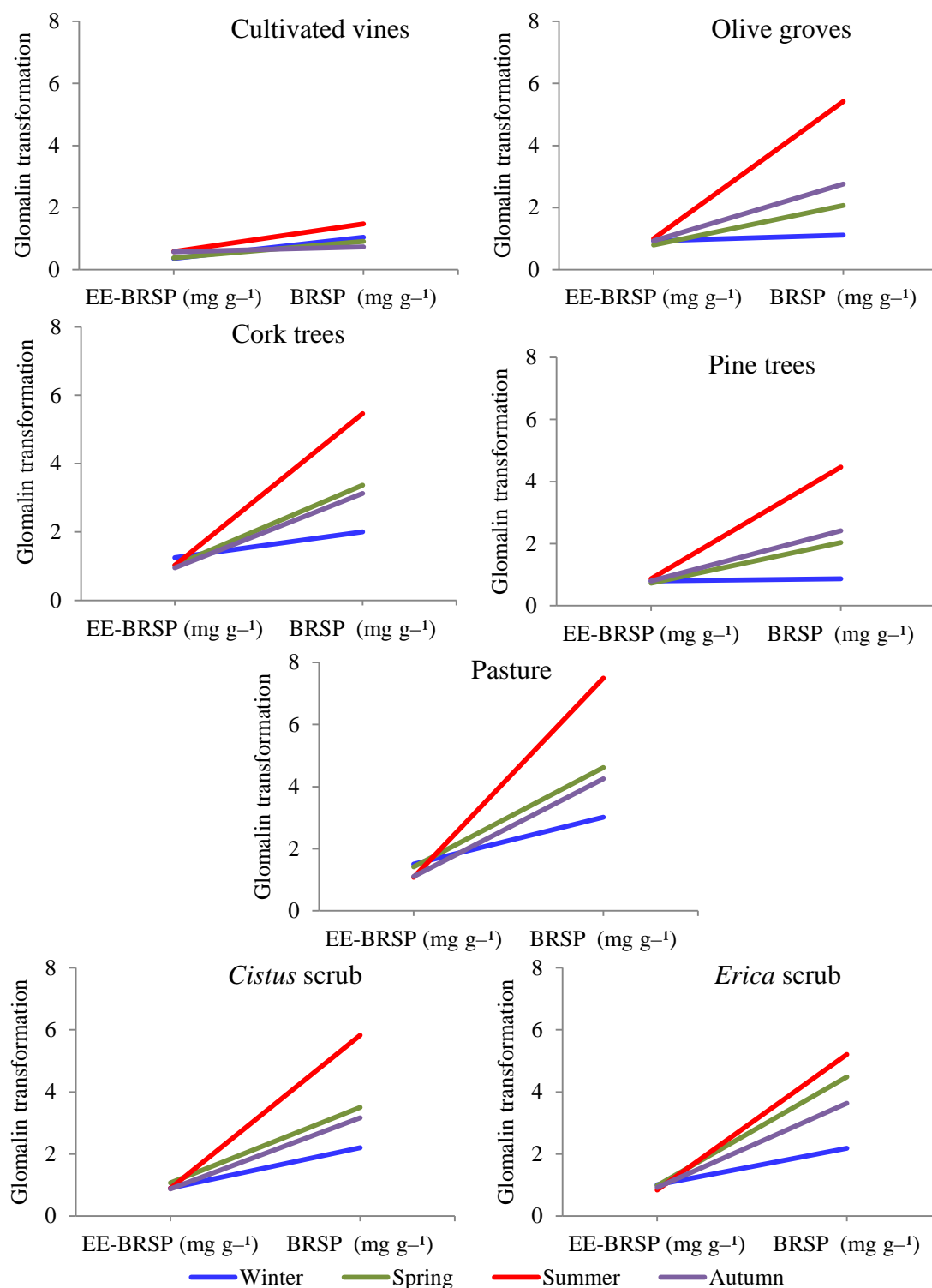


Figure 113. Patterns of seasonal glomalin fractions at all soil environments.

However, the two types of glomalin extracted can indicate the differences in carbon storage capacity among the studied soils differing in use and age of abandonment. The so called freshly produced glomalin (EE-BRSP) and the total glomalin (BRSP) were then compared. The more recalcitrant glomalin fraction cited by Nichols and Wright (2005) was not detected by our methods. The glomalin increase in

soils can be interpreted by focusing upon the seasonal increasing patterns of both glomalin fractions at each soil environment (Figure 113). The supposed glomalin transformation seems to be related also to the type of management and changes. Thus, the two fractions studied may be able to highlight the differences in glomalin pools in these peculiar soils.

Accordingly, it can be postulated that the “fresh” glomalin ratio may be conceptually seen as the ratio of the EE-BRSP to the BRSP that is: EE-BRSP/BRSP ratio. The “old” fraction (recalcitrant) of glomalin produced and deposited along the time should be identified in this study as the “non-easily or old” extractable glomalin i.e. $\text{NON EE-BRSP} = \text{BRSP} - \text{EE-BRSP}$, that is: NON EE-BRSP/BRSP ratio. This theoretical model of transformation/deposition of glomalin pools is shown by plotting the ratios of fresh and older fractions of glomalin as a function of BRSP, as a total glomalin pool (Figure 114).

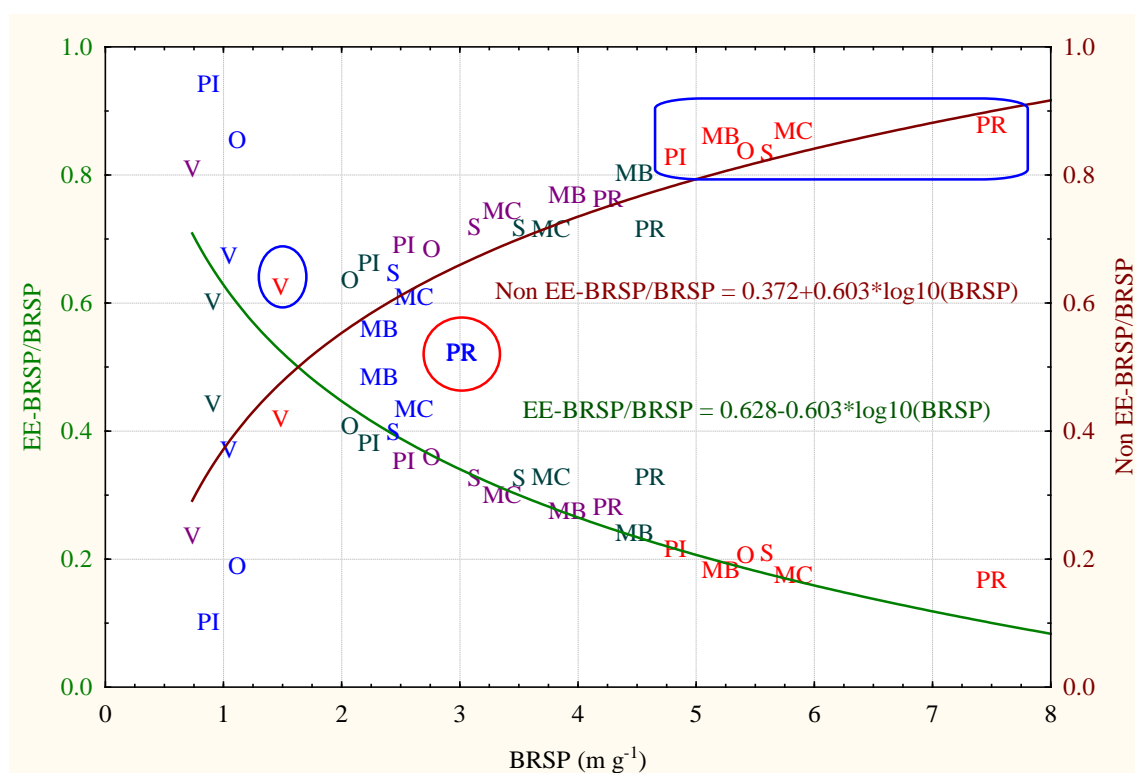


Figure 114. The seasonal distribution of the two ratios (fresh EE-BRSP/BRSP and old NON EE-BRSP/BRSP) of glomalin pools for all soil environments. Blue color: Winter; Dark green color: Spring; Red color: Summer; Violet color: Autumn.

Significant negative and positive correlations have been obtained for “fresh” and “old” glomalin ratios respectively. The equation for the fresh glomalin ratio was: EE-

$BRSP/BRSP=0.654-0.089BRSP$, $r=-0.768$, $p<0.001$. The linear regression equation for the old glomalin ratio was: $NON\ EE-BRSP/BRSP = 0.346+0.089BRSP$, $r=+0.768$, $p<0.001$. Despite the significant linear correlations obtained, the logarithmic equations fitted well with the seasonal distribution patterns of both glomalin ratios for all soil environments. If higher fresh glomalin may be produced in winter in almost all soil environments, the ageing process to form more recalcitrant glomalin may occur in summer, when higher values of BRSP are recorded. As can be observed in the graph the red ring indicates that similar ratios of $EE-BRSP/BRSP=0.5$ and $NON\ EE-BRSP/BRSP=0.5$ were found in the soils under pasture in winter. The 1:1 ratio between the two fractions may indicate that lower recalcitrant amounts of glomalin is formed in winter and may increase through seasons as $NON\ EE-BRSP/BRSP$ with ratios of 0.69, 0.86, and 0.74 were found for spring, summer, and autumn respectively. In summer, more than 80% of the “old” fraction may be formed in all soils but only 60% in vines.

3.7.5. Factors affecting the deposition of both glomalin fractions

3.7.5.1. *Atmospheric CO₂*

The increase in atmospheric CO₂ is one of the most relevant manifestations of global climate changes also affecting the glomalin production as changes in land use and age of abandonment do. In earlier studies, Wright and other scientists from the University of California at Riverside and Stanford University (Rillig and Allen, 1999; Rillig et al., 1999; Rillig et al., 2001a; Treseder et al., 2003; Treseder, 2005; Treseder and Turner, 2007) showed that higher CO₂ levels in the atmosphere stimulate the fungi to produce more glomalin. They did a 3-year study on semiarid shrub land and a 6-year study on grasslands in San Diego County, California, using outdoor chambers with controlled CO₂ levels. When atmospheric CO₂ reached 670 parts per million (ppm), the level predicted by mid to late 20th century, hyphae grew three times as long and produced five times as much glomalin as fungi on plants growing with today CO₂ level of 370 ppm. Elevated atmospheric CO₂ can thus enrich glomalin concentrations, as illustrated also by Rillig et al. (1999) for IRSP in serpentine and sandstone grasslands in northern California and in chaparral in southern California. Similar responses have been observed for EE-BRSP and BRSP in a natural CO₂ spring in New Zealand (Rillig et al., 2000) and for EE-BRSP in a free-air CO₂ enrichment experiment in a Arizonan

sorghum [*Sorghum bicolor* (L.) Moench] field, Maricopa, USA (Rillig et al., 2001a). These effects of CO₂ on glomalin are consistent with increases in AMF abundance observed in many field manipulations of CO₂ (Treseder, 2004), and they may be caused by the augmentation of investment by plants in AM fungi as photosynthate becomes more available and soil nutrients become more limiting (Treseder and Turner, 2007). Likely, the same trend is observed at field condition in soils with a higher range of CO₂ concentration. We used the infrared gas analyzer method (IRGA) to measure the atmospheric CO₂ concentration at each soil sampling date together with glomalin (EE-BRSP and BRSP) determinations. All data were recorded in triplicates for each soil environment. At field conditions the seasonal mean of atmospheric CO₂ concentrations increased from 252±24 ppm in winter to 467±15 ppm in summer. Results showed a positive significant linear correlation between the BRSP and atmospheric CO₂ concentration in the seven studied soil environments (Figure 115).

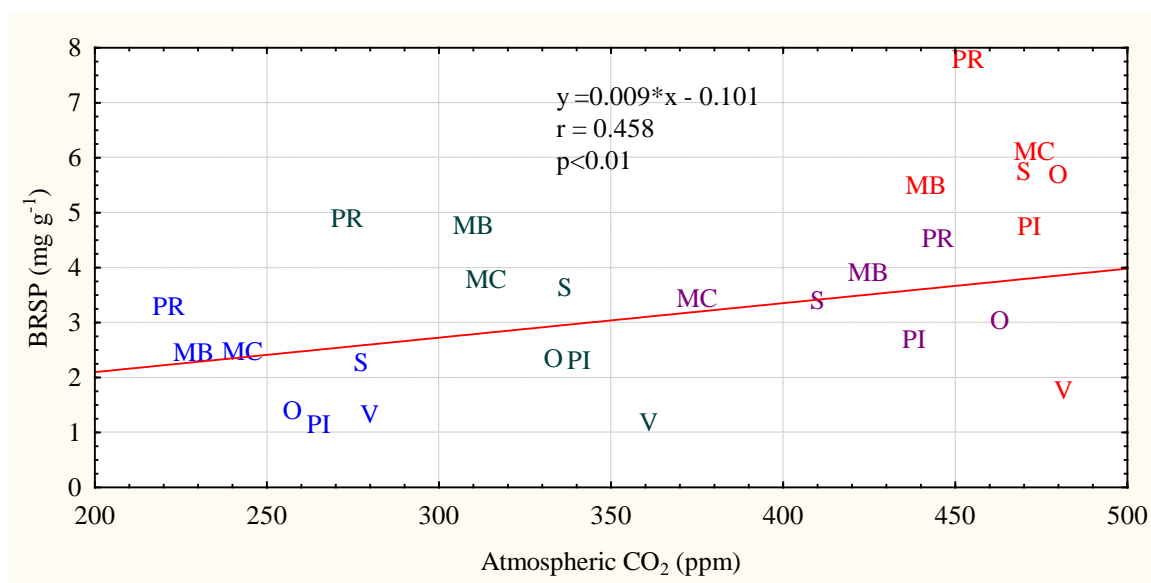


Figure 115. Significant linear regression equation between the BRSP and atmospheric carbon dioxide concentrations along the seasons for all soil environments. Blue color: Winter; Dark green color: Spring; Red color: Summer; Violet color: Autumn.

A positive correlation was also found between the EE-BRSP and atmospheric CO₂ by using only data obtained from all soil environments during summer and autumn (Figure 116). The environments included in the square were not used. We assumed that when more soil nutrients become available to the host plants, like in winter and spring (red square on the graph), plants may not allocate more nutrients to their AM symbionts and therefore the EE-BRSP production increases (Treseder, 2004; Treseder and Turner,

2007). Elevated atmospheric CO₂ consistently increases AMF abundance (Treseder, 2004) either because plants are more N or P limited, photosynthate is more readily available, or both (Treseder and Turner, 2007). Perhaps the most widely studied CO₂ effect on fungi is the response of mycorrhizal groups. Since these symbionts receive carbohydrates directly from plants, they should be directly influenced by the increased photosynthetic capacity of their hosts under elevated CO₂. Nitrogen (N) or phosphorus (P) tend to become more limiting to plant growth under these conditions, so plants may allocate excess carbon (C) to mycorrhizal fungi in order to improve nutrient acquisition through their production of glomalin (Treseder, 2005).

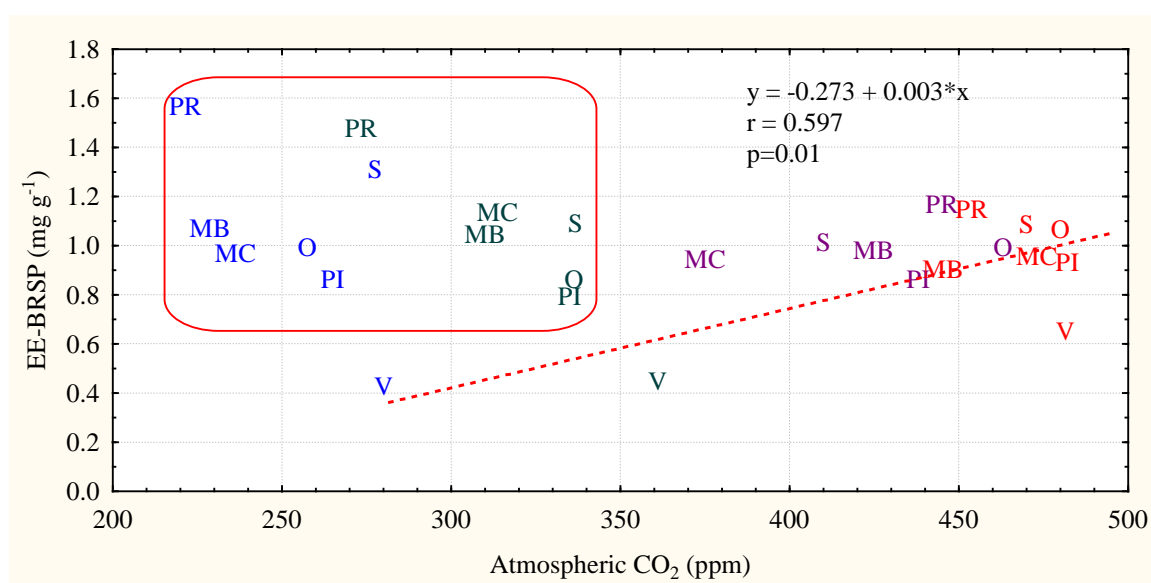


Figure 116. The relationship between the EE-BRSP and atmospheric carbon dioxide concentrations for all soil environments. Blue color: Winter; Dark green color: Spring; Red color: Summer; Violet color: Autumn.

3.7.5.2. Soil organic reserve

Nutrients are generally cycled in soil through the interactions of biological, physical, and chemical components. The rhizosphere, the location of the greatest flow of energy and minerals among these components (Wright and Millner, 1994), is the highly productive region where a vital symbiosis, AMF, exists between roots of 80% of all vascular plant species (Wright et al., 2006). AMF help plants to capture nutrients such as carbon, nitrogen, phosphorus, sulfur and micronutrients from soil. When soil nutrients are limited and plants are non-mycorrhizal, these plants may produce exudates to acquire as many of these nutrients as possible. The opportunistic biochemical

transportations of soil minerals between plant roots and AM fungi through the mutualistic symbiotic relationship can influence the biochemical metabolic pathways of the geochemical nutrient cycles in the rhizosphere. Glomalin, the most manifested character of soil AMF in association with the root of the majority of land plants, may provide plant available nutrients through the principle of substitutability.

Different authors have identified glomalin as an N-linked glycoprotein (Wright and Upadhyaya, 1998; Wright et al., 1998) because of the presence of N-linked oligosaccharides on AMF hyphae. Lovelock et al. (2004) have quantified C and N in glomalin within a range of 28-42% and 3-5% respectively. Nichols and Wright (2006) have assessed the amount of C and N in five USA soils of the extracted glomalin pools for an average of 73% and 31% of the total C and N, respectively. Glomalin accounted for 25% and 52% of total C in the mineral soils and organic soil respectively (Schindler et al., 2007).

3.7.5.2.1. *Organic carbon*

As resulted that carbon is forming a major part of glomalin, glomalin is highly produced in soils rich in their carbon content and in the rhizosphere zone of the plants more adequated to capture the atmospheric carbon dioxide and convert it into organic carbon by photosynthetic processes. Therefore, a positive relationship between SOC and glomalin is found among soil ecosystems differing in their organic carbon content (Emran et al., 2012b). Highly significant positive correlations were found when seasonal SOC values were plotted against both EE-BRSP and BRSP seasonal values recorded in the studied soils (Figure 117A and B).

The linear regression equations are representing the clear increase of glomalin and soil carbon along different soil environments, depicting the soil evolution after abandonment, from cultivated soils to soils under stands of cork and pine trees, soils under abandoned scrubs and under pasture, especially with successful soil/plant species interactions. This trend strengthens the behavior of both parameters in soil environments, indicating that the labile glomalin fraction (EE-BRSP) may be partly easily mineralized and partly transformed into more recalcitrant carbon. The clear increase of glomalin along different soil environments may probably depend on the

maturity of soil and its interaction with plant species (Seguel et al., 2008). Findings suggested that glomalin production was lower in soil environments with unsuited cultivation practices (V, O) and wildfire perturbation (PI, S, MC) where soil evolution might be affected, with respect to environments (PR, MB) showing better soil conditions. The dynamics of glomalin transformation into more recalcitrant fraction is likely to occur in summer season than in spring > autumn > winter (Emran et al., 2012b).

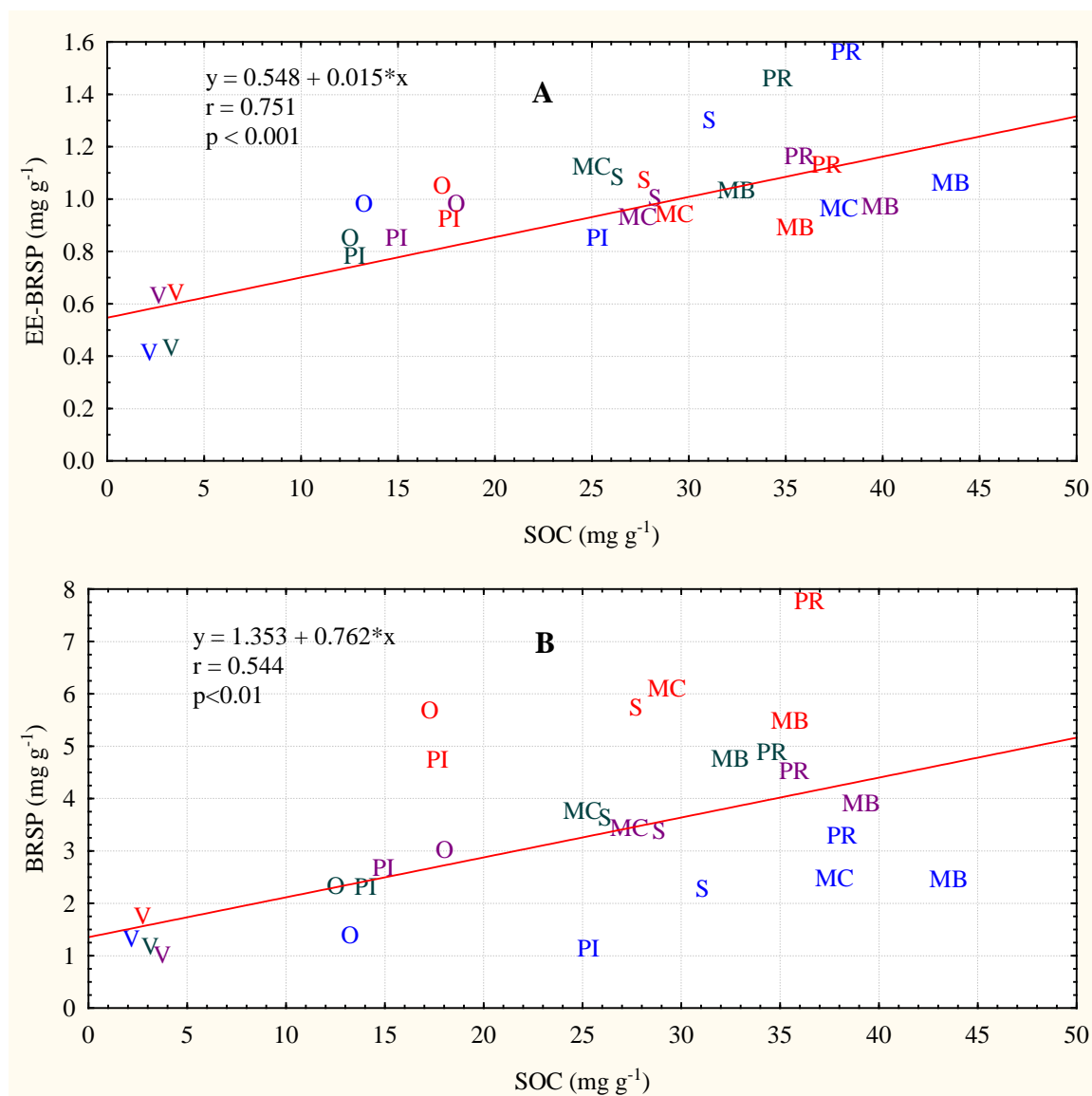


Figure 117. Linear regression equations, correlations, and significant levels between the mean seasonal values of both glomalin fractions (A: EE-BRSP and B: BRSP) and soil organic carbon (SOC) contents for the selected soil environments during 2009. Blue color: Winter; Dark green color: Spring; Red color: Summer; Violet color: Autumn.

However, the glomalin production and its relationship with soil organic carbon seemed to be particularly different within soil ecosystems. During the observed period,

the carbon resource used for building glomalin units may have been either soil carbon or photoassimilated carbon or both depending on the environmental and soil condition at any period of the year. For example, Veresoglou et al., (2011) reported that in the absence of P fertilization, there are extensive flows of photoassimilated carbon into the rhizosphere that support diverse root associated microbial communities, including AMF. Typically, between 12% to 27% of photoassimilated carbon is given to AMF (Johnson et al., 1997; Nichols, 2003). Conceptually, the production of glomalin in soils with low carbon content and low plant colonization may be more dependent on soil organic carbon pool, while glomalin production may be less dependent from SOC in soils with high organic carbon content because of abundance of plant species able to photoassimilate more carbon to be utilized by symbiotic AM fungal groups (Nichols, 2003). The photoassimilated carbon may be much more easily captured in summer (Palmqvist, 2002), when the highest amount of glomalin is produced. An example of this assumption may be seen in Figure 118.

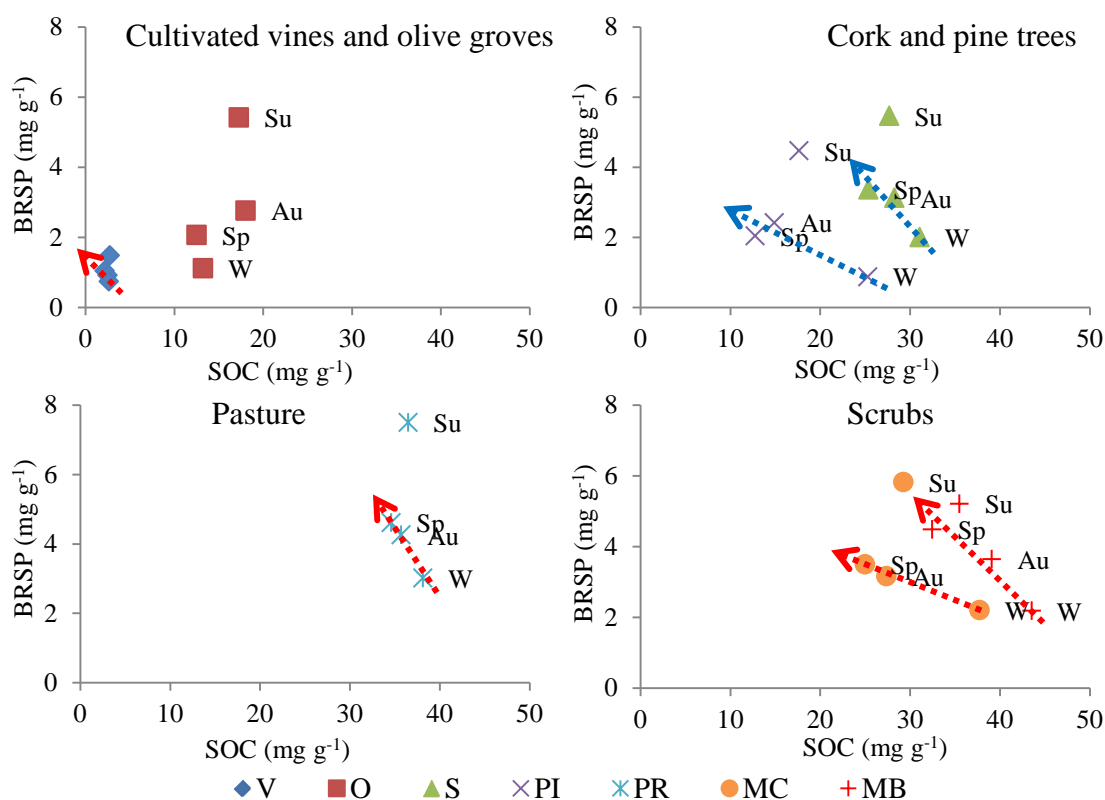


Figure 118. Relationships between total glomalin (BRSP) production and soil organic carbon (SOC) for the selected soil environments during the four seasons of 2009.

Cultivated soils (O) showed that BRSP concentration increases with increasing SOC whilst a different trend is shown by other soils (V, S, PI, PR, MC, and MB). It was

postulated that during winter, spring and autumn, SOC stock was mainly used by AMF to form BRSP, but the whole system was also subjected to loss of carbon dioxide with the result of SOC amount decrease when BRSP amount increase. We may assume that this mechanism seems to be reversed in summer, when AMF may use photoassimilated carbon which in turn may produce an increase of SOC. Effectively, by excluding the summer measurements, significant negative correlations (Table 73) were obtained in V, PR, MC, and MB soil environments (dotted red arrow lines in Figure 118 are showing the increasing of glomalin production from winter to spring and autumn excluding summer values). No significant correlations were observed in S and PI soil environments though the same trend was maintained (dotted blue arrow lines Figure 118 are showing the increasing of glomalin production from winter to spring and autumn excluding summer values). This interpretation may suggest a hypothesized balance between BRSP formation and SOC consumption.

Table 73. Linear regression equations between total glomalin production and soil organic carbon during winter, spring, and autumn 2009 for the selected soil environments.

ENV	equations	r	p-level
V	$y = -0.602x + 2.359$	0.955	<0.05
O	$y = 0.205x - 1.010$	0.741	NS
S	$y = -0.238x + 9.561$	0.935	NS
PI	$y = -0.111x + 3.732$	0.922	NS
PR	$y = -0.461x + 20.623$	0.995	<0.01
MC	$y = -0.099x + 5.934$	0.997	<0.01
MB	$y = -0.202x + 11.182$	0.965	<0.05

ENV: Soil environments; V: Soils under vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub.

3.7.5.2.2. Total nitrogen

Glomalin has reported to have also a relevant pool of soil nitrogen (Nichols and Wright, 2006) estimated to be nearly 5% of total N. This nitrogen should be related to the N-glycosidic bonds and amino acids forming the glycoprotein molecules (Schindler et al., 2007). Thus the requirements of nitrogen to build up glycoprotein units within glomalin molecules have favored significant positive correlations between total nitrogen and both glomalin fractions (Figure 119A and B).

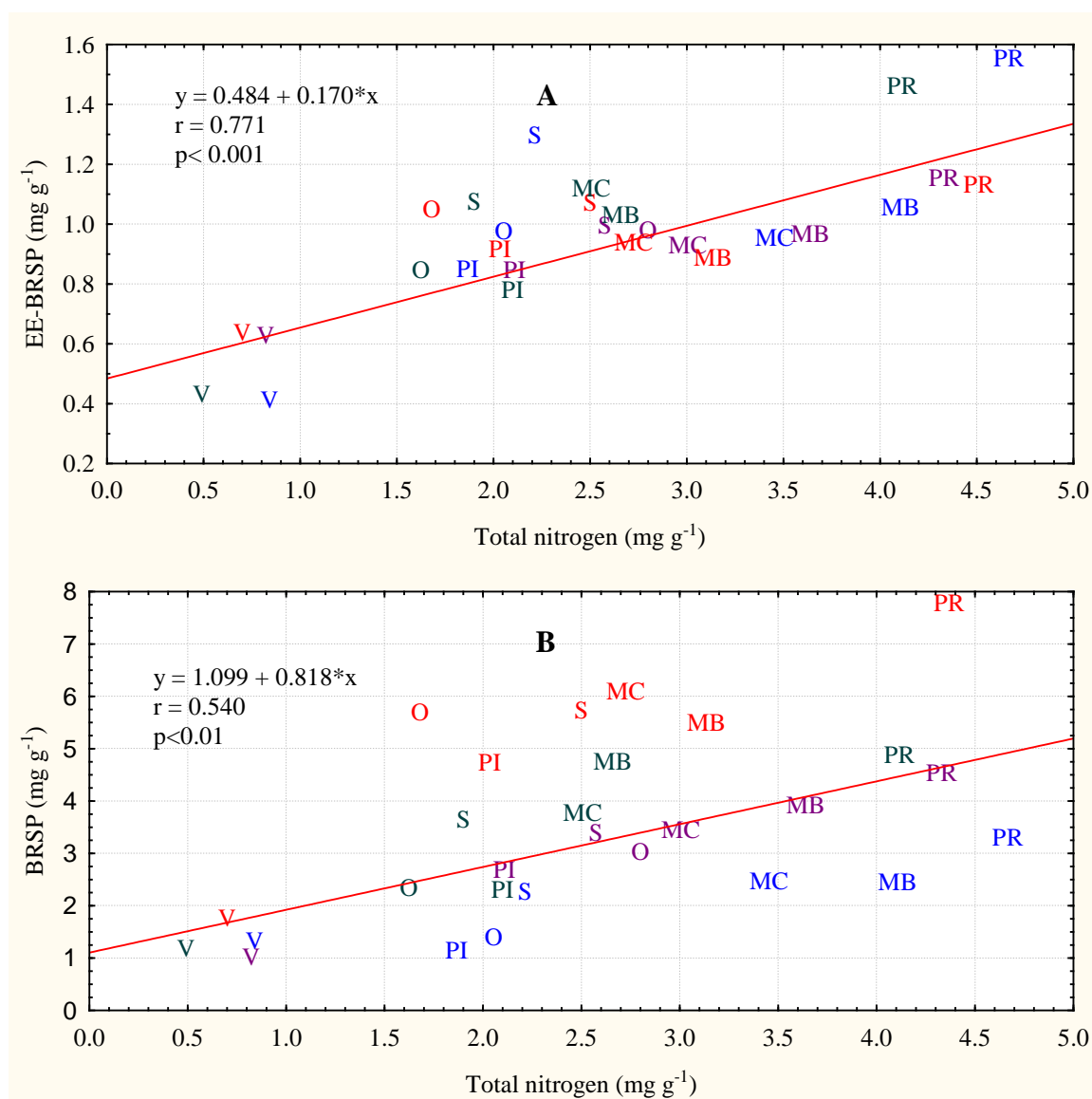


Figure 119. Linear regression equations, correlation, and significant levels between the mean seasonal values of both glomalin fractions (A: EE-BRSP and B: BRSP) and total nitrogen content for the selected soil environments during 2009. Blue color: Winter; Dark green color: Spring; Red color: Summer; Violet color: Autumn.

The positive and negative trends in BRSP production as a function of soil nitrogen may be observed in Figure 120. Glomalin production in soils under V, O, S, and PI environments showed a rather unclear trend. Conversely glomalin production seemed to depend on soil nitrogen content in those soils with high total nitrogen content (PR, MC, and MB environments), therefore depicting negative trend.

By excluding the summer measurements because of supposed higher photosynthetic N assimilation that may be utilized for metabolic microbial activity (Vasconcelos et al., 2001), significant negative correlations (Table 74) were also obtained

in soils under pasture and *Cistus* scrubs (dotted red arrow lines in Figure 120 are showing the increasing of glomalin production from winter to spring and autumn excluding summer values). Though no significance was found in the negative trend observed in MB soil environment, dotted blue arrow line in Figure 120 showed the increasing of glomalin production from winter to spring and autumn excluding summer values.

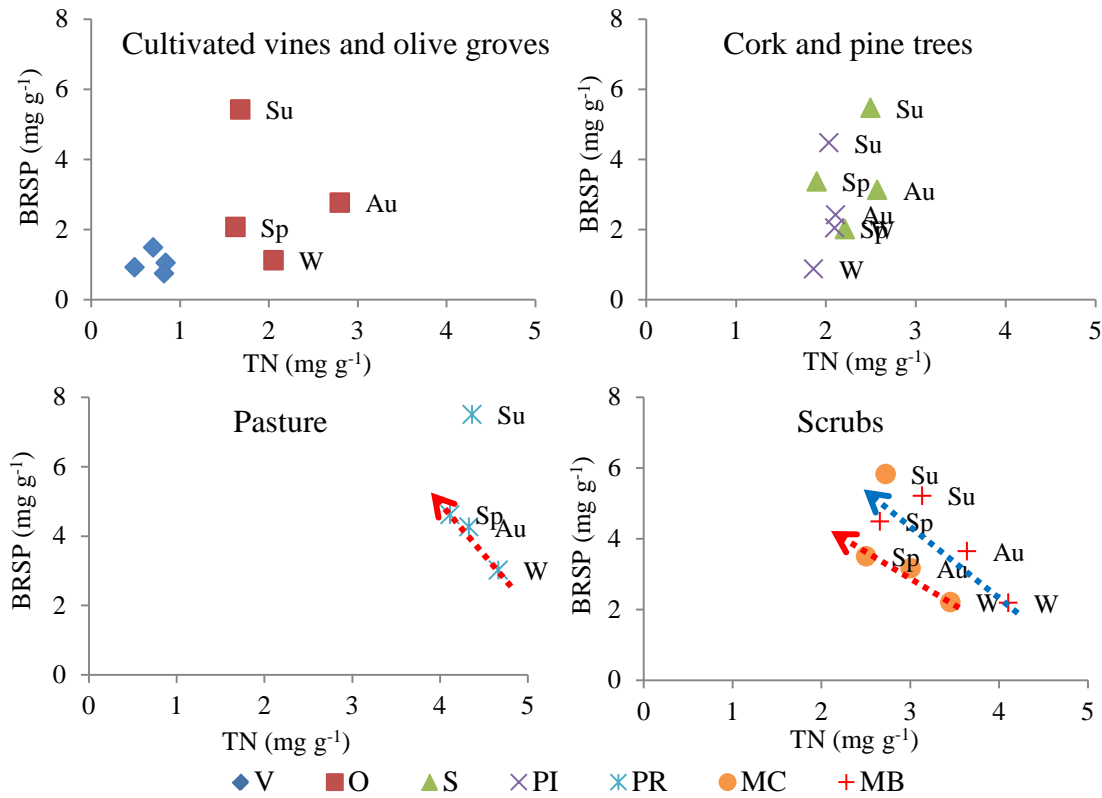


Figure 120. Relationships between total glomalin (BRSP) production and total nitrogen (TN) for the selected soil environments during the four seasons of 2009.

Table 74. Linear regression equations between total glomalin production and total nitrogen during winter, spring, and autumn 2009 for the selected soil environments.

ENV	Equation	r	p-level
V	$y = -0.019x + 0.911$	0.022	NS
O	$y = 0.768x + 0.323$	0.553	NS
S	$y = -0.276x + 3.444$	0.128	NS
PI	$y = 5.731x - 9.833$	0.980	<0.01
PR	$y = -2.967x + 16.931$	0.983	<0.01
MC	$y = -1.358x + 7.011$	0.954	<0.05
MB	$y = -1.478x + 8.561$	0.938	NS

ENV: Soil environments; V: Soils under vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub.

3.7.5.2.3. Total phosphorus

As reported by Bücking and Shachar-Hill (2005) an increase in carbon supply by plant to AMF increased the phosphorus uptake and transfer from fungi to plant. Species of AMF differ in their abilities to supply the plant with phosphorus (Smith et al., 2003). The significant positive correlation observed when plotting the seasonal means of the freshly produced glomalin fraction (EE-BRSP) against the total phosphorus content in the studied soils (Figure 121) may suggest the relevance of phosphorus in this kind of mechanisms. The seasonal relationship between BRSP and total phosphorus was not clear.

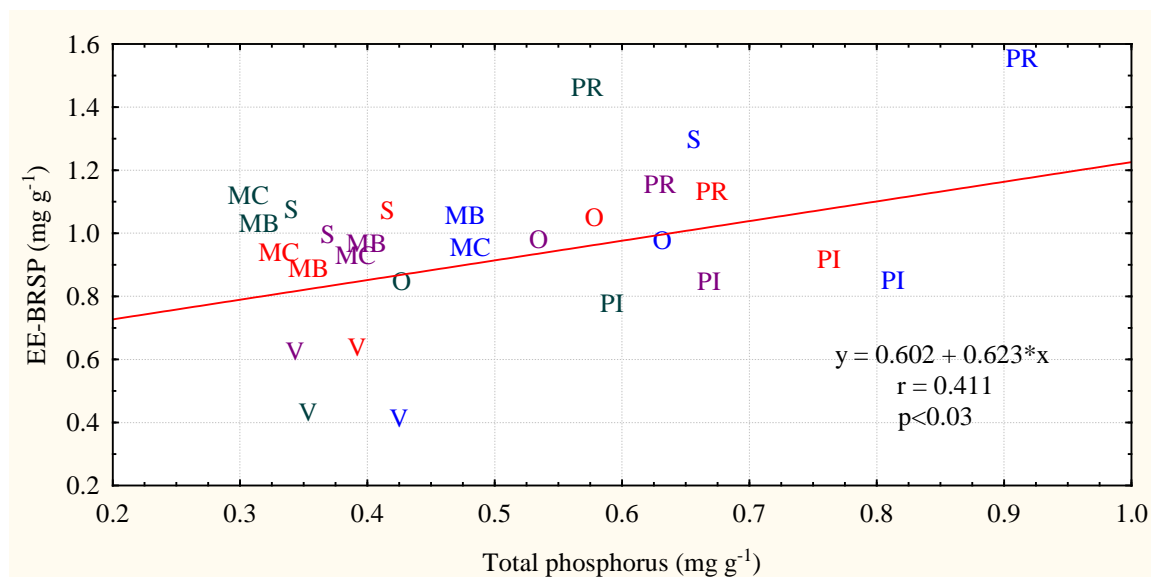


Figure 121. Linear regression equation, correlation, and significant level between the mean seasonal values of freshly produced glomalin fraction (EE-BRSP) and total phosphorus content for the selected soil environments during 2009. Blue color: Winter; Dark green color: Spring; Red color: Summer; Violet color: Autumn.

The behavior of each fungal group at each soil environment may be different because of the variations in plant cover colonization (Figure 122). Soils under pasture had high P and higher glomalin content while soils under pine trees had high P and low glomalin content. Soils with low fertility (V, O, and PI) have shown that produce less glomalin with respect to the other investigated soils. Significant negative correlations (Table 75) were obtained in S, PR, MC, and MB soil environments (dotted red lines in Figure 153 are showing the increasing of glomalin production from winter to spring and autumn excluding summer values) and TP decreasing, while no significance was found in soils under olive groves and stands of pine trees (dotted blue lines in Figure 122 are

showing the increasing of glomalin production from winter to spring and autumn excluding summer values). The data sets of winter, spring, and autumn may have indicated that glomalin is highly produced as a result of stimulating the AMF by root plants to supply the required nutrients (TP) from soils to plants.

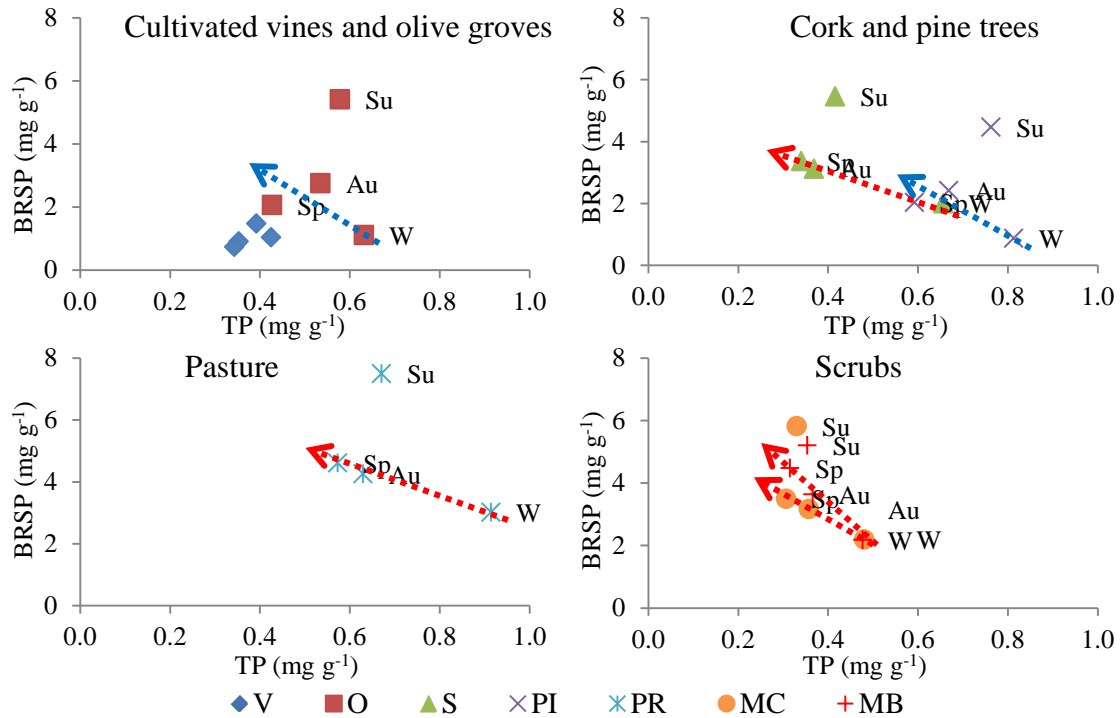


Figure 122. Relationships between total glomalin (BRSP) production and total phosphorus (TP) for the selected soil environments during the four seasons of 2009.

Table 75. Linear regression equations, correlations, and significant levels between total glomalin production and total phosphorus during winter, spring, and autumn 2009 for the selected soil environments.

ENV	Equation	r	p-level
V	$y = + 0.309x - 0.259$	0.887	NS
O	$y = - 0.446x + 4.352$	0.553	NS
S	$y = - 0.415x + 4.719$	0.996	<0.01
PI	$y = - 0.599x + 5.912$	0.835	NS
PR	$y = - 0.457x + 7.189$	0.998	<0.01
MC	$y = - 0.753x + 5.827$	0.999	<0.01
MB	$y = - 1.407x + 8.865$	0.998	<0.01

ENV: Soil environments; V: Soils under vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub.

A correlation matrix was carried out by using the mean seasonal values of both glomalin fractions, pH, electrical conductivity (EC), soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), C/N ratio, C/P ratio, and N/P ratio (Table 76).

Negative correlations were observed between soil pH and both glomalin fractions with lack of significance. However, significant positive correlations were obtained with the total dissolved salts (EC) among the studied soils. The highly significant positive correlations between EE-BRSP and BRSP and SOC, TN and TP strengthened the relationships between glomalin production and soil nutrients.

Table 76. Correlation matrix between the seasonal means of some soil characteristics and both glomalin fractions during 2009 for the studied soil environments.

Glomalin fractions	pH	EC	SOC	TN	TP	C/N	C/P	N/P
EE-BRSP	-0.339 p=0.077	0.552 p=0.002	0.751 p=0.000	0.771 p=0.000	<i>0.411</i> <i>p=0.030</i>	0.548 p=0.003	0.459 p=0.014	0.472 p=0.010
BRSP	-0.282 p=0.146	0.500 p=0.007	0.544 p=0.003	0.540 p=0.003	-0.018 p=0.929	<i>0.394</i> <i>p=0.038</i>	0.530 p=0.004	0.551 p=0.002

EC: Electrical conductivity; SOC: Soil organic carbon; TN: Total nitrogen; TP: Total phosphorus; C/N: Carbon to nitrogen ratio; C/P: Carbon to phosphorus ratio; N/P: Nitrogen to phosphorus ratio. Bold: $p < 0.01$; Italic: $p < 0.05$.

3.7.6. Soil fertility and deposition of glomalin fractions

A principal component analysis (PCA) was used to test the relationship between the soil characteristics and the glomalin fractions. In the PCA analysis each eigenvector consists of the eigenvalue of each variable. The contribution of each soil characteristic to each eigenvector is shown in Table 77. The first eigenvector explained 51.86% of the total variation in variables (Table 77) and was characterized by high negative contributions of pH and exchangeable Al and very low negative contribution of exchangeable Ca and K. The other soil variables contributed positively to this vector. The highest positive contribution was from SOC, TN, and exchangeable Mg probably due to their average higher concentrations in those soils favoring higher fertility levels. EC, CEC, C/N, N/P, C/P were also positively loaded. Very low loading was found in soil phosphorus (TP) due to the very low concentrations found in all the studied soils, though high positively loaded in the third vector. The first vector can better explain the chemical soil fertility though the second and third vector should be also taken into account. Accordingly, glomalin data were related to the factor score values of the first axis. Factor scores are the scores of each case (environments) on each factor and may be positive or negative according to the positive or negative contribution of that environment to the whole environment's system. The values of factor scores for a given factor are then considered as how each environment can contribute to the whole fertility

of the studied area. Some of soil environments such as V, O, and PI resulted in a negative contribution to soil fertility, while S, PR, MC and MB resulted to positively contribute to fertility. When glomalin fractions BRSP and EE-BRSP, together with the theoretically calculated fraction of glomalin (NON-EE-BRSP) were plotted against factor scores significant positive correlations were found, indicating a close negative or positive relationship with the environments (Figure 123).

Table 77. The first three eigenvector extracted by the principal component analysis (PCA) for the soil properties related to fertility.

Variable	Eigenvector I	Eigenvector II	Eigenvector III
pH	-0.29	-0.08	-0.37
Electrical Conductivity (EC)	0.24	-0.20	0.12
Soil organic carbon (SOC)	0.36	-0.02	0.04
Total nitrogen (TN)	0.33	0.11	0.22
Total phosphorus (TP)	0.01	0.02	0.63
C/N	0.29	-0.29	-0.15
N/P	0.33	0.06	-0.22
C/P	0.32	-0.05	-0.27
Cation exchange capacity (CEC)	0.25	-0.28	0.21
Exchangeable Ca	-0.07	-0.49	0.02
Exchangeable Mg	0.35	0.06	-0.04
Exchangeable Na	0.22	0.17	-0.44
Exchangeable K	-0.09	-0.51	-0.11
Exchangeable Al	-0.25	0.21	-0.07
Base saturation	0.14	0.44	0.05
Explained variance			
Total variance (%)	51.86	19.16	13.30
Cumulative variance (%)	52.86	71.02	84.32

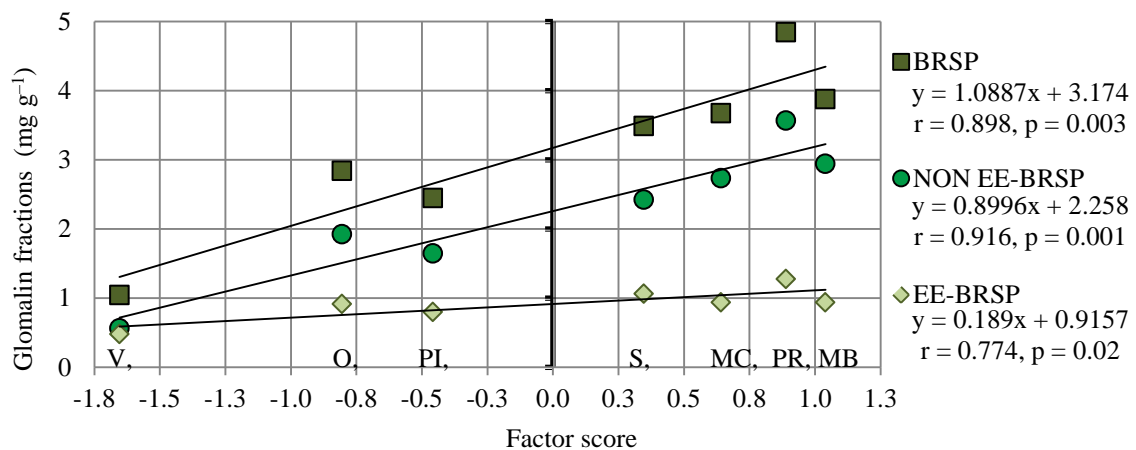


Figure 123. Linear regression equations, correlations, and significant levels between glomalin fractions (BRSP, NON-EE-BRSP, and EE-BRSP) and scores related to soil environments linked with the chemical variables loaded to the first eigenvector.

This graph summarized that the distribution of produced glomalin fractions from each soil environment was significantly correlated with the distribution patterns of soil fertility indices. Moreover, it was shown that glomalin is highly produced in those soils with higher soil fertility. The ratios of each glomalin fraction (NON-EE-BRSP/BRSP and EE-BRSP/BRSP) were plotted against the factor score values (Figure 124). The two ratios showed positive and negative significant correlations respectively. This can strongly suggest that glomalin is more transformed to the recalcitrant fraction in those soils with high fertility levels than other with low fertility. Patterns can also indicate that turnover cycles of fresh glomalin fractions in the more fertile soils is faster than in the less fertile soils due to their healthy conditions favoring the metabolic activities of soil microorganisms at the studied horizons.

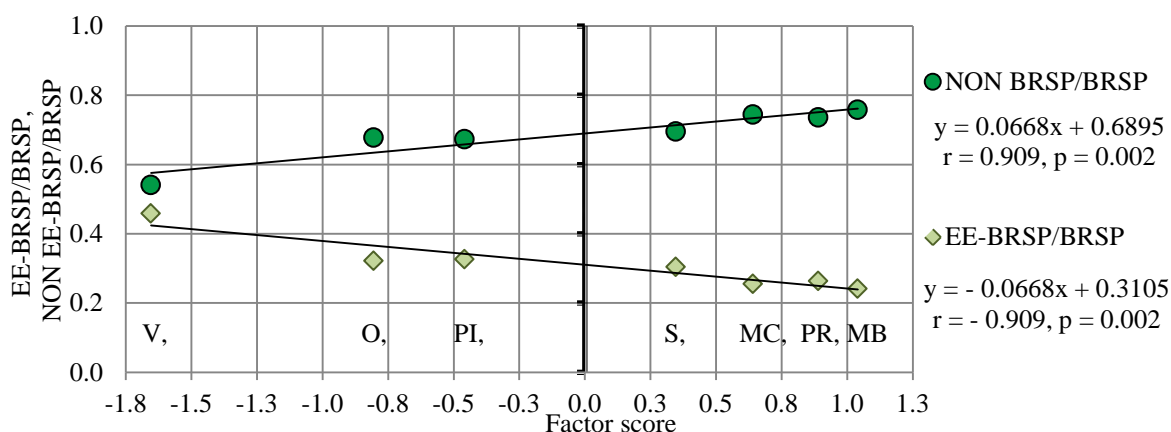


Figure 124. Linear regression equations, correlations, and significant levels between NON-EE-BRSP/BRSP and EE-BRSP/BRSP ratios and factor score values.

3.7.7. Carbon loss and glomalin concentrations

As reported elsewhere the hypothesized fast turnover in EE-BRSP and BRSP in our soils may also justify the positive significant correlation found between EE-BRSP and C-CO₂ under laboratory estimation of CO₂ emission ($r=0.449, p<0.05$), indicating that part of the metabolized labile carbon originates from EE-BRSP (Figure 125). Preger et al. (2007) reported that EE-BRSP was lost most rapidly under prolonged arable cropping in sandy soils of the South African Highveld, whilst BRSP content were not reduced below a certain steady-state level, despite potentially negative management effects. Several authors (Rillig et al., 2003b; Nichols and Wright, 2006) have stated that some glomalin may be in the slow or recalcitrant soil carbon fraction. We did not find

significant correlation between BRSP and C-CO₂, under laboratory estimation of CO₂ respiration, mainly because of the highest BRSP values recorded in summer which corresponded to the lowest C-CO₂ values. When processing seasonal BRSP and C-CO₂ data, excluding summer measurements (environments in the square of the graph Figure 126), a significant positive correlation was found ($r=0.467$, $p<0.05$) indicating that BRSP, during winter, spring and autumn, may also largely contribute to active soil organic carbon pool.

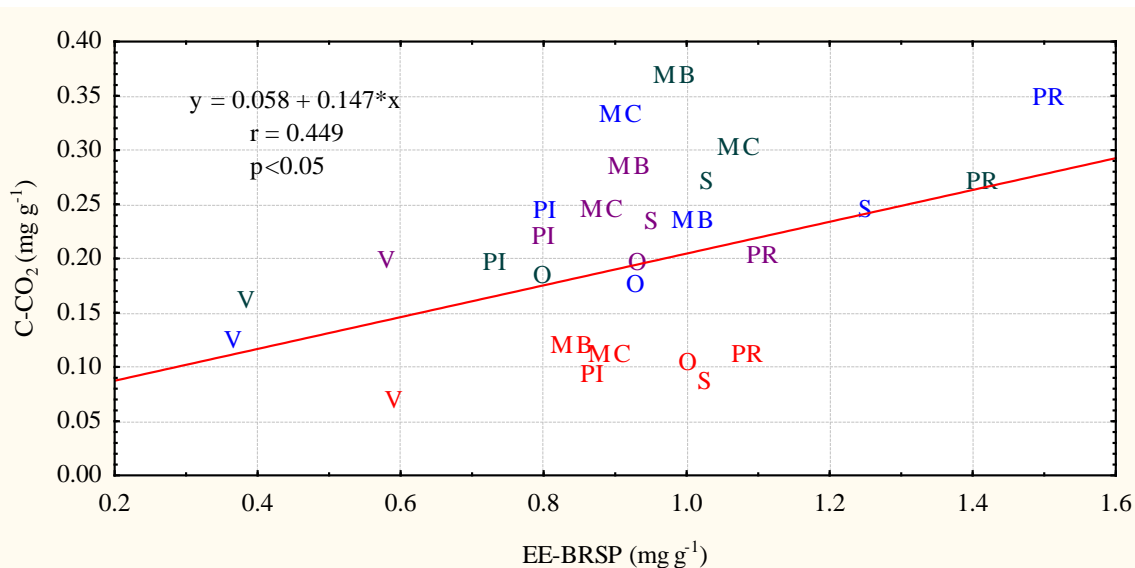


Figure 125. Linear regression equation between C-CO₂ laboratory estimations and easily extractable glomalin (EE-BRSP) for the studied soil environments during 2009. Blue color: Winter; Dark green color: Spring; Red color: Summer; Violet color: Autumn.

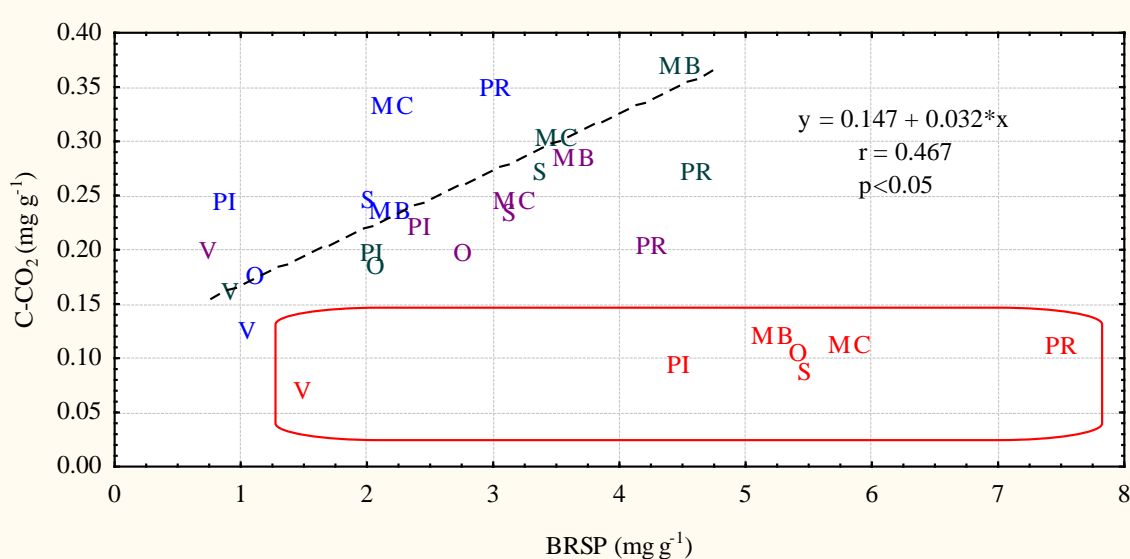
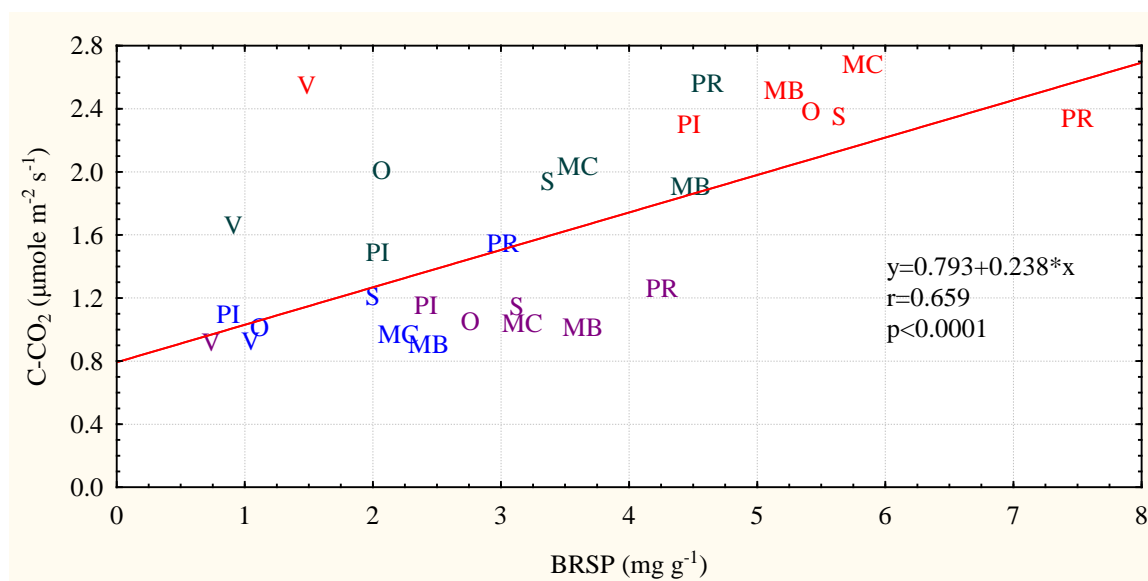


Figure 126. Relationship between C-CO₂ laboratory estimations and BRSP for the studied soil environments during 2009. Blue color: Winter; Dark green color: Spring; Red color: Summer; Violet color: Autumn.

Carbon loss estimated by the laboratory method was carried out on the bases that soil microorganisms are metabolizing the active fraction of soil organic matter with limited water content and somewhat adequate soil temperature (25 °C) that is: different from natural conditions. When checking this relationship with C-CO₂ field estimation, a significant positive correlation was found with BRSP, during the observed period, further proving that BRSP can significantly contribute to active soil organic carbon pool (Figure 127).



the most significant variables used for analysis of variance and their partition in the k-means grouping are presented in Figure 128.

Table 78. Analysis of variance (ANOVA) of selected soil parameters for all soil environments by using the k-means grouping method.

Variables	SS between groups	df	SS within groups	df	F-test	p level
BD (g cm^{-3})	0.383	2	0.514	25	9.316	0.001
SOC (%)	26.256	2	15.340	25	21.395	0.000
TN (%)	0.171	2	0.185	25	11.583	0.000
EE-BRSP (mg g^{-1})	0.843	2	0.892	25	11.813	0.000
BRSP (mg g^{-1})	67.536	2	14.210	25	59.407	0.000
C-CO ₂ (mg g^{-1})	0.065	2	0.119	25	6.834	0.004

BD: Bulk density; TN: Total nitrogen; SOC: Soil organic carbon; EE-BRSP: Easily extractable Bradford reactive soil protein; BRSP: Bradford reactive soil protein; C-CO₂: Carbon-Carbon dioxide; df: Degree of freedom.

Table 79. List of groups resulting from the k-means analysis to emphasize significant differences in soil parameters and related environments along the period of observation.

Seasons	Group I	Group II	Group III
Winter	S, MC, MB, PR		V, O, PI
Spring	S, MC	PR, MB	V, O, PI
Summer		O, S, PI, MC, MB, PR	V
Autumn	O, S, MC, MB	PR	V, PI

Analyzed soil environments V: Soils under vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub; PR: Soils under pasture.

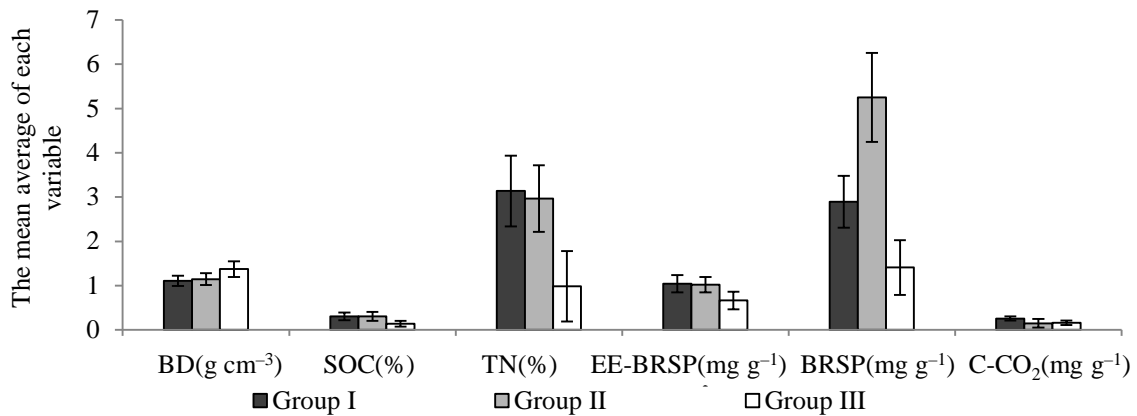


Figure 128. Mean values and error bars of the most significant variables used for analysis of variance and their partition in the k-means grouping. BD: Bulk density; TN: Total nitrogen; SOC: Soil organic carbon; EE-BRSP: Easily extractable Bradford reactive soil protein; BRSP: Bradford reactive soil protein; C-CO₂: Carbon-Carbon dioxide.

The k-means grouping is a simple way to summarize the underlying mechanisms occurred during the seasons. As may be observed from Table 79 the soil environments

S, MC, MB, PR are included in group I, winter, because this season favored lowest bulk density, highest organic carbon and total nitrogen, high EE-BRSP, low BRSP and higher C-CO₂ values. No environments were included in group II because of the absence of intermediate values in these parameters. However, soil environments V, O, and PI were included in group III for their highest bulk density and lower SOC, TN, EE-BRSP, BRSP, and C-CO₂ values (Figure 128). During spring the MB and PR environment shifted to group II because of their increase in BRSP content alone, whereas V, O, P remained in group III (Figure 128). In summer season the dominance of high BRSP content and lower C-CO₂ loss displaced all environments in group II but vines. In autumn all environments shifted again in group I, but V and PI in which lower amounts of SOC, TN, EE-BRSP and BRSP were recorded. The PR environment was found in group II for its still higher BRSP values (Emran et al., 2012b).

3.7.9. Glomalin as a reserve of soil carbon pools

Many authors have reported that glomalin C may have between 27.9% and 43.1% carbon, depending on the extraction method (Rillig et al., 2003b; Nichols and Wright, 2005) and may increase to 52% in organic soils (Schindler et al., 2007). The carbon content of glomalin has been determined by Lovelock et al. (2004), and resulted in a range of 28%-42% (mean 36%±0.83) when working with Ultisols and Inceptisols of a Costa Rica tropical wet forest. Nichols and Wright (2005) found similar values (mean 28.4%±3.5) investigating eight native US soils. Similarly, our carbon estimation in the extracted glomalin solutions has showed similar range across the studied soil environments (Table 80). These values were used to have an indicative estimation of glomalin (BRSP) contributions to soil organic carbon (SOC) and to compare the relationships between SOC, glomalin stocks, and carbon loss (C-CO₂) along different land use and abandonment during the period of study. All data sets of SOC and BRSP were expressed in Mg ha⁻¹ year⁻¹ by using the mean average measurements of bulk density. Values of C-CO₂ (Mg ha⁻¹ year⁻¹) were calculated from CO₂ emission by considering the 12/44 ratio (the molecular weight of carbon to CO₂). Table 80 showed that soil organic carbon pool (Mg ha⁻¹ year⁻¹) had a reduced seasonal variability within each environment but was progressively increasing according to land use and abandonment as indicated by the following annual means 5.81±0.72, 30.78±5.74, 48.02±4.71, 31.19±8.52, 60.21±3.07, 52.10±10.10, 53.97±7.42 Mg ha⁻¹ year⁻¹ for V, O,

S, PI, PR, MC, and MB soil environments respectively. BRSP exhibited the same trend showing annual means of 2.42 ± 0.76 , 5.76 ± 3.81 , 6.01 ± 2.72 , 4.42 ± 2.82 , 8.17 ± 3.60 , 6.49 ± 3.04 , 5.56 ± 1.92 Mg ha⁻¹ year⁻¹ for the same soil order. The accumulation of organic matter within the soil is balanced between the continuous input of residues and their subsequent loss by metabolic activity of microorganisms. Thus, increasing amounts of SOC and glomalin in soil may be mainly attributed to the immobilization of organic matter fractions (Nichols, 2003) along the soil sequence under study.

Table 80. Seasonal means of soil organic carbon (SOC), glomalin (BRSP), percent of Glomalin-Carbon (G-C), carbon-carbon dioxide (C-CO₂), and the proportion of Glomalin-Carbon and carbon-carbon dioxide to soil organic carbon for the studied soil environments.

ENV	Season	SOC (Mg ha ⁻¹ year ⁻¹)	BRSP (Mg ha ⁻¹ year ⁻¹)	G-C (Mg ha ⁻¹ year ⁻¹)	G-C/SOC (%)	C-CO ₂ (Mg ha ⁻¹ year ⁻¹)	C-CO ₂ /SOC (%)
V	Winter	4.84±0.19	2.35±0.57	0.28±0.07	5.77	8.06±0.89	166.49
	Spring	5.73±0.36	2.09±0.18	0.22±0.02	3.79	15.18±6.27	265.11
	Summer	6.45±1.90	3.50±1.57	0.59±0.26	9.12	23.76±17.28	368.39
	Autumn	6.24±2.55	1.74±0.30	0.15±0.03	2.35	7.99±0.84	128.11
O	Winter	26.16±8.14	2.20±0.33	0.28±0.04	1.07	8.90±1.68	34.03
	Spring	25.47±4.11	4.21±1.55	0.98±0.36	3.86	18.52±6.62	72.71
	Summer	35.32±6.80	11.09±6.92	6.76±4.21	19.13	22.15±18.89	62.71
	Autumn	36.15±7.25	5.54±0.35	1.72±0.11	4.77	9.23±3.68	25.54
S	Winter	53.50±4.86	3.44±0.22	0.81±0.05	1.52	10.79±1.56	20.18
	Spring	42.66±3.32	5.66±2.15	2.34±0.89	5.48	17.86±7.97	41.87
	Summer	49.93±11.80	9.84±5.58	6.75±3.83	13.52	21.83±20.08	43.73
	Autumn	46.01±9.55	5.09±0.19	1.94±0.07	4.22	10.21±3.49	22.19
PI	Winter	42.58±0.50	1.47±0.79	0.15±0.08	0.34	9.72±1.35	22.82
	Spring	23.28±5.55	3.71±2.63	0.85±0.60	3.67	13.49±6.78	57.95
	Summer	32.56±11.38	8.24±5.59	4.14±2.81	12.72	21.37±19.73	65.63
	Autumn	26.32±4.50	4.26±0.15	1.16±0.04	4.41	10.26±2.52	39.00
PR	Winter	60.42±1.88	4.78±0.16	1.87±0.06	3.10	14.06±5.70	23.28
	Spring	59.44±5.30	7.92±1.95	4.46±1.10	7.51	23.87±10.63	40.16
	Summer	64.20±3.87	13.20±5.32	11.55±4.66	17.99	21.75±17.54	33.88
	Autumn	56.80±17.05	6.77±0.20	3.55±0.11	6.26	11.29±3.08	19.88
MC	Winter	65.50±7.58	3.82±0.35	1.00±0.09	1.52	8.44±2.67	12.88
	Spring	44.25±4.04	6.19±1.26	2.67±0.54	6.03	18.77±7.70	42.41
	Summer	54.30±5.18	10.80±4.20	7.92±3.08	14.59	25.02±20.70	46.07
	Autumn	44.34±4.17	5.13±0.19	1.99±0.07	4.48	9.13±1.06	20.59
MB	Winter	63.35±2.97	3.17±0.88	0.82±0.23	1.30	7.88±1.65	12.44
	Spring	45.32±12.52	6.25±3.58	3.49±2.00	7.71	17.56±6.38	38.74
	Summer	52.53±2.50	7.71±6.93	5.04±4.53	9.59	23.49±21.15	44.71
	Autumn	54.68±6.51	5.09±0.07	2.28±0.03	4.18	8.88±1.12	16.23

ENV: Soil environments; V: Soils under vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub; PR: Soils under pasture.

An important indication of carbon immobilization in soil is shown by the highly significant correlations between the percentage of Glomalin-Carbon (G-C) determined in the glomalin extraction solution and total glomalin (BRSP) in Figure 129A and soil organic carbon (SOC) in Figure 129B.

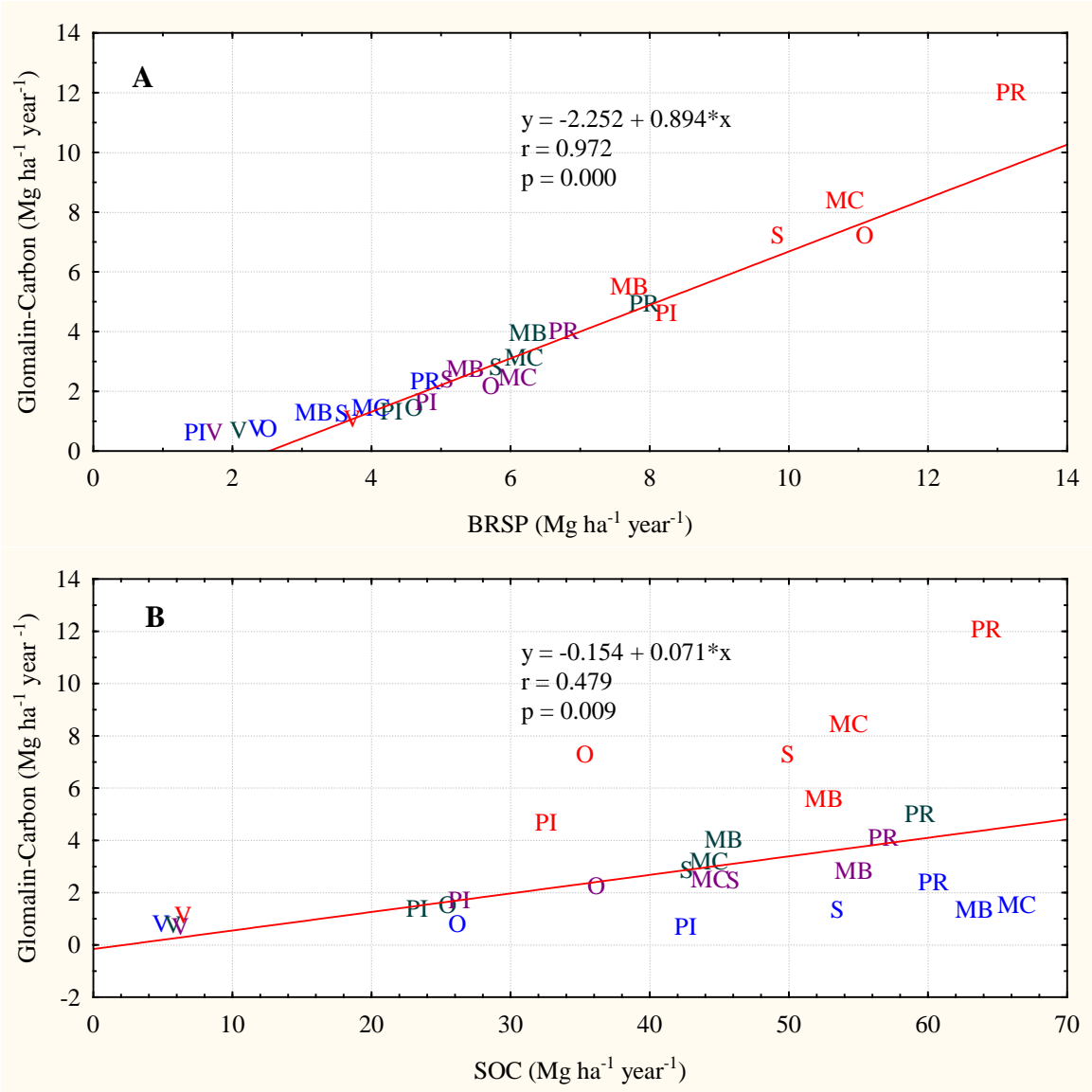


Figure 129. Linear regression equations, correlations, and significant p levels between the seasonal means of the percent of Glomalin-Carbon in the glomalin extraction solution and total glomalin (A) and SOC (B) for the selected soil environments. Blue color: Winter; Dark green color: Spring; Red color: Summer; Violet color: Autumn.

Values of G-C were found to be highly dependent of seasonal conditions. Despite the very high fit of the regression equation, values of each environment were laying in different position on the equation line, emphasizing the seasonal effects. The accumulation of the immobilized glomalin carbon seemed to be high in the abandoned

scrubs and pasture than in soils under stands of pine and cork trees. The lower per cent of glomalin carbon was found in soils under cultivated vines and olive groves corroborating their low contribution on the carbon pools. The estimation of soil carbon loss was reported as $C-CO_2$ ($Mg\ ha^{-1}\ year^{-1}$) and results showed rather similar values along the land use and abandonment sequence (Table 80), which indicated that soil C is lost in comparatively lower amounts in those soils containing higher organic carbon stocks. This trend is also proved by the $C-CO_2/SOC$ ratio. A further indication of the SOC and glomalin dynamics is shown in Figure 130A and B. Both trends of the G-C and $C-CO_2$ to SOC ratios in the studied soils suggested that glomalin carbon extracted from soils under vines may be prevalently formed by the labile glomalin fraction and most easily mineralized, thus more abundant in $C-CO_2$ lost by soil respiration with respect to other soils.

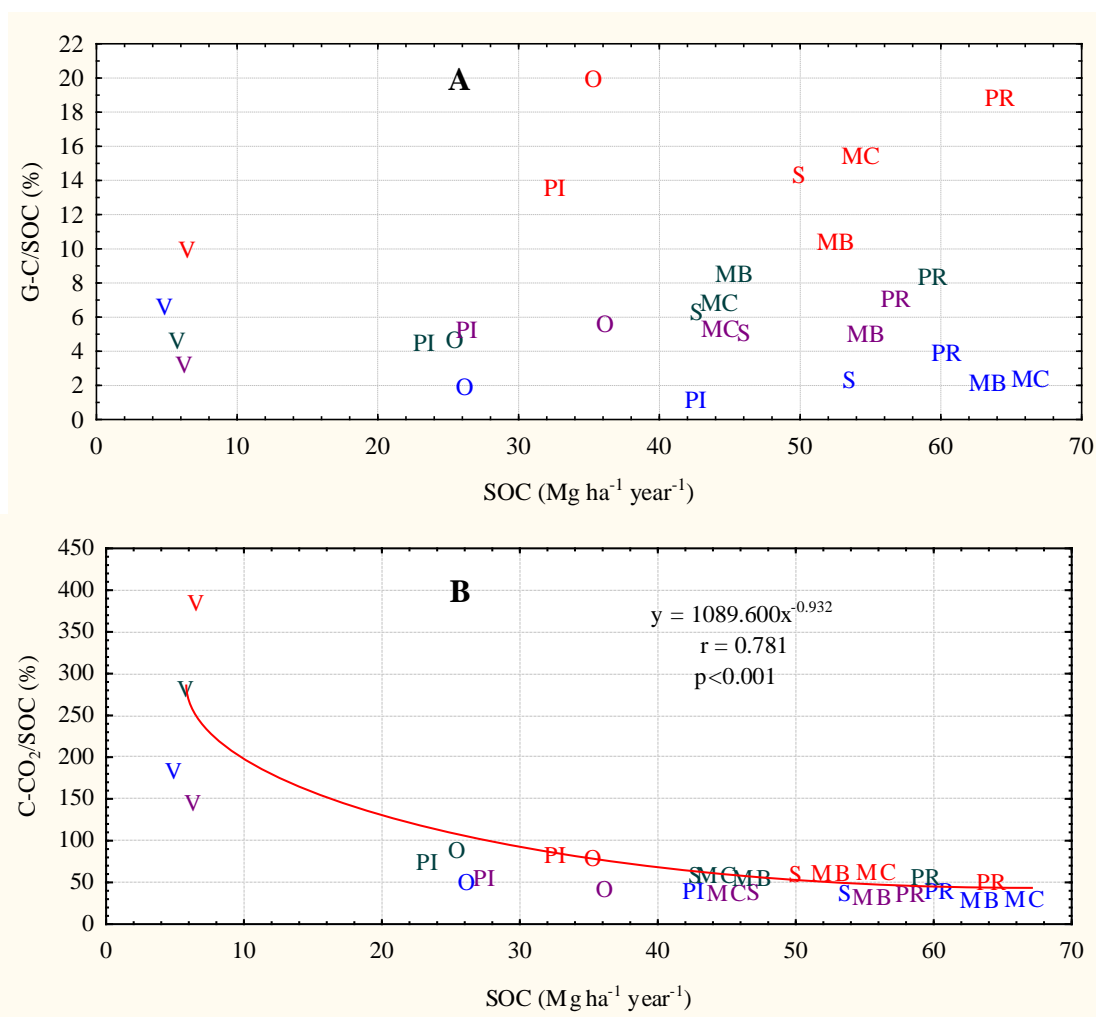


Figure 130. Relationships between the seasonal means of the proportion of G-C (A) and $C-CO_2$ (B) to SOC along the studied soil environments. Blue color: Winter; Dark green color: Spring; Red color: Summer; Violet color: Autumn.

The proportional amounts of C-CO₂/SOC as a function of SOC would decrease drastically from vines to pasture due to partial ageing process to labile fraction (Figure 130B). However, the proportion of G-C/SOC as a function of SOC may increase from vines to pasture due to the addition of glomalin to the recalcitrant carbon pool (Figure 130A). In addition, these processes of carbon storage/loss dynamics would have more relevance in summer than in spring→autumn→winter. This assumption may be not speculative because the EE-BRSP/BRSP ratio had indicated the same trend. The rapid turnover of labile glomalin carbon in EE-BRSP into more stable C form was rather clearly proved. Accordingly, total glomalin (BRSP) resulted more coherently related to stable organic compounds, contributing to both recalcitrant carbon pool enrichment and particle aggregation.

3.8. Stabilization mechanisms of soil organic carbon

As resulted from the literature total glomalin estimated as BRSP may have more significant role in the conservation of organic carbon pools. Different authors (Preger et al., 2007; Wright et al., 2007; Hontoria et al., 2009; Wu et al., 2008; 2012) have reported that BRSP showed also good relationships with soil aggregate stability and carbon pools under diverse soil types and use. Glue-like properties have been attributed to glomalin and it has been described as an abundant component of soil organic matter.

In recent decades, soil aggregation has been associated with arbuscular mycorrhizal fungi (AMF), mutualistic symbionts with the roots of the majority of land plants (Wright et al., 2006). They are hypothesized to be much effective at the soil macroaggregate level, where direct hyphae involvement is thought to be most pronounced (Miller and Jastrow, 2000; Rillig and Mummey, 2006). Wright et al. (2007) have reported that glomalin may contribute to binding micro and macro aggregates in Acrisols in the Mid-Atlantic region of the United States. Hontoria et al. (2009) found that glomalin was twice as high in stable than in unstable aggregates under different land management and abandonment. After deposition of glomalin on soil particles soil micro and macro aggregates are progressively stabilized, so that higher levels of glomalin produce better structure that in turn increase soil porosity, water storage and root development, and a more consistent resistance to surface crusting and sealing, hence erosion (Wright and Anderson, 2000). Moreover, the ability of glomalin to maintain a stable structure may preserve organic compounds in soil micro sites able to protect organic carbon from mineralization processes, thereby favoring carbon sequestration against carbon dioxide production (Rovira and Vallejo, 2003).

The structural stability of aggregates in soils and their relationships with soil organic carbon and glomalin have been seasonally determined during 2009. The role of glomalin as stabilizing agent of soil structure was investigated in two aggregate fractions of the selected soils together with their capability to protect organic carbon from mineralization, in view to consider abandoned soils as potential organic carbon sink (Emran et al., 2012b).

3.8.1. Seasonal patterns of organic carbon (SOC), glomalin (BRSP), and aggregate stability (WSA)

Two-dimensional classes of aggregates were investigated (0.25-2.00 mm, and 2.00-5.60 mm) in order to detect trends in soil structural stability and its correlation with SOC and BRSP content measured in the same dimensional classes. Experiments were conducted in the seven soil environments, analyzing data on seasonal basis. The mean values of structural stability of aggregates, Bradford reactive soil protein, and soil organic carbon are listed in Table 81 with related standard deviation numbers.

We found the structural stability of aggregates always higher in summer in both the 0.25-2.00 mm and 2.00-5.60 mm aggregate fractions in all environments. According to the lowest rainfall amount recorded in this season we postulated that both aggregates classes may have shown higher resistance to aggregate stability tests owing to a steadier soil crumb arrangement achieved in driest conditions. Conversely, the lowest WSA values were found in winter, in the 2.00-5.60 mm aggregate fractions, and in spring, in the 0.25-2.00 mm aggregate fractions, for the same environments. These patterns may be ascribed to different moisture conditions at sampling, higher in winter (range 16-29%) and lower in spring (range 7-18%). The antecedent moisture conditions and the laboratory aggregates air-drying process, previous structural stability tests, may have weakened the structure of larger aggregates (2.00-5.60 mm) and smaller aggregates (0.25-2.00 mm) respectively. It was noticed that the WSA values in summer were slightly higher in the 2.00-5.60 mm aggregate fraction than the other fraction in each environment, probably indicating higher resistance due to stronger assemblage of soil mineral and organic particles. WSA values found in summer in the 0.25-2.00 mm and 2.00-5.60 mm fractions were 22.17%, 19.09%, 12.13%, 24.79%, 16.38%, 10.90%, 10.14% higher than spring values and 304.00%, 99.14%, 10.29%, 27.67%, 13.01%, 6.47%, 27.61% higher than winter values respectively in V, O, S, PI, MC, MB, and PR soils. Generally, lowest percentages indicated a stronger stability of structure and better biological activity to withstand climatic conditions. More organic soils (S, MC, MB, PR) were more structurally stable by 44% in 0.25-2.00 mm soil aggregate fraction and 88% in 2.00-5.60 mm soil aggregates fraction with respect to less organic soils (V, O, PI) in the same aggregate classes (Emran et al., 2012b).

Table 81. Seasonal means (\pm standard errors) of water stable aggregates (WSA), Bradford reactive soil Protein (BRSP), and soil organic carbon (SOC) in the two aggregates classes investigated during the observed period.

ENV.	Season	n	0.25-2.00 mm aggregates			2.00-5.60 mm aggregates		
			WSA (%)	BRSP (mg g ⁻¹)	SOC (mg g ⁻¹)	WSA (%)	BRSP (mg g ⁻¹)	SOC (mg g ⁻¹)
V	Winter	3	75.58 \pm 0.81	0.46 \pm 0.18	2.18 \pm 0.09	22.78 \pm 16.69	0.85 \pm 0.09	2.00 \pm 0.46
	Spring	3	71.11 \pm 3.01	0.10 \pm 0.09	0.75 \pm 0.54	25.63 \pm 11.84	0.50 \pm 0.18	0.90 \pm 0.45
	Summer	3	86.88 \pm 11.14	0.63 \pm 0.18	1.44 \pm 0.09	92.18 \pm 0.89	0.37 \pm 0.27	1.69 \pm 0.25
	Autumn	3	70.85 \pm 3.49	0.85 \pm 0.36	1.81 \pm 0.57	29.84 \pm 8.98	0.97 \pm 0.21	2.01 \pm 0.38
O	Winter	3	84.50 \pm 6.06	2.51 \pm 0.09	8.12 \pm 0.20	48.67 \pm 9.45	3.54 \pm 0.39	8.12 \pm 1.75
	Spring	3	81.50 \pm 7.40	2.20 \pm 0.14	8.44 \pm 1.09	81.80 \pm 7.21	2.56 \pm 0.22	11.46 \pm 0.80
	Summer	3	97.06 \pm 0.44	2.72 \pm 0.28	10.45 \pm 0.90	96.92 \pm 1.41	3.21 \pm 0.37	13.23 \pm 2.69
	Autumn	3	92.00 \pm 3.12	2.28 \pm 0.25	13.67 \pm 2.44	92.57 \pm 5.43	3.47 \pm 0.40	10.35 \pm 1.74
S	Winter	3	94.70 \pm 0.83	4.95 \pm 0.16	27.05 \pm 0.84	86.38 \pm 6.29	3.74 \pm 0.19	25.32 \pm 1.28
	Spring	3	84.35 \pm 3.91	4.37 \pm 0.43	26.73 \pm 6.61	88.39 \pm 1.47	3.19 \pm 0.35	22.51 \pm 1.73
	Summer	3	94.64 \pm 0.94	3.52 \pm 0.39	18.71 \pm 0.34	95.27 \pm 3.95	3.99 \pm 0.51	21.79 \pm 0.42
	Autumn	3	93.98 \pm 2.21	2.99 \pm 0.19	20.50 \pm 1.98	95.80 \pm 2.34	3.09 \pm 0.14	23.62 \pm 1.37
PI	Winter	3	90.44 \pm 3.05	2.85 \pm 0.04	21.19 \pm 4.95	77.09 \pm 7.20	3.59 \pm 0.09	19.58 \pm 0.57
	Spring	3	76.41 \pm 15.71	3.13 \pm 0.16	17.29 \pm 2.18	80.17 \pm 15.39	3.09 \pm 0.33	28.94 \pm 2.20
	Summer	3	95.35 \pm 0.18	2.68 \pm 0.15	8.86 \pm 4.79	98.42 \pm 0.51	3.46 \pm 0.18	29.05 \pm 3.84
	Autumn	3	94.97 \pm 1.57	2.93 \pm 0.16	20.80 \pm 5.67	96.09 \pm 1.38	3.13 \pm 0.26	24.52 \pm 3.40
PR	Winter	3	89.85 \pm 2.10	5.95 \pm 0.43	47.00 \pm 4.32	76.30 \pm 1.85	6.02 \pm 0.68	29.76 \pm 0.33
	Spring	3	87.11 \pm 3.85	5.33 \pm 0.20	31.96 \pm 5.75	89.24 \pm 3.64	4.29 \pm 0.27	33.97 \pm 10.02
	Summer	3	95.94 \pm 0.09	5.17 \pm 0.29	35.62 \pm 1.50	97.37 \pm 1.13	5.03 \pm 0.04	21.89 \pm 0.22
	Autumn	3	93.21 \pm 2.55	4.10 \pm 0.16	35.58 \pm 12.99	96.77 \pm 1.29	4.90 \pm 0.30	26.83 \pm 0.63
MC	Winter	3	90.72 \pm 0.69	4.75 \pm 0.03	37.98 \pm 4.95	86.91 \pm 7.85	4.56 \pm 0.84	39.91 \pm 2.60
	Spring	3	82.22 \pm 6.94	3.96 \pm 0.23	29.75 \pm 4.02	92.44 \pm 5.05	3.18 \pm 0.60	32.16 \pm 3.32
	Summer	3	95.69 \pm 1.71	4.03 \pm 0.12	33.43 \pm 4.18	98.22 \pm 0.77	3.88 \pm 0.14	36.62 \pm 4.19
	Autumn	3	89.20 \pm 6.41	3.48 \pm 0.34	26.03 \pm 4.83	89.39 \pm 6.04	3.40 \pm 0.25	25.33 \pm 1.57
MB	Winter	3	94.60 \pm 1.12	3.93 \pm 0.01	40.88 \pm 0.06	93.49 \pm 1.86	4.42 \pm 0.47	27.62 \pm 0.13
	Spring	3	85.58 \pm 7.68	4.63 \pm 0.18	43.02 \pm 4.84	92.47 \pm 3.38	3.22 \pm 0.37	19.50 \pm 2.51
	Summer	3	94.91 \pm 0.61	3.84 \pm 0.49	27.06 \pm 4.64	98.47 \pm 0.43	4.51 \pm 0.53	27.26 \pm 1.42
	Autumn	3	95.58 \pm 0.74	3.49 \pm 0.19	28.44 \pm 3.77	97.60 \pm 1.22	3.50 \pm 0.40	24.22 \pm 1.70

ENV: soil environments V: Soils under vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub; PR: Soils under pasture; n: Number of seasonal replications.

As reported by Wright et al. (2007), glomalin is able to strongly influence soil structural stability by acting as glue, binding together soil mineral particles. Though the BRSP data reported in Table 81 did not show a clear trend to fully support WSA data, significant relationships were found when WSA was plotted against BRSP (Figure 131A and B). BRSP trend was different in the two aggregate fractions and not always the highest glomalin values were found in summer as in the 0-2.00 mm fraction. This fraction covers the microaggregates (0-0.25 mm) and part of macroaggregates (0.25-

2.00 mm) size which micropores may represent a more suitable habitat for hyphae to produce larger glomalin amounts during driest conditions in summer (Emran et al., 2012b).

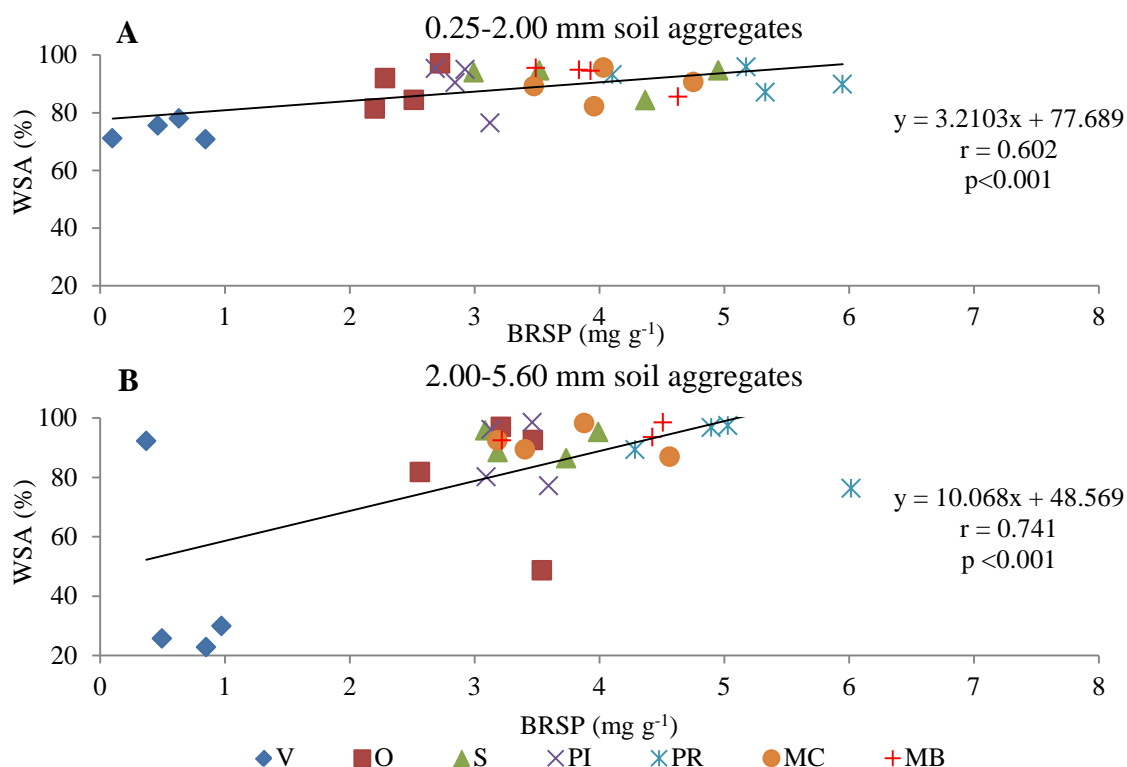


Figure 131. Linear regression equations (correlation coefficient and p-level) between per cent of water stable aggregates (WSA) and Bradford reactive soil protein (BRSP) in the two aggregate classes tested.

The increasing trend in water stable aggregates was coherent with glomalin content along the studied soils especially in those soil environments with higher BRSP and SOC content. According to the linear regression equation, 7.34 mg g^{-1} and 5.05 mg g^{-1} of BRSP were considered to be necessary to reach 100% of soil aggregate stability in the 0.25-2.00 mm and the 2.00-5.60 mm aggregate class respectively, when processing all environments. Similarly, significant relationships were found when plotting the WSA values against the SOC values in the two aggregates classes investigated (Figure 132A and B). By using the linear regression equation reported in Figure 132 A and B, 63.67 mg g^{-1} and 33.84 mg g^{-1} of SOC will provide 100% structural stability in the 0.25-2.00 mm and the 2.00-5.60 mm aggregate class respectively when processing the same environments. Despite the unrealistic significance of the extrapolated values of BRSP and SOC, they may provide an indication of the theoretical amounts to reach an ideal maximum aggregate stability in these conditions.

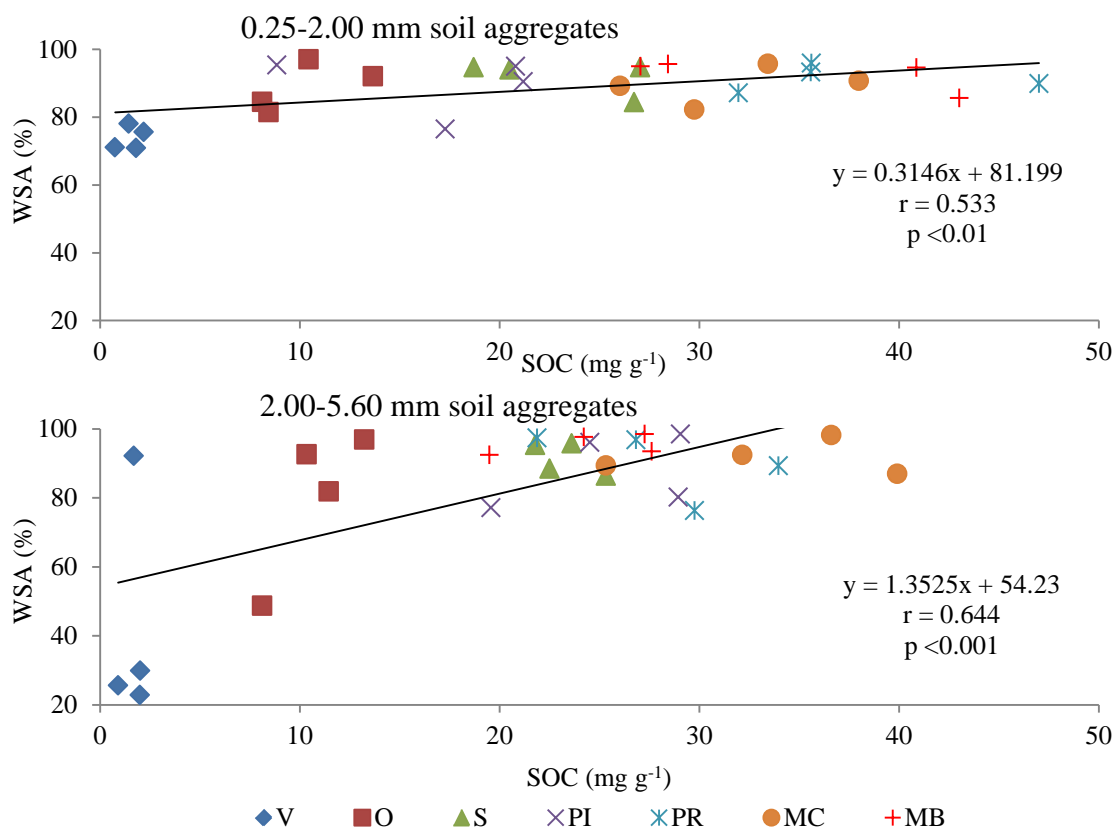


Figure 132. Linear regression equations (correlation coefficient and p-level) between per cent of water stable aggregates (WSA) and soil organic carbon (SOC) in the two aggregate classes tested.

3.8.1.1. ANOVA for checking data variability

Analysis of variance was performed on WSA, BRSP, and SOC data in order to check the significance of seasonal values within each environment and between environments. ANOVA results are presented in Table 82, indicating high significant variations in each environment and between environments during the four seasons examined in 2009.

Significant variations at $p < 0.01$ and $p < 0.05$ are listed in bold and italic respectively. According to the sampling protocol we analyzed three samples per season and later on checked the seasonal significance of data running twelve values (three per season) of $WSA_{(0.25-2.00 \text{ mm})}$, $WSA_{(2.00-5.60 \text{ mm})}$, $BRSP_{(0.25-2.00 \text{ mm})}$, $BRSP_{(2.00-5.60 \text{ mm})}$, $SOC_{(0.25-2.00 \text{ mm})}$, $SOC_{(2.00-5.60 \text{ mm})}$ for V, O, S, PI, MC, MB and PR respectively. When searching the significance of data variations between environments, twenty one values (seven per season) of the same parameters were run.

Table 82. Analysis of variance (ANOVA) for data variability significance in the two dimensional classes of aggregates within and between soil environments.

	WSA (%)				BRSP (mg g ⁻¹)				SOC (mg g ⁻¹)			
	0.25-2.00 mm		2.00-5.60 mm		0.25-2.00 mm		2.00-5.60 mm		0.25-2.00 mm		2.00-5.60 mm	
	F	p	F	p	F	p	F	p	F	p	F	p
Within soil environments												
V	4.356	<i>0.033</i>	46.970	0.000	6.628	0.008	6.991	0.010	12.095	0.001	4.439	<i>0.028</i>
O	9.328	0.003	45.577	0.000	5.213	<i>0.018</i>	4.981	<i>0.026</i>	16.257	0.000	5.953	<i>0.012</i>
S	17.878	0.000	4.903	<i>0.024</i>	38.639	0.000	6.779	0.007	7.652	0.005	6.063	<i>0.011</i>
PI	4.972	<i>0.023</i>	7.163	0.007	7.325	0.006	4.159	<i>0.042</i>	4.993	<i>0.020</i>	15.320	0.000
PR	10.826	0.002	76.617	0.000	20.697	0.000	9.489	0.002	4.362	<i>0.030</i>	17.451	0.000
MC	6.020	<i>0.013</i>	3.819	<i>0.046</i>	35.936	0.000	4.085	<i>0.036</i>	5.186	<i>0.018</i>	24.428	0.000
MB	5.717	<i>0.015</i>	9.418	0.003	12.215	0.001	7.156	0.006	21.935	0.000	4.377	<i>0.029</i>
Between soil environments												
Winter	17.594	0.000	25.733	0.000	19.952	0.000	14.480	0.000	27.984	0.000	15.647	0.000
Spring	2.844	<i>0.050</i>	24.805	0.000	46.183	0.000	32.136	0.000	6.274	0.002	4.650	0.008
Summer	25.359	0.000	10.611	0.000	41.639	0.000	47.583	0.000	55.648	0.000	8.435	0.001
Autumn	20.499	0.000	83.900	0.000	6.710	0.002	33.147	0.000	5.000	0.006	6.730	0.002

V: Soils under vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub. Bold: p<0.01; Italic: p<0.05.

Findings showed that the structural stability of aggregates in our soils varied significantly (Table 82), with values ranging within 70%-95%, all along the seasons per each environment. Soils under vines and olive trees, presenting higher seasonal fluctuations in the WSA_(2.00-5.60 mm), showed significant variability. The wet sieving test apparatus used (Kemper and Rosenau, 1986) may give different values according to the cycling rate (we used 34 cycles min⁻¹; Green *et al.*, 2005 used a rate of 35 cycles min⁻¹) (Emran *et al.*, 2012b). Although the soil aggregates are representing the more stable part of soil profiles, seasonal variability has been found among each data set of the analyzed soil parameters at the two studied soil aggregate fractions. Total glomalin estimated as BRSP significantly fluctuated through seasons by 750%, 24%, 66%, 17%, 45%, 36%, and 33% in the 0.25-2.00 mm aggregate fraction and by 162%, 38%, 29%, 16%, 40%, 43%, and 40% in the 2.00-5.60 mm aggregate fraction, these numbers referring to the lowest and highest average concentrations for V, O, S, PI, PR, MC, and MB soil environments respectively.

The Intraclass Correlation Coefficient (ICC) was tried by using the mean squares between and within groups obtained by ANOVA (Table 83). This additional statistical check was tried to ensure that data were significantly distributed. High ICC values were

found for all soil environments and seasons when testing the water stable aggregates (WSA), soil organic carbon (SOC), and total glomalin (BRSP) at the two aggregate fractions (0.25-2.00 mm and 2.00-5.60 mm) corroborating the above mentioned assumptions.

Table 83. Intraclass Correlation Coefficient (ICC) by using the mean squares between and within groups obtained by ANOVA.

	WSA (%)		SOC (mg g ⁻¹)		BRSP (mg g ⁻¹)	
	0.25-2.00 mm	2.00-5.60 mm	0.25-2.00 mm	2.00-5.60 mm	0.25-2.00 mm	2.00-5.60 mm
Within soil environments						
V	0.456	0.920	0.765	0.467	0.583	0.599
O	0.676	0.918	0.796	0.551	0.511	0.500
S	0.808	0.494	0.625	0.556	0.904	0.590
PI	0.498	0.606	0.500	0.781	0.603	0.442
PR	0.711	0.950	0.456	0.805	0.832	0.680
MC	0.557	0.413	0.512	0.856	0.896	0.436
MB	0.541	0.678	0.839	0.458	0.738	0.607
Between soil environments						
Winter	0.703	0.779	0.794	0.677	0.730	0.658
Spring	0.208	0.777	0.430	0.343	0.866	0.816
Summer	0.777	0.579	0.886	0.515	0.853	0.870
Autumn	0.736	0.922	0.363	0.450	0.450	0.821

V: Soils under vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub.

3.8.2. Statistical analysis for determining trends of SOC and glomalin pools within the studied aggregates

3.8.2.1. Correlation matrix

A correlation matrix was tried between the analyzed parameters at the two aggregates fractions (Table 84). WSA, BRSP, and SOC resulted with a total of 15 high positive significant correlations ($p < 0.01$) between each one at the two aggregate soil fractions (i.e. WSA_{0.25-2.00 mm} vs WSA_{2.00-5.60 mm}, BRSP_{0.25-2.00 mm} vs BRSP_{2.00-5.60 mm}, SOC_{0.25-2.00 mm} vs SOC_{2.00-5.60 mm}) and between each parameter to others. That glomalin contributes to soil organic carbon was also indicated by the linear regression equations tried between BRSP and SOC data in the two analyzed soil aggregate fractions (Figure

133A and B). The linear pattern outlined once more the importance of soil evolution to reach a steady state which was further showed from vines to pasture.

Table 84. Correlation matrix between water stable aggregates (WSA), Bradford reactive soil protein (BRSP), and soil organic carbon (SOC) at 0.25-2.00 mm and 2.00-5.60 mm aggregates for all soil environments.

	WSA (%) 0.25-2.00 mm	WSA (%) 2.00-5.60 mm	BRSP (mg g ⁻¹) 0.25-2.00 mm	BRSP (mg g ⁻¹) 2.00-5.60 mm	SOC (mg g ⁻¹) 0.25-2.00 mm
WSA (%) 2.00-5.60 mm	0.772 p=0.000				
BRSP (mg g ⁻¹) 0.25-2.00 mm	0.602 p=0.001	0.615 p=0.000			
BRSP (mg g ⁻¹) 2.00-5.60 mm	0.741 p=0.000	0.600 p=0.001	0.890 p=0.000		
SOC (mg g ⁻¹) 0.25-2.00 mm	0.533 p=0.003	0.551 p=0.002	0.911 p=0.000	0.814 p=0.000	
SOC (mg g ⁻¹) 2.00-5.60 mm	0.616 p=0.000	0.644 p=0.000	0.901 p=0.000	0.831 p=0.000	0.868 p=0.000

Bold: $p < 0.01$.

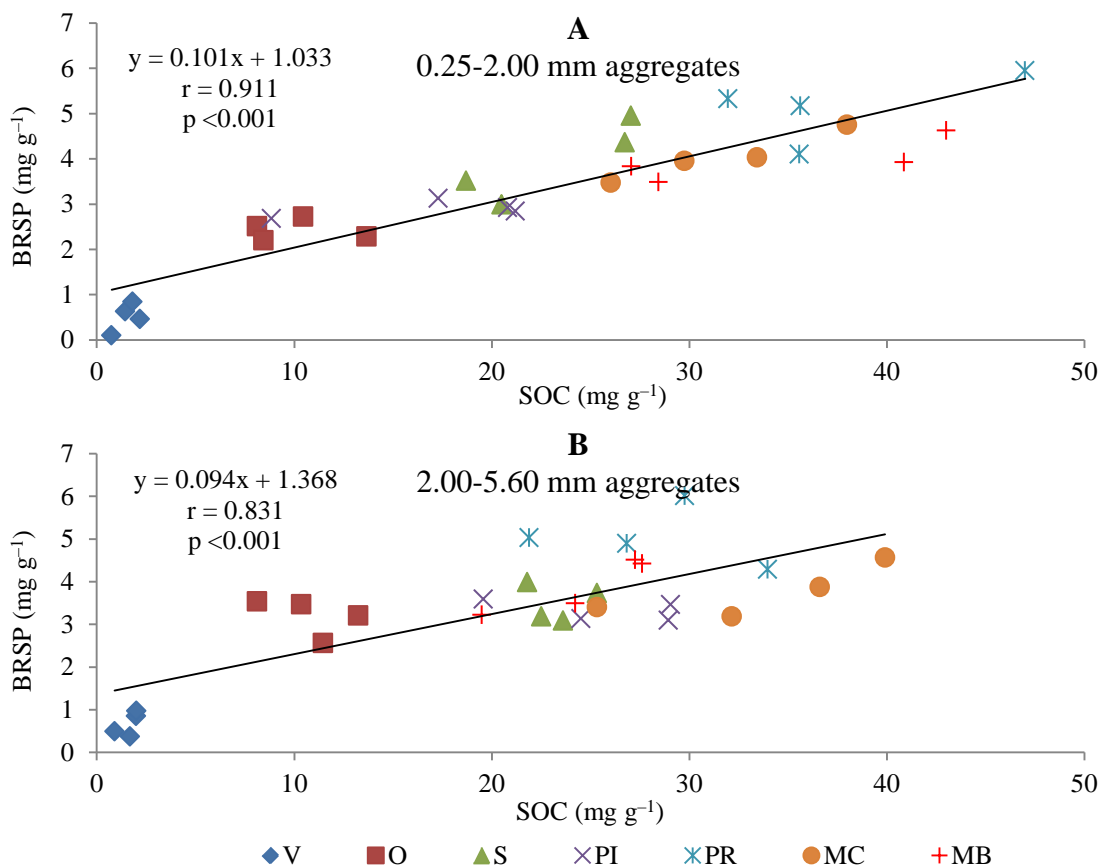


Figure 133. Linear regression equations (correlation coefficient and p-level) between Bradford reactive soil protein (BRSP) and soil organic carbon (SOC) at 0.25-2.00 mm (A) and 2.00-5.60 mm (B) aggregates for all soil environments.

Likewise, the following linear regression equations, correlations, and significant levels were found between WSA (Figure 134A), BRSP (Figure 134B), and SOC (Figure 134C) for the two fractions of soil aggregates.

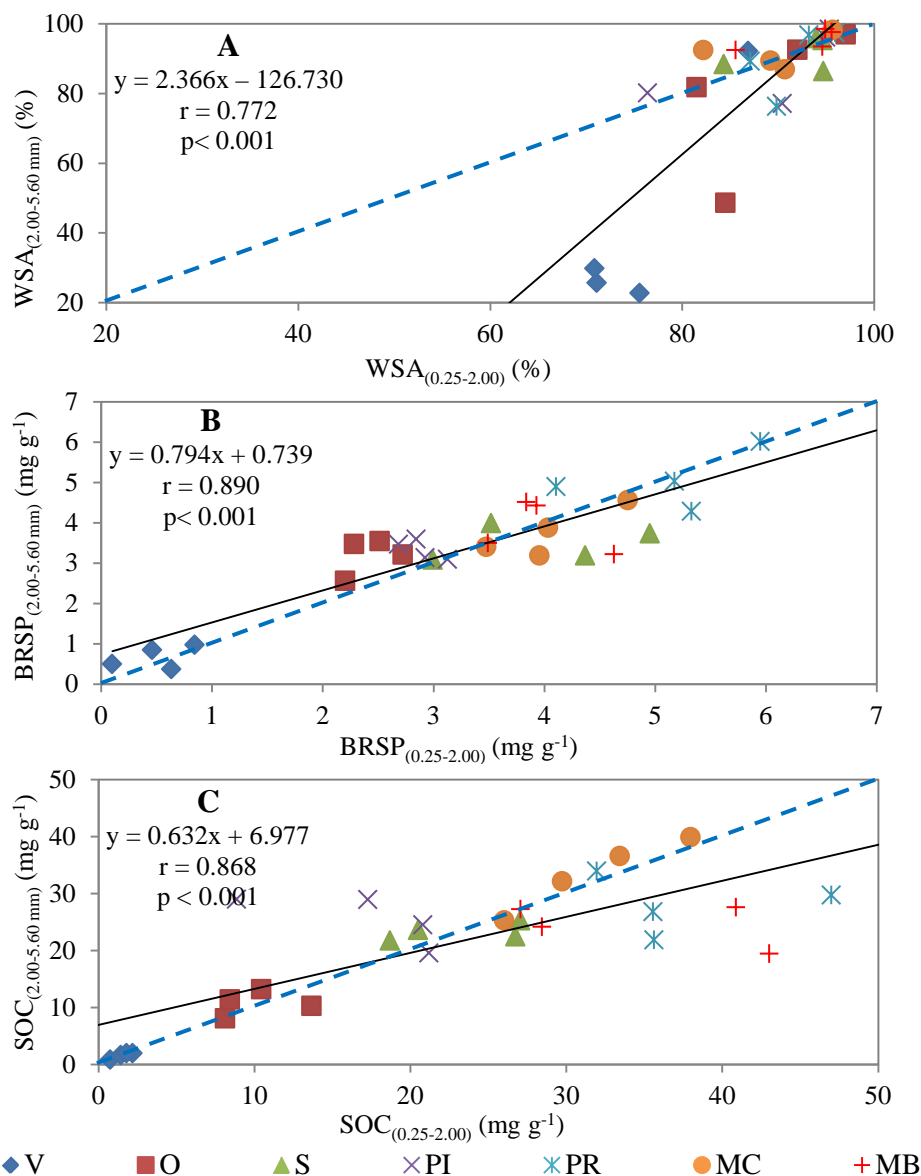


Figure 134. Linear regression equations (correlation coefficient and p-level) between percent of water stable aggregates (WSA) at 0.25-2.00 mm and at 2.00-5.60 mm aggregates (A), Bradford reactive soil protein (BRSP) at 0.25-2.00 mm and at 2.00-5.60 mm aggregates (B), and soil organic carbon (SOC) at 0.25-2.00 mm and at 2.00-5.60 mm aggregates (C) for all soil environments.

Highly significant positive correlations were found indicating an important role of soil carbon dynamics and glomalin deposition at the two studied aggregate fractions and moreover their similar impact on structural stability improvement. This assumption was suggested by the significant reciprocal increases in BRSP, SOC, and WSA values

($p < 0.001$) recorded along the land use and abandonment sequence and depending on climatic and soil conditions. Specifically, the terms of $WSA_{(2.00-5.60 \text{ mm})}$ to $WSA_{(0.25-2.00 \text{ mm})}$ equation (Figure 134A) may be erroneously interpreted because of the slope forced by lower WSA values in soils under cultivated vines during the four seasons and in soils under olive groves in winter 2009 with respect to 1:1 dotted line. In spite of the significant seasonal variability of WSA within and between environments in each of the two aggregate classes, the majority of soils showed quite similar WSA values in the two aggregate fractions (Emran et al., 2012b).

3.8.2.2. Analysis of variance (ANOVA)

The data set for WSA in both aggregate fractions were run separately as dependent variable by ANOVA using BRSP and SOC for the same aggregates fraction as independent variables. The results obtained are shown in Table 85. Highly significant variability was observed associated with high F values for both aggregate fractions corroborating that the role of glomalin was enhanced by the structural stability of aggregates (WSA), investigated in two aggregate fractions (0.25-2.00 mm and 2.00-5.60 mm), also indicating beneficial effect on carbon storage.

Table 85. Analysis of variance (ANOVA) by using the seasonal means of water stable aggregates (WSA) as dependent variables against total glomalin (BRSP) and soil organic carbon (SOC) measured at 0.25-2.00 mm and 2.00-5.60 mm soil aggregates for all soil environments.

Dependent variables	Aggregate fraction	Independent variables			
		BRSP (mg g^{-1})		SOC (mg g^{-1})	
		F	p	F	p
WSA (%)	0.25-2.00 mm	14.762	<0.001	10.342	<0.01
	2.00-5.60 mm	14.651	<0.001	18.442	<0.001

3.8.2.3. Environmental trends of soil carbon loss and storage

Seasonal glomalin values were found to be correlated with soil C-CO₂ values as a proportion of SOC content in the two aggregate fractions, fitting a power law equation (Figure 135A and B). After log/log transformation, the linear regression equations for the two aggregates fractions depicted some patterns which may also refer to the environments better favoring carbon storage (Figure 136A and B).

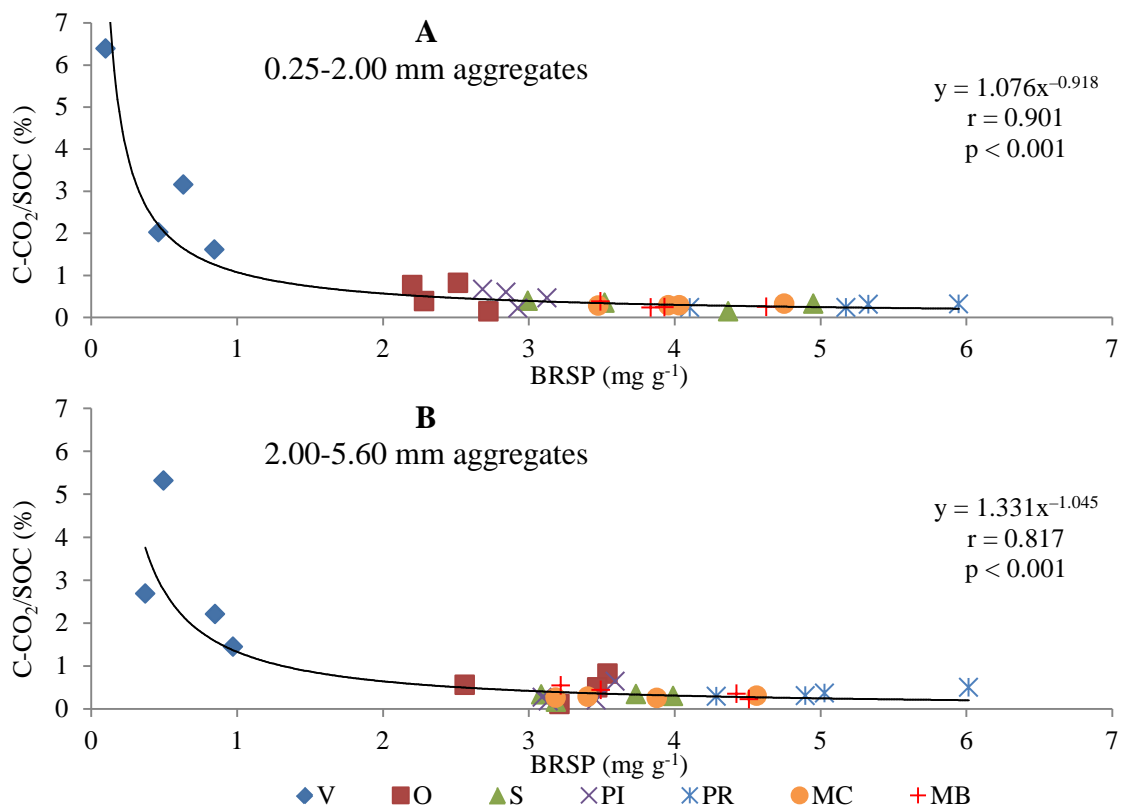


Figure 135. Power law equations found between the percent of C-CO₂/SOC and Bradford reactive soil protein (BRSP) at A) 0.25-2.00 mm aggregates and B) 2.00-5.60 mm aggregates for the selected soil environments.

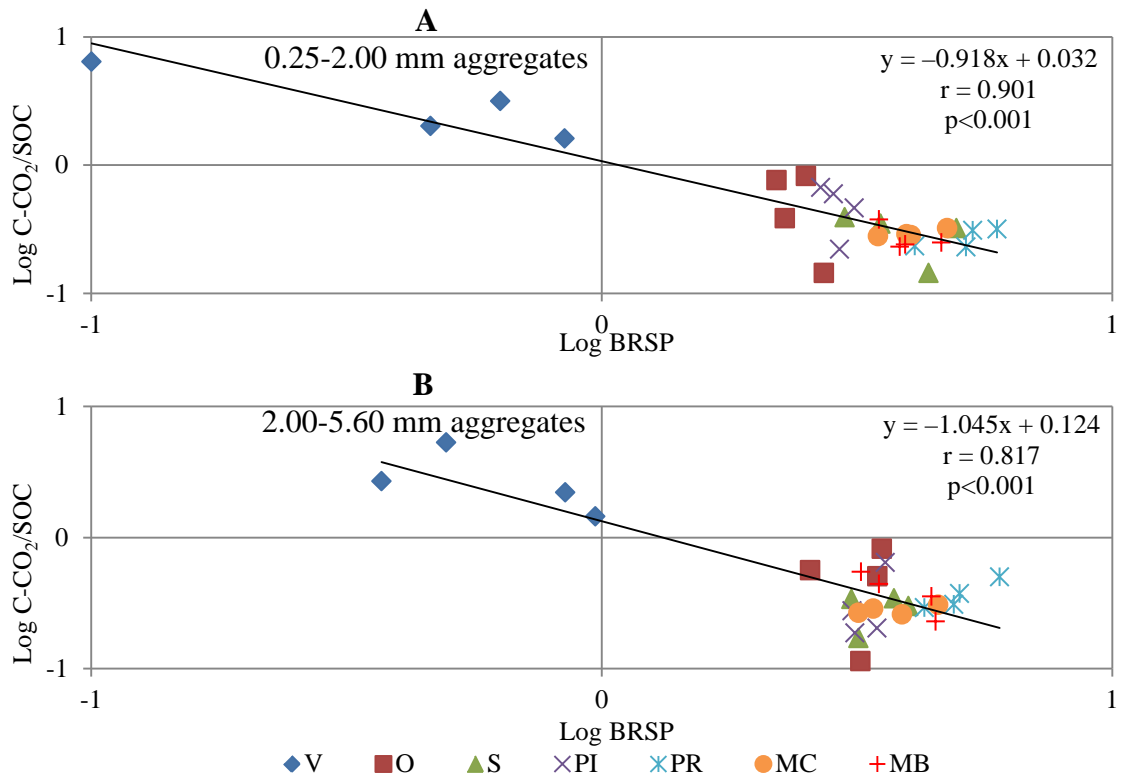


Figure 136. Log/log transformation of data showing the relative seasonal contribution of soil environments to BRSP pool and carbon loss. A) 0.25-2.00 mm aggregates; B) 2.00-5.60 mm aggregates.

Much larger carbon dioxide losses are produced by soils under vines (regardless of the lower SOC content) which contemporarily showed a negative contribution to glomalin formation in both aggregates fractions. Other soil environments showed an opposite trend, reflecting a more conservative action towards carbon loss. All exhibited positive relationships with glomalin and negative with C-CO₂/SOC ratio though to a different extent. These results may help in understanding the carbon dynamics and turnover time in soil, and its role in the formation and stability of aggregates. It was postulated that relatively stable glomalin carbon in more aged soil environments exerts a stronger protection to soil aggregates which in turn may favor carbon sequestration. Nevertheless, we found that both EE-BRSP and BRSP may vary largely along the year depending on climatic conditions, land management and age of abandonment (Emran et al., 2012b). Further research is needed to establish these relationships especially in soils under abandonment and fire occurrence, where more complex dynamics in both soil organic carbon and structure occur.

The structural stability of aggregates was found to be greatly influenced by this dynamics. However, critical structural conditions may occur in unwell structured soils (V, O, and even PI) unable to withstand high interseasonal environmental variations. The relevance of glomalin, especially as a component of the SOC pool has been also verified for its capability to contrast carbon loss processes. The overall findings have suggested that increasing structural stability creates favorable mechanisms of soil carbon protection in very shallow soils, preserving C from biological and biochemical dynamics, thus minimizing soil carbon loss (Emran et al., 2012b). Glomalin content may be therefore considered as a reliable indicator of healthy soil conditions even though further studies need to clarify the real nature of this soil component.

3.9. Enzymatic activities

Biological activity and soil structure are two relevant soil properties mutually interacting and their dynamic favoring the improvement of soil health and plant productivity in soil ecosystems (Bertiller et al., 2009). Soil ecosystem productivity and ecological functionality may be based on the function of soil organic matter by its relation with other chemical and biophysical properties following soil biological activity upon mineralizable and decomposable primary organic materials (Masciandaro et al., 1997; Marinari et al., 2000; 2007). Soil microorganisms play therefore an essential role in the cycling of soil nutrients, stabilization of organic compounds and improvement of soil structure. The large community of soil organisms such as bacteria and fungi involves a wide range of specific enzymes for processing the metabolic transformation of soil organic compounds (Nannipieri et al., 2002) and soil enzymes act as catalysts of important metabolic functions. Thus, soil enzymes were taken into account as indicators of soil microbiological characteristics, and are very important in detecting soil quality and health early warning indicators. Many long-term studies have shown that soil enzymes are sensitive in discriminating among soil management practices, such as manure or agrochemical application and minimum versus traditional tillage practices (García-Ruiz et al., 2008). As reported by Acosta-Martínez et al. (2007), soil enzymes can be used to describe changes in soil quality because they are considered pre-sensitive indicators of changes in soil properties as a response to soil management and other external effects such as adverse climate and environmental stress. Enzyme activities play key roles in the biochemical functioning of soils, including formation and degradation of organic matter, and cycling of soil nutrients, allowing the evaluation of the overall soil microbial activity (Alef and Nannipieri, 1995) and its intensity. The efforts made in understanding the behavior of soil biological activity and its function in processing the metabolic transformations in the studied soil environments have taken into account the enzymatic activities due to its importance in the evaluation of soil quality and productivity and moreover its sensitivity to climatic changes, soil use and management, and age of abandonment.

3.9.1. Soil enzymes

Three selected soil enzymes were investigated in order to establish their specific role in organic matter decomposition and nutrient cycling (Alef and Nannipieri, 1995; Makoi and Ndakidemi, 2008) along the studied soils. β -glucosidase, protease, and phosphatase, closely related to C, N, and P cycles in soil ecosystem respectively, play important roles in the hydrolytic processes of soil organic matter. A better understanding of these activities will potentially provide a unique opportunity for an integrated biological assessment in soil due to their crucial role in several biological interactions and their rapid response to changes in soil management practices (Bandick and Dick, 1999). The values of the three enzymatic hydrolase activities with respect to the natural soil substrate can be seen in Table 86. A clear seasonal trend with an increasing in the activities of β -glucosidase, phosphatase, and protease enzymes during certain periods gave indications on the soil response to seasonal climatic variations and according to the diverse soil use. It may be observed that the three soil enzymes under study differ consistently between the seven soil environments ranging from low values recorded always in soils under vines (V) along the 4 seasons, to intermediate values in soils under stands of pine (PI) and cork trees (S), soils under *Cistus* (MC) and *Erica* (MB) scrubs, and under olive groves (O), until the highest values observed in soils under pasture (PR). A number of long-term studies have shown that soil enzyme activities have the ability to discriminate among soil management practices and the distribution of root biomass (Bandick and Dick, 1999; García-Ruiz et al., 2009). In this study, the variation in soil enzymes was assumed to depend on the diversity of soil uses: from minimum agricultural practices to different stages of land abandonment, creating soil-plant succession interactions. Some plant species may stimulate enzyme activities providing successful biochemical interactions with soil nutrients and structure because of their positive effects on microbial activity (Mungai et al., 2005). These species-specific differ in their dynamic interactions due to the difference in the quality and quantity of the deposited organic materials that also depends on the soil history. The low organic matter and nutrient contents in soils under vines (V) is then probably the main factor responsible for lowering soil enzymatic activities. Other studies indicated that long-term viticulture activity reduced considerably the quality and functionality of soil due to the addition of inorganic fertilizers required for healthy plant growth and to resist mildew on vines (Fernández-Calviño et al., 2010).

Table 86. The mean results (\pm standard errors) of the three enzymatic activities along 2009 for all the selected environments.

ENV Season		β -glucosidase (mg pNP kg ⁻¹ h ⁻¹)					Protease (mg NH ₃ kg ⁻¹ h ⁻¹)					Phosphatase (mg pNP kg ⁻¹ h ⁻¹)				
		Min	Max	\bar{x}	σ	CV	Min	Max	\bar{x}	σ	CV	Min	Max	\bar{x}	σ	CV
V	Winter	23.58	48.37	35.48	3.12	8.62	0.64	1.69	1.30	0.08	6.27	96.28	141.26	121.84	11.85	10.16
	Spring	7.45	8.53	8.14	0.60	7.39	0.65	1.19	0.71	0.14	20.20	83.74	159.28	155.73	5.02	3.22
	Summer	25.25	27.24	26.19	1.00	3.83	0.75	1.87	0.94	0.05	5.05	93.17	98.83	95.17	3.17	3.34
	Autumn	16.47	23.63	20.85	3.84	18.41	0.80	1.40	1.11	0.07	6.29	147.07	182.01	161.86	18.08	11.17
O	Winter	356.38	479.59	410.57	25.08	6.00	11.47	15.11	13.25	1.50	11.35	471.90	899.33	668.98	25.89	3.18
	Spring	208.30	275.68	242.41	33.70	13.90	12.18	15.21	13.93	1.55	11.15	633.23	742.49	678.71	56.88	8.38
	Summer	299.48	411.78	305.51	8.53	2.79	10.63	13.73	13.50	1.61	11.92	610.55	735.33	664.53	64.07	9.64
	Autumn	228.35	250.81	240.97	11.48	4.77	10.30	12.40	11.43	1.20	10.50	839.45	913.77	867.92	40.09	4.62
S	Winter	104.99	508.70	266.48	49.77	16.17	10.76	14.54	12.23	1.43	11.67	491.64	716.26	572.53	72.21	12.38
	Spring	149.99	153.90	151.46	2.13	1.40	10.86	13.36	12.16	1.27	10.42	613.32	903.38	670.17	80.39	12.00
	Summer	176.56	211.09	189.74	18.66	9.83	11.76	14.29	12.23	1.40	11.42	375.79	756.24	681.09	106.28	15.60
	Autumn	151.02	188.46	166.14	19.73	11.88	11.24	15.00	13.36	1.30	9.73	789.74	851.30	812.85	33.53	4.12
PI	Winter	96.38	207.27	138.03	20.29	12.09	5.80	9.72	6.89	0.51	7.47	355.32	643.10	614.10	55.18	8.99
	Spring	86.02	111.31	97.61	12.78	13.09	5.21	8.78	6.14	1.00	16.29	301.69	415.94	868.06	59.32	6.83
	Summer	172.36	229.35	198.57	28.77	14.49	10.54	14.51	12.68	0.71	5.59	576.61	746.87	738.91	93.87	12.70
	Autumn	87.60	118.71	102.93	15.56	15.12	5.30	8.65	5.93	1.00	16.88	472.57	504.73	989.76	16.20	1.64
PR	Winter	387.67	698.90	617.56	28.37	4.59	33.25	43.04	36.41	2.11	5.79	859.23	4016.71	2325.29	284.42	12.13
	Spring	206.93	242.70	220.65	19.28	8.74	11.98	17.24	13.80	1.97	14.31	1184.63	1585.05	1446.40	226.83	15.68
	Summer	340.74	478.00	414.50	69.20	16.70	17.54	23.54	21.75	0.86	3.93	1613.89	1638.29	1623.87	12.79	0.79
	Autumn	298.73	325.35	308.02	15.02	4.88	15.30	19.87	16.11	1.40	8.69	2016.73	2083.28	2053.70	33.89	1.65
MC	Winter	247.82	300.20	274.77	11.89	4.40	12.14	17.07	13.48	1.94	14.41	608.94	1151.63	882.69	93.17	11.10
	Spring	304.72	313.87	308.05	5.06	1.64	12.95	15.10	14.21	0.65	4.56	885.30	1001.21	925.97	65.23	7.04
	Summer	238.96	300.10	274.45	31.74	11.56	11.54	16.93	13.65	1.38	10.10	610.44	773.49	702.69	83.61	11.90
	Autumn	234.17	300.31	271.33	33.82	12.46	13.33	16.87	15.46	1.50	9.70	1141.65	1275.86	1197.48	69.89	5.84
MB	Winter	164.96	428.59	233.41	25.65	10.75	10.73	15.32	14.16	0.88	6.19	960.19	1696.57	1213.94	147.70	11.20
	Spring	230.72	278.14	259.98	25.58	9.84	9.75	13.21	12.01	0.84	7.02	1735.18	2052.41	1556.83	171.06	9.21
	Summer	147.61	212.09	176.73	32.69	18.49	8.98	12.00	10.60	0.74	7.00	721.03	1070.98	748.76	39.22	5.24
	Autumn	231.97	272.73	250.96	20.52	8.18	13.52	17.54	16.47	1.45	8.80	1411.02	1655.04	1525.27	122.75	8.05

V: Soils under cultivated vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub.

Enzymes are highly variable over short distances, and their activity is linked to the distribution of other soil properties such as moisture and organic matter content. Ushio et al. (2010) reported that seasonal variations in soil enzyme activity are attributed to patterns of temperature and moisture. An increase in soil enzymes activity in response to soil moisture increase is corroborating the establishment of better conditions for microbial growth and activity. Moreover, the rate of soil respiration (CO₂ emission) has been found to relate closely to soil microbial biomass and activity (McCulley et al., 2004; Wang et al., 2006; Jin et al., 2009). In order to explore the variability among the studied soils, descriptive statistical analysis by using the seasonal means of the studied soil enzymes activity to check the intra-annual variability was carried out (Table 87). The results showed that soils under *Cistus* (MC) and *Erica* (MB) scrub showed an intra-annual variability lower than 20% for both β -glucosidase and protease enzyme activities (Table 87), suggesting a protective effect of these plant species from seasonal climate variations. Moreover, the average coefficient of intra-annual variation of protease and phosphatase enzyme activities was lower than 20% in soils under olive groves (O) and stands of cork trees (S). In the other ecosystems, soil enzyme activities varied more consistently among the different seasons (Table 87).

Table 87. Intra-annual variability for data of β -glucosidase, protease and phosphatase activities among the studied soils during the observed period in 2009.

	V	O	S	PI	PR	MB	MC
β -glucosidase							
\bar{x} (mg pNP kg ⁻¹ h ⁻¹)	22.67	299.86	193.45	134.28	390.18	282.15	230.27
σ (mg pNP kg ⁻¹ h ⁻¹)	11.42	79.70	51.17	46.45	171.06	17.33	37.36
CV (%)	50	27	26	35	44	6	16
Protease							
\bar{x} (mg NH ₃ kg ⁻¹ h ⁻¹)	1.02	13.03	12.50	7.91	22.01	14.20	13.31
σ (mg NH ₃ kg ⁻¹ h ⁻¹)	0.25	1.10	0.58	3.21	10.16	0.90	2.57
CV (%)	25	8	5	41	46	6	19
Phosphatase							
\bar{x} (mg pNP kg ⁻¹ h ⁻¹)	133.65	720.03	684.16	802.71	1862.32	927.21	1261.20
σ (mg pNP kg ⁻¹ h ⁻¹)	31.11	98.77	98.70	162.18	400.34	204.48	375.04
CV (%)	23	14	14	20	21	22	30

V: Soils under cultivated vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub; \bar{x} : Mean; σ : Standard deviation; CV: Coefficient of variation.

One-way ANOVA was used in order to explore the interseasonal variations of data within and between soil environments (Table 88). The data set of β -glucosidase

activity showed highly significant seasonal variability ($p < 0.01$) within V, O, S, PI, and PR soil environments indicating significant response of the soil biological ecosystems to changes in climatic conditions. Similarly data of soils under *Erica* scrub showed significance at $p < 0.05$. Conversely the lack of significance in soils under *Cistus* scrub may be related to the lower microbial populations and organic substrates unable to reveal significant changes in soil microbial activities with respect to soils under *Erica* scrub. The data set for protease activity showed significant seasonal variability only in soils under vines (V), stand of pine trees (PI), and pasture (PR) due to the variability of nitrogen addition in those soils even by chemical fertilizers (V soil environment) or by periodical grazing activities (PI and PR soil environments). Phosphatase activity in all the studied soils was found to vary significantly due to its natural mechanisms upon soil phosphorus that showed higher oscillations in winter and summer seasons in all soil environments. High significant variability ($p < 0.01$) was found between the studied soil environments at each season (Table 88), indicating the significant diverse response of soil microbial activity in soils under study to changes in land use, soil management, and age of abandonment.

Table 88. ANOVA results for exploring significance of seasonal variability within and between soil environments.

	β -glucosidase (mg ρ NP kg ⁻¹ h ⁻¹)		Protease (mg NH ₃ kg ⁻¹ h ⁻¹)		Phosphatase (mg ρ NP kg ⁻¹ h ⁻¹)	
	F	p	F	p	F	p
Within soil environments						
V	60.514	0.000	22.623	0.000	23.110	0.000
O	38.710	0.000	1.676	0.248	12.171	0.002
S	9.762	0.005	0.548	0.663	4.852	<i>0.033</i>
PI	15.746	0.001	44.637	0.000	20.183	0.000
PR	56.719	0.000	112.264	0.000	14.389	0.001
MC	1.556	0.274	1.155	0.385	20.223	0.000
MB	5.977	<i>0.019</i>	0.994	0.444	24.937	0.000
Between soil environments						
Winter	145.469	0.000	185.449	0.000	86.491	0.000
Spring	97.122	0.000	54.454	0.000	49.444	0.000
Summer	38.224	0.000	95.27	0.000	128.333	0.000
Autumn	86.506	0.000	68.349	0.000	304.875	0.000

V: Soils under cultivated vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under stands of pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub. Bold: $p < 0.01$; Italic: $p < 0.05$.

3.9.1.1. *β-glucosidase activity*

The β -glucosidase is a common and predominant enzyme in soil (Tabatabai, 1994) named according to the type of bond that it hydrolyses. It plays an important role in soils because it is involved in catalyzing the hydrolysis and biodegradation of various β -glucosides present in plant debris while decomposing in the ecosystem. Thus it indicates the carbon cycle, as it catalyzes the hydrolysis of cellobiose (dimer resulting from the degradation of cellulose) to glucose (Alef and Nannipieri, 1995). The annual average of β -glucosidase activity in the studied soils ordered the soil environments according to their ability to hydrolyze the organic carbon substrates as following: PR> O> MC> MB> S> PI> V soil environments. The high values of β -glucosidase activity in soils under olive groves was probably due to a higher cellulose content that act as suitable substrate for this enzyme, as reported by Navas Vázquez et al. (2009). Different authors (Makoi and Ndakidemi, 2008) stated that a significant fraction of enzyme activity measured in soil may originate from the immobilized enzymes of microbial origin sorbed to clays or humic colloids. We found the higher values of β -glucosidase activity in soils under pasture, olive groves, *Cistus* and *Erica* scrubs indicating that β -glucosidase can be used to detect changes in soil properties along management and abandonment. This has suggested its adoption as biochemical indicator of soil quality (Bandick and Dick, 1999), mainly based on the type of organic matter content and related biological and biochemical parameters (Doran and Parkin, 1994). The patterns observed in our soils may be in agreement with previous results on organic carbon dynamics, where soil organic carbon losses, estimated by means of CO₂ emission, were considerably higher in soils under vines than soils under pasture (Emran et al., 2012a). Independently of the order of susceptibility to carbon loss: V> O> PI> S> MC> MB> PR, the highest CO₂ emissions from more organic horizons resulted however in a higher carbon storage capacity, because CO₂ concentrations were negligible with respect to total SOC content. It may be postulated that results of enzyme activity reinforced previous observations inferring that more biologically and biochemically active soil are able to process labile organic compounds and temporarily protect more stable SOC fractions (Pardini and Gispert, 2006). A considerable variability was observed within each environment as can be seen in Figure 137.

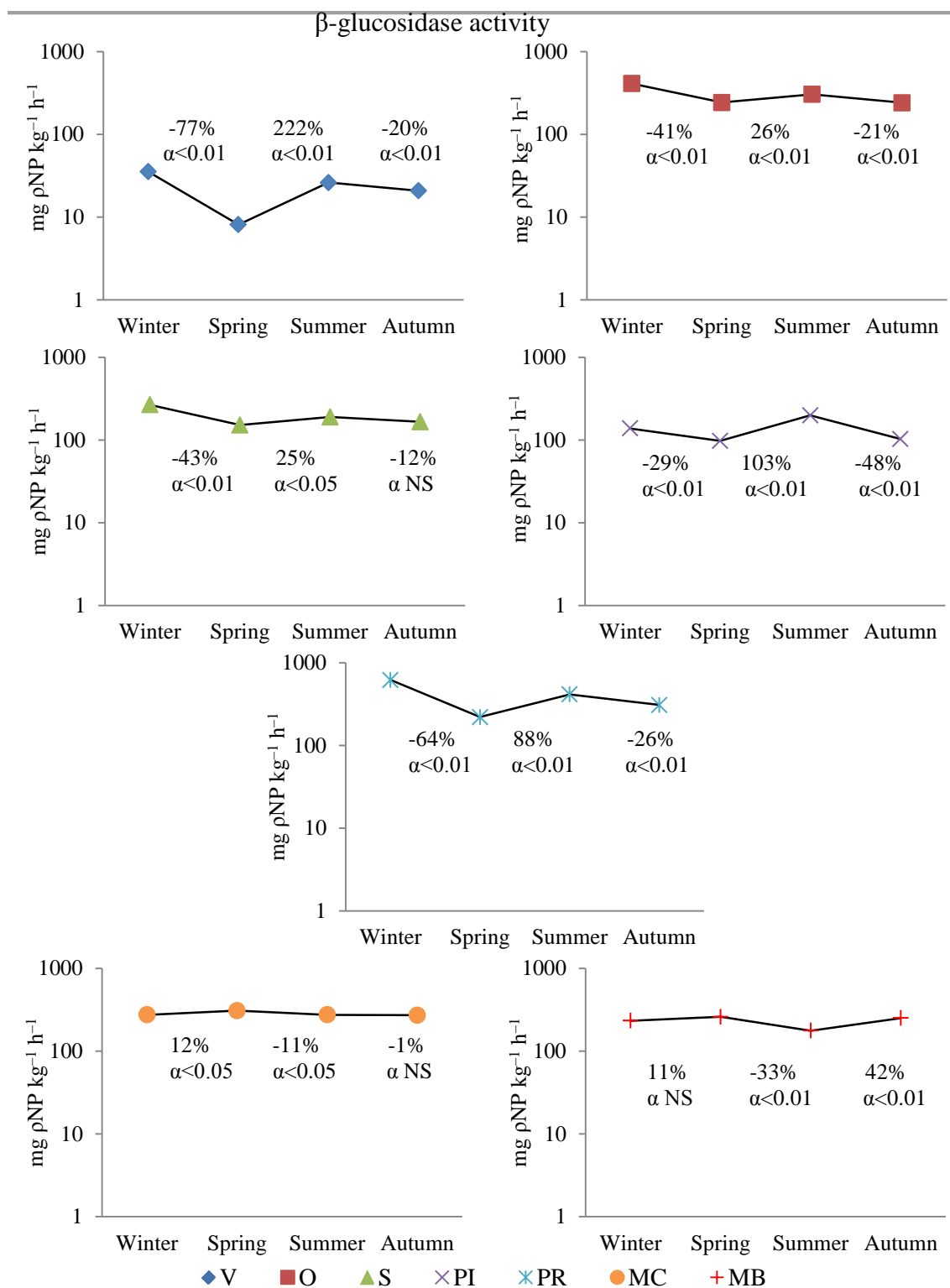


Figure 137. Seasonal fluctuations and their Tukey's significance level (α) of β -glucosidase activity data for each soil environment during the observed period.

A significant trend of seasonal fluctuations in β -glucosidase activity was found in V, O, S, PI, and PR soil environments which showed a decrease from winter to spring then an increase from spring to summer followed by a decrease again in autumn. The literature reports that lignocelluloses degrading enzymes such as β -glucosidase are

regulated by substrate availability (Boerner et al., 2005). In our study sites, soil organic matter increases were generally recorded in summer and winter. Thus, it is logical that β -glucosidase activity increases in these seasons as a reflection of a change in substrate availability. The seasonal fluctuation was presented as a percent of change between each seasonal mean with respect to the previous one as can be seen in Figure 137. Tukey's HSD (Q) test was used for exploring the multiple significant comparisons of seasonal changes by calculating the difference for each seasonal mean with respect to the mean square (MS) of data obtained for each soil environment by one-way ANOVA (Elfstrand et al., 2007). The Q values were calculated by using the following equation:

$$M_1 \text{ versus } M_2 = \frac{M_1 - M_2}{\sqrt{MS(\frac{1}{n})}}$$

where M_1 is the seasonal mean, M_2 is the previous seasonal mean, MS is the statistical mean square obtained by one-way ANOVA analysis by using the four seasons, and n is the total observations for each environment (3samples \times 4season=12). The Q data were employed to show which seasonal data variability was significantly differing from the statistical mean (MS) of each soil environment. The Q values were obtained by comparing each seasonal mean to the mean of the previous season for all soil environments: spring versus winter, summer versus spring, and autumn versus summer. These season-to-season fluctuations in soil enzymes activity may be due to the effect of natural processes undergone by soil microbial activity i.e. climatic conditions during the observed period (winter, spring, summer, and autumn). Data significance was calculated according to the Tukey's significance/probability table (Table 89), taking ng as the number of groups or seasons (ng=4) and $df_{\text{within}}=(n-ng)=12-4=8$, being n in the total observation for each environment (3samples \times 4season=12). According to the Table 89 the alpha significance was presented in Figure 137, as α =NS (not significant) ($Q>6.20$), $\alpha<0.05$ ($Q\leq 6.20$), or $\alpha<0.01$ ($Q\leq 4.53$), to explore the significance level of the seasonal fluctuations for each soil environment. An example can be seen in soils under vines that showed significant decrease ($\alpha<0.01$) by -77% from winter to spring, then significant ($\alpha<0.01$) increase by 222% from spring to summer followed by significant ($\alpha<0.01$) decrease by -20% from summer to autumn. Soil response to seasonal changes differed according to current soil use and cover change with a higher variability in V, O, PI, and PR soils along all seasons. Soils under *Cistus* and *Erica* scrub showed a rather similar trend with higher β -glucosidase activity in spring and autumn.

Table 89. The Q values for Tukey's HSD test corresponding to alpha (α) <0.05 (top values) and α <0.01 (bottom values).

Degree of freedom (df)	ng = Number of groups															
	2		3		4		5		6		7		8		9	
	α <0.05	α <0.01	α <0.05	α <0.01	α <0.05	α <0.01	α <0.05	α <0.01	α <0.05	α <0.01	α <0.05	α <0.01	α <0.05	α <0.01	α <0.05	α <0.01
5	3.64	5.70	4.60	6.98	5.22	7.80	5.67	8.42	6.03	8.91	6.33	9.32	6.58	9.67	6.80	9.97
6	3.46	5.24	4.34	6.33	4.90	7.03	5.30	7.56	5.63	7.97	5.90	8.32	6.12	8.61	6.32	8.87
7	3.34	4.95	4.16	5.92	4.68	6.54	5.06	7.01	5.36	7.37	5.61	7.68	5.82	7.94	6.00	8.17
8	3.26	4.75	4.04	5.64	4.53	6.20	4.89	6.62	5.17	6.96	5.40	7.24	5.60	7.47	5.77	7.68
9	3.20	4.60	3.95	5.43	4.41	5.96	4.76	6.35	5.02	6.66	5.24	6.91	5.43	7.13	5.59	7.33
10	3.15	4.48	3.88	5.27	4.33	5.77	4.65	6.14	4.91	6.43	5.12	6.67	5.30	6.87	5.46	7.05
11	3.11	4.39	3.82	5.15	4.26	5.62	4.57	5.97	4.82	6.25	5.03	6.48	5.20	6.67	5.35	6.84
12	3.08	4.32	3.77	5.05	4.20	5.50	4.51	5.84	4.75	6.10	4.95	6.32	5.12	6.51	5.27	6.67
13	3.06	4.26	3.73	4.96	4.15	5.40	4.45	5.73	4.69	5.98	4.88	6.19	5.05	6.37	5.19	6.53
14	3.03	4.21	3.70	4.89	4.11	5.32	4.41	5.63	4.64	5.88	4.83	6.08	4.99	6.26	5.13	6.41
15	3.01	4.17	3.67	4.84	4.08	5.25	4.37	5.56	4.59	5.80	4.78	5.99	4.94	6.16	5.08	6.31
16	3.00	4.13	3.65	4.79	4.05	5.19	4.33	5.49	4.56	5.72	4.74	5.92	4.90	6.08	5.03	6.22
17	2.98	4.10	3.63	4.74	4.02	5.14	4.30	5.43	4.52	5.66	4.70	5.85	4.86	6.01	4.99	6.15
18	2.97	4.07	3.61	4.70	4.00	5.09	4.28	5.38	4.49	5.60	4.67	5.79	4.82	5.94	4.96	6.08
19	2.96	4.05	3.59	4.67	3.98	5.05	4.25	5.33	4.47	5.55	4.65	5.73	4.79	5.89	4.92	6.02
20	2.95	4.02	3.58	4.64	3.96	5.02	4.23	5.29	4.45	5.51	4.62	5.69	4.77	5.84	4.90	5.97
24	2.92	3.96	3.53	4.55	3.90	4.91	4.17	5.17	4.37	5.37	4.54	5.54	4.68	5.69	4.81	5.81
30	2.89	3.89	3.49	4.45	3.85	4.80	4.10	5.05	4.30	5.24	4.46	5.40	4.60	5.54	4.72	5.65
40	2.86	3.82	3.44	4.37	3.79	4.70	4.04	4.93	4.23	5.11	4.39	5.26	4.52	5.39	4.63	5.50
60	2.83	3.76	3.40	4.28	3.74	4.59	3.98	4.82	4.16	4.99	4.31	5.13	4.44	5.25	4.55	5.36
120	2.80	3.70	3.36	4.20	3.68	4.50	3.92	4.71	4.10	4.87	4.24	5.01	4.36	5.12	4.47	5.21
Infinity	2.77	3.64	3.31	4.12	3.63	4.40	3.86	4.60	4.03	4.76	4.17	4.88	4.29	4.99	4.39	5.08

3.9.1.2. Protease activity

Protease plays a significant role in N mineralization (Ladd and Jackson, 1982), an important process regulating the amount of plant available N and plant growth. It hydrolyzes the most nitrogen forms in soil and plays an important role in the N cycle (Alef and Nannipieri, 1995). Data of protease enzyme activity were very low in soils under vines and increased to intermediate values in PI, S, and O soil environments followed by slightly higher values in soils under *Cistus* and *Erica* scrubs. The higher values were found in soils under pasture which also showed the highest data oscillations. The trends of protease activity in the studied soils were similar to the trends of TN content in all soil environments during the same observed period. Previous studies have reported that protease activities may occur partly in soils as a carbohydrate-enzyme or humo-carbohydrate complex from arable soil and from forest or permanent grassland soils (Nannipieri et al., 1980; Alef and Nannipieri, 1995; Shukla and Varma, 2011). The amount of this extracellular enzyme activity may be indicative not only of the biological capacity of soil for the enzymatic conversion of the substrate, which is

independent of the extent of microbial activity, but might also have an important role in the ecology of microorganisms in the ecosystem (Makoi and Ndakidemi, 2008).

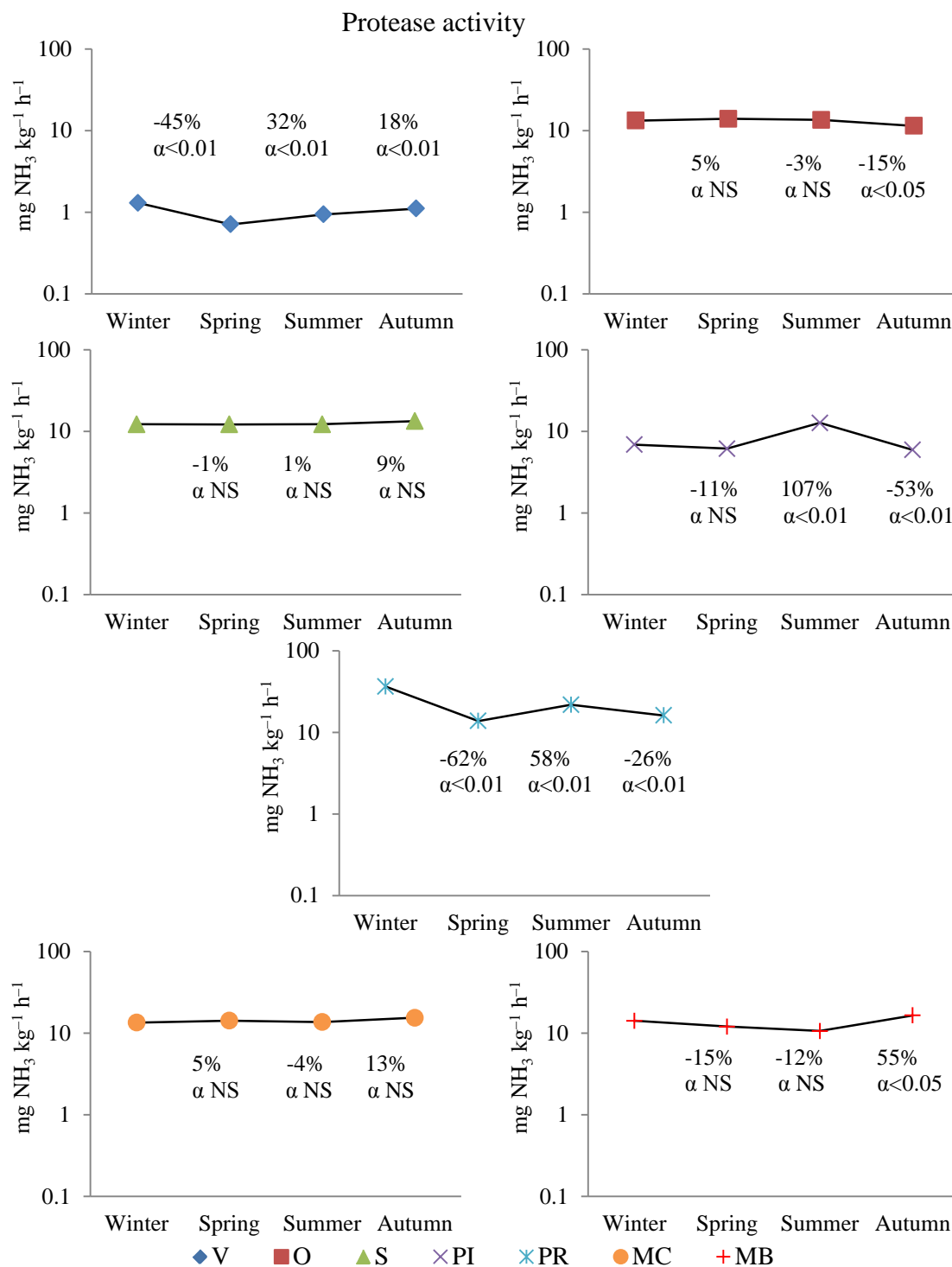


Figure 138. Seasonal fluctuations and Tukey's significance level (α) of protease activity data for each soil environment during the observed period.

The seasonal fluctuations, tested by Tukey's HSD, were highly significant in soils under vines (higher activity in winter), soils under pines (higher activity in

summer), and soils under pasture (higher activity in winter) (Figure 138). In soils under stands of pine trees, showing generally poorer soil properties, the increase in protease activity may be ascribed to a decrease in moisture which in other season may maintain more acid conditions; it was postulated that counterions, i.e. proton activity, may be reduced in drier conditions when proton adsorption at the solid/liquid interface is reduced, producing a weak increase in pH and probably in microbial biomass activity. Effectively, enzyme activities were always higher in summer season when pH increased in V, PI, and also PR soils, probably stimulated by more favorable conditions. By contrast, O, S, MC, and MB soils showed almost no significant variability.

3.9.1.3. *Phosphatase activity*

The phosphatase catalyzes the hydrolysis of phosphate esters to phosphate and therefore is an enzyme believed to play critical roles in P cycle. Compared with values of yearly means of total phosphorus found in soils under *Cistus* scrub (0.37 mg g⁻¹), *Erica* scrub (0.38 mg g⁻¹), vines (0.38 mg g⁻¹), corks (0.45 mg g⁻¹), olives (0.54 mg g⁻¹), pasture (0.70 mg g⁻¹) and pines (0.71 mg g⁻¹), the values of yearly means of phosphatase activity were in the following ascending order: 133.65, 684.16, 720.03, 802.71, 927.21, 1261.20, and 1862.71 mg pNP kg⁻¹ h⁻¹ for V, S, O, PI, MC, MB, and PR soil environments respectively. Previous studies suggested that phosphatase activity may increase in agro-ecosystems and forest ecosystems under the influence of optimal environmental conditions for microbial growth and activity (García-Ruiz et al., 2009). Other authors (Selmants and Stephen, 2010) have reported that the decline in total phosphorus, primary mineral P and labile inorganic P, is often accompanied by an increase in phosphatase activity, a result consistent with greater ecosystem-level P demand on more active soil substrates. Accordingly, the above results of TP and phosphatase may be discussed as follows: We had three groups of TP values 0.377 mg g⁻¹ (MC, MB, and V), 0.495 mg g⁻¹ (S, O) and 0.705 mg g⁻¹ (PR, PI) with very diverse phosphatase activity. Soils under *Cistus* and *Erica* scrub may probably stimulate the increase of acid phosphatase activity as it tends to increase with P deficiency (Li et al., 2004). For example, when P deficiency is detected in the soil, acid phosphatase secretion from plant roots is increased to enhance the solubilization and remobilization of phosphate, thus influencing the ability of the plant to cope with P-stressed conditions (Makoi and Ndakidemi, 2008). Many authors (Li et al., 2004) reported that the amount

of acid phosphatase exuded by plants roots has shown to differ between plant species probably justifying our higher values in soils under *Erica* (richer in plant heterogeneity and with better soil properties) than in soils under *Cistus* scrub (reduced plant heterogeneity, worse soil conditions) during the observed seasons. The extremely low phosphatase activity in soils under vines was therefore attributed to both lack of active substrates and periodical uses of P fertilizer which may be slowly metabolized due to currently very poor soil conditions. Soils under olive groves and stands of cork trees may have indicated some equilibrium mechanisms related to P demand and availability. By contrast, in soils under PI (high TP value, lower phosphatase value) P transformations in available P forms may have not occurred at a proper extent at the investigated depth due to reduced biological activity probably caused by hardly mineralizable pine needles and acid pH value (Zhao and Zeng, 2006). In soils under pasture (acid) the same process should have occurred, but is often mediated by manure addition giving the highest phosphatase values.

The values of soil phosphatase activity were generally higher in spring and autumn in all soil environments (Figure 139) probably related to the growing season and the end of vegetation cycle. Soils under pasture showed higher values in winter and autumn, corroborating that during the grazing period (winter) the soil is P enriched lowering phosphatase activity in spring but increasing it in autumn at the end of the vegetation cycle. Tukey's HSD (Q) test showed significant seasonal fluctuations in phosphatase activities in all the studied soils, however no significance was found in soils under O and S from winter to summer, under PR from spring to summer, and under MC from winter to spring in agreement with what above exposed.

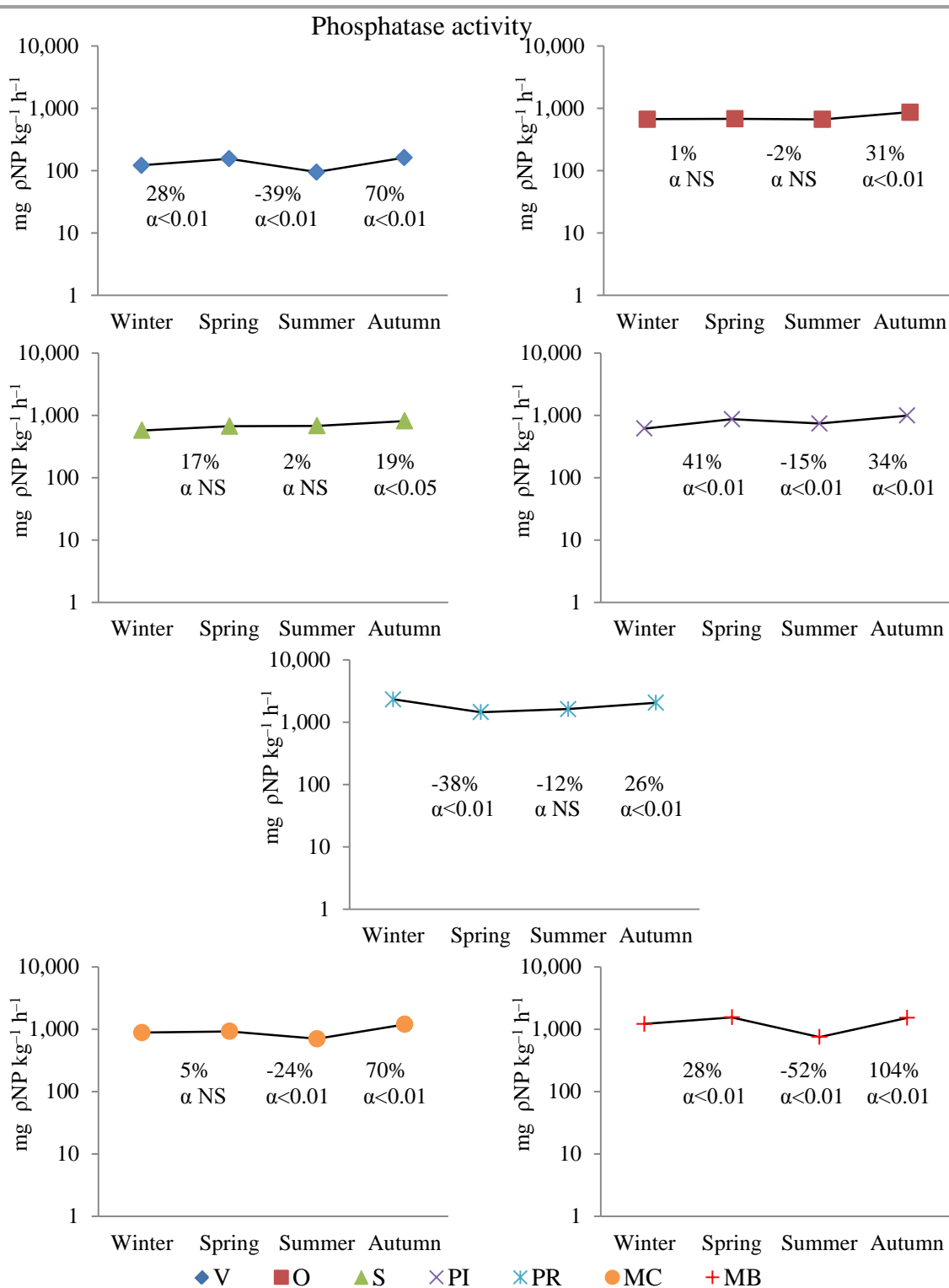


Figure 139. Seasonal fluctuations and Tukey's significance level (α) of phosphatase activity data for each soil environment during the observed period.

3.9.2. Soil enzymes and their relations to the most relevant soil properties

A correlation matrix was obtained by using all data of the main soil physical, chemical, biological, and biochemical parameters simultaneously and results are shown

in Table 90. Highly significant correlations were found when plotting the three studied hydrolytic enzymatic activity against the main soil physical, chemical, and biological soil parameters. Firstly it was observed that β -glucosidase, protease, and phosphatase were highly positively correlated with clay content. Though in minor amount in our soils, clay may enable the formation of clay-humus and clay-enzyme complexes (Pinnavaia et al., 1986; Alther, 2003). In general, sandy soils tend to exhibit lower rates of enzyme activity because of their naturally lower organic matter content and poorer water holding capacity, which in turn results in lower microbial biomass and therefore lower enzyme activity. In our cases, the organic matter content had a relevant role for its implications in a major part of soil reactivity, progressively higher in more organic soils. The negative significant correlations found between the three enzymes and the bulk density (BD) may emphasize the contribution of organic matter content in creating more spongy structure along the studied soils.

Table 90. Correlation matrix between the most relevant soil parameters against the seasonal means of soil β -glucosidase, protease, and phosphatase activities in 2009.

Soil parameters	β -glucosidase (mg pNP kg ⁻¹ h ⁻¹)		Protease (mg NH ₃ kg ⁻¹ h ⁻¹)		Phosphatase (mg pNP kg ⁻¹ h ⁻¹)	
	r	p	r	p	r	p
Clay (%)	0.627	0.000	0.734	0.000	0.881	0.000
BD (g cm ⁻³)	-0.476	0.010	-0.590	0.001	-0.722	0.000
SM (%)	0.545	0.003	0.579	0.001	0.608	0.001
WHC (%)	0.593	0.001	0.676	0.000	0.722	0.000
pH	-0.286	0.140	<i>-0.376</i>	<i>0.048</i>	-0.600	0.001
ST (°C)	-0.179	0.362	-0.210	0.284	-0.351	0.067
CO ₂ at field (μ mole m ⁻² s ⁻¹)	0.062	0.755	0.097	0.623	-0.031	0.876
CO ₂ at laboratory (mg g ⁻¹)	0.336	0.081	<i>0.383</i>	<i>0.044</i>	0.514	0.005
SOC (mg g ⁻¹)	0.616	0.000	0.714	0.000	0.745	0.000
TN (mg g ⁻¹)	0.717	0.000	0.800	0.000	0.893	0.000
TP (mg g ⁻¹)	<i>0.440</i>	<i>0.019</i>	<i>0.410</i>	<i>0.030</i>	<i>0.377</i>	<i>0.048</i>
Bacteria (cfu g ⁻¹)	0.569	0.001	0.682	0.000	0.705	0.000
Fungi (cfu g ⁻¹)	0.553	0.002	0.680	0.000	0.755	0.000

BD: Bulk density; SM: Soil moisture; WHC: Water holding capacity; ST: Soil temperature; CO₂: Carbon dioxide production; SOC: Soil organic carbon; TN: Total nitrogen; TP: Total phosphorus; SOM: Soil organic matter; Bold: p<0.01; Italic: p<0.05.

The activity of the three enzymes under study were positively correlated with soil moisture content (SM) and water holding capacity (WHC), which interact directly with the soil microbiota and organic matter. Only protease and phosphatase activities were found correlated with soil pH corroborating that soil enzymes have varying optimum pH values at which they function most effectively on their own substrate. For

instance, the activity of phosphatase involved in the phosphorus cycle is strongly correlated to variations in soil pH values (Yadav and Tarafdar, 2001). All soil enzymes showed no correlations with soil surface temperature (ST) because the soil is a heterogeneous complex system where each enzyme has specific an optimum temperature to reach its perfect complex activity. The lack of significance may also be related to the higher soil temperature producing drier soil conditions often recorded in summer which can reduce the soil microbial productivity. Similarly, no significant correlations were found with the carbon dioxide emission estimated at field conditions. However, significant correlations with protease and phosphatase were found when related to the estimated carbon dioxide emission under laboratory conditions where the soil temperature was relatively constant, (20 °C). No significant correlation was found between β -glucosidase and CO₂ emission at laboratory conditions. By excluding the summer measurements of CO₂ production, a high significant positive correlation was found ($r=+0.545$, $p=0.01$) between these parameters accounting for some changes in laboratory conditions in summer. Likewise, when data of β -glucosidase and CO₂ recorded in spring and summer at field were excluded, significant correlation ($r=+0.663$, $p<0.01$) was obtained, confirming a higher sensitivity of β -glucosidase activity to seasonal changes. Both trends are shown in Figure 140A (at laboratory conditions) and Figure 140B (at field conditions). From these results we may postulate that soil moisture and temperature may be relevant factors affecting the soil enzymes activity.

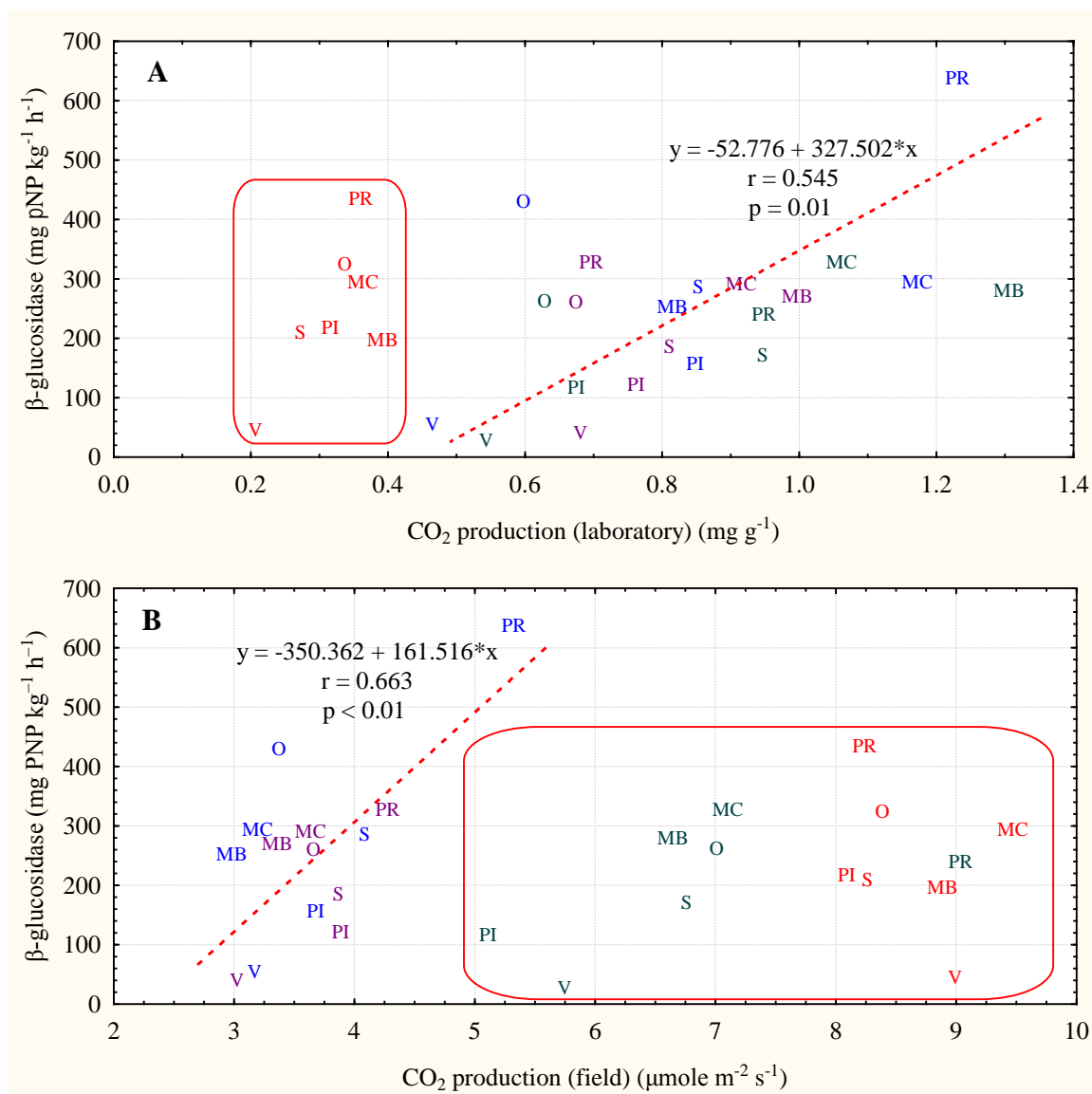


Figure 140. Relationships between seasonal means of β -glucosidase activity in soil and CO₂ production at laboratory (A) and field (B) conditions in 2009. Blue color: Winter; Dark green color: Spring; Red color: Summer; Violet color: Autumn.

β -glucosidase, protease, and phosphatase are highly positively correlated with the soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP) contents in soils. The relationships between each soil enzyme and its substrate in soil can be seen in Figure 141A, B, and C respectively.

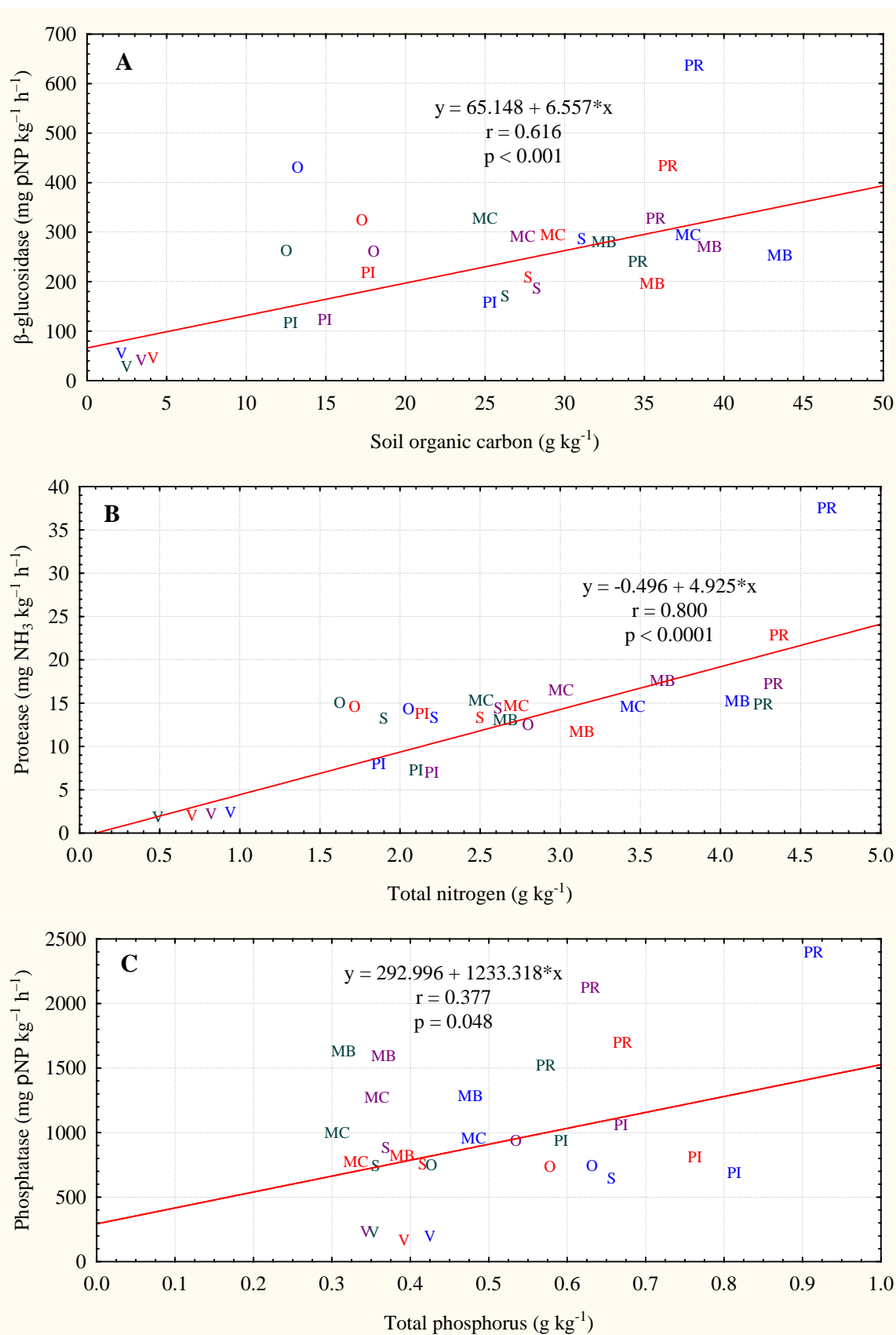


Figure 141. Relationships between the seasonal means of β -glucosidase, protease, and phosphatase versus soil organic carbon (A), total nitrogen (B) and total phosphorus (C) contents respectively for all soil environments in 2009. Blue color: Winter; Dark green color: Spring; Red color: Summer; Violet color: Autumn.

Soils under vines showed effectively the most striking results along the observed seasons which may be related to the extremely low SOC content and β -glucosidase activity. When SOC content increased 6.09, 11.22, 7.05, 14.47, 11.91 and 15.03 times in soils under O, PI, S, PR, MC, and MB with respect to vines β -glucosidase increased 13.23, 8.54, 5.92, 17.21, 12.45, and 10.16 times for the same environments. Likewise, the mean annual average of total nitrogen (TN) content in soils under O, PI, S, PR, MC, and MB resulted noticeably higher 2.86, 3.22, 2.84, 6.13, 4.10, and 4.75 times with respect to soils under vines exhibiting an increasing of 12.82, 12.29, 7.78, 21.66, 13.97, 13.10 times in protease activities in the same soil order. Both β -glucosidase and protease activity patterns showed significant correlations with SOC (Figure 141A, $r=0.616$, $p<0.001$) and TN (Figure 141B, $r=0.800$, $p<0.0001$) respectively. Total phosphorus (TP) values showed a different distribution along the soil environments, suggesting that this parameter alone may be less informative with respect to phosphatase activity. However, a progressive increase in phosphatase was recorded in soils under pasture, *Cistus*, and *Erica* scrubs showing also higher organic carbon and nitrogen content thus more active substrates (Figure 141C).

When β -glucosidase, protease, and phosphatase plotted against SOM on seasonal basis (Figure 142A,B,C), significant positive correlations were found showing how the hydrolytic enzymes are increasingly contributing to soil dynamics from vines to pasture. Similarly, positive relationships were found when plotting each data set of β -glucosidase, protease, and phosphatase against each data set of bacteria and fungi (Figure 143A,B,C and 144A,B,C respectively) indicating that fungal and bacterial populations may directly affect the cycling of soil nutrients because they form a major part of active fraction of organic compounds. They carry out the immobilization and mineralization of organic materials through diverse metabolic pathways by using specific enzymes to transform the organic substrate into the target compounds. Thus soil microbiota and soil enzymes may be considered integrative signals of the microbial significance in soils because they are biologically meaningful, easily measurable, and sensitive to different management intensities (Pajares et al., 2009). For that reason, the relationship between each hydrolytic enzyme and microbial group in soil can interpret its significant contribution to metabolic activity in soil biological ecosystems.

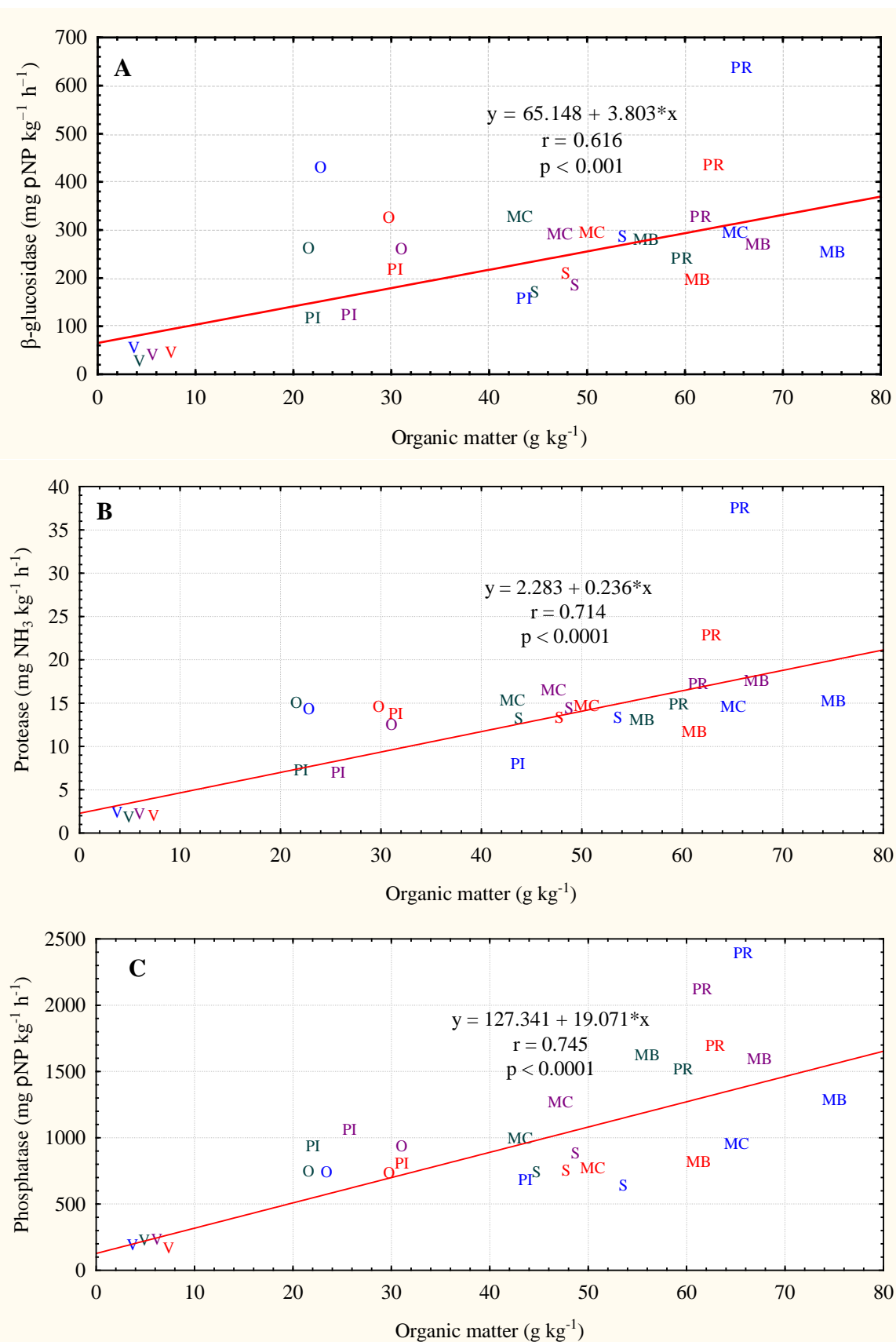


Figure 142. Linear regression equations (correlations and p level) between the seasonal means of the β -glucosidase (A), protease (B), and phosphatase (C) activities against organic matter in soil during 2009. Blue color: Winter; Dark green color: Spring; Red color: Summer; Violet color: Autumn.

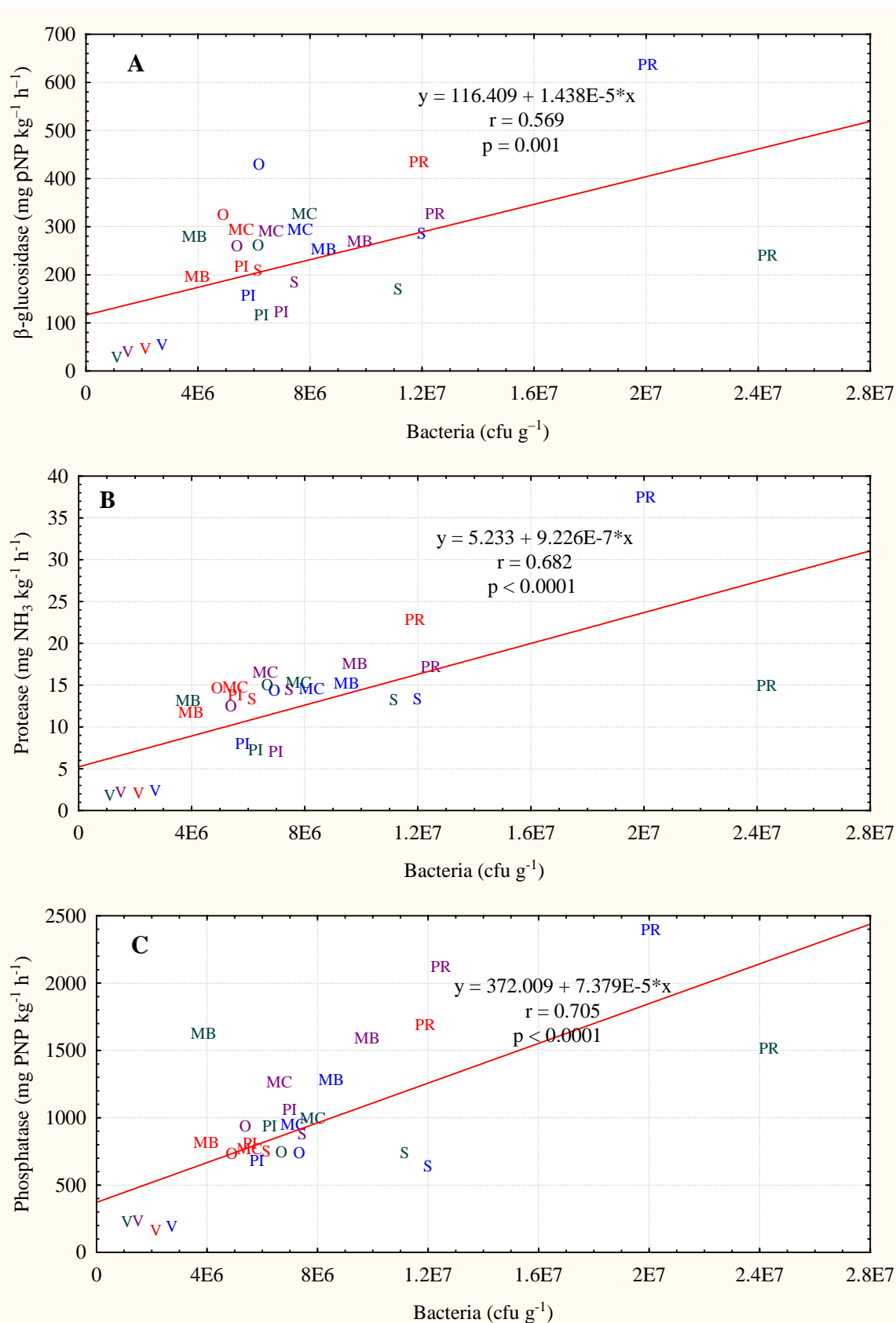


Figure 143. Linear regression equations (correlations and p level) between the seasonal means of the β -glucosidase (A), protease (B), and phosphatase (C) activities against bacterial populations in soil during 2009. Blue color: Winter; Dark green color: Spring; Red color: Summer; Violet color: Autumn.

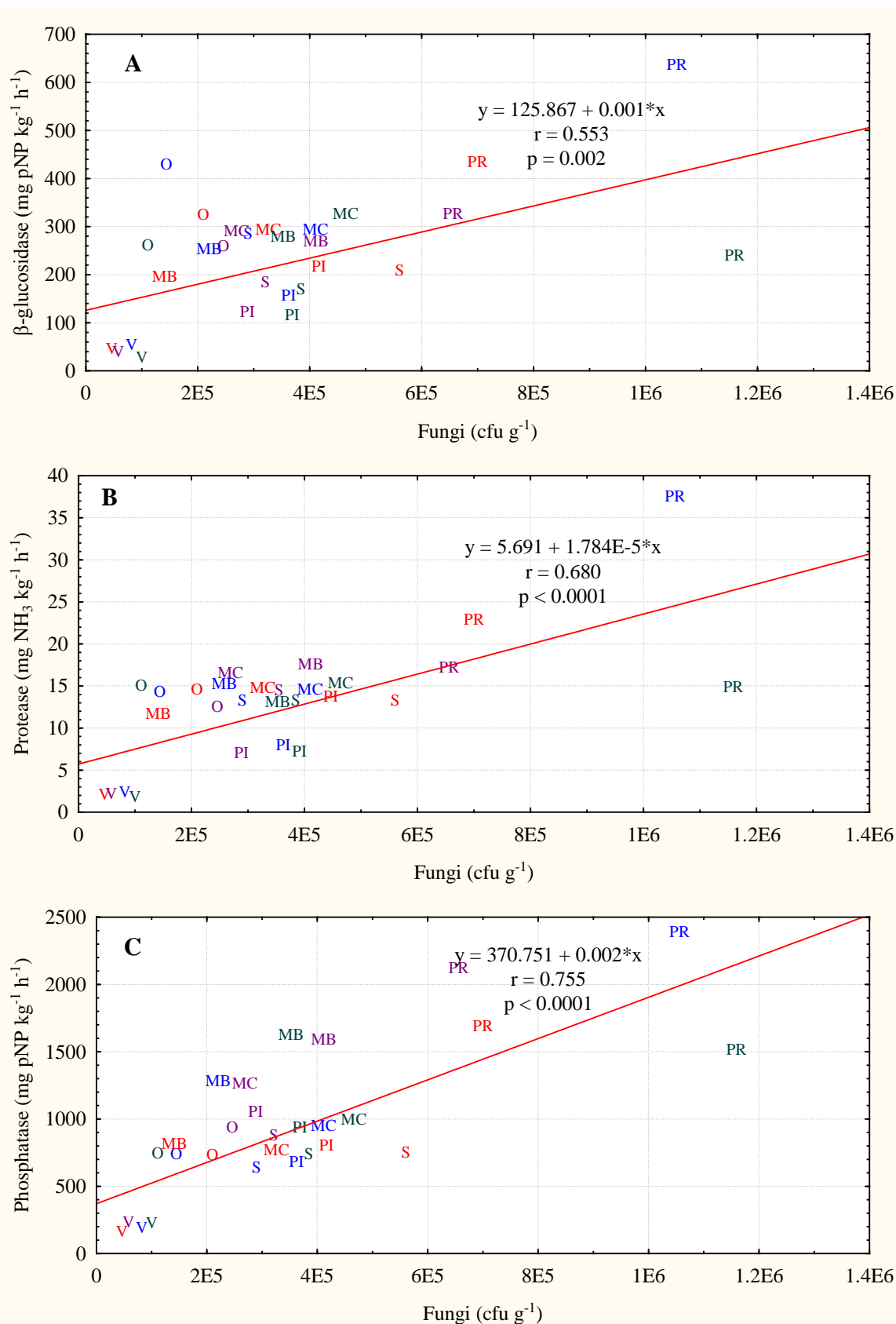


Figure 144. Linear regression equations (correlations and p level) between the seasonal means of the β -glucosidase (A), protease (B), and phosphatase (C) activities against fungal populations in soil during 2009. Blue color: Winter; Dark green color: Spring; Red color: Summer; Violet color: Autumn.

The three enzymes showed similar patterns confirming the higher productivity and activity of soil biological ecosystems during the observed period at each soil environment. Their activities vary according to the intensity of soil management and age of abandonment and moreover differ along the studied period indicating the impact of soil climatic conditions upon the soil microbiota (bacteria and fungi) and enzymatic activity.

3.9.3. Role of soil enzymes on glomalin production and structural stability

In most soils the microbial populations and their activities are fundamental for maintaining soil nutrients within stable structures by mediating the immobilization of organic compounds (Doran and Parkin, 1994). Bai et al. (2009) have devoted considerable efforts in understanding the biotic relationships among AM fungi, glomalin and soil enzymes. Soil organic carbon dynamics occurring in soils of Cap de Creus Peninsula may also be explained through glomalin, soil structure, and soil enzymes interactions and their role in soil nutrient cycling and metabolic transformations. Linear regression equations, correlations, and significance level are presented in Table 91 between β -glucosidase, protease, and phosphatase activity against both glomalin fractions (EE-BRSP and BRSP) determined at 0.00-2.00 mm soil fraction, and water stable aggregates (WSA), soil organic carbon (SOC), total glomalin (BRSP) at both 0.25-2.00 mm and 2.00-5.60 mm soil aggregate fractions. Equations were all positive indicating that β -glucosidase, protease, and phosphatase play meaningful roles in transforming soil organic C, N, and P to soil available nutrients. At this regard, many authors (Wu et al., 2008; 2012) have reported that the relationships between soil enzymes involved in carbon and nitrogen transformations in soil, such as β -glucosidase and protease, and glomalin which contain both C and N, may contribute to the release of glucose maintaining metabolically active microbial biomass in soil while contributing to a stable structure. As soil phosphatase activity represents the ability of utilizing organic phosphorus, this concept may demonstrate that the spatial distribution of glomalin may be significantly affected by AMF, β -glucosidase, protease, and phosphatase in soil. The strong relationships found between the three soil enzymes under study against both glomalin fractions at 0.00-2.00 mm (EE-BRSP and BRSP), WSA, SOC and BRSP contents at both 0.25-2.00 mm and 2.00-5.60 mm aggregate

fractions indicated that AM fungal colonization may be a key component for glomalin production in soil.

Table 91. Linear regression equations, correlations, and significance level between the three soil enzymes and easily extractable glomalin (EE-BRSP), total glomalin (BRSP), soil organic carbon (SOC), and aggregate stability (WSA) measured in the two aggregate classes for all soil environments.

Soil parameters	Equations	r	p-level
β-glucosidase (y axis)			
EE-BRSP (mg g ⁻¹)	$y = -149.930 + 405.989 x$	0.779	0.000
BRSP (mg g ⁻¹)	$y = 118.323 + 32.615 x$	0.429	<i>0.023</i>
WSA _{0.25-2.00 mm} (%)	$y = -514.622 + 8.348 x$	0.503	0.006
WSA _{2.00-5.60 mm} (%)	$y = 48.843 + 2.091 x$	0.355	0.064
SOC _{0.25-2.00 mm} (mg g ⁻¹)	$y = 84.084 + 61.739 x$	0.631	0.000
SOC _{2.00-5.60 mm} (mg g ⁻¹)	$y = 73.405 + 70.421 x$	0.582	0.001
BRSP _{0.25-2.00 mm} (mg g ⁻¹)	$y = 14.313 + 63.270 x$	0.715	0.000
BRSP _{2.00-5.60 mm} (mg g ⁻¹)	$y = -39.985 + 78.277 x$	0.789	0.000
Protease (y axis)			
EE-BRSP (mg g ⁻¹)	$y = -9.035 + 22.968 x$	0.823	0.000
BRSP (mg g ⁻¹)	$y = 5.679 + 1.991 x$	0.490	0.008
WSA _{0.25-2.00 mm} (%)	$y = -30.822 + 0.485 x$	0.547	0.003
WSA _{2.00-5.60 mm} (%)	$y = 0.299 + 0.141 x$	0.449	<i>0.017</i>
SOC _{0.25-2.00 mm} (mg g ⁻¹)	$y = 3.738 + 3.702 x$	0.708	0.000
SOC _{2.00-5.60 mm} (mg g ⁻¹)	$y = 2.286 + 4.607 x$	0.712	0.000
BRSP _{0.25-2.00 mm} (mg g ⁻¹)	$y = -0.057 + 3.675 x$	0.777	0.000
BRSP _{2.00-5.60 mm} (mg g ⁻¹)	$y = -2.719 + 4.400 x$	0.830	0.000
Phosphatase (y axis)			
EE-BRSP (mg g ⁻¹)	$y = -541.407 + 1588.328 x$	0.735	0.000
BRSP (mg g ⁻¹)	$y = 449.420 + 146.074 x$	0.464	<i>0.013</i>
WSA _{0.25-2.00 mm} (%)	$y = -1932.533 + 32.256 x$	0.469	<i>0.012</i>
WSA _{2.00-5.60 mm} (%)	$y = -35.217 + 11.461 x$	0.470	<i>0.012</i>
SOC _{0.25-2.00 mm} (mg g ⁻¹)	$y = 185.726 + 325.967 x$	0.805	0.000
SOC _{2.00-5.60 mm} (mg g ⁻¹)	$y = 80.209 + 395.118 x$	0.788	0.000
BRSP _{0.25-2.00 mm} (mg g ⁻¹)	$y = 2.988 + 277.454 x$	0.757	0.000
BRSP _{2.00-5.60 mm} (mg g ⁻¹)	$y = -163.822 + 321.947 x$	0.784	0.000

Bold: $p < 0.01$; Italic: $p < 0.05$.

3.9.4. Statistical approach for interpreting the dynamics of soil microbial activities in the studied soils

PCA was used to underline the interaction mechanisms of soil enzymes with the most relevant soil physical, chemical, biological, and biochemical properties (Table 92).

Table 92. PCA loadings followed by varimax rotation of physical, chemical, biological and biochemical properties determined in the different soil environments. Values below 0.50 are omitted.

Variables	PC 1	PC 2	PC 3	Communality
Clay (%)	0.92			0.90
BD (g cm ⁻³)	-0.86			0.90
SM (%)	0.63		0.59	0.92
WHC (%)	0.92			0.93
pH	-0.50	-0.50		0.40
EC (dS m ⁻¹)	0.57			0.58
SOC (mg g ⁻¹)	0.95			0.96
TN (mg g ⁻¹)	0.94			0.88
TP (mg g ⁻¹)		0.68		0.63
C/N	0.64			0.66
C/P	0.71	0.59		0.94
N/P	0.72	0.50		0.81
EE-BRSP (mg g ⁻¹)	0.87			0.81
BRSP (mg g ⁻¹)	0.60			0.90
WSA _{0.25-2.00 mm} (%)	0.69			0.64
WSA _{2.00-5.60 mm} (%)	0.66			0.72
SOC _{0.25-2.00 mm} (mg g ⁻¹)	0.92			0.90
SOC _{2.00-5.60 mm} (mg g ⁻¹)	0.91			0.82
BRSP _{0.25-2.00 mm} (mg g ⁻¹)	0.95			0.90
BRSP _{2.00-5.60 mm} (mg g ⁻¹)	0.93			0.87
ST (°C)			-0.68	0.71
Fungi (cfu g ⁻¹)	0.76			0.81
Bacteria (cfu g ⁻¹)	0.75			0.79
C-CO ₂ (μmole m ⁻² s ⁻¹)			-0.67	0.59
β-glucosidase (mg pNP kg ⁻¹ h ⁻¹)	0.74			0.62
Protease (mg NH ₃ kg ⁻¹ h ⁻¹)	0.83			0.76
Phosphatase (mg pNP kg ⁻¹ h ⁻¹)	0.87			0.82
Explained variance				
Total (%)	58.34	10.86	9.87	
Cumulative (%)	58.34	69.20	79.07	79.07

BD: Bulk density; SM: Soil moisture; WHC: Water holding capacity; EC: Electrical conductivity; SOC: Soil organic carbon; TN: Total nitrogen; TP: Total phosphorus; EE-BRSP: Easily extractable Bradford reactive soil protein; BRSP: Bradford reactive soil protein; WSA: Water stable aggregates; ST: Soil temperature; C-CO₂: Carbon loss as CO₂; C/N: Carbon/Nitrogen; C/P: Carbon/phosphorus; N/P: Nitrogen/phosphorus.

Eigenvalues from this analysis indicated that the first three principal components (PCs) accounted for 79.07% of the total variance of data (PC1: 58.34%, PC2: 10.86%, PC3: 9.87%). Within the component's structure, PC1 showed that clay, soil moisture (SM), water holding capacity (WHC), electrical conductivity (EC), soil organic carbon (SOC_{0.00-2.00 mm}, SOC_{0.25-2.00 mm}, and SOC_{2.00-5.60 mm}), total nitrogen (TN), total phosphorus (TP), C/N, C/P, N/P ratios, easily extractable Bradford reactive soil protein (EE-BRSP_{0.00-2.00 mm}), Bradford reactive soil protein (BRSP_{0.00-2.00 mm}, BRSP_{0.25-2.00 mm}, and BRSP_{2.00-5.60 mm}), water stable aggregates (WSA_{0.25-2.00 mm} and WSA_{2.00-5.60 mm}), fungal and bacterial populations, and hydrolytic enzyme activities were all significantly and positively correlated, whilst bulk density (BD) and soil pH exhibited negative loads (Table 92). This component was able to underline the relation between soil hydrolytic enzymes and soil nutrients supported by the most relevant soil characteristics to provide the biological metabolic transformation processes in soil. In the PC2, total phosphorus (TP) showed high positive loadings while soil pH had negative load. The third component (PC3) showed that carbon loss estimated by CO₂ emission was negatively correlated to this component together with soil temperature (ST) against soil moisture (SM). The communality values showed that SOC, with the highest value, is the reliable soil parameter able to control different mechanisms occurred in soil ecosystems especially those indicated by the first three components such as the metabolic transformations of soil nutrients (PC1), nutrient limitations (PC2), and carbon loss dynamics (PC3).

In addition, PCA analysis provided information about the relationships between treatments (environments and seasons) and variables to improve knowledge on the overall dynamics in the area of study. The PCA plot diagram (Figure 145) was generated to show the environment position through seasonal observations at the orthogonal space defined by the two first PCs. The contribution along the PC1 accounted for 58.34% of the total variance and may be mainly associated to the changes in soil use and management and abandonment. As before, the lowest contribution was recorded in soils under vines (negative part of the plot diagram) with worse structure, weak aggregates particularly susceptible to detachment, low clay amount and organic matter content, low enzyme activities, scarce microbial populations, and reduced glomalin production. Negative intermediate contributions were established for soils under olive groves (O) and stands of pine trees (PI) along the observed periods.

Increasing positive contribution to soil properties was found in soils under stands of cork trees (S) and *Cistus* scrub (MC), whereas the highest positive contribution to the PC1 was ascribed to soils under *Erica* scrub and pasture (PR) which always presented the best soil characteristics. PC2 accounted for 10.86% of the total variance and arranged the environments taking into account the seasonal climatic variability. For instance, all soil environments were grouped in summer season (red color) probably associated with pH variation whereas TP was mainly related to environments like PI, O, MC.

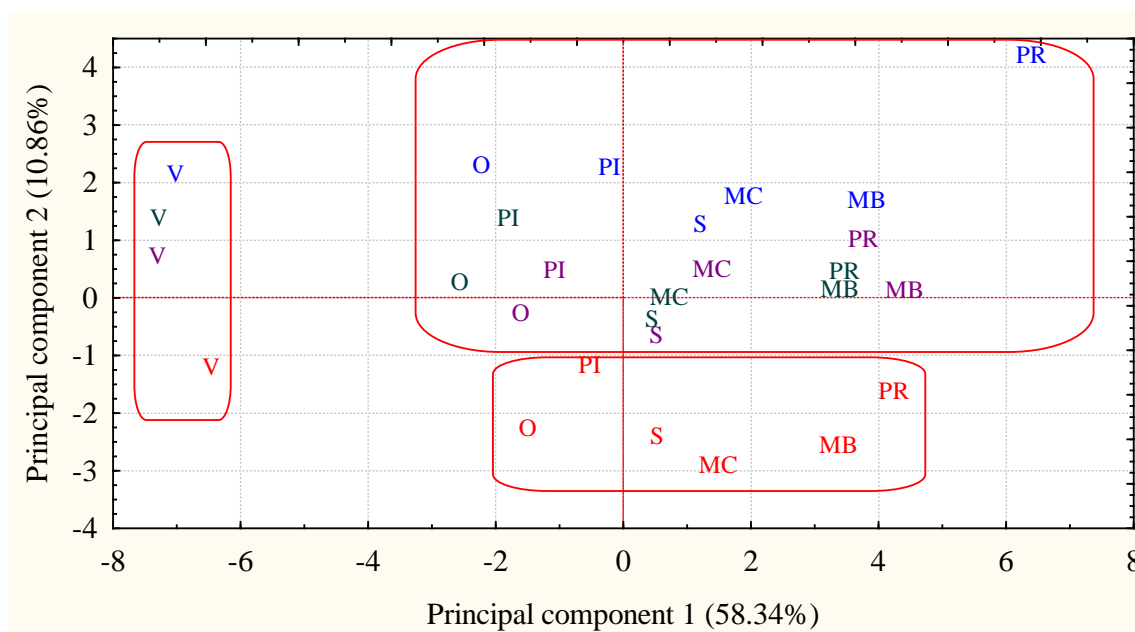


Figure 145. PCA plot diagram of the first two principal components. Blue color: Winter; Dark green color: Spring; Red color: Summer; Violet color: Autumn.

As a general view for the data plotted in the graph between the first two components, three different groups were separated: one including all the summer observations from all soils except soils under vines, a second group formed by those soils sampled in winter, spring, and autumn except soils under vines (V), and a third group containing all seasons for soils under vines (V). The three pictures may help in interpreting higher positive or negative impact of soil use and management and abandonment along with the seasonal changes in climatic conditions. At this regard, the results may give an advice of early warning indicators of soil health and be used for post abandonment and sound management when used for carbon sinks. Soil enzymes resulted then positively used as indicators of soil metabolic activity (Garcia and Hernandez, 1997) and as additional diagnostic indices of soil fertility and soil quality (Yakovchenko et al., 1996).

3.10. Structural composition of soil organic matter

The structural composition of soil organic matter (SOM) has been taken into account due to its importance in the evaluation of soil quality and productivity, its role to control the integration study between soil ecosystems, and its sensitivity to climatic changes, soil use and management, and age of abandonment. SOM can be divided into two major pools based on their relative susceptibility to biological decomposition: a more resistant pool (humic substances) and an easily metabolized pool (non-humic or labile fractions of SOM). On the one hand, humic substances, make up a significant portion of the total organic carbon and nitrogen in soil, are complex polymeric organic compounds of high molecular weight comprising polyphenols, proteins, active enzymes, lipids, and polysaccharides, which are more resistant to decomposition than the non-humic materials (Ceccanti et al., 1986; Ceccanti et al., 2007). Humic substances are also considered an important fraction of SOM as they regulate nutrients availability associated with mineral particles and immobilized compounds (Ceccanti and Garcia 1994). On the other hand, labile fractions of SOM consist of partially decomposed roots and non-harvested shoots materials and have a rapid turnover in soil (Masciandaro and Ceccanti, 1999). The non-humic substances contribute to soil ecosystem functionality by providing metabolically labile organic C and N sources, such as low molecular weight aliphatic and aromatic acids, carbohydrates, amino acids, and their polymeric derivatives such as polypeptides, proteins, polysaccharides, and waxes (Page et al., 1986; Schnitzer, 1991). Biological properties and labile fractions of SOM may be considered much more sensitive to soil management than soil physical and chemical properties (Nannipieri et al., 1990; Gil-Sotres et al., 2005; Pajares et al., 2009). The labile fractions are important because readily undergoing modifications by decomposing processes, being the most susceptible to be lost by mineralization, which besides that, provides mineral nutrients for microorganisms and plants. This concept may help for understanding the dynamic mechanisms of mineralization and immobilization of SOM governed by soil microbial activity, carbon availability, and climatic factors (Hassink and Whitmore, 1997).

3.10.1. Chemico-structural properties of organic matter

Py-GC was used for the determination of humic substances in our soils under different land use and abandonment (Ceccanti et al., 1986; Ceccanti and Garcia, 1994; Masciandaro et al., 1998).

3.10.1.1. *Pyrolytic fragments of SOM*

Pyrograms are interpreted by the quantification of seven peaks corresponding to the major volatile pyrolytic fragments (Bracewell and Robertson, 1984; Ceccanti et al., 1986; 2007):

- **E1:** Acetonitrile is derived from the pyrolysis of amino acids, proteins, and microbial cells.
- **AA:** Acetic acid is preferentially derived from pyrolysis of lipids, fats, waxes, cellulose, and carbohydrates (Bracewell and Robertson, 1984) and represents relatively less-degraded lignocellulose material.
- **B:** Benzene comes from several sources, but indicates the state of condensation (humification) of aromatic humic molecules.
- **PY:** Pyrrole is derived from nitrogenous compounds such as nucleic acids, proteins, microbial cells, and condensed humic structures (Bracewell and Robertson, 1984).
- **E3:** Toluene is basically derived from condensed aromatic structures of stable (humified) organic matter with aliphatic chains, albeit short.
- **FF:** Furfural is mostly derived from carbohydrates, ligno cellulosic materials, proteins and other aliphatic organic compounds (Bracewell and Robertson, 1984), indicating the presence of rapidly metabolizable organic substances.
- **Y:** Phenol is derived from amino acids, tannins and fresh or condensed (humic) ligno cellulosic structures. Its evolution is related to the hydrolytic processes of decomposition and humification of fresh or condensed ligno cellulosic (humic) structures.

Peak areas were normalized, so that the area under each peak referred to the percentage of each of the selected seven peaks (relative abundances). The alphanumeric code used was conventional and was already employed by different authors (Ceccanti et al., 1986; Bonmati et al., 1998; Ceccanti et al., 2007).

The assessment of Py-GC fragments normally gives evidence of micro-structural differences in SOM. The relative abundances of the main pyrolytic fragments are reported in Table 93 together with descriptive statistical analysis. All soils were characterized by a greater abundance of aromatic compounds (benzene, toluene, pyrrole and phenol) than aliphatic compounds (acetic acid, furfural and acetonitrile) indicating higher humic than labile organic substances at all the studied soils. The annual average of acetonitrile (E1) values was higher in V and PI environments and progressively lower in O, S, MC, MB, and PR. The combined pyrrole (PY) and acetonitrile (E1) fragments derived from both aromatic and aliphatic nitrogenous humic substances (Bracewell and Robertson, 1976; Masciandaro et al., 1998) showed the lowest average values in soils under cultivated vines ($8.97\%PY + 12.73\%E1 = 21.70\%$) followed by soils under stands of pine trees ($9.66\%PY + 12.38\%E1 = 22.04\%$), soils under *Cistus* ($11.18\%PY + 10.94\%E1 = 22.13\%$) and *Erica* ($11.64\%PY + 10.92\%E1 = 22.56\%$) scrub, soils under pasture ($12.61\%PY + 10.31\%E1 = 22.91\%$), soils under stands of cork trees ($11.87\%PY + 11.32\%E1 = 23.18\%$), and the highest average was in soils under olive groves ($12.12\%PY + 11.88\%E1 = 24.00\%$). This suggested that soil organic matter in MB, PR, S, and O soil environments were enriched in nitrogenous organic compounds relatively resistant to biodegradation than V, PI, and MC soil environments.

Furfural (FF), which is probably derived from proteins and microbial cells, showed increasing amounts from vines (V) to *Erica* scrub (MB) ($V \rightarrow O \rightarrow PI \rightarrow S \rightarrow MC \rightarrow PR \rightarrow MB$), indicating an order of abundance of nitrogenous microbial compounds. The presence of aliphatic cellulosic organic compounds indicated by the combined fragments of acetic acid and furfural showed similar trend with the lowest values in soils under cultivated vines ($11.92\%AA + 9.47\%FF = 21.39\%$). This values increased in soils under olive groves ($13.06\%AA + 12.36\%FF = 25.42\%$), soils under stands of pine ($13.41\%AA + 13.28\%FF = 26.68\%$) and cork ($13.00\%AA + 14.48\%FF = 27.47\%$) trees, soils under *Cistus* scrub ($14.10\%AA + 15.65\%FF = 29.75\%$), and soils under pasture ($14.10\%AA + 16.15\%FF = 30.25\%$). The highest combined average values were found in soils under *Erica* scrub ($14.83\%AA + 16.94\%FF = 31.77\%$). Furfural (FF) and acetic acid (AA), resulting from the pyrolysis of cellulose, proteins and lipid hydrocarbons, had lower values at the cultivated soils (V and O) and soils under forests (S and PI), indicating a marked mineralization of labile organic matter probably due to minimum and insufficient agricultural management. The combined values of FF and AA volatile

fragments had higher values in soils under scrubs (MC and MB) and pasture (PR) suggesting their enrichment in condensed organic compounds following land abandonment.

The presence of more stable humic compounds in soils under study was confirmed by the trend of phenol (Y) and furfural (FF), both arising predominantly from ligno cellulosic materials, carbohydrates, and proteins (Bracewell and Robertson, 1976; Masciandaro et al., 1998; Ceccanti et al., 2007). The average values of the combined fragments of phenol (Y) and furfural (FF) showed the same soil order as follows: V (20.19%) → O (22.15%) → PI (23.07%) → S (24.45%) → MC (25.48%) → PR (25.59%) → MB (26.89%). These pyrolytic compounds were found significantly lower in the cultivated soils, in which metabolizable organic substances were probably lost more rapidly than in other soils. This picture, which showed lower values in PI and MC with respect to S and MB respectively, can infer that wildfire occurrence has influenced the stability of both aromatic as well as aliphatic organic compounds.

Other fragments, benzene (B) and toluene (E3), which are considered mostly arising from condensed aromatic structures of stable (humified) organic matter (Bracewell and Robertson, 1976; Masciandaro et al., 1998), seemed to characterize different soil order: V (24.31%B+21.89%E3= 46.20%) > PI (22.58%B+18.91%E3= 41.49%) > O (20.42%B+20.38%E3= 40.80%) > S (19.15%B+20.22%E3= 39.37%) > MC (19.89%B+18.41%E3= 38.29%) > PR (18.63%B+18.77%E3= 37.40%) > MB (18.28%B+17.44%E3= 35.72%) soil environments. Higher abundance of benzene (B) and toluene (E3) in those soils with low agricultural management may be related to the presence of higher aromatic humic substances ratio with respect to non-humic substances. This seemed to reflect the higher ratio of native SOM rather than fresh, easily decomposable organic C that may have faster turnover times due to the higher mineralization processes.

Table 93. Relative abundances (%) of main pyrolytic fragments: acetonitrile E1, acetic acid AA, benzene B, pyrrole PY, toluene E3, furfural FF, and phenol Y, for all soil environments during the observed seasons.

ENV	Season	E1 (%)	AA (%)	B (%)	PY (%)	E3 (%)	FF (%)	Y (%)
V	Winter	12.51	11.46	24.17	7.72	22.90	10.99	10.25
	Spring	12.21	12.45	23.19	9.10	21.84	10.71	10.51
	Summer	13.22	13.02	24.59	9.45	20.27	8.94	10.51
	Autumn	12.96	10.76	25.27	9.61	22.56	7.24	11.60
	$\bar{x} \pm \sigma$	12.73 \pm 0.45	11.92 \pm 1.01	24.31 \pm 0.87	8.97 \pm 0.86	21.89 \pm 1.17	9.47 \pm 1.74	10.72 \pm 0.60
	CV	3.55	8.45	3.58	9.59	5.34	18.39	5.61
O	Winter	12.20	12.48	25.08	11.03	20.48	7.85	10.88
	Spring	11.35	13.64	17.94	11.93	20.00	15.66	9.48
	Summer	11.14	12.48	19.01	12.83	20.55	14.23	9.76
	Autumn	12.84	13.62	19.65	12.67	20.48	11.70	9.04
	$\bar{x} \pm \sigma$	11.88 \pm 0.79	13.06 \pm 0.66	20.42 \pm 3.19	12.12 \pm 0.82	20.38 \pm 0.25	12.36 \pm 3.42	9.79 \pm 0.78
	CV	6.61	5.09	15.60	6.79	1.25	27.70	8.02
S	Winter	11.14	12.48	19.01	12.83	20.55	14.23	9.76
	Spring	11.04	12.89	19.33	11.21	19.89	14.79	10.85
	Summer	11.10	13.50	19.15	11.67	20.26	15.12	9.19
	Autumn	11.98	13.11	19.11	11.76	20.18	13.77	10.09
	$\bar{x} \pm \sigma$	11.32 \pm 0.45	13.00 \pm 0.43	19.15 \pm 0.13	11.87 \pm 0.69	20.22 \pm 0.27	14.48 \pm 0.60	9.97 \pm 0.69
	CV	3.93	3.28	0.70	5.78	1.34	4.13	6.95
PI	Winter	12.30	13.30	18.85	11.07	19.46	15.44	9.58
	Spring	12.55	12.88	24.92	9.52	18.72	11.28	10.14
	Summer	11.25	14.20	21.99	9.33	19.03	14.70	9.49
	Autumn	13.42	13.24	24.57	8.71	18.41	11.69	9.97
	$\bar{x} \pm \sigma$	12.38 \pm 0.89	13.41 \pm 0.56	22.58 \pm 2.81	9.66 \pm 1.00	18.91 \pm 0.45	13.28 \pm 2.10	9.80 \pm 0.31
	CV	7.22	4.19	12.45	10.39	2.37	15.80	3.17
PR	Winter	10.05	13.49	20.57	12.32	19.12	13.83	10.61
	Spring	10.30	14.66	17.82	11.98	17.08	18.34	9.82
	Summer	9.67	14.57	17.16	12.84	19.72	17.39	8.66
	Autumn	11.20	13.69	18.97	13.28	19.15	15.02	8.69
	$\bar{x} \pm \sigma$	10.31 \pm 0.65	14.10 \pm 0.60	18.63 \pm 1.49	12.61 \pm 0.57	18.77 \pm 1.16	16.15 \pm 2.08	9.45 \pm 0.95
	CV	6.31	4.24	8.02	4.54	6.17	12.89	10.01
MC	Winter	11.25	14.20	21.99	9.33	19.03	14.70	9.49
	Spring	10.89	14.34	19.28	12.00	16.86	16.58	10.04
	Summer	9.79	14.25	18.81	12.22	19.17	15.87	9.88
	Autumn	11.84	13.60	19.46	11.18	18.57	15.44	9.92
	$\bar{x} \pm \sigma$	10.94 \pm 0.86	14.10 \pm 0.34	19.89 \pm 1.43	11.18 \pm 1.31	18.41 \pm 1.06	15.65 \pm 0.79	9.83 \pm 0.24
	CV	7.88	2.39	7.19	11.75	5.77	5.03	2.42
MB	Winter	11.67	15.00	17.44	11.43	20.43	15.10	8.93
	Spring	9.88	15.20	18.06	11.43	15.63	18.99	10.80
	Summer	10.83	14.15	19.18	11.57	17.11	17.04	10.13
	Autumn	11.31	14.98	18.43	12.13	16.58	16.63	9.94
	$\bar{x} \pm \sigma$	10.92 \pm 0.78	14.83 \pm 0.47	18.28 \pm 0.73	11.64 \pm 0.33	17.44 \pm 2.09	16.94 \pm 1.60	9.95 \pm 0.77
	CV	7.10	3.14	3.98	2.86	11.97	9.45	7.77

ENV: Soil environments; V: Soils under vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub; \bar{x} : Mean; σ : Standard deviation; CV: Coefficient of variation.

The results in Table 93 showed rather low interseasonal variability within each environment as indicated by the coefficient of variation values, whilst higher differences were detected between the soil environments (treatments). Data were then run by two-way ANOVA in order to assess their significance along treatments and seasons (Table 94).

Table 94. Two-way ANOVA by using the seasonal values of the seven pyrolytic fragments for the studied soil environments.

Pyrolytic fragments	Treatments		Seasons	
	F	p	F	p
Acetonitrile E1	11.845	0.000	8.094	0.001
Acetic acid AA	10.487	0.000	1.622	0.219
Benzene B	5.402	0.001	0.502	0.685
Pyrrole PY	9.543	0.000	0.711	0.558
Toluene E3	10.366	0.000	3.980	<i>0.024</i>
Furfural FF	8.521	0.000	2.657	0.080
Phenol Y	1.345	0.289	0.859	0.480

Bold: $p < 0.01$; Italic: $p < 0.05$.

All the analyzed pyrolytic fragments of SOM showed significant variability ($p < 0.001$) along the changing conditions imposed to soils by different environments representing the sequence of land use and abandonment. Only phenol (Y), derived from aromatic condensed/humified substances originated from ligno cellulosic structures, did not show significant variability. Significant variability ($p < 0.01$) was found within the acetonitrile values may be due to the seasonal influences upon the addition of fresh/labile organic substances. Moreover, significant changes ($p < 0.05$) were found within the values of toluene, indicating a possible seasonality impact upon the aromatic nitrogenous compounds.

3.10.1.2. *Pyrolytic indices*

Ratios between relative abundances of the SOM chemical components were determined (Ceccanti et al., 1986; 2007; Marinari et al., 2007) to highlight the following information on organic matter:

- Furfural/Pyrrole (FF/PY): Mineralization index of labile soil organic matter. This index expresses the ratio between furfural (FF), which is the pyrolytic product arising from polysaccharides, and pyrrole (PY), which derives from nitrogenated

compounds, humified organic matter, and microbial cells. The highest the ratio, the lowest the mineralization of the organic matter, that means a high concentration of polysaccharides may be present.

- Pyrrole/Phenol (PY/Y): Mineralization index of more stable organic matter. The highest the ratio, the highest the mineralization of organic matter, because pyrrole derives from nitrogenous compounds and microbial cells and phenol (Y) mostly derives from polyphenolic compounds.
- Benzene/Toluene (B/E3): Humification index. The highest the ratio, the highest the condensation of the organic matter, because benzene (B) derives mostly from the pyrolytic degradation of condensed aromatic structures, while toluene (E3) comes from uncondensed aromatic rings with aliphatic chains.
- Aliphatic/Aromatic compounds (AL/AR): Index of “energy reserve”. The index of AL/AR expresses the ratio between the sum of aliphatic products (acetic acid, furfural, and acetonitrile) and the sum of aromatic hydrocarbons (benzene, toluene, pyrrole, and phenol). This index may give a "reserve of energy" in extreme terrain poor in organic matter because it considers the labile and stable component of humic substances.

Differentiation of pyrolytic peaks was better shown by the pyrolytic indices of mineralization (FF/PY, PY/Y), humification (B/E3), and preserved energy (AL/AR). The performance of these indices is shown in Table 95. As for the pyrolytic fragments, there is no tendency in the seasonal variation among the representative mineralization, humification or energy reserve indices.

The FF/PY and PY/Y mineralization indices gave evidence of the existence of mineralization of labile and more stable fraction of organic substances, respectively. It was said (Ceccanti et al., 1986; 2007; Marinari et al., 2007) that highest values of FF/PY indicate lowest mineralization index while highest values of PY/Y correspond to highest mineralization index. Therefore, relatively high mineralization of labile organic compounds were indicated by FF/PY ratio (cellulosic aliphatic compounds/nitrogenous aromatic compounds) in soils under cultivated vines and olive groves, whereas intermediate values were found under stands of pine and cork trees and under pasture, and a lower mineralization index was recorded in soils under *Cistus* and *Erica* scrub (Table 95).

Table 95. Pyrolytic indices of mineralization (furfural/pyrrole FF/PY, pyrrole/phenol PY/Y), humification (benzene/toluene, B/E3), the sum of aliphatic products (acetic acid, furfural, and acetonitrile) and the sum of aromatic hydrocarbons (benzene, toluene, pyrrole, and phenol), and aliphatic/aromatic (AL/AR) ratios.

ENV	Season	FF/PY	PY/Y	B/E3	AL (%)	AR (%)	AL/AR
V	Winter	1.42	0.75	1.06	34.95	65.05	0.54
	Spring	1.18	0.87	1.06	35.36	64.64	0.55
	Summer	0.95	0.90	1.21	35.18	64.82	0.54
	Autumn	0.75	0.83	1.12	30.95	69.05	0.45
O	Winter	0.71	1.01	1.22	32.54	67.46	0.48
	Spring	1.31	1.26	0.90	40.66	59.34	0.69
	Summer	1.11	1.31	0.93	37.85	62.15	0.61
	Autumn	0.92	1.40	0.96	38.16	61.84	0.62
S	Winter	1.11	1.31	0.93	37.85	62.15	0.61
	Spring	1.32	1.03	0.97	38.71	61.29	0.63
	Summer	1.30	1.27	0.95	39.73	60.27	0.66
	Autumn	1.17	1.16	0.95	38.85	61.15	0.64
PI	Winter	1.40	1.16	0.97	41.05	58.95	0.70
	Spring	1.19	0.94	1.33	36.71	63.29	0.58
	Summer	1.58	0.98	1.16	40.16	59.84	0.67
	Autumn	1.34	0.87	1.33	38.34	61.66	0.62
PR	Winter	1.12	1.16	1.08	37.38	62.62	0.60
	Spring	1.53	1.22	1.04	43.31	56.69	0.76
	Summer	1.35	1.48	0.87	41.63	58.37	0.71
	Autumn	1.13	1.53	0.99	39.91	60.09	0.66
MC	Winter	1.58	0.98	1.16	40.16	59.84	0.67
	Spring	1.38	1.20	1.14	41.81	58.19	0.72
	Summer	1.30	1.24	0.98	39.92	60.08	0.66
	Autumn	1.38	1.13	1.05	40.88	59.12	0.69
MB	Winter	1.32	1.28	0.85	41.77	58.23	0.72
	Spring	1.66	1.06	1.16	44.08	55.92	0.79
	Summer	1.47	1.14	1.12	42.02	57.98	0.72
	Autumn	1.37	1.22	1.11	42.92	57.08	0.75

ENV: Soil environments; V: Soils under vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub.

The seasonal effect on soil environments and their FF/PY values is more clearly indicated in Figure 146. Higher FF/PY values are generally found in those soils with higher organic matter content except vines in winter. It was suggested that in winter soils under MB, PR, S, and O environments were sensitive to lose labile organic compounds due to higher biological activity. By contrast in spring, summer and autumn, higher FF/PY values may indicate best regulations in the mineralization/humification dynamics.

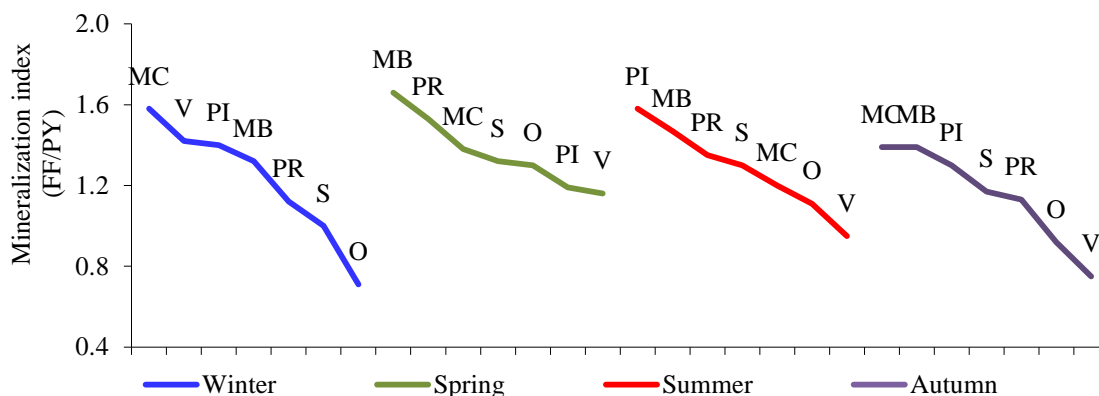


Figure 146. Seasonal trends of mineralization index (FF/PY) of labile organic compounds for the selected soil environments.

The mineralization of more stable organic compounds indicated by PY/Y ratio (nitrogenous aromatic compounds/cellulosic aliphatic compounds) showed an inverse trend. This picture may suggest that the nitrogenous aromatic compounds are proportionally higher with respect to cellulosic and easily decomposable organic substances. Effectively by observing Figure 147 more structured soils presented PY/Y values higher than less structured soils almost along all seasons.

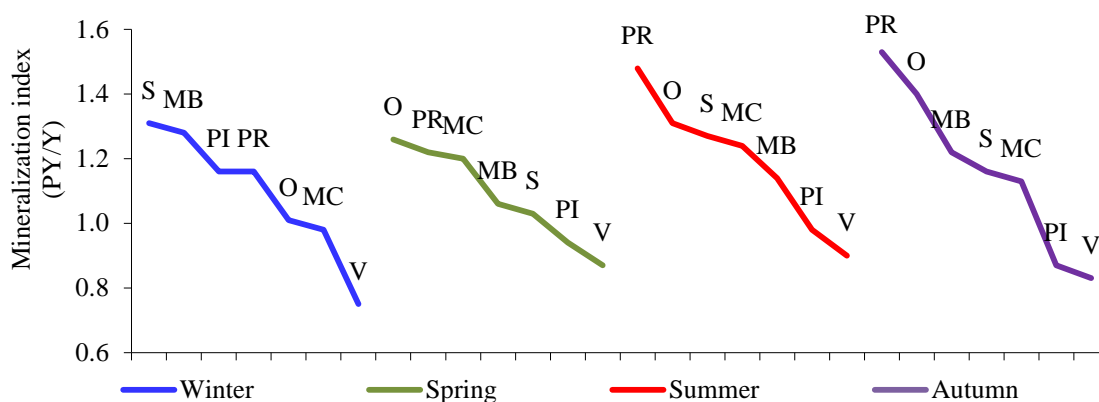


Figure 147. Seasonal trends of mineralization index (PY/Y) of stable organic compounds for the selected soil environments.

Moreover, except winter season, soils under pines show lower PY/Y values which would be not surprising, considering the hardly mineralizable compounds of its vegetal debris. FF/PY and PY/Y mineralization indices may thus give an idea of biodegradation of aliphatic and aromatic organic structures respectively (Ceccanti et al., 1986). The index of mineralization FF/PY expresses the disappearance of the labile fraction of polysaccharides, producing furfural, compared to the less labile fraction of pseudo-type phenolic or humic acid. The index PY/Y, tend to assume lower values at

more disturbed soils, evidently due to the low biological degradation of aromatic structures.

The index of humification B/E3 (Ceccanti et al., 1986; 2007; Marinari et al., 2007) is the structural condition of "stable aromatic nucleus (condensed)" of humic substances (Table 95). Masciandaro and Ceccanti (1999) and Ceccanti et al. (2007) reported that the humification index may be relevant in assessing the organic matter condensation and stability in different soil ecosystems. Higher index indicates a higher stability of humic compounds. Following this assumption, soil organic matter under scrubs was found to be more stable than other soils. The surprisingly high humification index in soils under cultivated vines (V) may refer to the presence of condensed aromatic structures in this soil though the values may be misleading because probably related to a higher ratio proceeding from the decline of labile organic compounds due to higher mineralization rates. When the FF/PY and PY/Y values are plotted against the ratio between aliphatic and aromatic compounds (AL/AR) and the humification index (B/E3) respectively (Figure 148A and B), positive and negative trend are obtained.

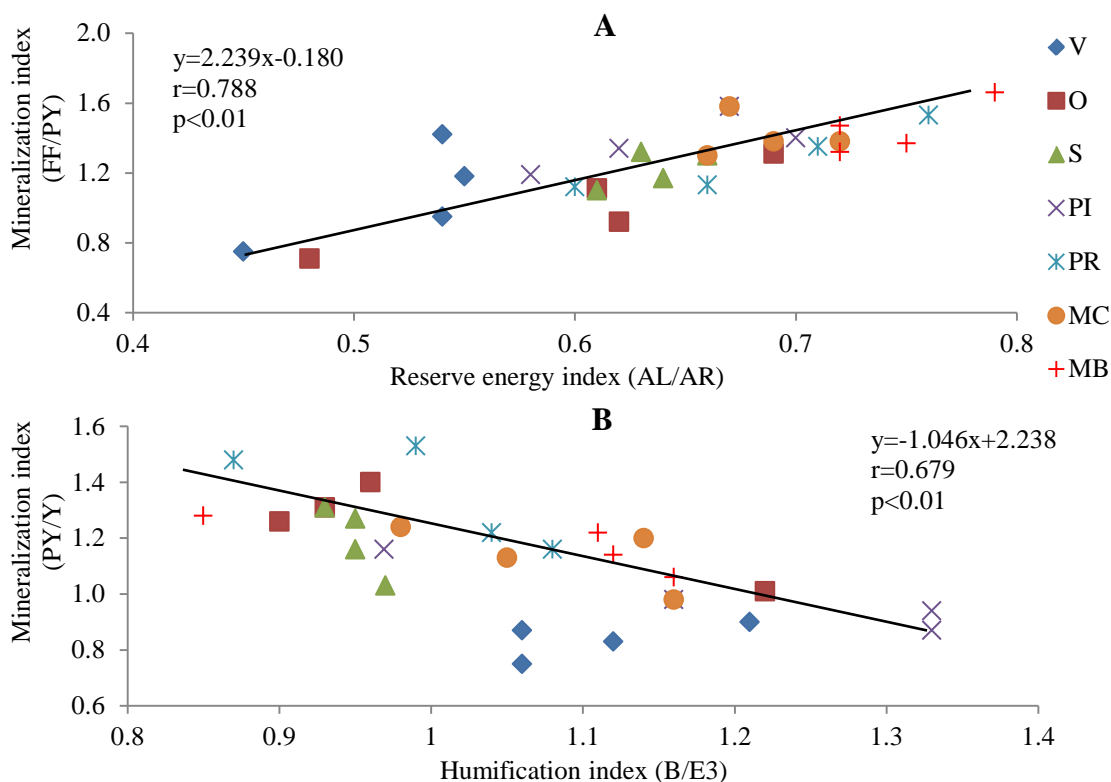


Figure 148. Relationships between mineralization indices (FF/PY and PY/Y) plotted against aliphatic and aromatic compounds (AL/AR) and the humification index (B/E3) respectively for the selected soil environments.

The positive one (Figure 148A) is in agreement with the fact that those environments showing better properties are progressively enriched with more stable organic compounds. The negative trend of the regression equation (Figure 148B) is also depicting those environments having a higher “energy reserve” which in turn show higher mineralization index of more stable organic compounds indicating that a regulated turnover of more stable humic compounds may be achieved.

AL parameter, i.e. the sum of the relative abundance of aliphatic compounds (acetonitrile E1, acetic acid AA, and furfural FF), originated from cellulosic and nitrogenous substances, considered as more easily decomposable organic compounds was found in decreasing order like MB > MC > PR > PI > S > O > V soil environments (Table 95). Conversely, the AR parameter, that is the sum of benzene B, pyrrole PY, toluene E3, and phenol Y fragments originated from condensed (humified) ligno cellulosic and nitrogenous stable organic substances showed an inverse trend with respect to AL. By using the ratio of aliphatic to aromatic compounds (AL/AR) the energy reserve index may be obtained, and soil environments were ordered as: V < O < S < PI < PR < MC < MB. The ratio AL/AR, between the aliphatic (labile) and the aromatic (stable) structures, is then higher in stable soils than in the unstable soils. It was postulated that the unstable part of the humic substances may be more promptly exhausted when the soil is under unstable or degrading conditions, and mechanisms may be proportional to the degree of soil stress. This may also indicate that mineralization and humification are complex mechanisms and that the proportion of labile to stable humic compounds depends on several interacting factors and, among them, the soil conditions, the changes in soil management and the age of abandonment (Schnitzer, 1991). This would indicate also that, despite the highest mineralization of stable organic matter found in soils under scrubs and pasture with respect to soils under forest and cultivated soils under olive groves and vines, the more stable soil ecosystems were able to self-regulating and preserve a significant amount of microbial resistant humic substances (Ceccanti and Masciandaro, 2003).

As a result, mineralization indices FF/PY and PY/Y resulted the pyrolytic parameters that better discriminate among the different soils, being involved in the dynamics of labile organic matter (furfural FF), linked to microbial metabolism, and pseudo-stable organic matter (phenol Y and toluene E3), related to the humic fraction.

The humification index did not discriminate clearly between soil environments probably due to the high condensed aromatic compounds in soils with low agricultural practices that may confuse the dynamic potential of soil microbial activity for the decomposition of organic structures. The energy reserve index was clearly classifying the soil environments as well as the mineralization index to be low in soils under cultivated vines (V) and olive groves (O), intermediate values in soils under stands of cork (S) and pine (PI) trees, and higher values in soils under *Cistus* scrub (MC) , *Erica* scrub (MB) and pasture (PR). The overall results of pyrolytic indices may demonstrate that C fractions could provide complementary indications of SOM quality under different agricultural management.

3.10.1.3. Similarity index (S_{ij})

It is a numeric index of similarity between the relative abundances (I) of chemical components obtained by PY-GC of the homologous peaks (k) in two pyro chromatograms from two different soil environments (i and j), and was calculated using the following expression:

$$S_{ij} = \sum n(I_i^k/I_j^k)/n \quad \text{with } I_i^k < I_j^k$$

where, I^k represents the relative abundance of pyrolytic fragments of pyrogram to compare (i and j), n the number of pyrolytic fragments (peaks), and k is the homologous of pyrolytic fragment. The index of similarity (S_{ij}) compares a pair of pyrograms without discriminating peaks. The index has a numerical range varying in the range of 0-1: the higher the value, the greater similarity. However, three conventional levels, high (>0.90), middle ($0.90-0.80$), and low (<0.80), have been suggested for the characterization of heterogeneous materials in SOM (Ceccanti et al., 1986; 2007).

By comparing pairs of pyro chromatograms it was possible to quantify the differences between the seven soil environments through the index of similarity (Table 96). The index of similarity explains the degree of similarity in the composition of organic compounds among the investigated soils. The index of similarity started in those soils from values higher than 0.75, probably indicating same similarity in the native organic compounds prior to transformation in agricultural lands. If this is the case the slight difference of S_{ij} among the studied soils may report for the effect of soil management and age of abandonment along the last decades. It is observed (Table 96)

that soils having better properties showed higher similarity in the structural composition of soil organic matter except soils under cultivated vines (V), probably due to fast SOM consuming agricultural practices such as tillage in a very poor soil with agrochemicals addition (Fernández-Calviño et al., 2010), and soils under stands of pine trees (PI) probably due to the impact of pine needles deposition rates on the decomposition of soil organic matter (Iovieno et al., 2006), with respect to other soils. A clear trend of dissimilarity was showed between soils under vines (V) and soils under pasture (PR) and *Erica* scrub (MB). Moreover soils under vines (V) showed a high similarity index with soils under pines. However the S_{ij} values of the studied environments testified the complex transformation of organic compounds along the sequence of soil use and abandonment. Soils under olive groves (O) showed high similarity with S, PI, PR, MC, and MB environments probably because of the presence of high cellulosic and nitrogenous compounds even with aromatic or aliphatic origin as it had higher values of Acetic acid (AA), benzene (B), pyrrole (PY), and toluene (E3) pyrolytic fragments.

Table 96. The pyrolytic index of similarity (S_{ij}) (\pm standard error) between the selected soil environments.

ENV	V	O	S	PI	PR	MC	Similarity index
O	0.84 \pm 0.10						
S	0.84 \pm 0.09	0.99 \pm 0.01					
PI	0.90 \pm 0.09	0.91 \pm 0.07	0.91 \pm 0.07				
PR	0.79 \pm 0.10	0.93 \pm 0.02	0.94 \pm 0.02	0.86 \pm 0.09			
MC	0.81 \pm 0.10	0.94 \pm 0.04	0.96 \pm 0.04	0.89 \pm 0.08	0.96 \pm 0.03		
MB	0.78 \pm 0.11	0.93 \pm 0.07	0.93 \pm 0.06	0.87 \pm 0.07	0.94 \pm 0.04	0.97 \pm 0.02	
							<0.80 Low
							0.80-90 Medium
							>0.90 High

ENV: Soil environments; V: Soils under vines; O: Soils under olive groves; S: Soils under stands of cork trees; PI: Soils under pine trees; PR: Soils under pasture; MC: Soils under *Cistus* scrub; MB: Soils under *Erica* scrub.

3.11. Statistical evidence of changing in environmental and soil conditions upon soil quality

A final PCA by using all the analyzed parameters along the period of study was carried out. The first two components were taken into account because of their higher eigenvalues explaining the most effective variation among the studied variables. The first component explained 49.96% of the total variance. It was observed that clay, mechanic impedance (MI), shear strength (SS), water holding capacity (WHC), soil organic carbon (SOC) at the three dimensional classes (0.00-2.00 mm, 0.25-2.00 mm, and 2.00-5.60 mm), total nitrogen (TN), easily extractable glomalin (EE-BRSP), total glomalin (BRSP) at the three dimensional classes (0.00-2.00 mm, 0.25-2.00 mm, and 2.00-5.60 mm), water stable aggregates (WSA) at the two dimensional classes (0.25-2.00 mm and 2.00-5.60 mm), bacterial and fungal populations, β -glucosidase, protease, phosphatase, mineralization index (PY/Y) of the stable organic compounds, and index of energy reserve (AI/AR) were positively correlated ($>+0.70$) with the first component. While soil erosion, sand content, bulk density (BD), and soil infiltration rate (IR) were negatively correlated (>-0.70) with the same component. This picture stress the importance of relevant soil physical characteristics such as clay, the potential resistance of soil surface to external stresses, which may be considered a pre-indicator of soil structural stability and their role, increased by enhancing the chemical and biological interactions among soil ecosystems. The role of soil organic carbon and glomalin was enhanced by the structural stability of aggregates (WSA), indicating the beneficial effect in aggregation and carbon storage. Soil enzymes, responsible of biochemical transformation of soil organic materials, were also positively correlated with soil nutrients such as carbon and nitrogen and with the bacterial and fungal populations in soils. The first component represents the overall soil interactions occurring for enhancing favorable soil conditions. The second principal component explained the 9.50% of the total variance, showing positive load ($+0.70$) in carbon dioxide (CO_2) estimated by soda lime at field condition (SLF) and soil temperature (ST) against the negative contribution of soil moisture (SM) (>-0.70). According to the data presented by the two component structure, soil carbon loss estimated as CO_2 can be ascribed to the vulnerability of soil evolution in reaching good soil health conditions, which was in contrasting to soil erosion processes.

The PCA plot shown in Figure 149 represents a compendium picture on the soil environments behavior along seasons and their contribution to soil variables, in order to improve the understanding of the dynamics of soil ecosystems at the area of study.

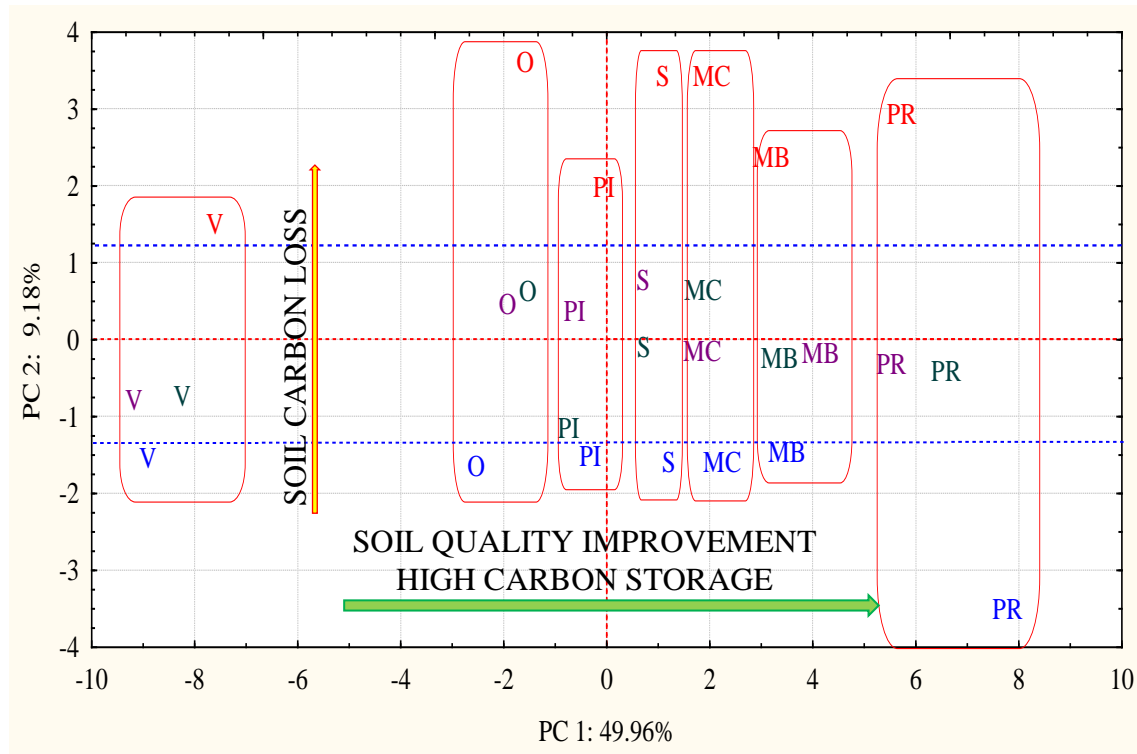


Figure 149. PCA plot diagram of the first two components obtained by using all soil parameters under study. Blue color: Winter; Dark green color: Spring; Red color: Summer; Violet color: Autumn.

The first component with 49.96% of explained variance represents the relevant variables for achieving better soil conditions associated to the changes in soil use and management and age of land abandonment. The progressive shift along PC1 from soils under vines (V) to soil under pasture represents a valuable indication of soil quality improvement through soil environments evolution and also can suggest the temporal step in which these ongoing dynamics occur. The projection of soil environments along PC2 axis represents the seasonal effect on organic compounds consumption and its indication deserves also special attention for organic carbon balance and monitoring in order to maintain proper carbon stocks in these fragile environments.

CHAPTER IV. CONCLUSIONS

Conclusions

The multiapproach study along the selected environments under different land use and abandonment has been consistent to evaluate soil attributes by the integration of physical, chemical, biological, and biochemical parameters recorded during two years of investigations. The combined research work under field and laboratory conditions deserves a special attention for time costing and the complexity of data collection and elaboration in such heterogeneous ecosystems. Many factors play important roles in soil quality evaluation such as vegetation cover, land use and land management, the extent and age of land abandonment, climatic changes, and periodical natural or induced perturbations like wildfire occurrence. The interrelationships of these factors, together with soil properties may produce different outcomes as degradation, especially when the soil systems response makes it vulnerable to sustain the external stresses. Although no clear evidence of degradation processes have been identified, the fragility of the soil system has been proved by different processes analyzed along the period of observation. Cultivated soils with scarce organic materials showed a reduced influence in their evolution to enhance the formation of organo-mineral complexes for improving soil surface resistance to erosion processes. Abandoned areas generally increased their vulnerability to erosion and nutrient depletion especially when wildfire occurrence throughout the dense and disorganized scrubland cover causes regressive dynamics in natural vegetation succession. However, when land abandonment is followed by spontaneous plant colonization, increasing of organic compounds occurs in the upper soil layers even in shallowest soils, though the organic layers may vary according to the age of abandonment. These processes may last decades and imply very complex biophysical and chemical interactions between soil and plant communities. Any time that wildfire devastates the plant-soil system the organic layer is decapitated and its regeneration is unlikely to occur in short time even though plant responding is fast. Soil properties at surface decrease especially affecting organic matter content and structural stability, thus reducing soil resistance to erosion. Therefore, low agricultural management and periodical perturbations of wildfire occurrence have increased the risk of degradation when affecting the organic carbon pools and soil nutrients, especially in those soils in worser conditions.

Accordingly, the nature of soil surface and plant cover under the effects of rainfall and soil conditions may have a major role in increasing soil erosion or protecting soil from erosion. When the removal of soil organic carbon and nitrogen is relevant, the depletion of soil nutrients through runoff erosion may be accentuated because of itself soil capacity to do not withstand adverse climatic conditions or anthropic soil mismanagement, leading to soil structural stability decrease, altering cycles of water, carbon, nitrogen and other nutrients, and causing adverse impact on biomass productivity, biodiversity and the environmental quality. This picture have arisen questions on the need of appropriate management of abandoned land and the recover of the landscape heterogeneity, in order to contrast threats of degradation. The trend in removal of SOC and TN, both dissolved (DOC, DN) and eroded (EOC, EN), with respect to runoff and erosion resulted in agreement with plant cover, soil structural condition, and organic matter content. Decline in DOC and EOC and DN and EN were proportionally higher in soils with higher organic matter content, though were fastly exhausted indicating that only partially humified organic debris may have been washed out from surface. The low amount of mineral particles in these soils indicated also a better structure. The highest organic carbon and nitrogen lost by erosion with respect to SOC and TN contents may however indicate that fractions of surface organic debris are progressively substracted from humification process. Soils under cultivated vines showed conversely the highest mineral particles removal, revealing a comparatively lower structural stability. In these soils mineral particles/nutrient depletion ratio may be more important and increase the progressive impoverishment of soil properties.

Soil infiltration and hydraulic conductivity rates were generally following the rainfall amounts and soil surface conditions. In some way they were considered as pre-sensitive physical indicators of the susceptibility to erosion as higher values resulted in lowering the runoff washing potential in sloping fields. Soil infiltration rates (IR) were higher in 2008 than 2009 due to the low and irregular rainfall precipitation in the former year. Consequently erosion rates were comparatively higher in 2009 and numbers were directly proportional but vines. This was because of rainfall irregularity. The erosion in vines was more abundant with respect to other environments (281% and 79% more for 2008 and 2009 respectively) but changed by 3% only in the two years (312 kg ha⁻¹ in 2008 and 301 kg ha⁻¹ in 2009). Olives, corks, pines, pasture, *Cistus* scrub, and *Erica* scrub increased erosion amount by 37%, 285%, 280%, 95%, 200%, and 24% with

respect to 2008 values also indicating those soils that more resisted the increased runoff washing action, on average 175% more runoff water was recorded in 2009 with respect to 2008. Others physical dynamic soil properties like BD, SM, MI, and SS varied according to rainfall amount and soil organic matter, differing considerably along the sequence of soil use and abandonment. Low managed soils and those affected by the natural or induced perturbations resulted during the two years with the worst soil conditions also because of the weakness of soil structure with respect to better structured soils. Nevertheless, the soil under *Cistus* scrub showed apparent suitable soil properties which was attributed to a relatively fast recovery after fire.

The overall soil behavior during the two observed years resulted coherently proved by statistics carried out separately for each year by using factor analysis corroborating the relationships between environmental conditions, soil management and abandonment and changes in soil properties. During 2008, a drier year, the factor analysis showed that the first factor, explained 35.14% of the total variance. Loadings on this factor were related to the soil organic reserve and water storage, associated by positive factor scores with PR, MB, MC, PI and S soil environments corroborating the relevant role of these environments to soil quality. The second factor explained 16.97% of the total variance and represented the soil erosion and surface hydraulic parameters with the highest factor scores found in soils under vines (V). The third factor explained 14.56% of the total variance and was highly loaded by the nutrient depletion parameters with the highest factor scores from soils under stands of pine (PI) and cork (S) trees. Factor analysis was run by using the same parameters recorded during 2009, a more rainy year. The first factor explained 41.52% of the total variance with positive loadings on organic reserve and water storage related parameters. Also in this year the factor scores were high and positive for PR, MB, MC, and S soil environments. Despite that, erosion increased in all environments though not significantly in terms of eroded sediments. In fact, only parameters related with nutrient depletion either eroded or dissolved were loaded on the second factor explaining 15.96% of the total variances. These parameters loaded in the third factor in 2008 moved to the second factor as to indicate a strong organic compounds removal capacity from surface runoff. The highest contribution to nutrient depletion was attributed to soil under vines by the factor scores. Loadings related to soil surface compaction parameters were found in the third factor, explaining 13.90% of the total variance. The higher rainfall precipitation during 2009

showed a higher impact on soil nutrients and may have also compacted the soil surface due to the progressive loss of partially humified organic debris from the upper soil horizon. Results obtained from the statistical treatment appointed that independently of rainfall amount the majority of soils in the area maintained good soil properties. In spite of that, rainfall may trigger progressively severe sediments and nutrients depletion when increases in amounts are recorded.

Land use and abandonment have also influenced the potential of soil respiration capacity (CO_2 emission) because of their impact upon microbial activity and soil organic carbon pools. The sequence of land use and abandonment showed significant variability in soil C fluxed to the atmosphere and C storage capacity in soil. Measurements of carbon dioxide, fluxed from the soil were therefore important in order to add information on the carbon dynamics and identify soils with aptitude of carbon sequestration or loss. A static soda lime method in the field (SLF) and laboratory (SLJ) and a dynamic infrared gas analyzer (IRGA) method at soil surface and at 5 cm depth under natural field conditions were proved successfully, also taking into account that these are the first data coming out from a sequence of both land use, cover change and abandonment in this part of NE Spain, where very peculiar and vulnerable soils exist.

Carbon dioxide measurements showed that soils with a better soil structure and more stable organic compounds were assumed to best preserve organic carbon against CO_2 emission, and this was proved by applying two conceptually different methods. Labile forms of organic carbon were likely to be most easily mineralized by the microbial activity, and released as CO_2 from the soil surface to the atmosphere. This process seemed to be less effective in soils under S, MC, MB, and PR environments with respect to soils under V, O and PI environments in terms of C- CO_2 loss. Data of C- CO_2 indicated a similar trend in soil carbon loss between the investigated soil environments along the experimental period and may appear surprising due to the difference in soil characteristics, especially the organic carbon content. These data were considered in contrast with soil carbon storage capacity. However, when these data were transformed as the proportion of the soil organic carbon pool, values allow to differentiating the carbon storage potential of each environment along the land use-cover change and abandonment. According to current land use, four groups may be proposed: The first includes current cultivation (V and O environments) showing the

highest loss of organic carbon in soils under vines with respect to organic carbon stock. To correct this trend, the soils under vines should be amended with organic compounds, as progressively increases in erosion rates have been generally recorded in this soil during the period of study. The second group refers to forest soils and shows higher C preservation potential existing in soils under *Quercus suber* (S) with respect to soils under *Pinus halepensis* (PI) probably due to a delay in forming stable organic compounds for the highest C/N ratio of decaying debris in the latter one. In the third group the two types of soils under scrubs (MC and MB) are included. Though the difference in C loss percentage is not high between these two soil environments, it may be ascribed to the historical effects of wildfire occurrence and the temporal regressive dynamics suffered by MC environment. Finally, the fourth group is formed by the soils under pasture (PR), representing the best situation as they may indicate the most favorable environment for carbon storage. At field conditions, the soil moisture and temperature play important roles in CO₂ emission dynamics together with soil structure and organic matter. The increase of organic compounds in soils changes with soil use, land management, and abandonment, and this sequence may control the efflux of CO₂ from soil surface to the atmosphere.

Findings of glomalin estimation in the soils under study showed that glomalin production was lower in soil environments insufficiently managed (V, O) and wildfire affected (PI, S, MC) where soil evolution towards a steady state may have been delayed, with respect to environments (PR, MB) showing better soil conditions. The overall data indicated that land use and abandonment have a beneficial effect on soil properties especially when organic carbon pool increases and it is maintained along the age of abandonment. Stable organic compounds in these shallow soils with very low clay content should be considered a valuable resource for its contribution to soil structure stability. In this study we have proved that both EE-BRSP and BRSP are subjected to a significant seasonal turnover among the studied soil ecosystems, in agreement with other findings reported in the literature. Seasonal fluctuations of both EE-BRSP and BRSP were higher in less stable soils suggesting more instability in mechanisms of glomalin formation and deficiency in carbon storage capacity. In spite of the high seasonal fluctuations, relatively constant glomalin pools may be expected in the dormant season where biological and root activity is low. However, additional glomalin pools may be captured at the beginning of the growing season. According to soil

conditions glomalin pools will increase gradually as follows: V→O→PI→S→MC→MB→PR. Soil moisture content seemed also to be the most relevant parameter controlling the metabolization processes of fungal biomass productivity. Moreover, soil organic carbon and total nitrogen were considered as fundamental nutrients powering the metabolic fungal activities under suitable pH level.

Data sets of soil carbon loss as CO₂ when related to glomalin pools showed that glomalin can significantly contribute to active as well as stable organic carbon pool. The labile glomalin fraction (EE-BRSP) was supposed to be partly mineralized by microbial activity to release soil carbon and partly transformed into recalcitrant glomalin fraction. This may confirm that along the sequence of land use, land use change, and land abandonment a progressive and increasing capability to preserve organic compounds against carbon loss as CO₂ may be expected from vines to pasture. Glomalin may thus be considered one of the most relevant carbon reserves in soil with the ability to further preserve stable organic compounds, enhancing aggregation and structure stability.

When statistical significance was sought through the k-means cluster analysis, three significant groups of soil environments were created: The group III included soils such as V, O, and PI which had shown some decline in soil properties along seasons. Soil environments MC, MB, S, and PR were distributed in groups I and II according to soil parameters relationships during seasons. In summer season all soil environments were located in group II mainly due to higher production of recalcitrant glomalin against carbon loss. The different behavior of soils under vines constrained it in the group III.

The chemical indicators of soil fertility related to soil nutrients (C, N, P), soil acidity (pH) and salinity (EC), and exchangeable cations (Ca, Mg, Na, K, Al), were also suggesting the following soil order: V, O, and PI soils resulted in a negatively contribution to soil fertility, while S, PR, MC and MB resulted in a positively contribution to soil fertility. This pattern was statistically proved with the data sets of glomalin and indicated that the turnover cycle of fresh glomalin fractions was faster in the more fertile soils than in the less fertile soils due to the former healthy soil conditions favoring metabolic activities of soil microorganisms at the studied horizons, and a comparatively higher accumulation of humic compounds.

The carbon in glomalin (G-C) was significantly increased along the sequence of soil use and abandonment in agreement with the increasing content of SOC and glomalin pools. As the increase in glomalin and SOC demonstrated an increase in stable organic compounds, the proportional amounts of G-C to SOC increased while carbon loss as C-CO₂ with respect to SOC pools decreased drastically from vines to pasture. This trend was indicative of a rapid turnover of labile glomalin carbon in EE-BRSP into more stable C form. Accordingly, total glomalin (BRSP) resulted more coherently related to stable organic compounds, contributing to both recalcitrant carbon pool enrichment and particle aggregation. In addition, these processes of the dynamics of soil carbon storage and carbon loss have more relevance in summer than in spring, autumn, or winter. The EE-BRSP/BRSP ratio was lower in soils under pasture and scrubs suggesting a rapid turnover of labile glomalin fraction (EE-BRSP) to be incorporated into the recalcitrant glomalin pools. This ratio showed the highest values in soils under cultivated vines (V) and olive groves (O) and intermediate values in soils under stands of pine (PI) and cork (S) trees once more reflecting the different soil conditions along the soil sequence under study. The EE-BRSP/BRSP ratio showed the same seasonal order, summer > spring > autumn > winter, along the investigated soils. Effectively, glomalin turnover into more stable C form was associated to a low EE-BRSP/BRSP ratio in better structured soils and BRSP resulted coherently related to organic carbon, suggesting contributions to both recalcitrant carbon pool and structure.

The structural stability of aggregates (WSA) was found to be greatly influenced by the higher accumulation of glomalin and SOC pools and moreover by the dynamic increases of the recalcitrant glomalin pools. Structural stability of aggregates increased significantly along the soil sequence as follows: V→O→PI→S→MC→MB→PR. The role of glomalin was enhanced by the structural stability of aggregates, investigated in two aggregate fractions (0.25-2.00 mm and 2.00-5.60 mm), indicating its beneficial effect in aggregation and carbon storage. Significant reciprocal increases in BRSP, SOC, and WSA values ($p < 0.001$) were recorded along the land use and abandonment sequence depending on climate and soil conditions. In summer, WSA values were higher than spring and winter in the Ø 0.25-2.00 mm and Ø 2.00-5.60 mm aggregates respectively and more organic soils were more structurally stable by 44% and 88% with respect to less organic soils in the same aggregate classes. The overall findings have suggested that increasing structural stability creates favorable mechanisms of soil

carbon protection in very shallow soils, preserving C from biological and biochemical metabolic activity, thus minimizing soil carbon loss. Glomalin content may therefore be considered as a reliable indicator of healthy soil conditions even though further studies need to clarify the real nature of this soil component.

The mechanisms between soil biological activity and structure through the biochemical interactions of soil enzymes with organic substrates and the soil nutrients cycling were focused to a better understanding of the potential differences in soil management and age of abandonment along the studied soils. The three hydrolytic enzymes activity differed consistently between the seven soil environments ranging from very low values recorded always in soils under vines (V) along the 4 seasons, to low values in soils under stands of pine trees (PI), intermediate values in soils under olive groves (O), stands of cork trees (S), *Cistus* (MC) and *Erica* (MB) scrubs, until the highest values observed in the soils under pasture (PR) in agreement with previous results on organic carbon dynamics. The β -glucosidase, protease, and phosphatase activity patterns closely indicated relevant relationships with SOC, TN, and TP contents in soils. The periodical wildfire occurrence and mismanagement of agricultural soils showed consistent effects on soil enzymes as well as carbon dynamic storage and carbon loss. Soils under stands of pine trees and *Cistus* scrub were also lower than soils under stands of cork trees and *Erica* scrub respectively in their enzymes activities. In all the studied soils, enzyme activities were higher in summer season corroborating that higher microbial activity may be coherently used to interpret the evidence of faster glomalin and organic carbon turnover in soils. The significant correlations between each soil enzyme and the most relevant soil physical, chemical, and biological properties were able to establish the role of these soil biochemical parameters in improving the comprehension of dynamic activities among soil ecosystems leading to a stable soil system.

The principal component analysis was tried to establish the interrelationships among the soil physical, chemical, biological, and biochemical characteristics. All the studied soil parameters related to better soil conditions resulted loaded in the first component explaining 58.34% of the total variance against carbon loss dynamics and related parameters such as soil moisture and temperature explained at the third factor (9.87% of the total variance). The first PC received a proportional contribution of soil

environments as follows: V→O→PI→S→MC→MB→PR. The general view of PCA plot, constructed by the first two factors, separated the treatments (soil environments and seasons) into three groups: one including all the summer measurements of different environments except soils under vines, a second group formed by measurements of spring and autumn except soils under vines, and a third group containing all seasons of the soils under vines (V). The statistical evidence summarized the impact of soil use and management and abandonment along with the seasonal changes in climatic conditions, attributing to each soil environment an increasing degree of contribution to biological ecosystems, through soil nutrient availability, enzymatic activities, accumulation of organic compounds, and consequently a scalar improving of better soil conditions from vines to pasture.

The structural composition of SOM carried out by pyrolysis gas chromatography (Py-GC) provided additional information about the humic or stable and non-humic or labile organic contents in the soils under study. On average the value of FF/PY ratio (furfural/pyrrole i.e. the highest ratio the lowest mineralization of labile organic compounds) indicated a higher mineralization of labile organic compounds in V and O soils, whereas intermediate values were found in PI, S, and PR soils, until a lower mineralization index found in MC and MB soils. Findings suggested that cellulosic aliphatic compounds may be proportionally higher with respect to nitrogenous aromatic compounds and thus more easily decomposable in poorer structured soils like V and O environments, than in intermediate soils like PR, S, and PI environments, or apparently more stable soils like MC and MB environments. However, the seasonal trend of this index revealed that climatic conditions may have a relevant role in the mineralization/humification process. By contrast, the ratio PY/Y (pyrrole/phenol) indicating the mineralization of more stable organic compounds (the highest the ratio the strongest mineralization rates), showed an inverse trend indicating that nitrogenous aromatic compounds (pyrrole) are proportionally higher with respect to polyphenolic compounds and are decomposable in more stable structured soils. This trend may also indicate that soils with more stable organic compounds need to mineralize part of them during the C cycle, in order to maintain a balance between nutrients input and structure stability. The question is different in V and O poorly structured soils that by showing loss of stable organic compounds, even present in reduced amounts, may trigger a worst soil response to degradation processes. The energy reserve index AL/AR (ratio between

aliphatic and aromatic compounds) showed the already experimented trend, arranging the soil environments as $V < O < S < PI < PR < MC < MB$ and therefore was able to strengthen the extent of soil response to sequential changes in soil use and abandonment and seasonal effects. The similarity index (S_{ij}) showed high similarity in the structural composition of SOM except V and PI soils with respect to other soils probably because these soils may be subjected for their peculiarities to diverse SOM dynamics. Moreover, both soils showed high similarity between them.

A cumulative PCA analysis was also tried by loading all data sets of soil erosion survey together with all data sets of soil physical, chemical, biological, and biochemical properties. The first component explained 49.96% of the total variance with high positive loadings on the most effective soil variables of soil quality adding information to the role of clay, shear stress, water holding capacity, nutrients, glomalin, water stable aggregates and their content of SOC and BRSP, bacterial and fungal populations, soil enzymes activity (β -glucosidase, protease, and phosphatase), mineralization index of stable organic compounds, and energy reserve index (AL/AR) against the high negative loadings on soil erosion and related soil physical parameters such as sand content, soil compaction, and infiltration rates. The second component explained 9.18% of the total variance mainly by soil carbon loss and related soil parameters such as soil moisture and temperature. The cumulative PCA plot diagram confirmed on statistical bases that the studied soil sequence represented a typical evolution of Mediterranean agro-silvo-pastural environments affected by progressive abandonment, where soil attributes are intimately related to post-abandonment vegetation succession. In the soils under study the multiapproach analysis detected the following order of environments with increasing contribution to soil health and associated to the first component: $PR > MB > MC > S > PI > O > V$, though this scaling assignment may be restricted to the period of study. Taking into account that this can be a logical picture of soil conditions it may be postulated that results gave useful indications on the future land managements to be performed to at least expand the surface occupied by meadows, also considering the proportional surface of land occupied by each soil environment. In addition if the scrubland or forest surface must be maintained at the current occupational extent of surface it should be worth to be subjected to severe prevention control in order to avoid wildfire occurrence risk. The second component, with relatively lower weight in the total variance explained, attributed to soil carbon loss a seasonal trend with a higher

contribution in summer, intermediate contribution in spring and autumn, and the lowest contribution in winter along all soil environments, corroborating the impact of seasonal climatic conditions upon soil carbon storage capacity.

The results showed in this work may give advice of early warning indicators of soil health and be used for post abandonment management with the aim to use these area as carbon sinks. More efforts of prevention and planning should be devoted to this topic instead of spectacular and costing interventions to extinguish wildfires. Soil use and management should be accurately planned and imbalance of CO₂ emission with respect to SOC content should be deeply studied in order to avoid land degradation processes. Land abandonment without contemporary management of abandonment is not a suggestible practice because of the risk of fire occurrence in a disorganized scrubland. Nevertheless, it is proved that progressive colonization of spontaneous vegetation in abandoned agricultural land may have a primary role in recovering and preserving stable organic compounds and a good soil structure against degradation processes. This will suggest that also land abandonment is worth of special management in order to recover the heterogeneous mosaic of land use and prevent environmental damages.

CHAPTER V. REFERENCES

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