Centre de Recerca Ecològica i Aplicacions Forestals
CREAF

Universitat Autònoma de Barcelona

Departament de Biologia Animal,
de Biologia Vegetal i d'Ecologia
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Exchange rates and content of VOCs in Mediterranean soils; their responses to drought and warming and their linkage with biotic factors

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Ph. D. Thesis

Mª Dolores Asensio Abella

Bellaterra, setembre del 2007

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Memòria presentada per:

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per optar al grau de Doctor

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Bellaterra, setembre del 2007

A Pandora i Enrique

A Adriano, per sobreviure

Todo momento, todo gesto, cada segundo, cada latido,
es una conversión de mí mismo.

Hermano Pastis

Óscar Jordán

Agraïments

De les cosetes bones que m'heu donat, conscient o inconscientment, i dels favors regalats, vull fer apreci:

Fer apreci és reconèixer el valor, el mèrit d'alguna cosa. Així, reconec lo bé que ha estat treballar amb **Josep Peñuelas** i el valor que té. No només perquè és un generador d'idees, de incansable energia, pràctic, clar, multi-tasca amb bon humor...és fantàstic vore tot això en marxa, he disfrutat molt i he tingut tots els privilegis científics i personals de pertànyer al ***Grup Peñu*** (gràcies a la secció "*grandes clásicos de hoy y de siempre*": Iola, Joan, Romà, Jordi, Marc...). Però lo curiós és que quan vaig arribar, jo no sabia res de tot això, ni tu, Josep, sabies res de mi. Llavors, gràcies per confiar en mi, donar-me l'oportunitat i dirigir-me el treball. I gràcies també, a l'atzar o necessitat, que van fer que jo vingués ací.

Ací, tot just arribada, em vaig trobar amb **Joan Llusià**. Joan és molt volàtil, per això arriba a tothom. El teu *savoir-faire* és sempre present a tot el que fas, des d'una pizza fins a un cromatògraf. Podria estar-me hores mirant com arregles un aparell espatllat o com fas extractes. Això és una manera bonica d'ensenyar les coses. Gràcies per tot això i per compartir amb mi pensaments, cines, massatges anti-estrès, menjars, xocolata, *parfums de Chine, ou bien de n'importe quoi*, aclarir-me dubtes...

Dubte sempre, això ho sé. Però per què? Potser em fa por equivocar-me, por al fracàs? O potser pense que no tinc prou informació per valorar?. Si no fos perquè **mon pare i ma mare** es van decidir a fotre'm canya, crec que encara estaria valorant les opcions de la primera decisió que vaig haver de prendre algun dia. Gràcies per fer-me llum, estar per mi i alhora deixar-me sempre ser, en sentit profund, jo.

Jo, sempre he sigut i seré, amb tesi o sense ella, **jo**. No és per reafirmar-me. Simplement *jo sóc altre*. Gràcies doncs, des de fora cap a endins. No tornaré, a currar els caps de setmana.

Però tots els caps de setmana, setmanes, mesos...anys! que porte al CREAF, han estat molt bé! Perquè **la gent al CREAF** fa tot més agradable. He estat com una reina, gràcies: Marta, Cristina, Charo, Magda, Carreño, Teresa i tots els que en conjunt, feu força.

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Coincidir amb les **persones**, trobar la sintonia, és una de les coses més agradables que hi ha. Des de el principi fins al final, ho he pogut comprovar. Hi ha un enteniment especial, després de tants cafès, sopars, estrès, viatges, Giraldillas, series dels 80, amor i desamor compartit, musica...uff..., amb els **frikibecaris, doctors i amics**, gràcies: Jara, Núria, Salvattore White Oak, Roger Dry, Stefania (quina dona...*mincra totus!*), Roser, Laia, Belén, Imma, Nacho, Xavi Domene, Carolina, Iola, Angi, Corina, Romà, Xavi Arnan, Rebeca, Miquel, José Luís, Gerardo...**ping-poneros irreductibles artístics guies**

***del más allá*:** Jordi...moltíssimes gràcies!; **cinèfils cràpules musicals bandarres:** Maria Deu, Gerard, Ivette, Charo, Dr. NO (és a dir, en Martínez que sempre em diu NO). **Futboleros** que mai em deixareu marcar un gol, **dones espectaculars:** Sue...quin escàndol!

És un escàndol que **Lo Vuestro** encara no tinga fet un vídeo ni una pàgina web...també és cert que hem assajat poc...No és *Summer time*, però sempre ens quedarà el youtube. **Salvattore, Nacho**: gràcies. *Encara que no deixeu que s'escolte el baix!*

En pis de baix de casa nostra, a Cerdanyola City, van eixir goteres. Tot venia de les nostres canonades instal·lades en el Pleistoceno Superior. Allà hem viscut 5 anys. Les panderoles viuen amb més dignitat a les clavegueres, **Senyora [...]** però gràcies, pels seus savis consells: "*Ves lo que pasa por ser bueno? Luego te engañan!*" .

I no m'enganye si dic que de tots els companys que he tingut de pis, el **Kuñi**, la **Reme** i la **Laura** són els més tarambanes, estrambòtics i carabasses que m'he trobat. Ens hem guanyat el cor de la Senyora [···] amb l'incansable frase: "*eso ya estaba roto cuando llegué*". La *festa de la creïlla*, les estones bones... gràcies. I ara que me'n vaig, fiquen l'ascensor?...no importa, el pis el fèiem nosaltres...

"*si nosaltres no poseïm la frescor de l'aire, ni la lluïssor de l'aigua, com ens ho podeu comprar?*" Gràcies **Aneta cutxareta** per regalar-me amb aquest llibre moltes de les teues idees i iniciar-me en l'art de la cullera. D'ací poc tot tornarà a funcionar com cal, ho sé.

Ja ho sé, tindre un origen comú o compartir material genètic no implica necessàriament l'estima. Però això ha fet que **Ibán** (amb B!), **Andrés** i jo cresquérem junts, amb estima i baralla, inevitablement. I per això, elements sempre presents, vos agraïsc...les impagables sessions d'Eugenio quan més ho he necessitat. Però sobre tot, per estar ací i allà...*repasate el manual? cada dia es un big-bang*...

Big-bang... sempre hi ha alguna cosa a l'inici. No em puc oblidar de Óscar Jordán, Àgueda Gras-*Lady Rostand*-, María, Núria...que sou amics al llarg del temps, gràcies.

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Bon profit!

CONTENTS

Introductory note and objectives

Biogenic Volatile Organic Compounds (BVOCs)

Over biological time the major gases exchanged with living organisms have included carbon dioxide (CO_2), oxygen (O_2) and nitrogen (N_2). These exchanges have altered the chemical composition and reactivity of the atmosphere (Peñuelas, 1993; Warneck, 1993). Most of the earth's atmosphere is made up of nitrogen (78% by volume) and oxygen (21% by volume). The remaining 1% of the atmospheric gases are known as trace gases because they are present in such small concentrations. Much attention has been given in the recent years to the measurements of fluxes of trace gases between the biosphere and the atmosphere, particularly the greenhouse gases CO_2, CH_4 and NO_2, because of their role in the global climate change. Living organisms also exchange other trace gases with the atmosphere. These biogenic volatile organic compounds (BVOCs) are very important for the global tropospheric chemistry and the global carbon cycle (Fehsenfeld et al, 1992; Singh and Zimmerman, 1992).

For the terrestrial biosphere, the principal non methane VOC sources come from vegetation. At the global scale it is estimated that vegetation emits 1.2×10^{15} g C per year (Guenther et al., 1995). Plants produce a variety of hydrocarbons of which the most representative and abundant group is isoprenoids (Table 1). Isoprene, monoterpenes and sesquiterpenes represent a small proportion of the diverse group of isoprenoid plant products. In addition to isoprenoids, several other volatile organic compounds are emitted by plants, for example methanol, methyl jasmonate, ethylene and many organic oxygenated carbon compounds.

Table 1. Nonmethane organic compounds emitted by vegetation. The molecular weight, boiling point and chemical structure also presented.

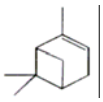
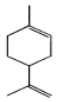
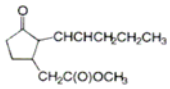
Compound name	Chemical formula	Molecular weight (g mol^{-1})	Boiling point (K)	Chemical structure
Isoprene	C_5H_8	68.12	307	
Camphene	$C_{10}H_{16}$	136.24	320	
Δ^3-Carene	$C_{10}H_{16}$	136.24	441	
α-Pinene	$C_{10}H_{16}$	136.24	428	
β-Pinene	$C_{10}H_{16}$	136.24	436	
Limonene	$C_{10}H_{16}$	136.24	448	
Myrcene	$C_{10}H_{16}$	136.24	440	
Terpineole	$C_{10}H_{16}$	136.24	459	
Sabinene	$C_{10}H_{16}$	136.24	437	
β-caryophyllene	$C_{15}H_{24}$	204.35	396	
α-Humulene	$C_{15}H_{24}$	204.35	396	

Table 1. *Continued*

Compound name	Chemical formula	Molecular weight (g mol^{-1})	Boiling point (K)	Chemical structure
Methyl chavicol	$C_{10}H_{12}O$	148.20	489	
Linalool	$C_{10}H_{16}O$	154.25	469	
Methyl jasmonate	$C_{13}H_{20}O_3$	224.30	383	
γ-Terpinene	$C_{10}H_{16}$	136.24	455	
α-Terpinene	$C_{10}H_{16}$	136.24	447	
β-Phellandrene	$C_{10}H_{16}$	136.24	446	
α-Phellandrene	$C_{10}H_{16}$	136.24	447	
p-Cymene	$C_{10}H_{14}$	134.22	450	
c-β-Ocimene	$C_{10}H_{16}$	136.24	373	
t-β-Ocimene	$C_{10}H_{16}$	136.24	373	
α-Copaene	$C_{15}H_{24}$	204.36	397	
α-Cedrene	$C_{15}H_{24}$	204.36	534	

Why do living organisms emit these volatile compounds to the atmosphere has continuously challenged research. Isoprene, monoterpenes and sesquiterpenes are not emitted by all plant species and so it has been suggested that there is not necessarily a specific role for every isoprenoid VOC emitted (Peñuelas and Llusia, 2004; Owen and Peñuelas, 2005). However, volatile isoprenoids avoid damages in cellular membranes under extreme conditions (Sharkey and Singsaas, 1995; Loreto and Velikova, 2001; Peñuelas and Llusià, 2003). So some plants emit isoprenoids that protect them from physiological stresses (water stress, high temperatures, oxidative stress or high irradiation). Monoterpenes and sesquiterpenes have also important ecological functions. Terpenes are used as defensive compounds in case of pathogens attack or herbivory (Croteau, 1987; Pichersky and Gershenzon, 2002; Peñuelas and Llusià, 2004, Rasmann et al. 2005), and also as antimicrobial compounds, as pollinator attractants (Kesselmeier and Staudt, 1999), and as allelopathyc compounds (Fischer et al., 1994, Peñuelas et al., 1996).

There are also multiple sinks of volatile compounds, i. e. processes that remove non methane biogenic VOCs from the atmosphere. Oceans, forests and agricultural sites are important sinks for many oxygenated VOCs due to the active uptake or passive adsorption processes (Chebbi and Carlier, 1996; Seco et al., 2007). VOCs emitted to the atmosphere are also removed by photochemical and dry and wet deposition processes (Atkinson and Arey, 2003). Photochemical reactions includes reactions with the hydroxyl radical (OH), photolysis, reactions with ozone and, during the night, with nitrate radicals (NO_3) (Warneke et al., 2004).

Biosphere and atmosphere show multiple regulation feedbacks in their processes. On one hand plants play an important role in the low atmosphere chemistry. The atmospheric photo-oxidation of the emitted VOCs leads to two important products in the lower atmosphere, ozone and organic aerosol, which have important consequences for air quality and climate. On the other hand, the earth's global climate, i. e. the changes in the variability or average state of the atmosphere over time, is affecting biosphere activities in turn.

Climate Change

Climate monitoring over the past century and long term reconstructions of climate over the past millennium indicate that the latter 20th century was anomalously warm and that the earth is actually warming up, as reported by the latest Intergovernmental Panel on Climate Change (IPCC 2007). For the next two decades a warming of about 0.2°C per decade is projected for a range of SRES emission scenarios (IPCC 2007). Continued greenhouse gas emissions at or above current rates would cause further warming and induce many changes in the global climate system during the 21st century that would very likely be larger than those observed during the 20th century. One of the direct observations on recent climate change (IPCC 2007) is that more intense and longer are droughts are occurring since the 1970s, particularly in the tropics and the subtropics. A general conclusion of most of the GCMs (global circulation models) shows that between 30º and 40º north and south latitudes the future decade's precipitations would be reduced, so Mediterranean communities would be affected by the enhanced drought, which is likely to affect soil water availability in the future (Fig. 1). Hence, it is important to estimate biogenic VOC fluxes to the atmosphere and their variations under the climate warming and the increased drought projected for the next decades by climatic (IPCC, 2007) and ecophysiological models such as GOTILWA (Sabaté et al., 2002, Peñuelas et al., 2005).

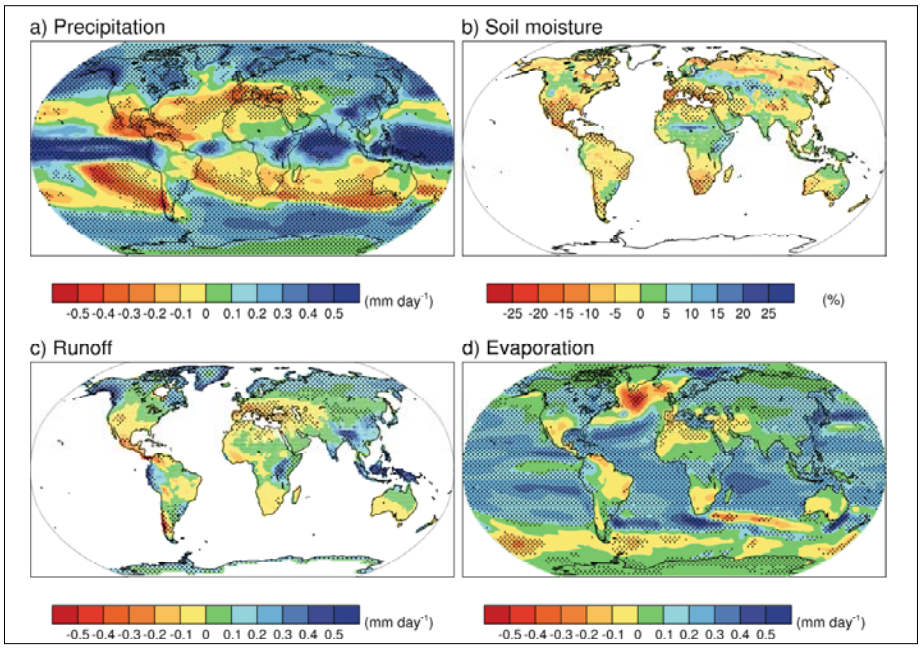


Figure 1. Multi-model mean changes in (a) precipitation (mm day−1), (b) soil moisture content (%), (c) runoff (mm day−1) and (d) evaporation (mm day−1). To indicate consistency in the sign of change, regions are stippled where at least 80% of models agree on the sign of the mean change. Changes are annual means for the SRES A1B scenario for the period 2080 to 2099 relative to 1980 to 1999. Soil moisture and runoff changes are shown at land points with valid data from at least 10 models. From IPPC 2007.

The soil VOCs

Most of the research about non methane VOC fluxes has focused on fluxes from the above-ground part of the plants at different levels, from canopy to foliar/flower/stem level. However, until the last years, information about VOC fluxes from the below-ground parts of the plants was very scarce. Firstly and apparently, the lack of information may be due to troubles studying the *hidden half* of the plants under field or roots' natural conditions. Many technologies have been developed to manage the methodologic inconveniences associated to roots growing in soil, for example the use of water culture as an alternative to soil as a growth medium. This research line has opened interesting new issues in the study of volatile and semi volatile root exudates and has shown remarkable ecological roles (Akiyama et al., 2005; Nishida et al., 2005). However, the main shortcoming of these laboratory techniques is that water culture

gives a homogeneous medium, whereas a fundamental character of soil is that it is heterogeneous at the scale of a root. Secondly, root VOCs under natural conditions may be less studied than VOCs from leaves because roots in soil establish multiple relationships with other soil living organisms increasing the complexity of the soil system. For example, the large capacity in soils for microbial oxidation of hydrocarbons and other organic species released by roots, largely prevents soil VOC emissions to the atmosphere, thus soil are thought to be a much weaker source of non methane VOCs than aboveground parts of terrestrial plants. But soil microorganisms produce also volatile compounds which are released to the soil atmosphere (Mackie and Wheatley, 1999; Ryu et al., 2003). Microbial populations associated to roots use roots' exudates as a growing carbon source and the effects of the organic matter degradation activity by microorganisms in soil becomes necessary to root growth. Lastly, roots and microorganisms activities are linked and herein the use of the term rhizosphere.

Soil nature and its gaseous phase

"Soil is essentially a natural body of mineral and organic constituents produced by solid material recycling during a myriad of complex processes of solid crust modifications, which are closely related to the hydrologic cycle" (from Mirsal, I. A. Soil Pollution, Origin, monitoring and remediation)

Plants and soil organisms are involved also in soil physical and chemical processes which lead through time to the transformation and evolution of rocks and minerals and the functioning of biogeochemical cycles of elements. These interactions, "plants-soil organisms and minerals" are mainly occurring in the upper soil horizons and in the rhizosphere. The rhizosphere receives large amounts of available energy as plant material (shoot and root litters, root exudates) and is where large populations of microorganisms (bacteria, fungi, algae and protozoa groups) are present and active. There are also other soil organisms grouped by their size in macrofauna (large molluscs, beetles, large insect larvae, etc.) and mesofauna (nematodes, arthropods, annelids and molluscs) which are also developing the soil profile by their activities.

Soil is a three dimensional system, made of a solid, a liquid and a gaseous phase, each one in amounts depending on the abundance of their constituents and their kinetic roles in the complex series of reactions leading to soil formation. Soil air or soil atmosphere is the characteristic name given to the mixture of gases moving in the

aerated zone and filling the soil pores where these are not already occupied by interstitial water. The mechanisms responsible for the transport of all soil gases are *diffusion*, resulting in a net movement of gas from a zone of higher concentration to one of lower concentration, and *mass flow*, where the whole gas mixture moves in response to a pressure gradient. Most gas movement is by diffusion; mass flow is important only when the pressure differences develop because of changes in barometric pressure, temperature, or soil water content. The movement occurs overwhelmingly in the air-filled pores, because diffusion in the gas phase is about four order of magnitude greater than through water. As air-filled porosity varies with soil water content and soil structure, these factors have a major effect on the rate of gas movement in soils. Gas exchange between soil and the atmosphere occurs also along temperature gradients.

Soils description

The experimental work was carried out on soils from Prades (41º13'N, 0º55'E), Garraf (41º18'N, 1º49'E) and Bellaterra (41º30' N, 2º6º E). Soils were classified according to Soil Taxonomy (Prades: Dystric Cambisol FAO, 1990; Garraf: Petrocalcic Calcixerept, Soil Survey Staff, 1998 and Bellaterra: Calcixerollic Xerochrept, Soil Taxonomy, 1975). Horizons description from the typical soil profile and some physico-chemical properties of the studied soils are in Tables 2, 3 and 4. Additional information about climate and vegetation are shown in chapters 1, 3 and 5.

Table 2. Soil horizons description and physico-chemical properties of the studied soil in Prades holm oak forest.

Prades	South slope soil (22º)
Oi (L)	9-3/5-2 cm. Thick horizon in some places. Identifiable leaves of Phyllierea and other dominant species
Oe (F)	5-2/3-1 cm. Well differentiated. Partly decomposed leaves. Fibrous structure. Abundant fungal mycelium. Many fine roots.
Oa (H)	3-1/0 cm. Humified material mixed with a mineral particles and leave debris
A1	0/5-8 cm. Texture sandy loam. Rich in organic matter, debris and humus.
A2	5-8/20-25 cm. Texture sandy loam. Moderate organic matter content.
A3	20-25/43-47 cm. Texture loamy sand. Few organic matter content.
Bw	43-47/75-90 cm. Texture sandy loam. Frequent fine to coarse roots.
R	>75-90 cm. Fracture methamorphic schists of Paleozoic.

Soil Type	Dystric Cambisol
Soil pH (water)	6.9
Soil texture	
% Sand	71.0
% Silt	21.6
% Clay	7.4
Mineral horizon (mean 0-15 cm)	
Mineral horizon bulk density (g cm^{-3})	1.0
Organic matter content (%)	7.3
Phosphorous (ppm)	13
Nitrogen (%)	0.2
Exchangeable cations	
K^+ (ppm)	263
Mg^{2+} (mequ/100g)	1.2
$EC_{25ºC}$ (dS/m) (1:5)	0.2

Table 3. Soil horizons description and physico-chemical properties of the studied soil in Garraf shrubland.

Garraf	South-South-East slope soil (13º)
O	3.5-0.5/0 cm. Gravels and stones layer of calcareous nodules.
A1	0/5-12 cm. Clay-loam. Moderate content of organic matter.
A2	5-12/32-37 cm. Clay-loam. Few organic matter.
B	32-37/35-70 cm. Petrocalcic horizon.

Soil Type	Petrocalcic Calcixerept
Soil pH (water)	8.1
Soil texture	
% Sand	42.9
% Silt	38.7
% Clay	18.4
Mineral horizon (mean 0-15 cm)	
Mineral horizon bulk density (g cm^{-3})	1.3
Organic matter content (%)	7.8
Phosphorous (ppm)	8
Nitrogen (%)	0.2
Exchangeable cations	
K^+ (ppm)	299
Mg^{2+}(mequ/100g)	1.2
EC $_{25ºC}$ (dS/m) (1:5)	0.2

Table 4. Soil horizons description and physico-chemical properties of the studied grassland soil in Bellaterra, Campus UAB (Barcelona). (Soil data from Alcañiz, 1980).

Bellaterra	Plain grassland soil
A1	0/3 cm. Texture clay loam. Moderate organic matter. Frequent medium roots and fine roots.
Ap	3/35 cm. Texture sandy clay loam. Very low organic matter. Few roots.
B	35/70 cm. Texture sandy clay loam. No organic matter. Few roots.
B/C_{ca}	70/90 cm. Texture sandy clay loam. No organic matter. Few medium roots, no fine roots.
C	90/±200 cm. Texture sandy clay loam. No organic matter. No roots.

Soil Type	Calcixerollic Xerochrept
Soil pH (water)	8.0
Soil texture	
% Sand	52.0
% Silt	22.3
% Clay	25.7
Mineral horizon (mean 0-20 cm)	
Mineral horizon bulk density (g cm^{-3})	1.3
Organic matter content (%)	2.1
Phosphorous (ppm)	41.3
Nitrogen (%)	0.1
Exchangeable cations	
K^+ (mequ/100g)	0.5
Mg^{2+}(mequ/100g)	2.8
EC $_{25ºC}$ (dS/m) (1:5)	1.0

A short introductory method description

In addition to the major constituents of the soil atmosphere (N_2 and O_2), other trace gases may occur in the soil air, produced within the soil body or introduced into the soil by rainwater, diffusion, uptake or other processes, from the atmosphere. We were interested in the soil atmosphere trace gas exchange of CO_2 and non methane biogenic VOCs. The principal method used to measure soil fluxes in this work is a variant of the enclosure method. We inserted a collar into the soil so that it protruded a few cm above the surface, and then sealed a lid onto the collar when making a measurement. The mode of operation was dynamic. A steady stream of air is pumped though the chamber, and the gas emitted from the soil is measured directly in the air stream or adsorbed in a suitable trapping material for subsequent release and analysis. We used the background values (VOCs measured on the atmosphere near the soil surface) to calculate the exchange rates on a mass balance basis. This way we measured either a positive soil VOC "*exchange rate*", i. e., a flux from the soil to the atmosphere (*"emission rate"*) or a negative soil VOC "*exchange rate*", i. e., flux from the atmosphere to the soil (*"uptake rate"*).

General objectives

Soils, being sources and sinks of non methane VOCs, have received little attention because of the complexity in studying soils and the assumption of the little soil source/sink strength compared with foliar non methane VOC fluxes. However, soil VOC fluxes really do happen and they are not well understood. Thereafter, the general objectives of this PhD thesis were 1) to characterize Mediterranean soil VOC contents and exchange, particularly monoterpenes because of their important role on ecology, plant physiology and atmospheric chemistry, but also other VOCs, and 2) to assess the possible changes in soil VOC and CO_2 exchange rates under the global environmental change towards more arid and warm conditions in the Mediterranean region.

Specific objectives

The general objectives were common to all chapters. In addition, the specific aims of each chapter are listed below:

Chapter 1. Seasonal soil CO_2 and VOC exchange rates in a Mediterranean holm oak forest and the responses to drought.

Since soil respiration and above-ground processes are linked processes (photosynthesis supplies carbon substrate for root metabolism and nutrition) we believe concurrent study of soil respiration and plant activity can provide more insight in the principal issue of this PhD, soil VOCs, and in the understanding of terrestrial carbon cycling and fluxes between the atmosphere and the terrestrial biosphere. Hence, we aimed:

Chapter 1.1. Seasonal soil leaf and CO_2 exchange rates and their responses to drought conditions.

1) To assess the seasonal CO_2 gas exchange from soils and plants.
2) To test their dependence on abiotic factors such as soil moisture and temperature.
3) To study the linkage between above- and below-ground exchange processes.
4) To study the response of soil and leaf CO_2 exchange to the lower soil water availability predicted for the next decades for Mediterranean ecosystems by IPCC and ecophysiological models.

Chapter 1.2. Seasonal soil VOC exchange rates and their responses to drought conditions.

1) To construct an inventory of the VOC (especially monoterpenes) exchange between soils and the atmosphere.
2) To investigate the seasonality of soil-atmosphere VOC exchange.
3) To investigate possible links to photosynthetic performance.
4) To study the response of soil VOC emissions to the lower soil water availability predicted for the next decades by GCM and ecophysiological models.
5) To investigate possible links between soil VOC and CO_2 exchanges and photosynthetic performance.

Chapter 2. Interannual and interseasonal soil CO_2 efflux and VOC exchange rates in a Mediterranean holm oak forest in response to experimental drought.

Given the importance of soil CO_2 efflux in the global carbon balance and the important role of soil monoterpene and VOCs in soil ecology, given the scarcity of knowledge on their responses to environmental conditions, and given the predicted climate change, we studied the responses of CO_2 and VOCs exchange to the more arid conditions forecasted for the Mediterranean region for the next decades. We performed a drought experiment by partially excluding rainfall and water runoff in a natural Mediterranean holm oak forest soil and we measured soil CO_2 and VOC exchange rates, under the predicted 15-20% lower soil water availability. Like in a previous study (Chapter 1) we studied the CO_2 and soil VOC exchange responses to the seasonal changes in soil moisture and temperature but now we also studied another year, the dry 2004-2005. Then, we aimed:

1) To study the effects of the important interannual variability in soil moisture and temperature characteristic of those Mediterranean ecosystems.
2) To measure the soil exchange of the whole range masses of volatile compounds with the PTR-MS technique.
3) To find possible linkages between monoterpenes and other VOC exchange rates and CO_2 efflux.

Chapter 3. Interannual and seasonal changes in the soil exchange rates of monoterpenes and other VOCs in a Mediterranean shrubland.

Similarly to Chapter 2, we conducted this study of soil VOC exchange rates in a Mediterranean shrubland at the Garraf Natural Park, in Catalonia. Our aims were:

1) To explore and quantify the soil VOC exchange rates, with special attention to monoterpenes, in a typical calcareous Mediterranean shrubland.
2) To investigate interannual and seasonal variations in soil VOC exchange rates.
3) To study soil VOC exchange rates' links to soil temperature, soil moisture and soil CO_2 efflux.

Chapter 4. On-line screening of soil VOCs exchange responses to moisture, temperature and root presence.

Many studies have shown the prominent role of VOCs on soil ecology, but the mechanisms controlling the exchange are not well understood. In this chapter we studied biotic and abiotic effects on soil VOCs exchange rates. We aimed:

1. To obtain information about changes in real time on the soil VOCs source/sink activity, taking advantage of the recent availability of analytical instrumentation to conduct these measurements.
2. To obtain information on soil VOCs exchange rates responses to root presence, to the soil warming and to the soil drought.

With these aims, we monitored soil VOCs exchange rates in real time with the PTR-MS technique in a controlled 'soil + plant'-atmosphere and in a 'soil'-atmosphere systems, and submitted both 'soil + plant' and 'soil' systems to moisture and temperature gradients.

Chapter 5. The distribution of volatile isoprenoids in the top soil horizons around *Pinus halepensis* trees in field conditions.

Due to the lack of information about terpenes concentrations in different horizons and on whether there is a natural root or litter source for soil, we aimed:

1. To quantify relative concentrations of terpenes in different soil layers (litter, organic layer, mineral layer directly underlying the organic layer, mineral layer 20 cm deep, and root material in the 2 mineral layers) and in a distance gradient from the trunk of a tree.
2. To quantify the pentane soluble fraction, i.e., the fraction likely to be colloid bound in the soil system or extractable from the air space.
3. To quantify the water soluble fraction, i. e. the fraction more directly available in aqueous solution to soil microfauna.
4. To investigate the effect of two variables that might affect the amount of volatile isoprenoid in the different soil horizons: (1) the fraction by weight of root material in the two mineral layers, and (2) the amount of litter per unit surface area.
5. To discern between roots and litter sources of soil BVOC concentrations.

Other VOC studies not presented in this PhD thesis

While conducting my PhD I also participated in other studies on plant VOCs to complement my scientific formation. As a result of that work I coauthored the following papers:

Llusià, J., Peñuelas J., Asensio D., Munné-Bosch S., 2005. Airbone limonene confers limited thermotolerance to *Quercus ilex*. Physiologia Plantarum 123, 40-48.

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Chapter 1

Seasonal soil CO_2 and VOC exchange rates in a Mediterranean holm oak forest and their responses to drought

1.1. Seasonal soil and leaf CO_2 exchange rates in a Mediterranean holm oak forest and their responses to drought conditions

Abstract.

We measured the soil and leaf CO_2 exchange in *Quercus ilex* and *Phillyrea latifolia* seasonally throughout the year in a representative site of the Mediterranean region, a natural holm oak forest growing in the Prades Mountains in South-Eastern Catalonia. In the wet seasons (spring and autumn), we experimentally decreased soil moisture by 30%, by excluding rainfall and water runoff in twelve plots 1 x 10 m and left twelve further plots as controls. Our aim was to predict the response of these gas exchanges to the drought forecasted for the next decades for this region by GCM and ecophysiological models.

Annual average soil CO_2 exchange rate was 2.27 ± 0.27 μmol CO_2 m^{-2} $s^{-1.}$ Annual average leaf CO_2 exchange rates were 8 ± 1 μmol m^{-2} s^{-1} and 5 ± 1 μmol m^{-2} s^{-1} in *Q. ilex* and *P. latifolia* respectively. Soil respiration rates in control treatments followed a seasonal pattern similar to photosynthetic activity. They reached maximum values in spring and autumn (2.5-3.8 μmol m^{-2} s^{-1} soil CO_2 emission rates and 7-15 μmol m^{-2} s^{-1} net photosynthetic rates) and minimum values (almost 0 for both variables) in summer, showing that soil moisture was the most important factor driving the soil microbial activity and the photosynthetic activity of plants. In autumn, drought treatment strongly decreased net photosynthesis rates and stomatal conductance of *Quercus ilex* by 44% and 53%, respectively. Soil respiration was also reduced by 43% under drought treatment in the wet seasons. In summer there were larger soil CO_2 emissions in drought plots than in control plots, probably driven by autotrophic (roots) metabolism. The results indicate that leaf and soil CO_2 exchange may be strongly reduced (by ca. 44%) by the predicted decreases of soil water availability in the next decades. Longer term studies are needed to confirm these predictions or to find out possible acclimation of those processes.

Key words: Soil CO_2 exchange, foliar net photosynthetic rates, Mediterranean holm oak forest, climate change, drought, roots, microorganisms.

1.1.1. Introduction

Soil respiration and foliar photosynthesis represent large natural fluxes in the dynamics of carbon exchange. While net primary production estimates are 50×10^{15} gC yr^{-1} (Field et al., 1998) carbon losses by soils are estimated at approximately 75×10^{15} gC yr^{-1} (Schlesinger and Andrews, 2000). Therefore, the study of soil respiration is important to understand the balance between biospheric and atmospheric carbon.

Soil respiration and above-ground processes are linked because photosynthesis supplies carbon substrate for root metabolism and nutrition. Root metabolism produces the release of exudates to the rhizosphere and these carbon-rich substances supply organic residues to decomposers (Schlesinger and Andrews, 2000; Ryan and Law, 2005). Concurrent study of soil respiration and plant activity (photosynthesis and conductance) can provide more insight in the understanding of terrestrial carbon cycling and fluxes between the atmosphere and the terrestrial biosphere.

Soil respiration includes two principal below ground processes: autotrophic and heterotrophic respiration (Hanson et al. 2000). The autotrophic respiration results from the growth and maintenance of roots and associated rhizosphere microorganisms (Pendall et al. 2004). The heterotrophic respiration is the sum of heterotrophic bacteria and fungi activity and soil faunal activity (Hanson et al. 2000). The proportion of soil respiration from autotrophic and heterotrophic contributions may vary seasonally and among ecosystems (Hanson et al. 2000) and may respond differently to environmental factors (Ryan and Law 2005).

We conducted a study in a typical Mediterranean holm oak forest. The dominant species, *Quercus ilex* L. and *Phyllirea latifolia* L. are widely distributed in the Mediterranean basin. Both species are well adapted to drought, although *P. latifolia* has been described as more drought resistant than *Q. ilex* (Tretiach 1993; Peñuelas et al., 2001; Ogaya and Peñuelas 2003).

The goals of our study were (i) to assess the seasonal CO_2 gas exchange from soils and plants (ii) to test their dependence on abiotic factors such as soil moisture and temperature, (iii) to study the linkage between above- and below-ground exchange processes, and (iv) to study the response of soil and leaf CO_2 exchange to the lower soil water availability predicted for the next decades for Mediterranean ecosystems by IPCC and ecophysiological models (IPCC 2001, Sabate et al. 2002, Peñuelas et al. 2005).

1.1.2. Material and methods

1.1.2.1 Sampling Site

This study was conducted between Spring 2003 and Spring 2004. Measurements were carried out in a natural holm oak forest growing in the Prades Mountain region, in Southern Catalonia (41º13'N, 0º55'E), on a south-facing slope (25% slope) at 930 m above sea level. The soil is a Dystric Cambisol (FAO, 1990) on a bedrock of metamorphic sandstone, and its depth ranges between 35 and 90 cm. The average annual temperature is 12 ºC and the annual rainfall 658 mm. Summer drought occurs approximately from mid-June to mid-September. The vegetation of the area is short holm oak forest characterized by 3 or 4-m tall trees and shrubs. This forest is dominated by *Quercus ilex* L. *Phillyrea latifolia* L. is also very abundant. *Arbutus unedo* L., some shrubs of *Erica arborea* L., *Juniperus oxycedrus* L. and *Cistus albidus* L. and occasional individuals of deciduous species (*Sorbus torminalis* L. Crantz and *Acer monspessulanum* L.) occur occasionally (Ogaya and Peñuelas, 2003).

Experimental design

Twenty-four 1 × 10 m plots were randomly distributed at the same altitude along the slope in the study area. Half of the plots were subjected to a drought treatment and the remainder plots were control plots. The drought treatment consisted of rainfall exclusion by suspending transparent PVC strips at a height of 0.5-0.8 m above the soil. In addition a 0.8-1 m deep ditch was excavated along the entire 1m top edge of the upper part of the treatment plots to intercept runoff water. Water intercepted by strips and ditches was drained to an area outside and downhill of the plots. Rainfall exclusion by plastic strips does not affect the light interception by the trees because the whole tree canopies are located above the plastic strips.

Litter-fall on the plastic strips was moved underneath them each month to sustain the humic composition of the soil. Therefore any nutrient differences below and outside the strips were due only to the change in water available for decomposition of this litterfall.

Drought treatment started in March 1999 and continues to the present.

1.1.2.2. Measurements of soil CO_2 flux, temperature and moisture.

Soil respiration was measured in situ using a flow-through chamber method and an infrared gas analyser system (EGM-4, PP Systems, Hitchin, Hertfordshire, England). A vented soil chamber system was performed with PVC collars (12.5 cm in diameter and 8 cm in height) installed permanently 3-4 cm into the soil. The collars were covered by a PVC lid with two outlets. One outlet was connected to the IRGA analyser by a tefflon tube. The other outlet was open to exterior air entry. Air inside the chamber was flowed (constant flux 0.4 L min^{-1}) to the CO_2 analyser by the EGM-4 integral DC pump. The flow was measured with a bubbler flowmeter. Equilibration of CO_2 concentration in the effluent stream occurred after 20 minutes. Before the collar was covered, we measured exterior air CO_2 concentrations. Net soil CO_2 fluxes were calculated by considering the stable difference in CO_2 concentration between the outlet air and the inlet air. Measurements were automatically corrected for temperature and pressure by the EGM-4 analyser. The accuracy of CO_2 measurements was estimated in 1%. Stability of the measurements were assured with the periodic "Auto-Zero" resulting in automatic correction for sample cell contamination, source aging, detector sensitivity variations and pre-amplifier gain changes.

Twelve collars in both control and drought plots (one collar per plot, n=12) were distributed randomly. The collars were installed in Winter 2002 and they were permanently placed into soil, in order to minimise possible effects of the mechanical disturbance during measurements. Before sampling litter recently fallen inside the PVC collars was removed to obtain CO_2 emissions only from soil roots and soil microorganisms. We measured one soil respiration value per collar.

Soil temperature and moisture were measured at 10 cm depth, just beside each PVC collar to avoid mechanical disturbances to the enclosed soil. Soil temperature above the soil surface (air temperature) was also measured. A soil digital thermometer was used to measure temperature (TO 15, Jules Richard instruments, Argenteuil, France) and a HH2 soil moisture meter connected to a ML2x soil moisture sensor (Delta-T Devices Ltd, Cambridge, England) was used to measure soil moisture.

1.1.2.3 Leaf CO_2 exchange rates and leaf water status measurements

Leaf net CO_2 exchange rates (A) and stomatal conductances (g_s) were measured in situ with a portable gas exchange system CIRAS2 (PP Systems, Hitchin, Hertfordshire, UK) at a 1500 μmol m^{-2} h^{-1} PPFD. Intact leaves were clamped in a Parkinson leaf cuvette (Std Broad 2.5, PP Systems, Hitchin, UK) connected to the CIRAS2.

Conductance for water vapor was calculated as: $g_s = 1 / r_s$ where r_s is the stomatal resistance to water vapor: $r_s = ((W_{leaf} - W_{an}) / \Delta W \times u_s) - r_b$, and where: $W_{leaf} = e_s / p$, e_s is the saturated vapor pressure at leaf surface, p is the atmospheric pressure, ΔW is the water vapor differential across leaf chamber, W_{an} is the water vapor concentration out of leaf chamber, r_b is the boundary layer resistance to water vapor and u_s is the mass flow of air per m^2 of leaf area.

Net photosynthetic rate and stomatal conductance were measured in one sunlit leaf of *Q. ilex* and one sunlit leaf of *P. latifolia* per plot. We conducted these measurements in 6 control and 6 drought plots which had accessible leaves to manual sampling. Sampled leaves had always the same age and similar position within the canopy.

Water potential was measured in one terminal twig of two different plants per species, in control and drought plots, using a Scholander pressure chamber (PMS, Corvallis, Oregon, USA). Relative water content (RWC) was measured early in the morning for 5 sunlit leaves of *Q. ilex* and *P. latifolia* in each plot. RWC was calculated as: $RWC = (M_F - M_D) / (M_T - M_D)$ where M_F is leaf fresh mass, M_D is leaf dry mass and M_T is leaf turgor mass, measured as water saturated leaf weight after 10-12 hours in water saturating conditions (petiole in water).

1.1.2.4 Sampling strategy

Measurement campaigns were carried out during 3 consecutive sunny days in each season: spring 2003 (April 22, 23 and 24), summer 2003 (August 12, 13 and 14), autumn 2003 (November 3, 4 and 5), winter 2004 (February 17 and 18) and spring 2004 (April 21, 22 and 23). Soil and leaf CO_2 exchange rates were measured during the mornings (from 7 a.m. to 11 a.m.). Soil respiration measurements in each plot took 20 min. The interval sample from plot to plot was 15 min. Net photosynthetic rates measurements took 15 min per plot. The interval sample between plots was 20 min.

Relative water content was measured early in the morning, and leaf water potential at midday.

1.1.2.5. Statistical analyses

Repeated measures analyses of variance (ANOVA) were conducted with soil CO_2 fluxes, soil moisture and temperature, leaf CO_2 exchange rates, relative water content and leaf water potential as dependent variables and with treatment and season as independent factors. Data was log transformed when necessary to meet the ANOVA assumptions. All analyses were performed with STATVIEW 5.01 software package (Abacus Concepts Inc., 1998).

1.1.3. Results

Annual average soil temperature and moisture during the sampling period were 13 ± 3 ºC and 17 ± 5 % respectively. We did not find significant differences in the soil surface temperature between control and drought plots (data not shown). Soil temperature at 10 cm depth was significantly higher in drought plots than in control plots only in November 2003 (Fig. 1.1.1), a season with no significant differences in soil respiration between treatments (Fig. 1.1.2). The lowest values of soil moisture were found in summer (1.6 ± 0.3 %), coinciding with maximum temperatures (25 ± 1 ºC). The drought treatment decreased soil moisture by 30% in spring and autumn ($p<0.0001$) when soil moisture was at maximum values for the year (Fig. 1.1.1).

Mean values of soil CO_2 efflux ranged from 2.00 to 2.53 μmol CO_2 m^{-2} s^{-1} for control treatment and from 1.64 to 1.92 μmol CO_2 m^{-2} s^{-1} for drought treatment (Fig. 1.1.2). There were seasonal variations in soil respiration during the year with the highest values in the springs (3.22± 0.49 and 3.76 ± 0.85 μmol CO_2 m^{-2} s^{-1}) and the lowest values in summer (0.13 ± 0.01 μmol CO_2 m^{-2} s^{-1}). Significant differences between drought and control plots in soil CO_2 fluxes were found only in the spring seasons when they were higher in control plots ($p<0.05$, $p< 0.1$ respectively), and in summer when CO_2 fluxes were higher in drought plots (control 0.13 ± 0.01 μmol m^{-2} s^{-1}, drought 0.75 ± 0.18 μmol m^{-2} s^{-1}; $p<0.01$) (Fig. 1.1.2).

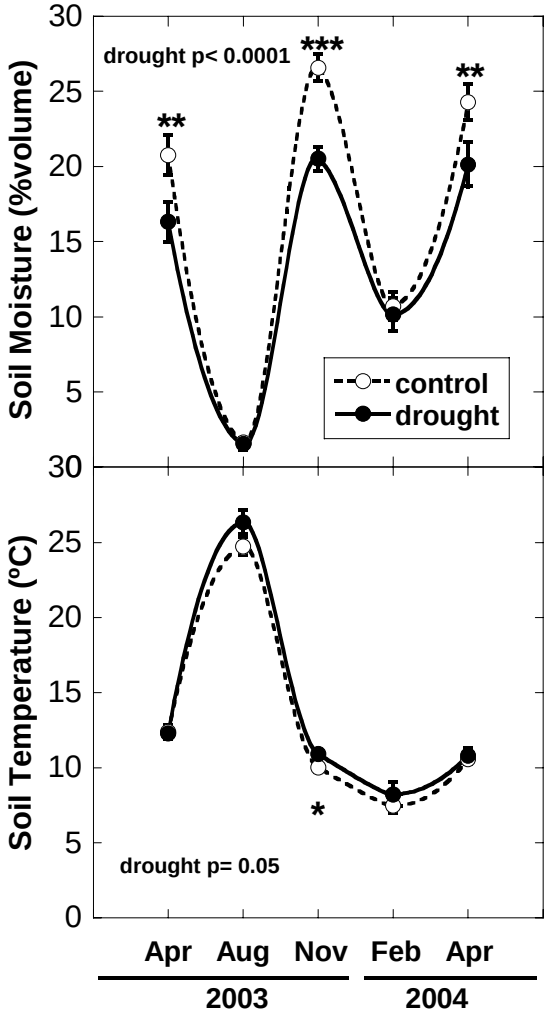

Figure 1.1.1. Seasonal course of soil moisture and soil temperature. Vertical bars indicate standard errors of the mean (n=12 plots). Significant differences between the two treatments in each season (ANOVA) are indicated with one asterisk ($p<0.1$), two asterisks ($p<0.05$) and three asterisks ($p<0.01$). Significance for the overall global effect of the treatment (repeated measurements ANOVA) is indicated inside the panels.

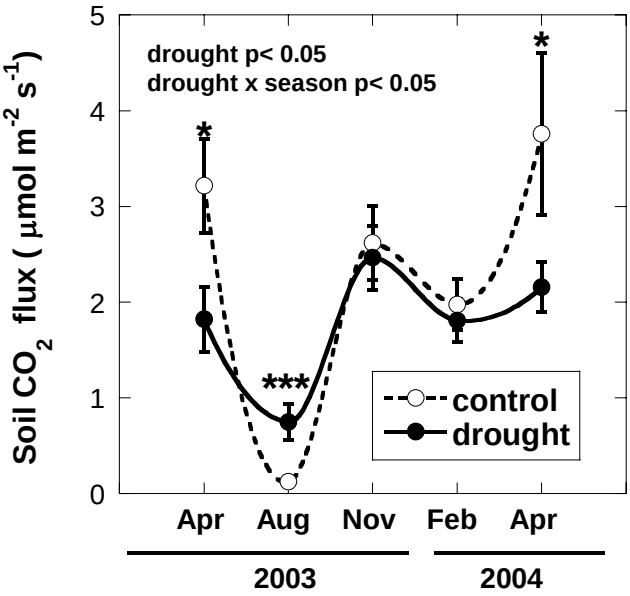

Figure 1.1.2. Seasonal course of soil CO_2 emission rates during the studied period. Error bars indicate standard error of the mean (n=12 plots per treatment and season). Significant differences between the drought and control treatments in each season are indicated with one asterisk ($p<0.1$), two asterisk ($p<0.05$) and three asterisks ($p<0.01$). Significance for treatment global effect on CO_2 emission rates from soil (repeated measurements ANOVA) is indicated inside the graph.

P. latifolia exhibited lower annual average of leaf water potential values than *Q. ilex* (-2.9 ± 0.2 and –2.1 ± 0.1 Mpa respectively). Both species had maximum values in spring and autumn, and minimum values in summer (Fig. 1.1.3 a and b). The summer drought response was greater in *P. latifolia*, with values that reached –6.7 ± 0.3 MPa, while values for *Q. ilex* only reached –3.1 ± 0.1 MPa. Slightly significant differences between treatments were found in spring 2004 in Q. ilex and P. latifolia ($p=0.056$ and $p=0.059$ respectively), (Fig. 1.1.3 a, b).

Leaf water potentials were strongly correlated with soil moisture (logarithmic regression: R= 0.92, n=10, p=0.0001 in *Q. ilex* and R= 0.94, n=10, p<0.0001 in *P. latifolia*).

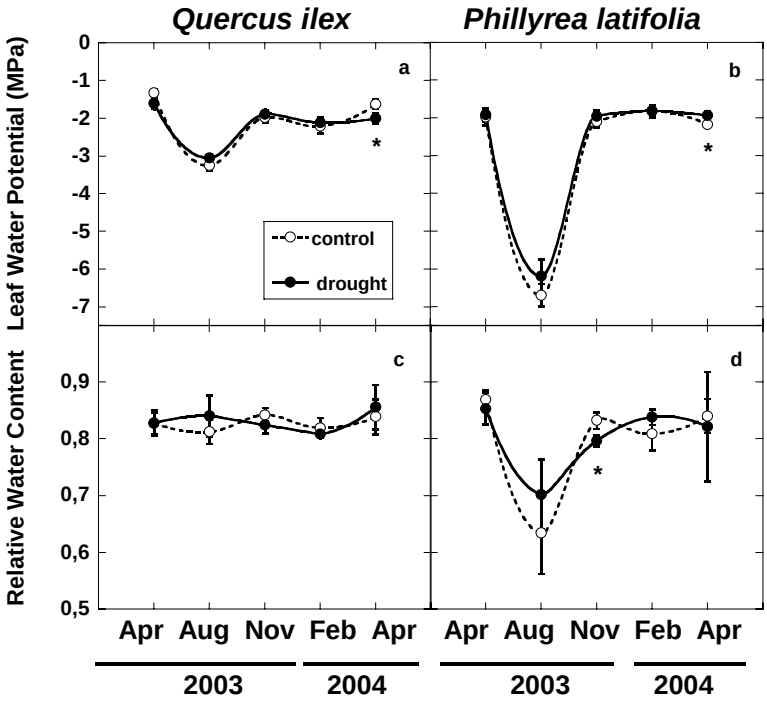

Figure 1.1.3 Seasonal course of leaf water potential in the two dominant plant species of the studied holm oak forest, *Q. ilex* (a) and *P. latifolia* (b). Seasonal course of relative water content in *Q. ilex* (c) in *P. latifolia* (d). Vertical bars indicate standard errors of the mean (n=8 samples per season, treatment and species). One asterisk indicates significant differences between the two treatments (p<0.1) in each season. No overall significant drought effect was found for any of the two species (repeated measurements ANOVA).

Annual mean values of RWC were similar in both species (0.83 ± 0.01 for *Q. ilex* and 0.80 ± 0.02 for *P. latifolia*). They showed a variation pattern similar to the LWP (Fig. 1.1.3 c, d), especially in control plants, although the range of variation in each species was different. *P. latifolia* minimum in summer (0.63 ± 0.07), and maximum in both spring and autumn, with slightly lower values in drought than in control plots treatments in the autumn season (p<0.1). For *Q. ilex* RWC values were constant throughout the year. No overall significant effects of drought treatment were detected (Fig. 1.1.3 c, d).

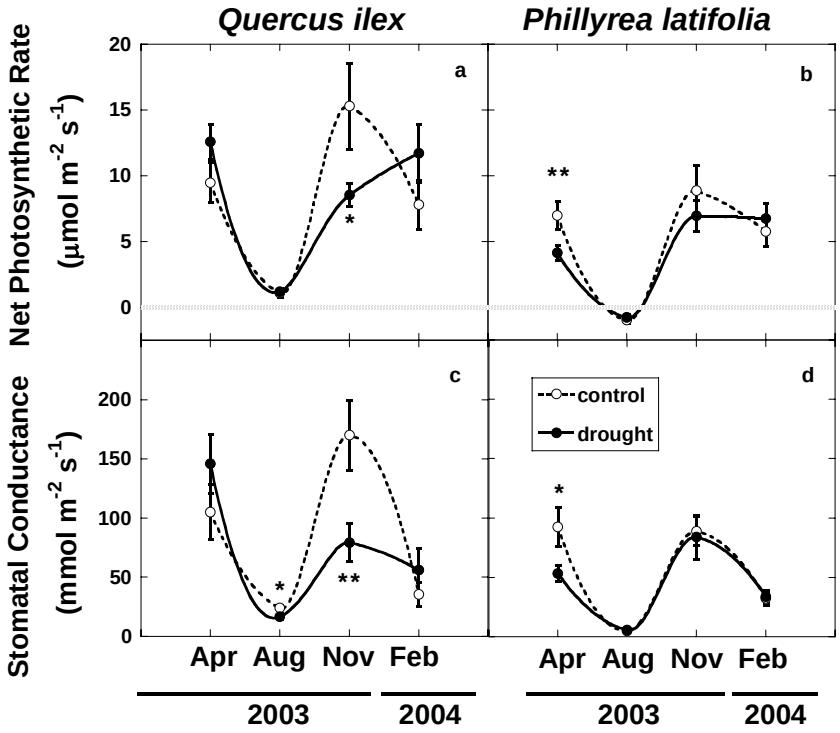

Figure 1.1.4. Seasonal course of net photosynthetic rates in sunlit leaves of *Quercus ilex* (a), and in sunlit leaves of *Phillyrea latifolia* (b). Seasonal course of stomatal conductance in sunlit leaves of *Q. ilex* (c), and in sunlit leaves of *P. latifolia* (d) during the studied period. Error bars indicate standard error of the mean (n=6 measures per treatment, season and species). One asterisk indicates ($p<0.1$) and two asterisk indicates ($p<0.05$) significant differences between the two treatments for the signalled species and season. No overall drought treatment effect (repeated measures ANOVA), was found for any of the two species.

Annual leaf CO_2 exchange rates in *Q. ilex* were higher than in *P. latifolia* (8 ± 1 and 5 ± 1 µmol m^{-2} s^{-1} respectively, Fig. 1.1.4 a and b). Both species' leaf CO_2 exchange was minimum in summer with values near to 0 or even slightly negative in *P. latifolia*. The maximum values in both species were found in spring and autumn, when the water availability was high (Fig 1.1.1).

Leaf CO_2 exchange rates were well correlated to leaf water potentials (logarithmic regression: $r^2= 0.62$, n=8, $p<0.05$ in *Q. ilex* and $r^2=0.83$, n=8, $p<0.005$ in *P. latifolia*). There were significant decreases (45%) of leaf CO_2 exchange in drought plots in spring (*P. latifolia* $p<0.05$) and in autumn (*Q. ilex* $p<0.1$), when there was rain to be

excluded, i. e. in the raining seasons, spring and autumn, and therefore there were significant effects of treatment on soil moisture (Fig. 1.1.1).

Stomatal conductances were usually higher in *Q. ilex* than in *P. latifolia* in all seasons (annual mean values 75 ± 9; 45 ± 9 mmol m^{-2} s^{-1} respectively) and varied with net photosynthetic rates (Fig. 1.1.4 c and d). Significant decreases in g_s in drought treatments for *Q. ilex* were found in summer and autumn ($p<0.1$ and $p<0.05$ respectively), whereas *P. latifolia* showed similar gs values in control and drought plots during the year, except for a slightly significant decrease in drought plots in spring ($p<0.1$) (Fig. 1.1.4 c, d).

1.1.4. Discussion and conlcusions

1.1.4.1. Seasonal course of soil CO_2 exchange rates in control and drought treatments

The 30% soil moisture decrease in the wet seasons and the lower soil CO_2 fluxes and photosynthetic rates measured in drought plots, show the effectiveness of the drought treatment. Root growth towards water sources beyond the influence of plastic strips in drought plots could be possible. However, the reduction of growth rates reported in this experimental system (Ogaya et al. 2003) in addition to our results, show that water availability is lower in drought plots and the whole plant is affected by this water reduction.

Soil respiration values coincided with values reported for other Mediterranean forests (Joffre et al. 2003) but they were higher than values reported for the same study area in previous reports (Piñol et al. 1995). The increase of the annual soil temperature in the 2003-2004 period compared with 1991-1992 may account for the differences.

In both spring and autumn, the wet seasons, the plant and microbial activities were high. This was also reflected in the seasonal patterns of leaf CO_2 uptake (Fig. 1.1.5). These results contrast with those reported for a southern boreal aspen forest (Griffis et al. 2004) where seasonal variability in soil respiration was mainly controlled by temperature with maximum rates in summer. In Mediterranean ecosystems, water is the principal factor controlling most of the aboveground and belowground processes resulting in a soil-moisture-dependent seasonal pattern for soil CO_2 emissions and leaf CO_2 uptake.

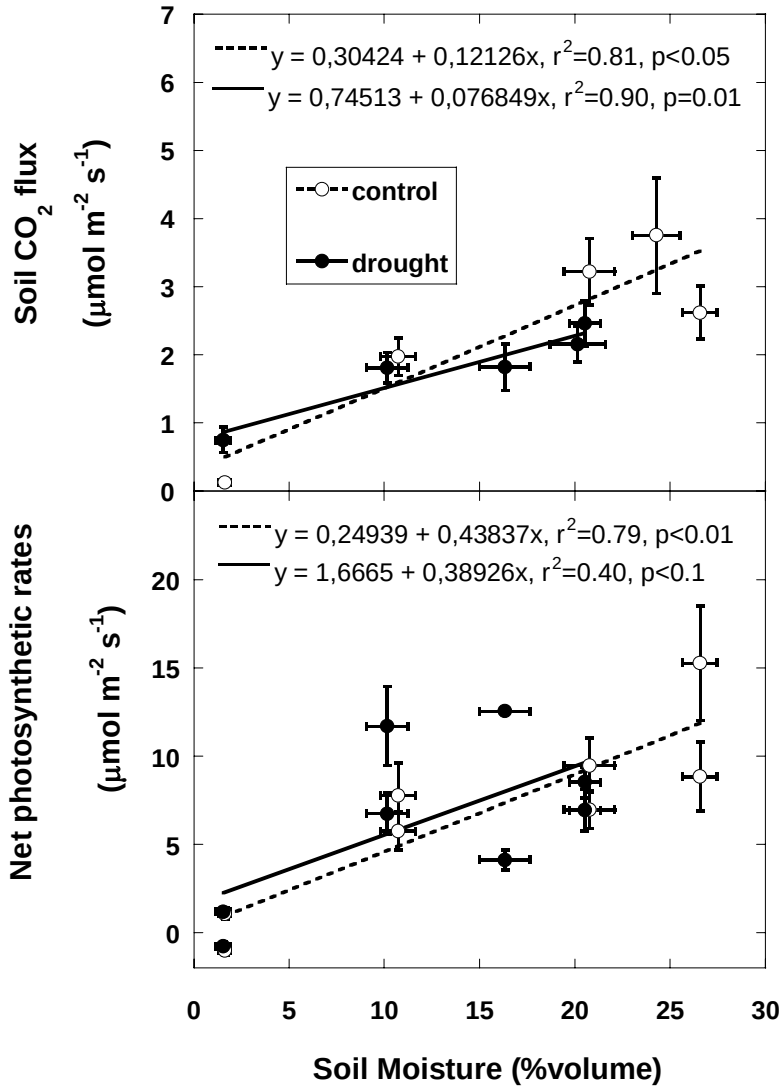

Figure 1.1.5. Relationship between the seasonal means of the soil CO_2 flux (n=12 in control and drought plots) and the corresponding seasonal means of soil moisture (n=12 on control and drought plots) and relationship between the seasonal means of the leaf CO_2 exchange (n=6 per species *Q. ilex* and *P. latifolia*, in control and drought plots) and the corresponding seasonal means of soil moisture (n=6 in control and drought plots). Error bars indicate ± standard error of the means.

Soil respiration is well correlated with microbial activity (Orchand et al. 1983). During summer drought, the physiological activity of microorganisms in response to the increase in temperature appears to be constrained by low soil moisture (Conant et al. 2004, Martin and Bolstad 2005). Similarly, drought treatment reduced soil respiration by 43% ($p<0.05$) during the rainy season in spring (Fig. 1.1.2). The reduction of soil respiration by excluding rainfall was found also by Borken et al. (1999, 2006) in similar studies on the soil respiration responses to experimental drought. To what extent is this reduction caused by lower microbial activity is unknown. Borken et al. (2006) suggest a stronger effect of drought in heterotrophs than in autotrophohs because they observed under drought smaller decreases in photosynthesis than in soil respiration. In our study photosynthetic rates decreased 40% in drought plots in spring only in *P. latifolia* (Fig. 1.1.4). Moreover, it is known that the root contribution to total soil respiration is higher during the growing season (Tang et al., 2005) thus, the reduction in the CO_2 efflux measured in drought plots in spring might be attributed to lower microbial activity.

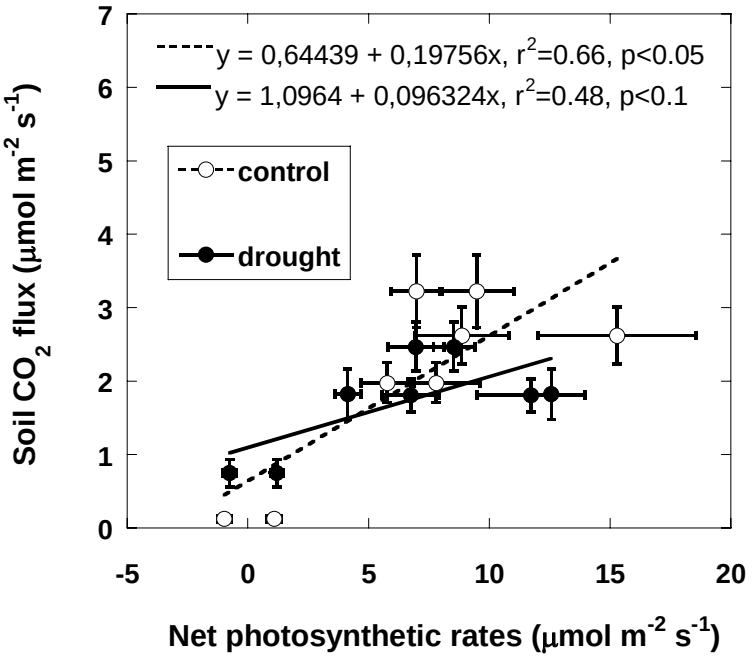

Figure 1.1.6. Relationship between the seasonal means of the soil CO_2 flux (n=12 per season in control and drought plots) and the seasonal means of the net photosynthetic rates in *Q. ilex* and *P. latifolia* (n=6 per season and species in control and drought plots). Error bars indicate ± standard error of the means.

Nevertheless results showed a different response of soil CO_2 emissions to drought treatment in wet spring compared with the dry summer. In summer, CO_2 emissions in drought plots were higher than in control plots which were practically zero (Fig. 1.1.2). Soil moisture in summer was very low in both treatments (Fig 1.1.1), but plants and microorganisms under drought treatment had been subjected to a lower water availability for a longer time than those in control plots. Prolonged low water availability in the drought treatment plots might have favoured root growth in those plots, resulting in enhanced root respiration. There are several reasons for high root respiration rate under severe drought stress (Li et al. 2004) in addition to the physical larger root surface. A change in the plant source-sink relationship may led to a greater proportion of assimilates transferring to roots, providing more substrate supply for root respiration. This could be a strategy to improve the nutrient availability and water uptake for the drought-stressed plant. Thus, in summer, CO_2 emissions in drought plots could be mostly driven by autotrophic metabolism as a result of changes in the importance of root versus soil microbial activity. Although this hypothesis needs further investigation, the significant correlation (r^2= 0.56, $p<0.05$) between leaf and soil CO_2 exchange in summer in drought plots, that was not found in control plots (r^2= 0.0001, p=0.76), provides support for the hypothesis that prolonged low water availability favour root growth.

1.1.4.2. Coupling between soil respiration and leaf CO_2 exchange.

Numerous studies of leaf gas exchange have demonstrated similar leaf responses to those described here of net photosynthetic rates decreasing from spring to summer (Fig. 1.1.4) with increasing drought (Oechel et al. 1981, Tenhunen et al. 1990, Peñuelas et al. 1998, Ogaya and Peñuelas 2003). For instance, in a Mediterranean maquis characterised by tall shrubs in Castelporziano (Italy), the seasonal variation of leaf CO_2 exchange rates and stomatal conductances (Gratani and Varone 2004) were very similar to those found at Prades. The study of soil and leaf CO_2 exchange throughout the year showed that soil moisture and temperature were the main factors driving CO_2 exchange.

Root respiration comprises a significant fraction of soil respiration (Irvine et al. 2005) and it strongly reflects plant metabolism (Ekblad and Högberg, 2001). We have found a significant correlation between photosynthetic rates and soil respiration rates (Fig. 1.1.6). However, this could be an indirect relationship resulting from seasonal variations of temperature, moisture and phenology. Moreover, there could be time lags

between the assimilation of carbon in leaves and the carbon transport to the roots (McDowell et al., 2004). Further studies considering separately autotrophic versus heterotrophic respiration in a high frequency of measurements over a number of days are needed to further confirm this correlation and gain knowledge on the relationships between aboveground and belowground plant processes. Although our experimental methods did not allow separation of the two components of the total soil respiration, seasonal data correlation between carbon fixation and soil respiration in control plots suggests a link between both variables.

The different soil respiration response to drought in spring and summer highlights the need of a better understanding of the contribution of autotrophic respiration to total soil CO_2 exchange. The results suggested changes in drought conditions towards a decrease in the microorganisms/roots ratio of activities in the rhizosphere, especially in summer.

Finally, coupled GCM and ecophysiology models predict a 20-30% decrease in water availability over the next three or four decades (IPCC 2001, Peñuelas et al., 2005). Our results suggest a 44% reduction in soil and foliar CO_2 exchange rates in wet seasons in response to this decrease in water availability, demonstrating the importance of considering climate change effects on soil CO_2 flux and foliar CO_2 uptake in the budgeting of carbon in the atmosphere and the biosphere. However, acclimation of soil respiration and photosynthesis to prolonged drought could occur and therefore long-term studies of soil and leaf CO_2 exchange are needed to discern the climate change effects on soil CO_2 fluxes.

Acknowledgements

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1.2. Seasonal soil VOC exchange rates in a Mediterranean holm oak forest and their responses to drought conditions

Abstract.

Available information on soil Volatile Organic Compound (VOC) exchange, emissions and uptake, is very scarce. We here describe the amounts and seasonality of soil VOC exchange during a year in a natural Mediterranean holm oak forest growing in Southern Catalonia. We investigated changes in soil VOC dynamics in drought conditions by decreasing the soil moisture to 30% of ambient conditions by artificially excluding rainfall and water runoff, and predicted the response of VOC exchange to the drought forecasted in the Mediterranean region for the next decades by GCM and ecophysiological models.

The annual average of the total (detected) soil VOC and total monoterpene exchange rates were 3.2 ± 3.2 and –0.4 ± 0.3 μg m^{-2} h^{-1} respectively, in control plots. These values represent 0.003 % of the total C emitted by soil at the study site as CO_2 whereas the annual mean of soil monoterpene exchange represents 0.0004 % of total C. Total soil VOC exchange rates in control plots showed seasonal variations following changes in soil moisture and phenology. Maximum values were found in spring (17 ± 8 μg m^{-2} h^{-1}). Although there was no significant global effect of drought treatment on the total soil VOC exchange rates, annual average of total VOC exchange rates in drought plots resulted in an uptake rate (-0.5 ± 1.8 μg m^{-2} h^{-1}) instead of positive net emission rates. Larger soil VOC and monoterpene exchanges were measured in drought plots than in control plots in summer, which might be mostly attributable to autotrophic (roots) metabolism.

The results show that the diversity and magnitude of monoterpene and VOC soil emissions are low compared with plant emissions, that they are driven by soil moisture, that they represent a very small part of the soil-released carbon and that they may be strongly reduced or even reversed into net uptakes by the predicted decreases of soil water availability in the next decades. In all cases, it seems that VOC fluxes in soil might have greater impact on soil ecology than on atmospheric chemistry.

Key words: Soil VOC exchange, soil CO_2 flux, Mediterranean holm oak forest, climate change, monoterpenes, drought

1.2.1. Introduction

Emissions of Biogenic Volatile Organic Compounds (BVOCs) from vegetation have been an area of research interest for several decades because of their important role in biology and in atmospheric chemistry (Fehsenfeld et al., 1992; Kesselmeier and Staudt, 1999; Peñuelas and Llusià, 2003, 2004). There are several roles hypothesized for VOC emission in plants, including various protective functions and intra- and inter-specific communication (Langenheim 1994, Owen and Peñuelas, 2005). There are many studies of VOC emissions from Mediterranean evergreen species, because the role proposed for VOCs could be important in these species which are typically exposed to high temperature and drought-stress summer conditions (Di Castri, 1973; Terradas and Savé, 1992) and because the Mediterranean vegetation is rich in plant species storing VOCs in specialized organs (Peñuelas and Llusià, 2001).

It is known that soils may be both VOC sources (Guenther, 1999a) and sinks (Van Ginkel et al. 1987; Bender et al., 1993), but the available information is scarce (Hayward et al., 2001), much more scarce than for vegetation, especially for the Mediterranean ecosystems. VOCs in natural soils may be derived from a variety of sources. The root system of plants (Janson 1993, Chen et al. 2004) and soil microorganisms (Scholler et al. 2002) seem to be the major sources of emission. Their production is strongly influenced by environmental conditions. For example, Stahl and Parkin (1996) studied the effect of different substrates and selective antibiotics on soil VOC emissions. They found that treatments dominated by different groups of microorganisms produced different kind of VOCs and the VOC composition was different at each sampling day and also changed within a given treatment. Consumption of VOCs by microorganisms has been demonstrated in laboratory conditions (Cleveland and Yavitt, 1998; Kleinheinz et al. 1999; Demyttenaere et al. 2000; Yoo and Day 2002) but, as far as we know, there are no reports on microbial consumption activity in natural conditions. Soil VOC exchange in natural and laboratory conditions could be different. The presence of roots and other below-ground biota in natural conditions may be synergistic or antagonistic factors affecting the VOC consumption/emission by microorganisms. For instance, Ryu et al. (2003) have reported bacterial volatiles that promote growth in plants, and Mackie and Wheatley (1999) described negative interactions between bacteria and fungi mediated by VOCs.

Yet, the role of soil VOCs is poorly understood in natural conditions due to the scarcity of field data (Hayward et al., 2001). We have little information about soil VOCs seasonality and also on their responses to the lower water availability predicted in the Mediterranean region by Global Change Modelling and ecophysiological models (IPCC, 2001; Peñuelas et al, 2005).

We conducted an study of soil VOC exchange in a typical Mediterranean holm oak forest. The dominant species, *Quercus ilex* L., is a tree well adapted to drought and widely distributed in the Mediterranean basin. *Phillyrea latifolia* L. is also codominant in this forest and frequent in the Mediterranean forests and shrublands. It is a tall shrub species associated with the holm oak forest. Both *Q. ilex* and *P. latifolia* emit significant amounts of monoterpenes and other VOCs, but do not appear to store them (Llusià and Peñuelas, 1998; Peñuelas and Llusià, 1999).

The goals of this study were (i) to construct an inventory of the VOC (especially monoterpenes) exchange between soils and the atmosphere, particularly for monoterpenes, (ii) to investigate the seasonality of soil-atmosphere VOC exchange, (iii) to investigate possible links to photosynthetic performance, (iv) to test dependence of VOC exchange on abiotic factors like soil moisture and temperature, and (v) to study the response of soil VOC emissions to the lower soil water availability predicted for the next decades by GCM and ecophysiological models (IPCC 2001, Sabate et al. 2002, Peñuelas et al. 2005).

1.2.2. Material and methods

1.2.2.1. Sampling site, experiment design and sampling strategy.

Full details of the sampling site and strategy and the effectiveness of the drought treatment are described before in this Chapter 1, section 1.1.2. In short, the study was conducted in a typical Mediterranean mountains environment in the Prades region of Southern Catalonia. Twelve 1x10 m plots were subjected to run off and rainfall exclusion, with a further 12 control plots with natural rain and drainage. Treatments started in March 1999 and measurements of soil VOC exchange were conducted in parallel to those of soil and foliar CO_2 fluxes (Chapter 1.1), during the four seasons of the year from spring 2003 to spring 2004.

1.2.2.2. Measurements of soil VOC exchange with the atmosphere.

Soil CO_2 flux and VOC exchange were sampled using a dynamic PVC soil cuvette system. One soil chamber per plot (n=12) was installed permanently since Winter 2002. Measurements of soil CO_2 flux and VOC sampling were conducted simultaneously. An IRGA non-dispersive CO_2 analyser (EGM-4, PP Systems, Hitchin, Hertfordshire, England) was connected to the outlet of the cuvette for determination of CO_2 (sampling interval and duration and flux calculation details in Chapter 1.1). Air from the cuvette flowed though a T system impelled by a pump towards a glass tube (11.5 cm long and 0.4 internal diameter) filled with VOC adsorbents Carbotrap C (300 mg), Carbotrap B (200 mg), and Carbosieve S-III (125 mg) (Supelco, Bellefonte, Pennsylvania) separated by plugs of quartz wool. The hydrophobic properties of the tubes minimized sample degradation by water. Standard compounds injected onto these tubes (3-hexen-1-ol, α-pinene, β-pinene, limonene and dodecane) did not deteriorate before analysis. These tubes were conditioned prior to use by heating at 350ºC for 3 minutes in a stream of purified helium.

The VOC flow from the soil chamber to the glass tube filled with adsorbents varied between 0.2 and 0.3 L min^{-1} depending on the glass tube adsorbent and quartz wool packing. Soil VOC were sampled during 5 min. and the flow was regulated with a peristaltic pump (Portable Escort Elf Pump, P/N 497701 S/N A2-31854; Mine Safety Appliances Company, Pittsburg, Pennsylvania, USA). The flow adjustment was determined with a bubble flowmeter. The interval sample from plot to plot was 15 min. Glass tubes were stored in a portable fridge at 4ºC and taken to the laboratory, where they were stored at -28ºC until analysis (within 1 week).

VOC analyses were conducted in a GC-MS (Hewlett Packard HP59822B, Palo Alto, California). Trapped VOCs were desorbed (Thermal Desorption Unit, Model 890/891; Supelco, INC, Bellefonte, Pennsylvania) at 250ºC during 3 min and injected into a 30 m x 0.25 mm x 0.25 mm film thickness capillary column (Supelco HP-5, Crosslinked 5% pH Me Silicone). After sample injection, the initial temperature (40°C) was increased at 30ºC min^{-1} up to 60ºC, and thereafter at 10ºC min^{-1} up to 150ºC, maintained for 3 min, and thereafter at 70ºC min^{-1} up to 250ºC, which was maintained for another 5 min. Helium flow was 1 mL min^{-1}. The identification of VOCs was conducted by GC-MS and comparison with standards from Fluka (Chemie AG, Buchs, Switzerland), and GCD Chemstation G1074A HP reference library. The reference

compounds used for the identification were: 3-hexen-1-ol, α-pinene, β-pinene, limonene and dodecane. Calibration with a range of solutions of these standards every 7 analyses was used for quantification. VOC calibration curves (N=5 different VOC concentrations) were always significant ($r^2 > 0.97$) in the relationship between signal and VOC concentration.

Calculations of VOC exchange rates were made on a mass balance basis, and they were also normalised to 30 ºC using the algorithm of Guenther et al. (1993): $E = E_s \exp(\beta (T - T_s))$, where E is the emission rate (μg m^{-2} h^{-1} in soil) at soil temperature T (K), E_s the emission rate at standard temperature T_s (K) and β (K^{-1}) is an empirically determined coefficient (0.09). Although mechanisms of release may be quite different between soil and plants, there was a significant relationship between monotepernes such as alpha-pinene and soil temperature. We calculated E_s = -1.31 μg m^{-2} h^{-1} and β = 0.10 for alpha-pinene fluxes.

1.2.2.3. Statistical analyses.

Repeated measures analyses of variance (ANOVA) were conducted with total soil VOC and soil monoterpene exchange rates as dependent variables and with treatment and season as independent factors. Data was log transformed when necessary to meet the ANOVA assumptions. Regression analyses model II were conducted with seasonal mean values of the studied variables. All analyses were performed with STATVIEW 5.01 software package (Abacus Concepts Inc., 1998).

1.2.3. Results

During the sampling period 2003-2004 the annual average soil moisture in the experimental station at Prades was 16.79 ± 4.66 %. The lowest values of soil moisture were found in summer (1.63 ± 0.31). Maximum values of soil moisture occurred in springs and autumn (20-26% volume), when the drought treatment decreased soil moisture by 30% ($p<0.0001$) (Chapter 1.1).

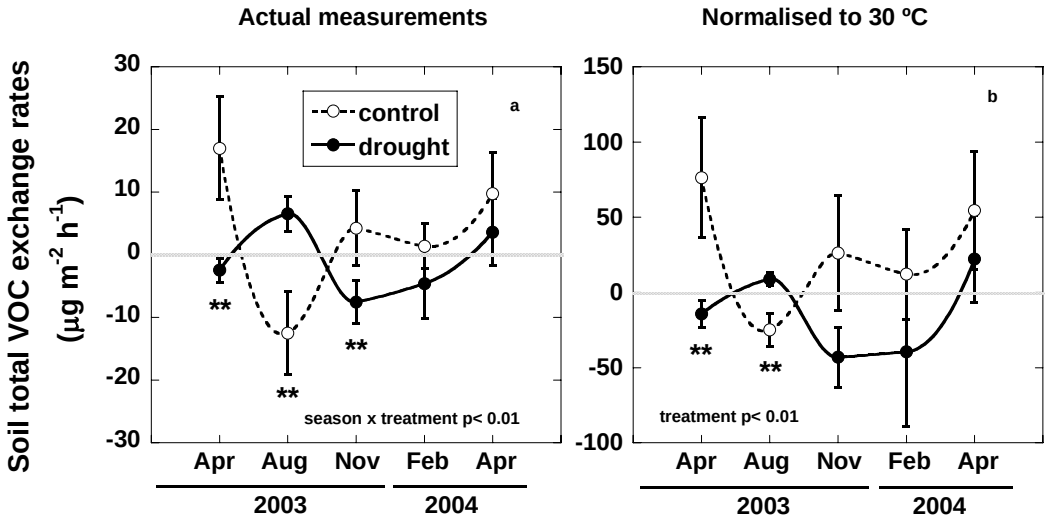

Figure 1.2.1. a) Seasonal course of total VOC exchange rates from soil and, b) seasonal course of total VOC exchange rates from soil normalised to 30 ºC. Normalised rates were calculated using the algorithm of Guenther et al. (1993). Error bars indicate standard error of the mean (n=12 plots per season and treatment). Significant differences between the drought and control treatments in each season are indicated with one asterisk ($p<0.1$) and two asterisks ($p<0.05$). Overall significance of the drought treatment effect on soil VOC emission rates (repeated measurements ANOVA) is indicated inside the panels.

The annual average VOC soil emissions was 3.15 (± 3.23) µg m^{-2} h^{-1} in control plots. Although soil VOC emissions prevail over the VOC uptake in the annual average values, we found significant seasonal variations including a high consumption activity during the summer (-12.49 ± 6.69 µg m^{-2} h^{-1} control plots, Fig. 1.2.1). Changes in soil moisture recorded during the sampling period (Chapter 1.1) partially explained the

seasonal variations in VOC soil exchange rates. Soil VOC exchange was positive in spring and autumn, resulting in overall emissions from the soil surface. There was a significant correlation between total soil VOC emission exchange rates and soil moisture in control plots (r^2= 0.64, p=0.1 Fig. 1.2.4).

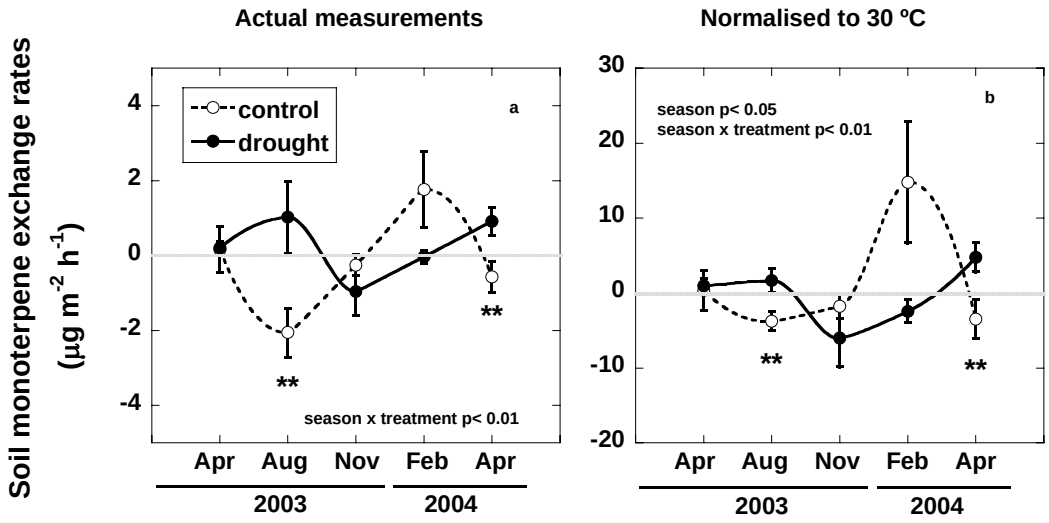

Figure 1.2.2 Seasonal course of monoterpene soil exchange rates (a) and monoterpene exchange rates normalised to 30 ºC (b). Normalised rates were calculated using the algorithm of Guenther et al. (1993). Error bars indicate standard error of the mean (n= 12 plots per season and treatment). Significance for the overall effect of drought on monoterpene emission rates from soil (repeated measurements ANOVA) is indicated inside the panels.

Total soil VOC emission rates tended to be higher in control than in drought treatments, especially in April 2003 and November 2003 ($p<0.05$ and $p<0.1$ respectively, Fig. 1.2.1 a) when there were emissions from control plots, and uptakes in drought plots. However, the overall treatment effect on total soil VOC emissions was only significant when considering soil emission rates normalised to 30ºC ($p<0.05$, Fig. 1.2.1 b).

In summer, there was uptake of VOC and monoterpenes by control plots and emissions from drought plots (Fig. 1.2.1 a and b; Fig. 1.2.2 a and 1.2.2 b). This was unexpected, because it was contrary to the annual exchange trends (i.e. emissions from control plots and uptake by drought plots). The spring response was more a seasonal or phenological response linked to soil moisture and phenological processes, e. g. high net photosynthetic rates in spring in *Q. ilex* and *P. latifolia* (9.5 ± 1.5 µmol m^{-2} s^{-1} and 7.0 ±

1.1 μmol m^{-2} s^{-1} respectively), rather than a temperature response, since it was still significant after standardizing to 30ºC (Fig. 1.2.1 b, 3.2.2 b).

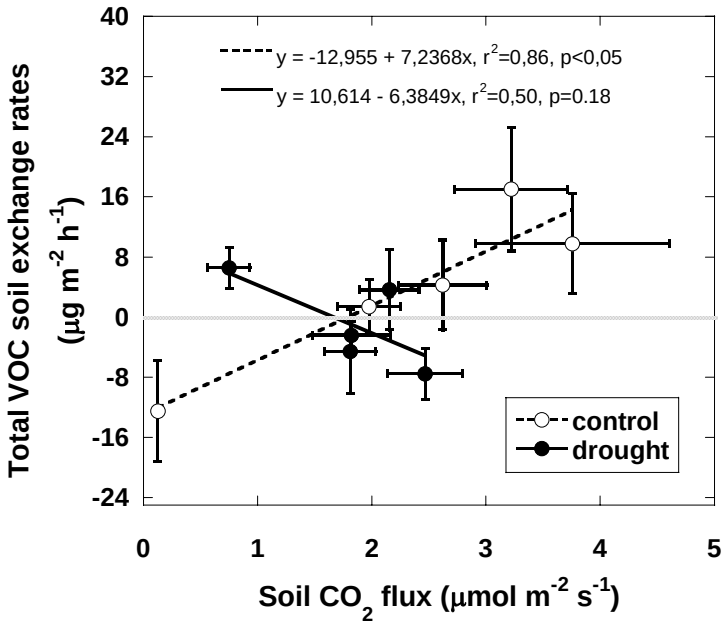

Figure 1.2.3. Relationships between the seasonal means of the soil total VOC exchange rates (n=12 in control and drought plots) and the corresponding seasonal means of soil CO_2 flux (n=12 in control and drought plots). Error bars indicate ± standard error of the means.

Emission rates of detected monoterpenes were low, and very variable (Fig. 1.2.2), and represented only a small percentage of the total VOC emissions from soil (8.21%). Contrary to the annual average of soil VOC exchange rates, annual soil monoterpene exchange rate in control plots was negative, with uptake of soil monoterpenes (-0.43 ± 0.30 μg m^{-2} h^{-1}) prevailing over the emission (Fig. 1.3.2).

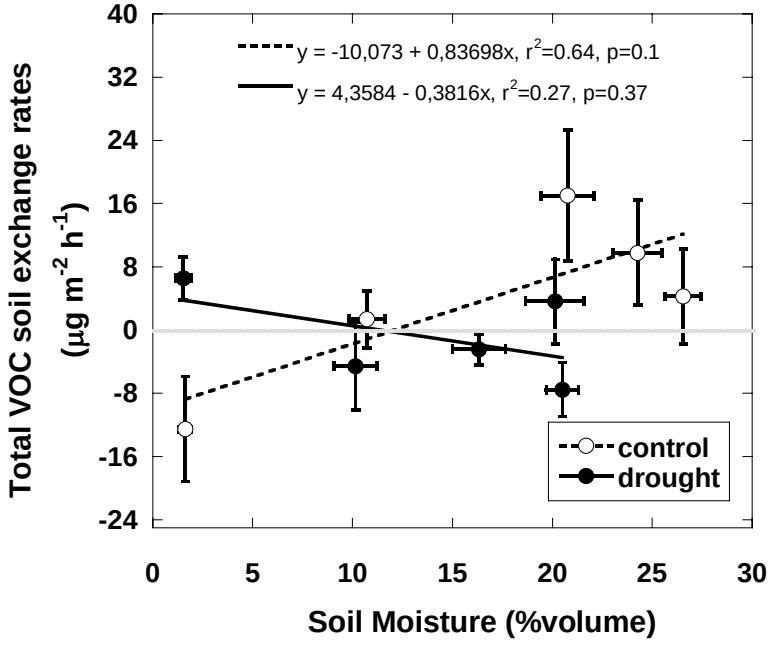

Figure 1.2.4. Relationships between the seasonal means of the soil total VOC exchange rates (n=12 in control and drought plots) and the corresponding seasonal means of soil moisture (n=12 in control and drought plots). Error bars indicate ± standard error of the means.

Soil monoterpene exchange rates in control and drought plots showed different seasonal variations. Monoterpene exchange pattern throughout the study period in control treatment showed maxima in February 2004 and minima in August 2003 (Fig. 1.2.2). Although the treatment global effect on soil monoterpene emissions was not significant, significantly higher emissions were measured in drought plots than in control plots in August 2003 (drought 1.02 ± 0.96 vs control –2.05 ± 0.66 µg m^{-2} h^{-1}, $p<0.05$) and April 2004 (drought 0.92 ± 0.37 vs control –0.57 ± 0.42 µg m^{-2} h^{-1}, $p<0.05$) (Fig. 1.2.2 a and 1.2.2 b). Emission rates normalized to 30 ºC showed an overall effect of season on monoterpene emissions ($p<0.05$) and an interaction with the drought treatment ($p<0.01$) (Fig. 1.2.2 b).

The variety of different identified compounds emitted or taken up was lower in drought than in control plots (Table 1.2.1) in concordance with the parallel decrease of emissions. The most common compounds observed were 3-cyclohepten-1-one, 3-octanone, nonanal, decanal and the monoterpenes α-pinene, limonene and camphene. Benzene and p-xylene soil uptake and toluene soil emissions occurred occasionally (Table 1.2.1). There was also a seasonal qualitative effect on the monoterpene emissions. Tricyclene and camphene dominated emissions in winter, and limonene and α-pinene in the warm seasons spring and summer (Table 1.2.1). Alpha-pinene and limonene were the monoterpenes with the highest uptake rates throughout all seasons.

1.2.4. Discussion

1.2.4.1. Soil VOC emission and uptake rates: compounds and amounts

The results show that soil emissions and uptake rates of VOCs in general, and monoterpenes in particular, are low in this Mediterranean ecosystem. The measured monoterpene soil emission rates in summer (1.67 ± 1.57 μg m^{-2} h^{-1} normalised to 30 ºC, Fig. 1.2.2 b) were lower than those described in temperate ecosystems, (range 8.97-16.4 μg m^{-2} h^{-1}) (Hayward et al. 2001). We also measured monoterpene emission rates in *Q. ilex* and *P. latifolia* leaves during the soil VOC sampling campaigns (data not shown). Mean annual monoterpene emissions showed significant differences between leaf and soil emission (about 1800 μg m^{-2} h^{-1} in Q. ilex and –0.4 μg m^{-2} h^{-1} in monoterpene soil exchange). However, leaf emissions and soil uptake followed the same seasonal pattern.

A greater diversity of compounds was exchanged in control plots than in drought plots (Table 1.2.1). In all cases, the diversity of monoterpenes (tricyclene, α-pinene, camphene, β-pinene and limonene) (Table 1.2.1) was slightly lower than reported for a temperate ecosystem (α-pinene, camphene, limonene, 3-carene, β-pinene, myrcene, α-phellandrene and α-terpinene) (Hayward et al. 2001). The drier conditions, the lower biomass and lower enzymatic activity linked to lower water availability may account for such differences. In fact, drought treatment strongly decreased the VOC emissions up to the point that soil became a sink more than a source for VOCs in most seasons of the year.

Although the annual mean of VOC and monoterpene emissions in Prades represents a very small percentage of the total C emitted as CO_2 (0.003% and 0.0004% respectively), they still might play an important ecological role on the soil and rhizosphere interactions. There are some studies which show these interactions. For example, root VOCs emissions induced by the attack of below-ground herbivores such the larvae of the black vine weevil, *Otiorhynchus sulcatus*, resulted in the attraction of soil-dwelling organisms such as nematodes (infective juveniles of *Heterorhabditis megidis*) that attack the below-ground herbivores (Boff et al. 2002). Mackie and Wheatley (1999) showed that VOCs may act as infochemicals in the interaction between microbial population and fungal activity in the rhizosphere and their surrounding. VOCs also have been reported to affect fungal mycelial growth by inducing hyphal branching in arbuscular mycorrhizal fungi (Akiyama et al. 2005) and to stimulate microbial respiration (Owens et al. 1969).

In a mixed-hardwood forest it has been showed that isoprene can be the growth substrate for some microorganisms (Cleveland and Yavitt 1998) and that consumption of this atmospheric isoprene by soil microorganisms may be a significant component of the global isoprene budget. However, in the studied Mediterranean holm oak forest soil, VOC exchange rates were very low and did not suggest a significant effect of soil VOC exchange on carbon budgets.

1.2.4.2. Contrasting seasonal course of CO_2 flux and VOC exchange rates in control and drought treatments

The effect of drought treatment on soil VOC emission and consumption was significant when VOC concentrations were normalised to 30ºC. There was a significant interaction effect between season and treatment in total soil VOC and monoterpene exchange ($p< 0.01$, Fig. 1.2.1 a and Fig. 1.2.2 a). Seasonal induction of VOC emissions by phenological processes must be considered as an additional factor which is likely to control VOC and monoterpene exchange between soil and atmosphere. Such processes, for example spring leaf emergence, which assumes an increase of physiological activity in plants, have been shown to influence foliar VOC emissions (Fuentes et al., 1998; Llusià and Peñuelas, 2000),.

Our results showed a different response in soil VOC emission and consumption between control and drought plots which might be explained by the changes in the importance of root versus microbial activity. VOC and monoterpene uptake depend on the physiological activity of the microorganisms (Demyttenaere 1998; Cleveland and Yavitt 1998). Thus soil VOC and monoterpene consumption should be linked to soil respiration. In fact, VOC exchange in control plots followed a similar seasonal pattern than CO_2 flux (r^2= 0.86, p< 0.05 Fig. 1.2.3) and soil moisture (r^2= 0.64, p= 0.1 Fig. 1.2.4). This suggests that under the current rainfall regime in this Mediterranean holm oak forest, soil moisture, in combination with plant phenology, regulate soil VOC exchange. Nevertheless, the trend tended to be inverse for soil VOC exchange relationship with CO_2 flux in drought treatment (r^2= 0.50, p= 0.18 Fig. 1.2.3), with maximum VOC and monoterpene emissions in summer and minimum in autumn (Fig. 1.2.1 and Fig. 1.2.2). When physiological activity of microorganisms was high (wet spring and autumn seasons and control plots), soil VOC emissions predominated. On the contrary, in the dry summer, the decrease in microorganism activity could have resulted in predominance of root emissions, if roots were less affected by the drought. Thus in summer, a considerable amount of detected VOCs (and monoterpenes) from soil could be produced by roots. Although monoterpene fluxes to atmosphere have been reported to originate mostly from the recently fallen litter on the soil surface (Hayward et al. 2001), a recent study in *Arabidopsis* shows that roots are also a non-negligible source of VOCs (Steeghs et al. 2004). These latter authors describe VOC emissions as induced specifically as a result of different compatible and non-compatible interactions between microbes with insects and *Arabidopsis* roots. We have also found higher soil CO_2 flux in the plots subjected to a prolonged drought than in the control plots in summer (Chapter 1.1), suggesting that autotrophic respiration (roots) is linked with soil VOC emissions. Like many of the processes that occur in the rhizosphere, the biological importance of these root emissions are not well understood, and need further studies to assess their ecological implications (Hirsch et al. 2003, Vivanco et al 2004;).

The responses of soil VOC emissions to changes in soil moisture and temperature, like those of soil respiration, are complex because of the heterogeneous nature of the soil matrix, the distribution of water, and the availability of substrates for microorganisms and roots, everything interacting in the process. In addition, there are still too few studies, of soil volatiles (root and microorganism volatiles) and their possible responses to abiotic stress, such as drought, warming and other stresses. For

example, one of the few studies on such issues reported the formation of some metabolic VOCs, such as acetaldehyde and ethanol in response to oxygen deficiency in roots of flooded soils (Drew, 1997). Further research is clearly warranted in this area of soil VOC exchanges.

1.2.4.3. Relationships with photosynthetic rates.

Foliar monoterpene formation and emission seem to be dependent on ATP, photosynthetic carbon availability and temperature (Loreto et al. 1996 a, b; Peñuelas and Llusià, 1999; Peñuelas and Llusià, 2001). Plant root emissions might also have those dependencies. This would account for the relatively high monoterpene emission rates found in winter sampling (Fig. 1.2.2), when the weather was unusually warm for the season, together with relatively high net photosynthetic rates, similar to those found in spring (Chapter 1.1).

Although the instantaneous monoterpene emission rates in leaves have been found to be correlated with net photosynthetic rates in many studies (Kesselmeier et al. 1996; Loreto et al. 1996c; Peñuelas and Llusià, 1999), other authors have reported a breakdown in this apparent relationship in a longer time-scale, because net photosynthesis and emission rates respond differently to stress conditions. There seems to be a stronger relationship between photosynthetic electron transport rates and reductive equivalents for net carbon assimilation (Niinemets et al. 2002). However, this correlation between photosynthetic electron transport rates and emission rates does not necessarily gives mechanistic insight into the physiology of monoterpene leaf emission nor on the monoterpene emission from underground parts of plants, which is much less understood. In fact, no significant direct relationships were found between the soil monoterpene exchange rates and the net photosynthetic rates in our study (r^2= 0.02, p= 0.62). Further studies are needed to gain knowledge on these linkages between photosynthetic rates and soil emission rates.

1.2.4.4. Dependence on soil temperature and moisture. Next decades prediction.

The results suggest that under Mediterranean climate with the current rainfall regime, VOC exchange rates were driven by the soil moisture, (r^2= 0.64, p=0.10 Fig. 1.2.4). Its effects, added to the phenology effects, resulted in a seasonal response in the total VOC soil emission rates (Fig. 1.2.1).

In spite of the relatively low photosynthetic activity of plants and microorganisms under the dry summer conditions, (even when the soil temperature effect was suppressed normalizing to 30º C emissions in August 2003, Fig. 1.2.1 b and Fig. 1.2.2 b), we found high VOC and monoterpene emission rates in drought plots. Because of this, there was no significant relationship of VOC and monoterpene exchange rates with soil moisture in the drought plots. Microorganisms might remain dormant, while the plants could switch on protective mechanisms involving the above-ground parts of the plant.

These results thus show that prolonged low water availability produces changes in the biological activity of soils under stress conditions. In view of the ca. 25% decrease in soil moisture forecasted by IPCC and the Gotilwa model for this Mediterranean region in the next three or four decades (IPCC 2001, Peñuelas et al 2005, Sabate et al. 2002), our results suggest that great differences can be expected in the patterns of VOC fluxes from Mediterranean soils, although acclimation of soil and plant processes to prolonged drought could occur, diminishing such expected differences. Long-term studies on these issues are needed to improve our knowledge on soil and plant responses to such expected climate changes.

1.2.5. Conclusions

This study constitutes a preliminary exploratory research for inventorying VOC and monoterpene soil exchange rates in the Mediterranean area. The results show that total VOC and monoterpene emission rates from soil surfaces are low. This supports the conclusions of Hayward et al. (2001) that there is a relatively low soil contribution to total biogenic monoterpene emissions to the atmosphere on a land area basis. In this Mediterranean holm oak forest, the potential significance of soil VOC emissions on atmospheric processes and carbon budgets seem to be low. In fact, in summer periods, uptake in control plots was predominant rather than emissions, and in drought plots net uptake was dominant over the year. The factors determining the sink potential and the timescales for sink/source reversal are unknown but these results warrant further research to solve these questions. Moreover, these results show, at least, that soil VOC exchange with the atmosphere might greatly change in the next decades in response to climate change.

There is a possible species composition effect on the diversity of identified volatile compounds in soil emissions, but to confirm this effect, and also to differentiate microorganisms and root contributions, further studies are needed. Meanwhile, these results indicate that multiple factors affect VOC emissions from soil. Soil moisture seems the most important one since it controls physiological processes in plants and microorganisms. Plant phenology also seems to affect VOC and monoterpene emissions from soil by increasing or decreasing availability of photosynthetic resources transferred to roots. The effect of these multiple factors results in seasonality of VOC soil emission rates.

The drought treatment simulated expected water availability in the next three or four decades in the GCM and ecophysiological model predictions (IPCC, 2001; Sabaté et al. 2002; Peñuelas et al. 2005). Total VOC emission rates were greatly reduced to the point that emission rates were reversed into uptake rates. The results also suggest changes towards a decrease in the ratio of microorganisms/roots activities in the rhizosphere, especially in summer. The ecological implications of these changes in soil VOC exchange rates need to be explored further, given the relatively smaller VOC emission rates from soils and the postulated connection to microbial, fungal, and herbivore activity there, it appears that cycling of VOCs in soils will have greater impact on soil ecology and foliar releases will have greater impact on the chemistry of the atmosphere.

Table 1.2.1. Seasonal means and standard error (n= 12) of soil exchange rates (μg m^{-2} h^{-1}) for detected VOCs from soil in the experimental site of Prades. Exchange rates values normalised to 30 ºC using the algorithm of Guenther et al. (1993) are indicated between brackets. Negative values correspond to uptake values. Not detected compounds are indicated as "nd". Different letters as superscripts indicate significant ($p<0.05$) differences between control and drought treatments (ANOVA).

		tricyclene	α-pinene	camphene	β-pinene	limonene	toluene	3-cyclo-hepten-1-one	ethyl-benzene	p-xylene	benzene	2-hepten-1-ol	3-octanone	nonanal	decanal	others
April 2003	control	nd ^{a}	-0.22±0.13 ^{a} (-1.25±0.70)^{a}	nd ^{a}	nd ^{a}	0.36±0.55 ^{a} (1.57±2.40) ^{a}	7.21± 3.10 ^{a} (33.5±15.0)^{a}	2.23±2.08 ^{a} (11.0±10.2) ^{a}	nd ^{a}	0.60±0.47 ^{a} (2.60±2.05) ^{a}	nd ^{a}	nd ^{a}	3.65±2.54 ^{a} (18.0±12.5)^{a}	nd ^{a}	nd ^{a}	1.97±3.52^{a} (5.9±16.2)^{a}
	drought	nd ^{a}	-0.12±0.12 ^{a} (-0.74±5.85)^{a}	nd ^{a}	nd ^{a}	0.32±0.14 ^{a} (1.67±0.73) ^{a}	nd ^{b}	0.24±0.24 ^{a} (1.20±1.20) ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.09±0.09 ^{a} (0.46±0.46) ^{a}	nd ^{a}	nd ^{a}	-3.13±1.97^{a} (-17.6±8.9)^{a}
August 2003	control	nd ^{a}	-0.72±0.18 ^{a} (-1.30±0.29)^{a}	0.03±0.01 ^{a} (0.05±0.03) ^{a}	-1.06±0.40 ^{a} (-1.92±0.75)^{a}	-0.30±0.16 ^{a} (-0.59±0.33)^{a}	0.80±1.99 ^{a} (0.45±3.08) ^{a}	nd ^{a}	-1.22±1.15 ^{a} (-2.6±2.1) ^{a}	-7.53±1.42 ^{a} (-13.3±2.7) ^{a}	-2.00± 0.51 ^{a} (-3.5±0.9) ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	-0.49±2.87^{a} (-0.5±5.6)^{a}
	drought	nd ^{a}	1.02± 0.96 ^{a} (1.67±1.57) ^{a}	nd ^{a}	nd ^{b}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.15± 0.15 ^{b} (0.27±0.27) ^{b}	nd ^{b}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	5.35±2.78^{a} (6.93±4.49)^{a}
November 2003	control	nd ^{a}	0.03±0.17^{a} (-0.07±0.96)^{a}	nd^{a}	nd ^{a}	-0.28±0.21^{a} (-1.69±1.23)^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd^{a}	nd^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	4.57±5.49^{a} (28.9±34.8)^{a}
	drought	nd ^{a}	-0.78±0.47 ^{a} (-4.82±2.87)^{a}	0.06±0.06 ^{a} (0.31±0.31) ^{a}	nd ^{a}	-0.25±0.14^{a} (-1.52±0.87)^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd^{a}	nd^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	-7.07±2.72^{a} (-39.8±15.8)^{a}
February 2004	control	1.13±0.53 ^{a} (9.51±4.16)	0.12±0.07 ^{a} (1.07±0.53) ^{a}	0.90±0.39 ^{a} (7.56±4.00) ^{b}	nd ^{a}	-0.40±0.13^{a} (-3.35±1.10)^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd^{a}	nd^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	-0.39±3.38^{a} (-2.7±27.9)^{a}
	drought	nd^{a}	nd^{a}	nd^{a}	nd ^{a}	-0.03±0.17^{a} (-0.25±1.52)^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd^{a}	nd^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	-4.55±5.54a (-39.3±48.8)^{a}
April 2004	control	1.12±0.06^{a} (0.69±0.36)	-0.28±0.09^{a} (-1.73±0.54)^{a}	0.12±0.06^{a} (0.69±0.36) ^{a}	-0.02±0.02^{a} (-0.14±0.10)^{a}	-0.50±0.37^{a} (-2.99±2.26)^{a}	nd ^{a}	9.32±1.94 ^{a} (53.0±11.4) ^{a}	nd ^{a}	nd^{a}	nd^{a}	1.27± 0.50^{a} (7.60±3.00) ^{a}	4.73±1.90^{a} (28.3±11.6) ^{a}	-0.64±1.16^{a} (-4.2±6.7) ^{a}	-2.29±2.15 ^{a} (14.3±12.4) ^{a}	-2.25±1.08^{a} (-13.4±6.6)^{a}
	drought	nd^{a}	0.46±0.09^{a} (2.42±0.51) ^{b}	0.28±0.28^{a} (1.57±1.57) ^{a}	0.03±0.03^{a} (0.12±0.12) ^{a}	0.15±0.15^{a} (0.66±0.66) ^{a}	nd ^{a}	4.61± 1.40 ^{a} (24.5±7.6) ^{b}	nd ^{a}	nd^{a}	nd^{a}	0.88± 0.57^{a} (5.44±3.74) ^{a}	2.86±1.02^{a} (16.4±5.6) ^{a}	-1.42±1.02^{a} (-6.4±6.1) ^{a}	3.28±2.33^{a} (-16.5±12.9)^{a}	-1.06±0.92^{a} (-7.37±5.34)^{a}

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Chapter 2

Interannual and interseasonal soil CO_2 efflux and VOC exchange rates in a Mediterranean holm oak forest in response to experimental drought

Abstract

Climate models predict drier conditions in the next decades in the Mediterranean basin. Given the importance of soil CO_2 efflux in the global carbon balance and the important role of soil monoterpene and VOCs in soil ecology, we aimed to study the effects of the predicted drought on soil CO_2, monoterpenes and other VOCs exchange rates and their seasonal and interannual variations. We decreased soil water availability in a Mediterranean holm oak forest soil by means of an experimental drought system performed since 1999 to the present. Measurements of soil gas exchange were carried out with IRGA, GC and PTR-MS techniques during two annual campaigns of contrasting precipitation. Soil respiration was twice higher the wet year than the dry year (2.27 ± 0.26 μmol m^{-2} s^{-1} and 1.05 ± 0.15 μmol m^{-2} s^{-1} respectively), and varied seasonally from 3.76 ± 0.85 μmol m^{-2} s^{-1} in spring, to 0.13 ± 0.01 μmol m^{-2} s^{-1} in summer. These results highlight the strong interannual and interseasonal variation in CO_2 efflux in Mediterranean ecosystems. The drought treatment produced a significant soil respiration reduction in drought plots in the wet sampling period. This reduction was even higher in wet springs (43% average reduction). These results show 1) that soil moisture is the main factor driving seasonal and interannual variations in soil respiration and 2) that the response of soil respiration to increased temperature is constrained by soil moisture. The results also show an additional control of soil CO_2 efflux by physiology and phenology of trees and animals. Soil monoterpene exchange rates ranged from –0.01 nmol m^{-2} s^{-1} to 0.004 nmol m^{-2} s^{-1}, thus the contribution of this Mediterranean holm oak forest soil to the total monoterpenes atmospheric budget seems to be very low. Responses of individual monoterpenes and VOCs to the drought treatment were different depending on the compound. This suggests that the effect of soil moisture reduction in the monoterpenes and VOCs exchange rates seems to be dependent on monoterpene and VOC type. In general, soil monoterpene and other VOCs exchange rates were not correlated with soil CO_2 efflux. In all cases, only a low proportion of variance was explained by the soil moisture changes, since almost all VOCs increased their emission rates in summer 2005, probably due to the effect of high soil temperature. Results indicate thus that physical and biological processes in soil are controlling soil VOCs exchange but further research is needed on how these factors interact to produce the observed VOCs exchange responses.

Key words: CO_2 efflux, soil monoterpenes, soil VOCs, soil moisture, soil temperature, drought, seasonality.

2.1. Introduction

Carbon dioxide produced in soils is an indicator of the biological activity of the entire soil biota including microorganisms (bacteria, fungi, algae, and protozoa), macroorganisms (such as earthworms, nematodes, and insects), and plant roots. Moreover it represents a large natural flux in the dynamics of carbon exchange with the atmosphere, approximately 75×10^{15} gC yr^{-1} (Schlesinger and Andrews, 2000). The study of soil respiration environmental controls is important to understand soil processes and their relationships with the global balance between biospheric and atmospheric carbon. This soil CO_2 efflux varies with substrate supply and root, micro and macroorganisms activity (Davidson et al., 1998) which in turn depends on soil physico-chemical conditions such as temperature, porosity or water content (Grant and Rochette, 1994). Soil CO_2 efflux is usually low in dry soils (Emmet et al., 2004; Asensio et al., 2007a) and soil temperature generally stimulates soil CO_2 efflux (Raich and Schlesinger, 1992).

Soil volatile organic compound (VOC) fluxes are less known. Soil may be both a VOC source (Guenther, 1999) and a sink (Van Ginkel et al. 1987; Bender et al., 1993; Asensio et al., 2007b). VOCs in natural soils may be derived from a variety of sources. The root system of plants (Janson 1993, Chen et al. 2004) and microorganisms (Scholler et al. 2002) seem to be the major sources of emissions. Above ground, litter is also an important source of VOCs emissions from soils, as reported in coniferous forests soils (Hayward et al., 2001; Schade and Goldstein, 2001), in a beech forests soil (Kesselmeier and Hubert, 2002) or in an orange plantation soil (Steinbrecher et al., 1999). Soil VOC uptake can occur by physical adsorption of VOCs by soil components (Pignatello and Xing, 1995) or by biodegradation by soil microorganisms (Van Roon et al., 2005). It has also been shown that some VOCs can be adsorbed and degraded by root tissue (Simonich and Hites, 1995; Newman et al., 1997; Cho et al., 2005).

Among other VOCs, monoterpenes have been reported to play different roles in soil processes. For example, they can alter the rates of nitrogen cycle (White, 1994; Smolander et al., 2006), or may act as potent biological inhibitors (Nishida et al., 2005)

or as attractant to the larvae of some pine weevil species (Nordenhem and Nordlander, 1994) and they can be used as growth substrate for bacteria (Misra et al., 1996). Several reports have also found soil volatiles to have interesting ecological roles, e. g. bacterial volatiles that promote growth in plants (Ryu et al., 2003), plant volatiles that induce hyphal branching in AM fungi (Akiyama et al., 2005) or plant exudates which act as an aphid colonization signal for nearby plants (Chamberlain et al., 2001). Negative interactions between bacteria and fungi mediated by VOCs have also been described (Mackie and Wheatley, 1999; Xu et al., 2004). However, some studies have found that their contribution to the total monoterpene and VOC atmospheric budget was low (Hayward et al., 2001; Asensio et al., 2007b).

Despite the important role soil VOCs seem to play on soil ecology, little is known about factors controling soil VOC emissions. Stahl and Parkin (1996) found that VOCs production and uptake in laboratory is strongly influenced by environmental conditions. However, information about soil moisture, temperature and their seasonal and interannual variations effects on soil VOCs exchange or the relationship between VOC exchange rates and the soil CO_2 efflux is still scarce (Asensio et al., 2007b).

The increasing global surface temperatures will probably change the amount, intensity and frequency of precipitations as well as evapotranspiration in most regions of the earth (IPCC 2001). Coupled GCM and ecophysiologycal models such as GOTILWA predict a 15-20% lower soil water availability and 1ºC warming for the next three decades in Mediterranean ecosystems (IPCC 2001; Sabaté et al. 2002; Peñuelas et al., 2005a), which are highly vulnerable to climate change (West et al., 1994, Peñuelas et al. 2002, 2004, 2005a; Peñuelas and Boada 2003).

Given the importance of soil CO_2 efflux in the global carbon balance and the important role of soil monoterpene and VOCs in soil ecology, given the scarcity of knowledge on their responses to environmental conditions, and given the predicted climate change, we studied the responses of CO_2 and VOCs exchange to the more arid conditions forecasted for the Mediterranean region for the next decades. We performed a drought experiment by partially excluding rainfall and water runoff in a natural Mediterranean holm oak forest soil and we measured soil CO_2 and VOCs exchange rates, under the predicted 15-20% lower soil water availability. Like in a previous study (Asensio et al., 2007a, b) we studied the CO_2 and soil VOCs exchange responses to the seasonal changes in soil moisture and temperature but now we also studied another year, the dry 2004-2005, to study the effects of the important interannual variability in

soil moisture and temperature characteristic of those Mediterranean ecosystems and we now measured the soil exchange of the whole range masses of volatile compounds with the PTR-MS technique. We conducted scans of all masses between 22 and 205 and monitored seasonally the exchange rate responses to drought and to soil moisture and temperature variations during the 2004-2005 period. During these studies, we also aimed to find possible linkages between monoterpenes and other VOC exchange rates and CO_2 efflux.

2.2. Material and methods

2.2.1. Study Site

This study was conducted between Spring 2003 and Summer 2005, in two campaigns from April 2003 to April 2004 and from November 2004 to July 2005. Measurements were carried out in a natural holm oak forest growing in the Prades Mountain region, in Southern Catalonia (41º13'N, 0º55'E), on a south-facing slope (25% slope) at 930 m above sea level. The soil is a Dystric Cambisol (FAO, 1990) on a bedrock of metamorphic sandstone, and its depth ranges between 35 and 90 cm. The average annual temperature is 12 ºC and the annual rainfall 687 mm. Summer drought occurs approximately from mid-June to mid-September. The vegetation of the area is short holm oak forest characterized by 3 or 4-m tall trees and shrubs. This forest is dominated by *Quercus ilex* L. and *Phillyrea latifolia* L. followed by *Arbutus unedo* L., *Erica arborea* L., *Juniperus oxycedrus* L. and *Cistus albidus* L. and occasionaly deciduous species (*Sorbus torminalis* L. Crantz and *Acer monspessulanum* L.) (Ogaya and Peñuelas, 2003).

2.2.2. Experimental design

Twenty-four 1 × 10 m plots were randomly distributed at the same altitude along the slope in the study area. Half of the plots were subjected to a drought treatment and the other ones were control plots. The drought treatment consisted of rainfall exclusion by suspending transparent PVC strips at a height of 0.5-0.8 m above the soil. In addition, a 0.8-1 m deep ditch was excavated along the entire 1m top edge of the upper part of the treatment plots to intercept runoff water. Water intercepted by strips and ditches was drained to an area outside and downhill of the plots. Rainfall exclusion by plastic strips

does not affect the light interception by the trees because the whole tree canopies are located above the plastic strips. There was practically no ground vegetation under the plastic strips, except for some *C. albidus* small shrubs growing under the plastic strip in some plots. In all cases the shrubs were wider than the plastic strip so their light environment was not hardly disturbed. Drought treatment started in March 1999 and continues to the present.

Litter-fall on the plastic strips was moved underneath them each month to sustain the humic composition of the soil. Therefore any nutrient differences below and outside the strips were due only to the change in water available for decomposition of this litterfall.

2.2.3 . Sampling strategy

Measurement campaigns were carried out during 3 consecutive sunny days in each season: spring 2003 (April 22, 23 and 24), summer 2003 (August 12, 13 and 14), autumn 2003 (November 3, 4 and 5), winter 2004 (February 17, 18 and 19), spring 2004 (April 21, 22 and 23), autumn 2004 (November 7, 8 and 9), winter 2005 (January 14, 15 and 20), spring 2005 (April 9, 10 and 11) and summer 2005 (July 25, 26 and 27). Soil CO_2 fluxes and soil VOC exchange rates were measured during the morning (from 7 a.m. to 12 a.m. solar time).

2.2.4. Measurements of soil CO_2 flux, temperature and moisture.

Soil respiration was measured in situ using a flow-through chamber method and an infrared gas analyser system (EGM-4, PP Systems, Hitchin, Hertfordshire, England). A vented soil chamber system was performed with PVC collars (12.5 cm in diameter and 8 cm in height) installed permanently 3-4 cm into the soil. The collars were covered by a PVC lid with two outlets. One outlet was connected to the IRGA analyser by a tefflon tube. The other outlet was open to exterior air entry. Air inside the chamber was flowed (constant flux 0.4 l min^{-1}) to the CO_2 analyser by the EGM-4 integral DC pump. The flow was measured with a bubbler flowmeter. Equilibration of CO_2 concentration in the effluent stream occurred after 20 minutes. Before the collar was covered, we measured CO_2 concentrations from external air. Net soil CO_2 fluxes were calculated by considering the stable difference in CO_2 concentration between the outlet chamber air and the inlet chamber air. Measurements were automatically corrected for temperature and pressure by the EGM-4 analyser. The accuracy of CO_2 measurements was estimated

to be 1%. Stability of the measurements were assured with the periodic "Auto-Zero" resulting in automatic correction for sample cell contamination, source aging, detector sensitivity variations and pre-amplifier gain changes.

Twelve collars in both control and drought plots (one collar per plot, n=12) were distributed randomly. The collars were installed in Winter 2002 and they were permanently placed into the soil, in order to minimise possible effects of the mechanical disturbance during measurements. Litter recently fallen inside the PVC collars was removed before sampling to obtain CO_2 emissions only from soil roots and soil microorganisms.

Together with each gas exchange measurement, soil temperature was measured with a soil digital thermometer (TO 15, Jules Richard instruments, Argenteuil, France) and soil moisture with a HH2 soil moisture meter connected to a ML2x soil moisture sensor (Delta-T Devices Ltd, Cambridge, England) at 10 cm depth, just beside each PVC collar to avoid mechanical disturbances to the enclosed soil. Temperature above the soil surface was also measured.

2.2.5. Measurements of soil VOC exchange with the atmosphere.

Measurements of soil VOC exchange were conducted immediately after those of soil CO_2 fluxes. Air from the cuvette flowed though a T system impelled by a pump towards a glass tube (11.5 cm long and 0.4 internal diameter) filled with VOC adsorbents Carbotrap C (300 mg), Carbotrap B (200 mg), and Carbosieve S-III (125 mg) (Supelco, Bellefonte, Pennsylvania) separated by plugs of quartz wool and treated as described by Llusià and Peñuelas (2000).

The VOC flow from the soil chamber to the glass tube filled with adsorbents varied between 0.2 and 0.3 l min^{-1} depending on the glass tube adsorbent and quartz wool packing and model of pump used. Soil VOCs were sampled during 5 min and the flow was regulated with a peristaltic pump (Portable Escort Elf Pump, P/N 497701 S/N A2-31854; Mine Safety Appliances Company, Pittsburg, Pennsylvania, USA). The flow adjustment was determined with a bubble flowmeter. Glass tubes were stored in a portable fridge at 4ºC and taken to the laboratory, where they were stored at -30 ºC until analysis (within 1 week).

VOC analyses were conducted in a GC-MS (Hewlett Packard HP59822B, Palo Alto, California) as described by Peñuelas et al. (2005b).

From November 2004 to July 2005 soil VOCs were additionally sampled in Tedlar bags in 4 control and 4 drought plots and analysed in laboratory with the PTR-MS technique. We scanned all masses between 22 and 205 to study seasonal variations and to find significant exchange rate responses to the drought effect. After equilibration of CO_2 concentration in the effluent stream, air from the cuvette was impelled by a pump towards a 3 l Tedlar bag. To minimize VOCs degradation by solar radiation in the field, Tedlar bags were enclosed into a dark bag and put into the shadow. In the laboratory, bags were stored in a dark cool chamber at 4ºC until analyses with PTR-MS (within a week).

The PTR-MS system (PTR-MS-FTD hs; Ionicon Analitik, Innsbruck, Austria) provides highly sensitive detection of the mass selected ions that are characteristic of the molecules of interest. Both PTR-MS and its use in VOC analysis have been described in detail in Lindinger et al. (1998) and Fall et al. (1999). Detection of the protonated volatiles is produced in a quadrupole mass spectrometer. We estimated the concentration of total monoterpenes as the concentration of ions with mass 137 multiplied by 2.17 (after testing α-pinene standard), because certain fragmentation of non-oxygenated monoterpenes in the drift tube occurred during ionization even under "soft" conditions and resulted in masses 67, 81 and 95 among others. This correction factor was corroborated by simultaneous PTR-MS and GC-MS (Carbotrap/Carbosive trapping) measurements of monoterpenes standards (Peñuelas et al., 2005b). Since different VOCs with the same mass cannot be separately measured, we combined PTR-MS with GC-MS techniques to determine the different monoterpenes.

The PTR-MS drift tube was operated at 2.1 mbar and 40ºC, with a drift field of 600 V cm^{-1}; the parent ion signal was maintained at ca. 3 x 10^6 counts per second during the measurements. We conducted scans of all masses between 22 and 205 and used the background values to calculate the exchange balance in each compound.

2.2.6 Statistical analyses

Repeated measures analyses of variance (ANOVA) were conducted with soil CO_2 fluxes, soil moisture and temperature as dependent variables and with treatment as independent factor. Repeated measures ANOVAs were conducted also for the exchange rate of every mass measured with the PTR-MS as dependent variable and with treatment as independent factor. Regression analyses were conducted with seasonal mean values, and therefore with values having known associated variance. Thus a model I regression

is not strictly appropriate and a model II regression was used. Although, there is not a consensus about the optimal technique to compute this model II regression, we used the reduced major axis method (Sokal & Rohlf 1995). All analyses were performed with STATVIEW 5.01 software package (Abacus Concepts Inc., 1998).

2.3. Results

2.3.1. Precipitation, soil moisture and soil temperature

The total rainfall during the first sampling period from August 2003 to April 2004 was much higher than the total rainfall during the analogous sampling period from November 2004 to July 2005 (893 mm and 265 mm respectively, Fig. 2.1 a).The difference between the two years is reflected in the average soil moisture in each sampling period (16.30 ± 1.30 % and 6.11 ± 1.04 % respectively, P <0.0001; Fig. 2.1 a). Average soil temperatures for both annual periods were similar (nearly 13ºC). Maximum soil moisture values during the wet year occurred in autumn 2003 and spring 2004 (26% and 24% respectively) while for the dry year, maximum soil moisture was measured in winter 2005 (12%). In both years, the highest soil temperatures were measured in summer (25ºC and 23ºC, Fig. 2.1) coinciding with the lowest soil moisture values in both sampling periods (2%). The drought treatment reduced soil moisture by 30% in spring and autumn during the first sampling period (P <0.0001) and by 45% on average in autumn, winter and spring during the second sampling period (P <0.0001). The drought treatment slightly increased soil temperature (mean values for the complete period 13.04 ± 0.63 and 13.54 ± 0.64 in control and drought plots, respectively). The soil temperature mean values for the complete period 2003-2005 were not significantly higher in drought plots than in control plots (13.54 ± 0.64 and 13.04 ± 0.63 respectively). However, the drought treatment increased soil temperature up to 2ºC in some seasons, such as summer 2003 and winter 2003 (P <0.005, Fig. 2.1.1).

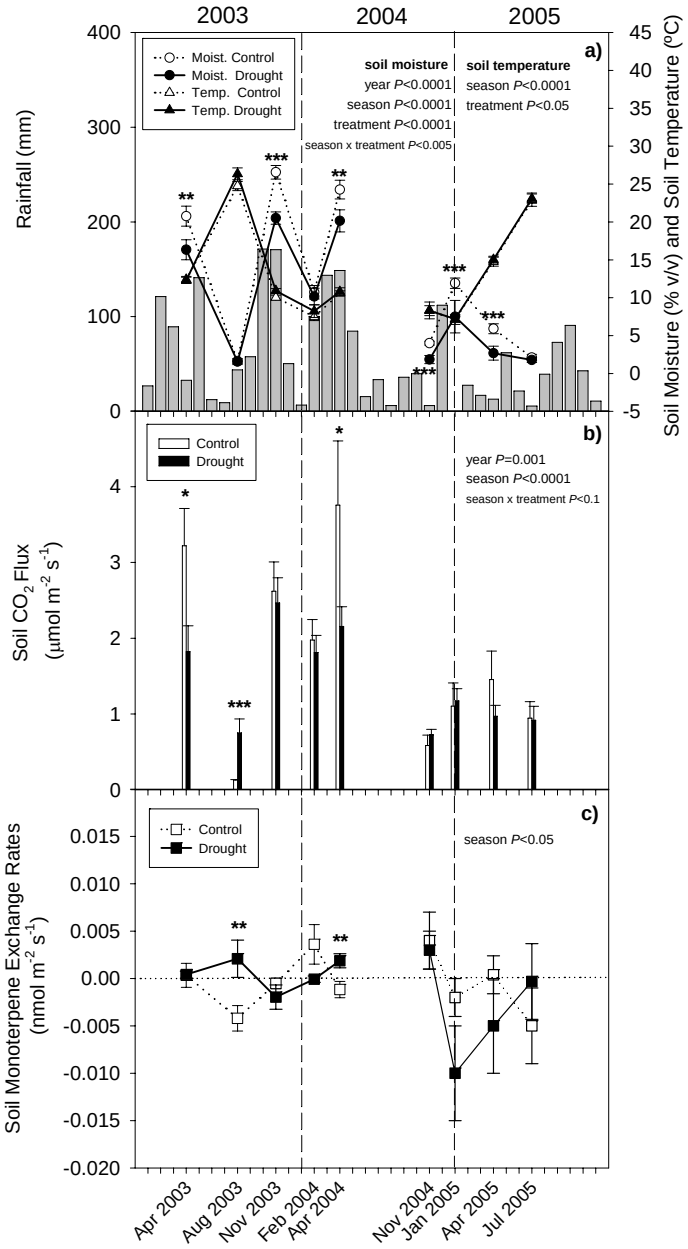

Figure 2.1. Seasonal course of rainfall, soil moisture and soil temperature measured in the two sampling annual periods, 2003-2004 and 2004-2005. a): Bar diagram represents total monthly rainfall. Dotted and full lines (control and drought plots respectively) represent soil moisture and temperature. b): Seasonal course of soil CO_2 efflux in control and drought plots. c): Soil monoterpene exchange rates in control and drought plots. Error bars indicate SEM for control and drought plots (n=12). Significant differences between the two treatments in each season (repeated measurements ANOVA) are indicated with one asterisk (P <0.1), two asterisks (P <0.05) and three asterisks (P <0.01). Significance for the overall global effect of the treatment (repeated measurements ANOVA) is indicated inside the panels.

2.3.2. Soil CO_2 efflux

Total average soil CO_2 efflux in the 2003-2004 sampling period was higher than total average efflux measured in the 2004-2005 period (2.27 ± 0.26 μmol m^{-2} s^{-1} and 1.05 ± 0.15 μmol m^{-2} s^{-1} respectively, *P*= 0.001, Fig. 2.1 b).

Drought treatment reduced soil respiration during the wet year 2003-2004, especially in springs (*P* <0.05, Fig. 2.1 b). There was no overall effect of the drought treatment on the soil respiration during the dry year (2004-2005), although soil respiration still was reduced in drought plots in spring 2005 (Fig.2.1 b).

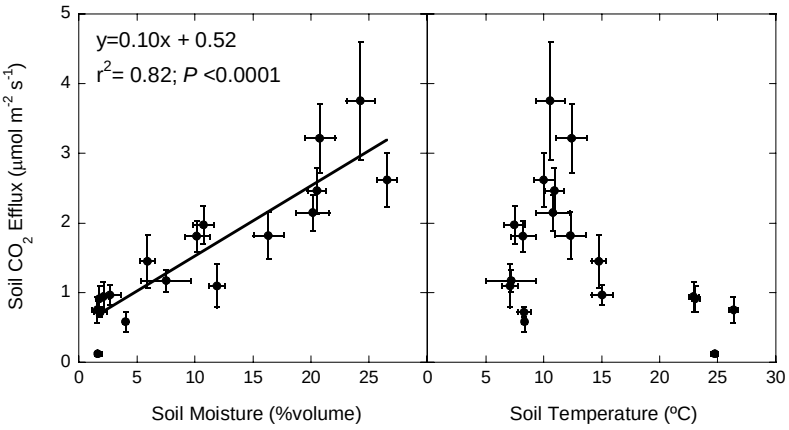

Figure 2.2. Relationships of the seasonal means of soil CO_2 flux with the seasonal means of soil moisture and soil temperature. Error bars indicate ± standard error of the mean (n= 12 for each season and treatment).

Seasonal soil CO_2 efflux variations followed soil moisture variations all over the sampling periods (overall season effect *P* <0.0001, Fig. 2.1 b). Soil respiration increased in the wet seasons, springs and autumn for the first sampling period. During the second sampling period, soil respiration was increasing from autumn to spring and it decreased in summer (Fig. 2.1 b). There was a significant relationship between seasonal CO_2 efflux mean values and soil moisture (r^2= 0.82, *P* <0.0001, Fig. 2.2), whereas no linear relationship was found between soil respiration and soil temperature.

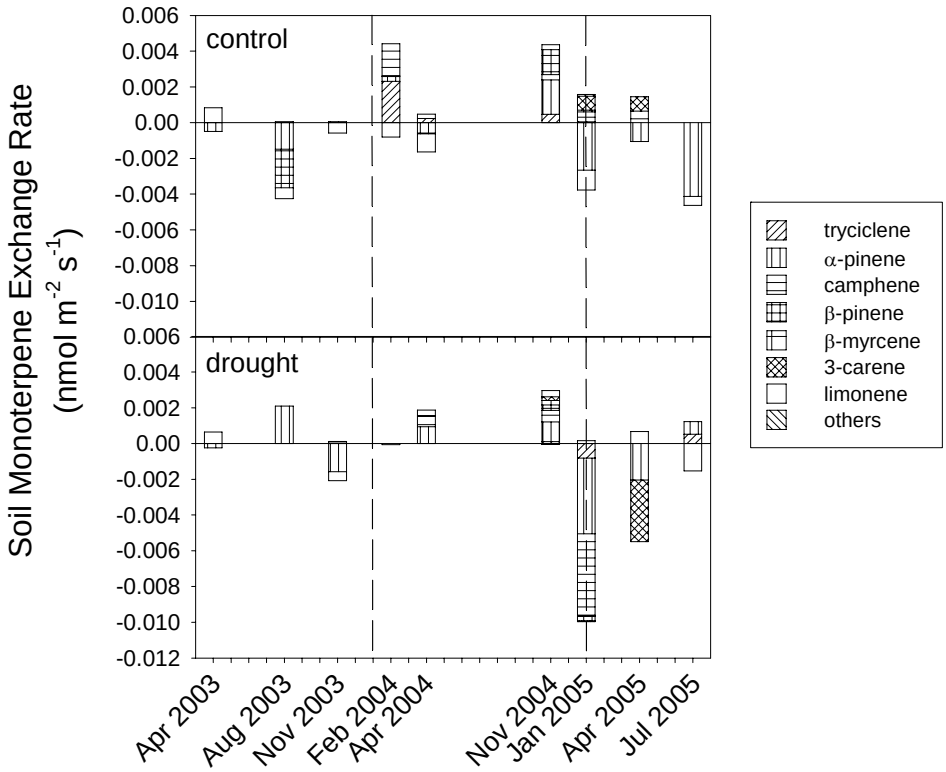

Figure 2.3. Soil monoterpene exchange rate of detected monoterpenes during the sampling annual periods 2003-2004 and 2004-2005 in control and drought plots.

2.3.3. Soil monoterpene exchange rates

Soil monoterpene exchange rates were very low; the total average exchange rate for both periods was nearly zero the first year or negative, the second one. Among several identified monoterpenes, camphene, α-pinene and Δ^3-carene showed the highest exchange rates (Fig. 2.3).

Soil moisture reduction in drought plots did not produce an overall significant change in the total soil monoterpene exchange rates, although significantly higher emissions in drought plots than in control plots were found in August 2003 and April 2004 (P <0.05; Fig. 2.1). Likewise, the soil moisture difference between the wet and the dry year did not produce a significant effect on monoterpene exchange rates. Moreover, they did not show significant correlations with soil moisture neither with the soil CO_2 efflux. However, a significant negative correlation between monoterpene exchange rate

and soil temperature was found for the total sampling period 2003-2005 (r^2=0.56, P <0.05), although this correlation was found only in control plots.

Among the monoterpenes detected, only camphene and limonene showed an overall effect of the drought treatment. The mean value of camphene exchange rate during all the sampling period 2003-2005 was an uptake of –0.0003 ± 0.0002 nmol m^{-2} s^{-1} in drought plots whereas it was an emission of 0.0004 ± 0.0001 nmol m^{-2} s^{-1} in control plots (P <0.005, Fig. 2.3). On the contrary, limonene was emited in drought plots whereas it was taken up in control plots (drought 0.00003 ± 0.0002 nmol m^{-2} s^{-1} and control –0.0004 ± 0.0002 nmol m^{-2} s^{-1}, P <0.1, Fig. 2.3).

Soil monoterpene exchange rates showed seasonal variations (overall season effect, P <0.05) from maximum emission rates in February 2004 and November 2004, in control plots (0.004 nmol m^{-2} s^{-1}, Fig. 2.1) to maximum uptake rates in January 2005 in drought plots (-0.01 nmol m^{-2} s^{-1}, Fig. 2.1).

2.3.4. Other soil VOCs exchange rates

Background air concentration for all masses with a significant exchange rate response to seasonal changes and drought treatment are shown in Table 2.1.

During the sampling period 2004-2005 all the masses which presented a significant effect of the drought treatment, showed higher emissions in drought plots than in control plots (Fig. 2.4). The greatest differences between treatments were found in January 2005 in M96 (monoterpenes fragment), M97 (heptanal), M125 (C9 aldehydes) and M139 (C10 aldehydes) and in July 2005 in M69 (isoprene), M71 (ethyl acetate), M96 (monoterpenes fragment), M97 (heptanal) and M123 (sesquitepenes) (Fig. 2.4).

Several masses which presented significant seasonal variations were increasing their emission rates from November 2004 to January 2005 and then decreasing them to April 2005, following the soil moisture changes from mid-autumn to mid-spring (Fig. 2.5 and Fig. 2.6). M45 (acetaldehyde), M57 ((E)-2-hexenal), M81 (monoterpenes) and M205 (sesquiterpenes) (Fig. 2.5) and M93 (toluene) and M136 (p-cymeme) (Fig. 2.6) showed this trend.

The uptake rates of some masses such as M35, M82 (monoterpene fragment), M127 (6-methyl-5-hepten-2-one, only in control plots) and M156 (monoterpene) varied seasonally, increasing with increasing soil moisture from November to January, and decreasing from January to May, in parallel to the soil moisture decrease (Fig. 2.1, 2.5)

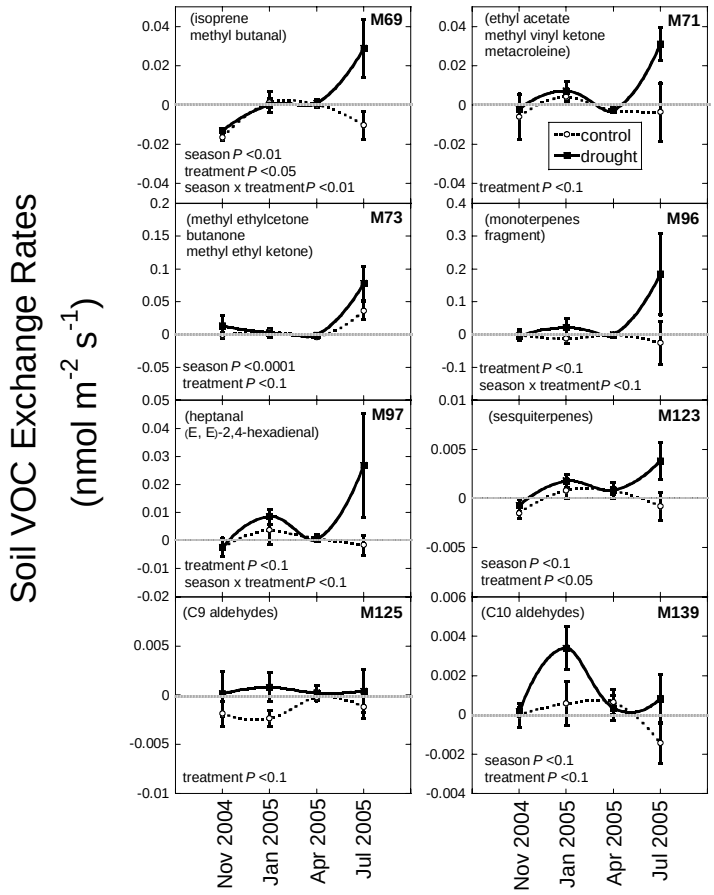

Figure 2.4. Soil VOC exchange rates detected during the 2004-2005 sampling period using PTR-MS technique that presented significant exchange rates responses to the drought treatment. Error bars indicate ± standard error of the mean (n=4 control, n=4 drought). Significances for the drought treatment overall effect or for seasonal and interaction effects (repeated measurements ANOVA) are indicated inside the panels. Between brackets it is indicated the most likely VOC corresponding to each mass based on standard calculations of the PTR-MS system and literature.

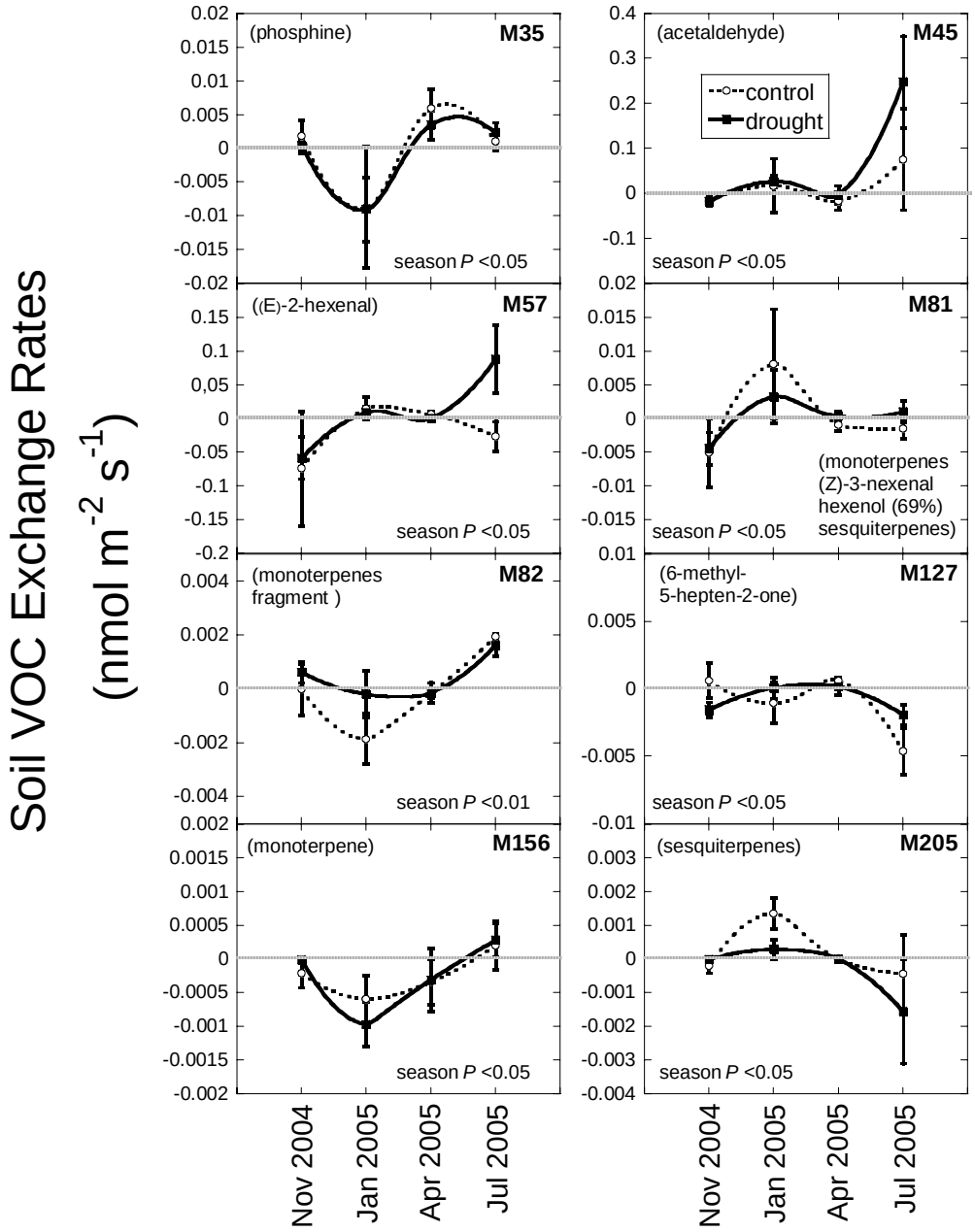

Figure 2.5. Soil VOC exchange rates detected during the 2004-2005 sampling period using PTR-MS technique that presented significant exchange rates differences among seasons. Error bars indicate ± standard error of the mean (n=4 control, n=4 drought). Significances for the overall season effect (repeated measurements ANOVA) are indicated inside the panels. Between brackets it is indicated the most likely VOC corresponding to each mass based on standard calculations of the PTR-MS system and literature.

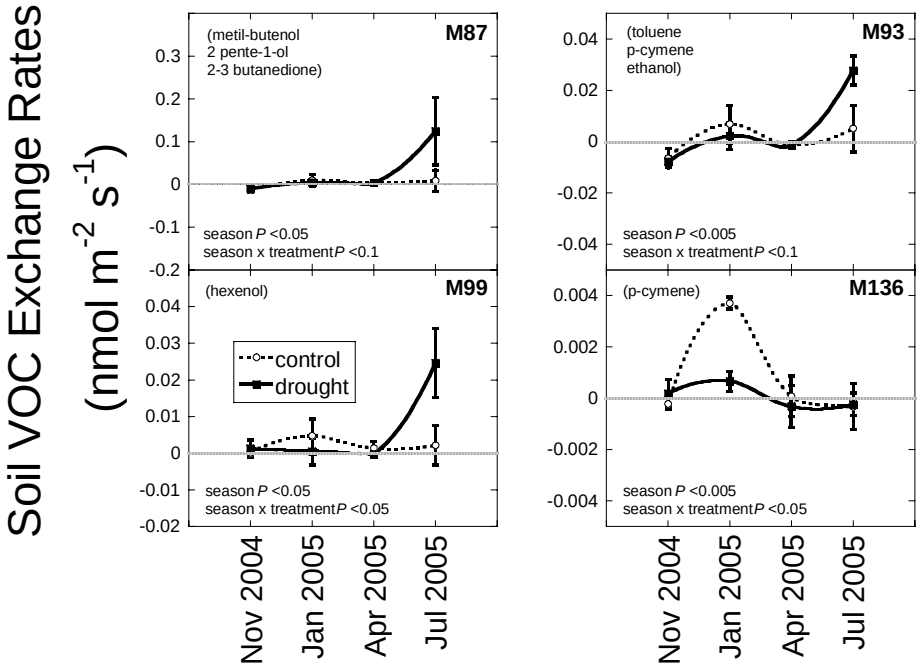

Figure 2.6. Soil VOC exchange rates detected during the 2004-2005 sampling period using PTR-MS technique that presented significant seasonal variations and significant interactions with the drought treatment. Error bars indicate ± standard error of the mean (n=4 control, n=4 drought). Significances for the overall season effect or the interaction between seasons and drought treatment effect (repeated measurements ANOVA) are indicated inside the panels. Between brackets it is indicated the most likely VOC corresponding to each mass based on standard calculations of the PTR-MS system and literature.

Many masses showed a common trend to increase their emissions in July 2005, especially in drought plots (M69, M73, M123, M71, M96, M97, M45, M57, M87, M93 and M99) (Figs 2.4, 2.5 and 2.6). There were also many other unidentified masses that showed significant responses to the drought treatment or significant seasonal variations (see table appendix A1 and A2).

Table 2.1.
Air concentrations of masses of volatile organic compounds in ppbv (1 ppbv = 1 part in 10^9 by volume) detected by PTR-MS during the 2004-2005 sampling period in Prades. Only those masses that were identified and that were significantly affected by season or treatment are shown. Data are means ± SEM; n=4. Between brackets it is indicated the most likely VOC corresponding to each mass based on standard calculations of the PTR-MS system and literature.

	Nov 2004	Jan 2005	April 2005	Jul 2005
M31 (formaldehyde)	3.89±0.26	3.66±0.11	26.93±3.91	9.68±1.14
M35 (phosphine)	0.07±0.01	1.09±0.14	0.18±0.02	0.09±0.01
M45 (acetaldehyde)	4.99±0.18	5.66±0.70	7.81±0.50	17.44±1.52
M57 ((E)-2-hexenal)	3.40±1.19	2.55±0.32	0.42±0.09	10.38±0.37
M69 (isoprene, methyl butanal)	0.75±0.06	0.49±0.04	0.09±0.02	2.38±0.23
M71 (ethyl acetate)	0.61±0.15	0.44±0.05	0.20±0.03	2.80±0.31
M73 (methyl ethyl cetone, butanone)	0.54±0.03	0.48±0.03	0.19±0.04	3.42±0.28
M81 (monoterpenes, hexenal, hexenol)	0.36±0.06	0.37±0.04	0.02±0.02	0.81±0.02
M82 (monoterpene fragment)	0.01±0.01	0.06±0.02	0.01±0.00	0.04±0.01
M87 (hexanol)	1.04±0.06	0.83±0.11	0.25±0.10	5.28±0.29
M89 (ethyl acetate)	6.04±0.43	7.66±0.73	0.21±0.05	37.98±2.38
M93 (toluene, p-cymene)	0.32±0.08	0.23±0.08	0.06±0.02	1.26±0.21
M96 (monoterpene fragment)	2.53±0.23	3.89±0.27	0.01±0.01	13.09±0.96
M97 (heptanal)	0.38±0.03	0.36±0.06	0.03±0.02	1.51±0.11
M99 (hexenol)	0.13±0.01	0.19±0.06	0.09±0.00	0.73±0.06
M109 (sesquiterpenes)	0.36±0.03	0.28±0.05	0.04±0.01	0.85±0.06
M123 (sesquiterpenes)	0.06±0.02	0.03±0.01	0.02±0.00	0.17±0.03
M125 (C9 aldehydes)	0.06±0.02	0.05±0.02	0.03±0.01	0.26±0.03
M127 (6-methyl-5-hepten-2-one)	0.08±0.02	0.07±0.01	0.01±0.01	0.36±0.03
M136 (p-cymene)	0.01±0.00	0.00±0.00	0.03±0.01	0.11±0.01
M139 (C10 aldehydes)	0.01±0.01	0.02±0.01	0.01±0.01	0.14±0.01
M143 (hexenyl acetales)	0.05±0.00	0.07±0.01	0.02±0.01	0.26±0.05
M156 (monoterpenes)	0.01±0.00	0.02±0.01	0.01±0.01	0.01±0.00
M205 (sesquiterpenes)	0.01±0.01	0.00±0.00	0.00±0.00	0.07±0.01

2.4. Discussion

2.4.1. Soil CO_2 Flux

Our results showed a decrease in the mean annual soil CO_2 efflux in response to the drier conditions during the 2004-2005 sampling period. This second sampling period, 2004-2005, was much drier than the first sampling period (2003-2004) (265 mm vs 893 mm respectively). Both annual periods, which exhibited very different climate regimes, showed total annual precipitation values far away from the average total annual precipitation for the last 7 years (634 ± 67mm). Other interannual soil respiration

studies have also found soil respiration to be depressed under drought conditions (Savage and Davidson, 2001) and in any case these results highlight the strong interannual variation in CO_2 exchange in Mediterranean ecosystems.

The predicted drier conditions of the next decades simulated by artificial drought induction by excluding rainfall and water run-off produced a significant soil respiration reduction in drought plots in the wet sampling period 2003-2004. This reduction was even higher in both springs in the first year (43% average reduction). Emmett et al. (2004) and Borken et al. (2006) found similar results with throughfall exclusion experiments which significantly decreased mean soil respiration rate in a Mediterranean shrubland and in a mixed deciduous forest respectively.

Our results show a high dependence of soil CO_2 efflux on soil moisture in this Mediterranean oak forest. Soil moisture is thus a good predictor of soil respiration rate. Soil respiration rates were instead not correlated with soil temperature (Fig. 2.2). There was a decrease in soil CO_2 efflux at the highest temperatures, which were registered when soil moisture values were lowest. These results show a soil moisture primary control seasonally and interannually on soil respiration in the Mediterranean forests and that the response of soil respiration to increased temperature is constrained by soil moisture. Other studies conducted in boreal forests, such a mixed-conifer subalpine forest (Scott-Denton et al., 2003) or a broad-leaved forest (Mo et al., 2005), showed that temperature was a much better predictor of soil respiration than soil moisture. Temperature is a primary control of the rates of all metabolic reactions. However, soil respiration responses to increases in soil temperature are constrained by soil moisture, due to the direct effects of soil moisture on microbial biomass and the indirect effects of moisture on the amount of photosynthate available as substrate for belowground root and rhizosphere respiration. Thus, typical seasonal changes in soil temperature and soil moisture will determine which is the limiting factor in each season.

The results also show an additional control of soil CO_2 efflux by physiology and phenology of trees and animals. For example, in April 2005, the increase of roots and microorganisms activities in spring (Sardans and Peñuelas, 2005; Tang and Baldocchi, 2005) coinciding not only with warmer temperatures than in winter but also with leaf emergence, probably enhanced soil respiration rates despite the lower soil moisture values than in winter (Fig. 2.1).

The seasonal pattern of soil respiration rate during the wet year 2003-2004 showed maximum CO_2 efflux in springs and autumn and minimum efflux during the

summer drought in August. This pattern is characteristic of the drought-stressed regions. Similar seasonal trends in soil respiration have been observed in other studies (Conant et al., 2000; Xu and Qi, 2001; Rey et al., 2002).

However, soil respiration during the dry year 2004-2005 did not respond significantly to the soil moisture seasonal variations neither did to the drought treatment, while these two responses were clearly evident during the wet year 2003-2004 (Fig. 2.1), suggesting that it is needed a minimum soil moisture to appreciate significant differences in soil respiration.

2.4.2. Soil VOCs exchange

Soil monoterpene exchange rates were very low. They ranged from –0.01 nmol m^{-2} s^{-1} to 0.004 nmol m^{-2} s^{-1} similarly to those measured in previous studies conducted with the same soil type (ranging from –0.004 nmol m^{-2} s^{-1} to 0.004 nmol m^{-2} s^{-1}, Asensio et al., 2007b). However, they were lower than values reported in a Sitka spruce forest soil (0.026 nmol m^{-2} s^{-1}, Hayward et al., 2001) and much lower than values reported for monoterpene foliar emission rates in *Q. ilex* (3.24 nmol m^{-2} s^{-1}; Llusià and Peñuelas, 2000). Results show thus that the contribution of this Mediterranean holm oak forest soil to the total monoterpenes atmospheric budget seems to be very low.

Responses of individual monoterpene to the drought treatment were different depending on the compound. For example, camphene uptake was significantly higher in drought plots than in control plots. Conversely, limonene uptake was significantly higher in control plots than in drought plots, whereas other monoterpenes such as α-pinene, β-pinene or Δ^3-carene, among others, did not show significant exchange rate responses to the drought treatment. This suggests that the effect of soil moisture reduction on the monoterpenes exchange rates seems to be dependent on monoterpene type.

In general, results seem to indicate that monoterpene exchange rates fluctuate randomly. However, since they showed significant seasonal variations, monoterpene exchange rates variations should be related to seasonal changes in soil moisture and temperature although we have not found a simple relationship for all the data set. The interaction of multiple factors could have produced non-linear responses resulting in a random-like fluctuation of soil monoterpene exchange rates. In addition, the low values and the high variability that may be explained by soil intrinsic heterogeneity and rhizosphere processes, e. g. root growth, water uptake, absorption, respiration and

exudation, which are responsible for spatial and temporal heterogeneities (Hinsinguer et al., 2005) made difficult to distinguish general trends in our results.

Soil monoterpene exchange rates during the complete sampling period 2003-2005 in control plots did not show a significant relationship with soil CO_2 efflux. Thus, the high monoterpene uptake rates measured in control plots in both summers (August 2003 and July 2005) can not be attributable to microbial monoterpene consumption activity. High monoterpene emission rates from *Quercus ilex* leaves have been described in summer in this Mediterranean holm oak forest (Llusià, personal communication), and our results show the low activity of soil microorganisms and roots during summer. Thus, those high monoterpene uptake rates measured in both summers in control plots might be the result of a monoterpene gradient between the rich-monoterpene air just above the soil surface and the poor-monoterpene soil air-space.

On the other hand, soil monoterpene exchange linkages with soil CO_2 efflux in drought plots were noticeable only during the dry year 2004-2005. High monoterpene uptake rates in drought plots coincided with high soil CO_2 efflux in this period (linear regression not shown, r^2=0.95, P <0.05). This suggests that soil monoterpene exchange rates during a dry year could be mostly driven by the microorganisms consumption activity.

Although the changes were small, the drought treatment increased the exchange rates of several other VOCs during the studied period 2004-2005 (Fig. 2.4 and appendix Table A1). These higher emissions observed in drought plots might be due to changes in soil physical properties induced by low soil moisture, more than changes in the rhizosphere or in the bulk soil microorganisms activity. Thus, when soil dries, larger pores are formed and VOCs exchange with the atmosphere can increase. Our results support this hypothesis because the 2004-2005 exchange rate patterns of most of the studied VOCs were not significantly correlated with the soil CO_2 efflux changes. At higher temperatures the gas phase concentrations of monoterpenes and other VOCs increased, leading to a larger volatilisation (Van Roon et al., 2005). Similarly to the physical effect of the drought treatment, this physical effect of high soil temperature over the soil exchange processes may also partially account for the high emissions measured in July 2005 in drought plots

Seasonal variations in the very low emission rates of VOCs revealed two different trends in responses to soil moisture changes during this dry period 2004-2005.

Most compounds increased their emission rates when soil moisture increased, but a minor number of compounds emissions tended to decrease with soil moisture increases (Fig. 2.5 and 2.6 and appendix Table A2). In all cases, only a low proportion of variance was explained by the soil moisture changes, since almost all compounds increased their emission rates in summer 2005, probably due to the effect of high soil temperature (Van Roon et al., 2005).

2.5. Conclusions

In this Mediterranean holm oak forest, temperature dependency of soil respiration was constraint by soil moisture. Phenology seemed to exert a secondary control over soil respiration. As a result, maximum rates of CO_2 efflux occurred in spring and autumn and the minimum values in summer.

Soil monoterpene exchange rates were extremely low. They were affected by seasonal variations in soil moisture, temperature and other factors throughout the years. High soil temperatures increased the monoterpene uptake in control plots. The response of each individual monoterpene to drought treatment and to the seasonal variations seems to be monoterpene specific.

High soil temperatures in summer increased VOC exchange rates. Drought treatment tended to increase the emission rates of several of these VOCs. Temperature seemed to affect the emission rates depending on the compound type.

The expected decrease in water availability in the next three or four decades by GCM and ecophysiological models such as GOTILWA (IPCC, 2001; Sabaté et al. 2002; Peñuelas et al. 2005a) will decrease soil respiration and may increase soil emissions of several volatile compounds.

Appendix-Table A1. Soil VOC exchange rates (nmol m^{-2} s^{-1}) detected during the 2004-2005 sampling period using PTR-MS technique that presented significant exchange rates responses to the drought treatment or to the interaction between season and drought treatment (S x T). Error bars indicate ± standard error of the mean (n=4 control, n=4 drought). Significance for the drought treatment overall effect or for season and interaction effects (repeated measurement ANOVA) is indicated for each mass.

	significance			November 2004		January 2005		April 2005		July 2005	
	Season	Treatments	S x T	control	drought	control	drought	control	drought	control	drought
M21			*P*<0.05	-0.2605±0.2765	-0.3599±0.5639	0.0552±0.2568	0.0743±0.0753	-0.0107±0.1484	-0.1224±0.4189	-1.8797±1.1548	2.6984±2.5534
M28		*P*<0.05		0.0023±0.0031	0.0041±0.0014	-0.0073±0.0057	0.0058±0.0060	-0.0010±0.0018	-0.0015±0.0018	-0.0061±0.0031	0.0040±0.0059
M30		*P*<0.1	*P*<0.05	0.5376±0.5461	-0.0709±0.5067	-0.9333±0.2533	-0.0998±0.3082	-0.0678±0.5778	-0.9870±1.3973	-7.0211±5.0306	7.5290±6.6176
M60		*P*<0.05	*P*<0.05	0.0007±0.0037	-0.0052±0.0029	-0.0097±0.0020	0.0052±0.0035	0.0004±0.0007	-0.0001±0.0012	-0.0091±0.0196	0.0516±0.0289
M75			*P*<0.05	-0.0009±0.0026	-0.0043±0.0009	-0.0002±0.0010	0.0007±0.0037	-0.0007±0.0016	-0.0010±0.0014	-0.0109±0.0062	0.0096±0.0101
M88			*P*<0.1	-0.9151±0.9192	-1.2952±0.5464	0.0306±1.3803	0.6966±1.5465	0.0011±0.0009	-0.0004±0.0009	-1.4723±4.4442	13.2305±8.4834
M105	*P*<0.05	*P*<0.1	*P*<0.005	-0.0031±0.0023	-0.0064±0.0033	-0.0017±0.0013	0.0001±0.0003	0.0010±0.0002	0.0001±0.0005	-0.0043±0.0029	0.0096±0.0038
M113			*P*<0.1	0.0000±0.0006	0.0004±0.0007	0.0007±0.0010	-0.0001±0.0009	0.0004±0.0009	0.0011±0.00003	-0.0008±0.0013	0.0032±0.0010
M141		*P*<0.05		-0.0004±0.0004	0.0004±0.0006	0.0000±0.0010	0.0007±0.0009	0.0004±0.0002	0.0006±0.0004	-0.0005±0.0010	0.0022±0.0006
M150			*P*<0.1	0.0004±0.0002	0.0002±0.0002	0.0004±0.0007	-0.0006±0.0003	-0.0002±0.0002	-0.0002±0.0002	-0.0008±0.0002	0.0003±0.0004
M193			*P*<0.05	0.0002±0.0002	0.0000±0.0000	-0.0003±0.0003	0.0009±0.0006	-0.0002±0.0002	-0.0002±0.0002	0.0001±0.0002	-0.0005±0.0003
M200			*P*<0.05	0.0002±0.0002	0.0000±0.0000	0.0004±0.0004	0.0003±0.0003	0.0002±0.0002	0.0002±0.0002	-0.0004±0.0001	0.0008±0.0002
M202	*P*<0.0001	*P*<0.0005	*P*<0.0001	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0003±0.0001	0.0007±0.0000

Appendix-Table A2. Soil VOC exchange rates (nmol m^{-2} s^{-1}) detected during the 2004-2005 sampling period using PTR-MS technique that presented significant exchange rates differences among seasons. Error bars indicate ± standard error of the mean (n=4 control, n=4 drought). Significance for season and season x treatment (S x T) interaction effects (repeated measurements ANOVA) is indicated for each mass.

	significance		November 2004		January 2005		April 2005		July 2005	
	Season	S x T	control	drought	control	drought	control	drought	control	drought
M22	*P*<0.1		0.2765±-0.0051	-0.0012±0.0016	0.0048±0.0025	0.0014±0.0019	-0.0004±0.0016	0.0023±0.0024	0.0008±0.0016	0.0016±0.0019
M36	*P*<0.1		0.0053±0.0023	0.0080±0.0050	-0.0114±0.0053	-0.0046±0.0043	0.0014±0.0020	0.0031±0.0027	0.0065±0.0069	0.0560±0.0478
M40	*P*<0.01		-0.0019±0.0006	-0.0015±0.0005	0.0004±0.0011	0.0003±0.0011	0.0002±0.0002	0.0001±0.0006	-0.0005±0.0007	-0.0007±0.0003
M44	*P*<0.1	*P*<0.05	-0.0219±0.0123	-0.0187±0.0045	-0.0001±0.0127	-0.0006±0.0107	0.0002±0.0014	-0.0004±0.0012	-0.0285±0.0163	0.0532±0.0398
M46	*P*<0.1		-0.0602±0.0184	-0.0653±0.0209	0.0086±0.0269	0.0112±0.0321	-0.0035±0.0038	-0.0012±0.0072	-0.0847±0.1409	0.3052±0.2188
M62	*P*<0.05		-0.0025±0.0015	-0.0003±0.0032	0.0011±0.0041	0.0005±0.0028	-0.0018±0.0005	-0.0008±0.0014	0.0085±0.0014	0.0308±0.0147
M70	*P*<0.1		-0.0025±0.0010	-0.0015±0.0012	0.0011±0.0012	0.0010±0.0005	0.0004±0.0003	0.0010±0.0006	0.0001±0.0015	0.0016±0.0024
M72	*P*<0.005		-0.0015±0.0019	-0.0030±0.0023	0.0007±0.0005	0.0011±0.0017	0.0001±0.0004	-0.0004±0.0004	0.0014±0.0015	0.0059±0.0007
M86	*P*<0.01		-0.0019±0.0007	0.0000±0.0001	0.0000±0.0015	0.0011±0.0004	-0.0014±0.0009	-0.0009±0.0010	0.0017±0.0015	0.0032±0.0009
M90	*P*<0.1		-0.0033±0.0050	-0.0020±0.0023	-0.0032±0.0033	0.0000±.0.0021	0.0007±0.0008	0.0002±0.0002	0.0003±0.0128	0.0407±0.0280
M94	*P*<0.05		-0.0003±0.0045	-0.0047±0.0079	-0.0048±0.0038	-0.0016±0.0011	0.0003±0.0005	0.0003±0.0005	0.0058±0.0104	0.0365±0.0229
M108	*P*<0.05		-0.0017±0.0019	-0.0024±0.0021	0.0023±0.0007	0.0000±0.0005	0.0004±0.0002	-0.0001±0.0003	0.0003±0.0008	0.0008±0.0006
M124	*P*<0.05		0.0012±0.0004	0.0019±0.0007	0.0010±0.0006	0.0010±0.0003	-0.0003±0.0004	-0.0002±0.0002	0.0010±0.0006	0.0001±0.00006
M126	*P*<0.05		0.0013±0.0007	0.0013±0.0009	-0.0006±0.0003	-0.0003±0.0003	-0.0001±0.0006	-0.0005±0.0005	0.0005±0.0006	0.0008±0.0010
M131	*P*<0.05		-0.0004±0.0006	-0.0010±0.0006	0.0005±0.0008	-0.0010±0.0004	-0.0002±0.0004	0.0001±0.0004	0.0007±0.0012	0.0021±0.0006
M145	*P*<0.05		-0.0006±0.0000	-0.0002±0.0008	0.0004±0.0008	0.0008±0.0005	0.0006±0.0008	-0.0002±0.0004	0.0004±0.0008	0.0035±0.0009
M163	*P*<0.1		-0.0006±0.0006	-0.0006±0.0006	0.0001±0.0006	0.0014±0.0011	-0.0002±0.0007	-0.0009±0.0005	0.0019±0.0012	0.0015±0.0019
M168	*P*<0.05		0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	-0.0002±0.0002	0.0000±0.0003	-0.0005±0.0003	-0.0007±0.0004
M173	*P*<0.1		0.0000±0.0004	-0.0004±0.0002	0.0010±0.0006	0.0014±0.0011	-0.0003±0.0005	-0.0005±0.0003	0.0007±0.0006	-0.0001±0.0005
M175	*P*<0.1		0.0008±0.0002	0.0002±0.0002	-0.0005±0.0003	-0.0009±0.0006	0.0000±0.0003	0.0000±0.0003	0.0006±0.0008	0.0002±0.0008
M177	*P*<0.05		0.0000±0.0007	0.0000±0.0003	-0.0008±0.0010	-0.0013±0.0005	0.0004±0.0002	0.0002±0.0002	0.0000±0.0009	0.0018±0.0005
M178	*P*<0.001		0.0000±0.0000	0.0002±0.0002	-0.0013±0.0005	-0.0013±0.0005	-0.0002±0.0002	-0.0002±0.0002	-0.0001±0.0003	0.0011±0.0004
M194	*P*<0.1		0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0003±0.0003	-0.0002±0.0002	-0.0002±0.0002	0.0002±0.0001	0.0004±0.0002

Appendix A

Table A.1 presents the soil VOC exchange rates (nmolm^2 s^{-1}) detected during the 2004–2005 sampling period using PTR-MS technique that presented significant exchange rates responses to the drought treatment or to the interaction between season and drought treatment (S_T).

Table A.2 presents the soil VOC exchange rates (nmolm^2 s^{-1}) detected during the 2004–2005 sampling period using PTR-MS technique that presented significant exchange rates differences among seasons.

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Chapter 3

Interannual and seasonal changes in the soil exchange rates of monoterpenes and other VOCs in a Mediterranean shrubland

Abstract

Information about soil VOC inventories and exchange rates in different soils is very scarce. Seasonality of soil VOC exchange rates is also largely unknown, despite the increasing interest in some soil volatile compounds, such as monoterpenes, because of their important role in soil ecology. We aimed to explore and quantify soil VOC exchange rates in a Mediterranean shrubland and their seasonality. Measurements of soil VOC exchange were taken using GC-MS and PTR-MS techniques, together with soil temperature, soil moisture and soil CO_2 efflux measurements, during two annual campaigns with contrasting precipitation.

M33 (methanol), M43 (acetic acid, ethyl acetate), M45 (acetaldehyde), M59 (acetone), M73 (C3 and C4 carboniles), α-pinene and limonene, showed the highest emission rates. Maximum soil monoterpene emission rates were very low (0.003 nmol m^{-2} s^{-1}) compared with foliar monoterpene emission rates. The emission rates of the other VOCs were also low (maximum 0.8 nmol m^{-2} s^{-1}) except for M33 (methanol, 2.5 nmol m^{-2} s^{-1}). Maximum soil uptake rates for some VOCs, such as M33 (methanol) and M42 (acetonitrile) (ranging from –0.1 nmol m^{-2} s^{-1} to –0.5 nmol m^{-2} s^{-1}) were, however, comparable with foliar uptake rates. Further studies are needed to corroborate these results and the possible importance of the soil VOC sink in regional chemistry-climate models.

Long-term severe drought increased soil monoterpene emission rates in this Mediterranean shrubland. The increases seem to be linked to changes in the soil's physical properties induced by low soil moisture. Unlike monoterpenes, other soil VOC emission rates decreased when soil moisture was low. The results suggest a strong seasonal control of soil temperature on the emission rates of monoterpenes and other VOCs. The emission rates increase with soil temperature. Positive correlations between the VOC exchange rates and the soil CO_2 fluxes suggest that phenology of roots and microorganisms also controls soil VOCs' seasonal changes in this Mediterranean shrubland.

Key words: Mediterranean shrubland, soil monoterpenes, soil VOCs, soil moisture, soil temperature, drought, seasonality.

3.1. Introduction

Volatile Organic Compounds (VOCs) are reactive trace gases in the troposphere which interact with other atmospheric trace compounds, affecting distributions of air pollutants such as NO_x, PANs, and particles (Atkinson and Arey, 2003) and playing a central role in tropospheric ozone formation (Fuentes *et al.*, 2000; Chen and Griffin, 2005). For this reason, considerable effort has been made to identify the sources and to quantify the amounts (Lamb *et al.*, 1987; Mueller, 1992).

VOCs originate from three main sources: anthropogenic activities, biomass burning and the biosphere. The biosphere is the largest source of VOCs, its emissions surpassing several times those from anthropogenic and biomass burning sources (Guenter *et al.*, 1995). Natural sources of VOC emissions to the atmosphere include marine and fresh water, soil and sediments, microbial decomposition of organic material, geological hydrocarbon reservoirs, plant foliage and woody material. Among terrestrial ecosystems, foliar emissions (mainly isoprene and terpenes) from woodlands are considered the largest source (Guenther *et al.* 1994; Fuentes *et al.*, 1996, Peñuelas and Llusia 2001). Isoprene and monoterpenes, and some partly oxygenated VOCs such as formaldehyde, acetaldehyde, acetone, methanol, ethanol, formic and acetic acids, are synthesized in plants and emitted in large amounts into the atmosphere (Guenther *et al.* 1994; Fall, 1999; Fall *et al.*, 1999, Seco et al 2007). Most studies have focused on these compounds, since they are highly reactive and have a greater ozone formation potential. These volatile compounds are also involved in numerous physiological and ecological interactions in forest ecosystems (Gershenzon, 1994; Lerdau *et al.*, 1995; Peñuelas *et al.*, 1995; Fall *et al.*, 1999; Heiden *et al.*, 2003; Peñuelas and Llusià, 2001, 2003, 2004).

In contrast to foliar VOC exchanges, non methane soil VOC exchanges with the atmosphere have received little attention, because they probably represent less than a few percent of total global VOC exchange (Lamb *et al.*, 1987; Guenther *et al.* 1995), though further investigation is needed to verify this assumption. In fact, there is still a lack of data on soil VOC inventories in different soil and ecosystem types. There are also many uncertainties about the soil VOC exchange process, since several factors are involved in the emission and uptake of VOCs by soils.

There is now evidence that soil may be both a VOC source (Guenther, 1999) and a sink (Van Ginkel *et al.*, 1987, Bender and Conrad, 1993; Asensio *et al.*, 2007a, b). Litter is the largest source of VOC emissions from natural soils (Hayward *et al.*, 2001,

Schade and Goldstein, 2001) followed by roots (Janson 1993, Chen *et al.* 2004; Lin *et al.*, 2007) and microorganisms (Scholler *et al.* 2002). Physical adsorption of VOCs to soil particles (Pignatello and Xing, 1996) and biodegradation by microorganisms (Van Roon *et al.* 2005b) or adsorption and degradation by root tissue (Simonich and Hites, 1995; Newman *et al.*, 1997¸ Cho *et al.*, 2005) are known to produce soil VOC uptake.

The role of monoterpenes and other VOCs in soil ecology is far from well understood. However, important roles such as the VOC-mediated interactions among bacteria/fungi and plants have been described in recent years. For example, Ruy *et al.* (2003) reported that 2,3-butanediol and acetoin are synthesized and emitted by plant growth-promoting *Bacillus* strains that enhance growth and induce systemic resistance of *Arabidopsis thaliana*. Splivallo *et al.* (2007) reported that fungal volatiles from truffles caused bleaching of A*rabidopsis thaliana* leaves, and/or inhibition of root and leaf development. Volatiles in soil also mediate plant-plant interactions (Nishida *et al.*, 2005) and plant-insect interactions (Nordenhem and Nordlander, 1994; Chamberlain *et al.*, 2001). Other authors have reported the potential for monoterpenes to alter rates of nutrient cycling since monoterpenes inhibit nitrification in soil (White, 1994; Smolander *et al.*, 2006) but serve as a carbon and energy source for soil microbes (Misra *et al.*, 1996; Owen *et al.*, 2007).

Climatic (IPCC, 2007) and ecophysiological models such as GOTILWA (Sabaté *et al.*, 2002, Peñuelas *et al.*, 2005) project increased drought in the near future in the Mediterranean Basin, which may affect soil VOC emission and uptake directly (Asensio *et al.* 2007a), or indirectly through its effects on plants and soil micro-organism activities (Ogaya and Peñuelas 2003; Ogaya *et al.* 2003; Emmet *et al.* 2004; Peñuelas *et al.* 1998; 2004; 2007; Sardans and Peñuelas, 2005).

Given the lack of information on soil-atmosphere VOC fluxes, the importance of soil VOCs on soil ecology, and the projections of increased drought in Mediterranean ecosystems, we conducted this study of soil VOC exchange rates in a Mediterranean shrubland at the Garraf Natural Park, in Catalonia. Our aims were (i) to explore and quantify the soil VOC exchange rates, with special attention to monoterpenes, in a typical calcareous Mediterranean shrubland (ii) to investigate interannual and seasonal variations in soil VOC exchange rates and (iii) to study soil VOC exchange rates' links to soil temperature, soil moisture and soil CO_2 efflux.

3.2. Material and methods

3.2.1. The study site and species description

The study was carried out in a dry shrubland (Rosmarino-Ericion) at the Garraf Natural Park, in Catalonia, North-East Spain (41º18'N, 1º49'E), at 210 m a.s.l., on a South-South-East slope (13º). The climate is typically Mediterranean (annual average temperature 15.1 ºC and annual average precipitation 455 mm. The site, which is located on terraces of abandoned vineyards, suffered severe fires in the summers of 1982 and 1994. The soil is a Petrocalcic Calcixerept (Soil Survey Staff, 1998), thin (12–37 cm), with a loamy texture and abundant calcareous nodules and soil pH is 7.7. Physico-chemical soil properties measured within 0-15 cm soil depth are shown in Table 3.1. Currently the vegetation covers 60-70% with a maximum height of 70 cm. The dominant species at the study site, *Erica multiflora* L., *Globularia alypum* L., *Pinus halepensis* L. and *Rosmarinus officinalis* L., are evergreen species that typically occur on basic soils of the western Mediterranean Basin, where they are common components of the coastal shrubland.

3.2.2. Sampling

Measurement campaigns were carried out on 2 consecutive sunny days in each season of two annual sampling periods (one with standard climate, between spring 2003 and winter 2004 and a dry one, between autumn 2004 and summer 2005): spring 2003 (May 9 and 10), summer 2003 (August 19 and 20), autumn 2003 (November 19 and 20), winter 2004 (January 27 and 28), autumn 2004 (November 9 and 10), winter 2005 (February 1 and 2), spring 2005 (May 25 and 26) and summer 2005 (August 4 and 5). Soil CO_2 fluxes and soil VOC exchange rates were measured during the morning (from 7 a.m. to 12 a.m. solar time).

3.2.3. Measurements of soil CO_2 flux, temperature and moisture.

Soil respiration was measured in situ using a flow-through chamber method and an infrared gas analyser system (EGM-4, PP Systems, Hitchin, Hertfordshire, England) as described by Asensio *et al.* (2007b).

Twenty seven collars were distributed randomly in a 1 ha field site. The collars were installed in winter 2002 and were permanently placed into the soil, in order to

minimise possible effects of mechanical disturbance during measurements. Litter recently fallen inside the PVC collars was removed before sampling to obtain CO_2 emissions only from mineral soil, roots and microorganisms.

Together with each gas exchange measurement, soil temperature was measured with a digital soil thermometer (TO 15, Jules Richard instruments, Argenteuil, France) and soil moisture with a HH2 soil moisture meter connected to a ML2x soil moisture sensor (Delta-T Devices Ltd, Cambridge, England). These measurements were taken at 10 cm depth, just beside each PVC collar to avoid mechanical disturbances to the enclosed soil. Temperature above the soil surface was also measured.

3.2.4. Measurements of soil VOC exchange with the atmosphere.

Measurements of soil VOC exchange were conducted immediately after those of soil CO_2 fluxes. Air from the cuvette was pumped though a T system to a glass tube (11.5 cm long and 0.4 internal diameter) filled with VOC adsorbents Carbotrap C (300 mg), Carbotrap B (200 mg), and Carbosieve S-III (125 mg) (Supelco, Bellefonte, Pennsylvania) separated by plugs of quartz wool and treated as described by Llusià and Peñuelas (2000).

The VOC flow from the soil chamber to the glass tube varied between 0.2 and 0.3 l min^{-1} depending on the adsorbent and quartz wool packing and model of pump used. Soil VOCs were sampled for 5 min and the flow was regulated with a peristaltic pump (Portable Escort Elf Pump, P/N 497701 S/N A2-31854; Mine Safety Appliances Company, Pittsburg, Pennsylvania, USA). The flow adjustment was determined with a bubble flowmeter. Glass tubes were stored in a portable fridge at 4ºC and taken to the laboratory, where they were stored at -30 ºC until analysis (within 1 week). VOC analyses were conducted in a GC-MS (Hewlett Packard HP59822B, Palo Alto, California) as described by Peñuelas *et al.* (2005b).

From November 2004 to August 2005 soil VOCs were additionally sampled in Tedlar bags in 9 PVC collars and analysed in laboratory using the PTR-MS technique as described by Asensio *et al.* (2007b). We scanned all masses between 22 and 205 to study seasonal variations. The PTR-MS system (PTR-MS-FTD hs; Ionicon Analitik, Innsbruck, Austria) provides highly sensitive detection of the mass selected ions that are characteristic of the molecules of interest. Both PTR-MS and its use in VOC analysis have been described in detail in Lindinger *et al.* (1998) and Fall *et al.* (1999). The protonated volatiles were detected in a quadrupole mass spectrometer. Since different

VOCs with the same mass cannot be separately measured, we combined PTR-MS with GC-MS techniques to determine the different monoterpenes.

The PTR-MS drift tube was operated at 2.1 mbar and 40ºC, with a drift field of 600 V cm^{-1}; the parent ion signal was maintained at ca. 3 x 10^6 counts per second during the measurements. We used the background values of all masses between 22 and 205 to calculate the exchange balance for each compound.

Table 3.1. Physico-chemical soil properties of the studied soil in Garraf shrubland. (Data for soluble ammonium, nitrate and phosphate are from Sardans *et al.* 2006 and Sardans *et al.*, 2007).

Soil Type	Petrocalcic Calcixerept
Soil texture	
% Sand	42.9
% Silt	38.7
% Clay	18.4
Mineral horizon (mean 0-15 cm)	
Mineral horizon bulk density (kg m^{-3})	0.00128
Carbon (%)	2.04
Nitrogen (%)	0.16
Carbon store (kg C m^{-2})	1.46
Nitrogen store (kg m^{-2})	0.24
Mean annual soil water content (% vol/vol)	18
Min (% wilting point)	5
Max (% field capacity)	26
Soluble ammonium NH_4^+ (mg kg^{-1} soil)	
Summer 04	1.45 ± 0.13
Autumn 04	1.25 ± 0.07
Winter 04-05	1.45 ± 0.09
Spring 05	1.18 ± 0.37
Soluble nitrate NO_3^- (mg kg^{-1} soil)	
Summer 04	13.57 ± 4.39
Autumn 04	10.33 ± 4.23
Winter 04-05	22.83 ± 2.54
Spring 05	9.10 ± 0.87
Olsen-Pi (mg g^{-1} soil)	
Summer 04	2.81 ± 0.25
Autumn 04	2.40 ± 0.28
Winter 04-05	1.90 ± 0.50
Spring 05	4.17 ± 0.64

3.2.5. Statistical analyses

Repeated measures analyses of variance (ANOVA) were conducted with soil CO_2 fluxes, soil moisture and temperature as dependent variables. Repeated measures ANOVAs were also conducted for the exchange rate of every mass measured with the PTR-MS as dependent variable. Regression analyses were conducted to explore the relationships of the exchange rates of monoterpenes and of every mass measured with the PTR-MS with soil CO_2 fluxes, soil moisture and temperature. All analyses were performed with STATVIEW 5.01 software package (Abacus Concepts Inc.).

3.3. Results

3.3.1. Soil temperature, moisture and soil CO_2 flux. Interannual and seasonal variations.

Total rainfall in the first annual sampling period (from May 2003 to January 2004) was 495.4 mm while total rainfall in the second annual sampling period (from November 2004 to August 2005) was 248.2 mm (Fig. 3.1). While the first annual sampling period was significantly wetter than the second (soil moisture 19.01 ± 1.53 vs 12.78 ± 1.01 %; P <0.005; Fig. 3.1), the mean soil temperatures in each period were not significantly different (soil temperature: 18.9 ± 1.3 vs 17.9 ± 1.3 ºC; Fig. 3.1). Soil CO_2 flux mean value did not differ significantly between the first and the second sampling periods (0.98 ± 0.11 vs 0.95 ± 0.07 μmol m^{-2} s^{-1}, Fig. 3.1).

Soil temperature, moisture and soil CO_2 flux showed seasonal changes throughout the years (overall season effect P <0.0001, Fig. 3.1). Soil respiration increased in the wet seasons, spring and autumn, for both sampling periods (Fig. 3.1).

During the first sampling period the highest soil temperature and the lowest soil moisture coincided in summer (August 2003, 30ºC and 8%, Fig. 3.1). During the second sampling period the highest soil temperature values were measured in spring and summer (May and August 2005, 26ºC and 24ºC respectively, Fig. 3.1). The lowest soil moisture values for this period were measured in winter (anomalously dry) and summer (February and August 2005, 9% and 10% respectively, Fig. 3.1).

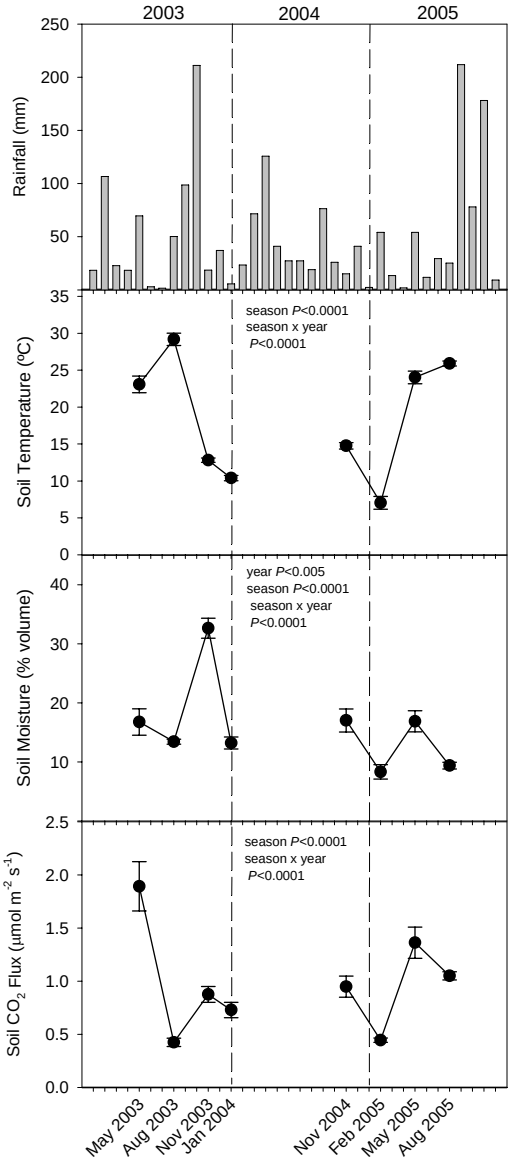

Figure 3.1. Seasonal course of rainfall, soil temperature, soil moisture and soil CO_2 flux measured in the two annual sampling periods, 2003-2004 and 2004-2005. Upper panel: Bar diagram represents total monthly rainfall. Middle panel: Soil temperature and moisture respectively. Lower panel: Seasonal course of soil CO_2 efflux. Error bars indicate SEM (n=27). Significances for the overall season effect, the sampling period effect (year) and the interaction between seasons and year effect (repeated measurements ANOVA) are indicated inside the panels.

3.3.2. Soil monoterpene exchange rates. Interannual and seasonal variations.

Total monoterpene exchange rates measured during the study years were very small and ranged from uptake to net emissions (from –0.003 ± 0.001 nmol m^{-2} s^{-1} to 0.003 ± 0.002 nmol m^{-2} s^{-1}, Fig. 3.2). Alpha–pinene and limonene were the most common monoterpenes detected with the highest exchange rates (Fig. 3.2) followed by camphene (Table 3.2). Beta-pinene, β-myrcene and Δ^3-carene were also detected in some seasons although with very low exchange rates (Table 3.2).

There were emissions of total monoterpenes, α–pinene and limonene only in the second, dry sampling period; in the first, wet period there was soil uptake of these monoterpenes except for a slight emission of total monoterpenes in spring 2003 (Fig. 3.2).

Soil monoterpene seasonal changes were small in most cases and no significant overall seasonal effect on total monoterpene or individual monoterpenes exchange rates was found (Fig. 3.2). However, total monoterpenes and limonene exchange rates increased their uptake rates from spring to winter, during the first sampling period (2003-2004, Fig. 3.2). During the second sampling period 2004-2005, maximum total monoterpene emission rates were recorded in autumn and summer (November 2004 and August 2005, Fig. 3.2).

Table 3.2. Seasonal course of soil exchange rates (nmol m^{-2} s^{-1}) of other detected monoterpenes during the sampling annual periods 2003-2004 and 2004-2005. Values are means ± SEM (n=27). Nd= non detected.

	camphene	β-pinene	β-myrcene	Δ^3-carene
May 2003	0.0000079±0.000036	0.000171±0.000159	Nd	Nd
Aug 2003	Nd	0.0000040±0.000004	Nd	0.00004±0.000034
Nov 2003	-0.000377±0.000164	-0.000092±0.000098	-0.000022±0.000150	0.000024±0.000060
Jan 2004	-0.000178±0.000125	-0.000030±0.000057	0.000119±0.000089	0.000015±0.000015
Nov 2004	0.000552±0.000386	0.000162±0.000179	0.0003±0.000409	0.000291±0.000226
Feb 2005	0.000129±0.000078	Nd	Nd	Nd
May 2005	0.000132±0.000132	0.000018±0.000018	Nd	Nd
Aug 2005	0.001127±0.000595	Nd	Nd	Nd

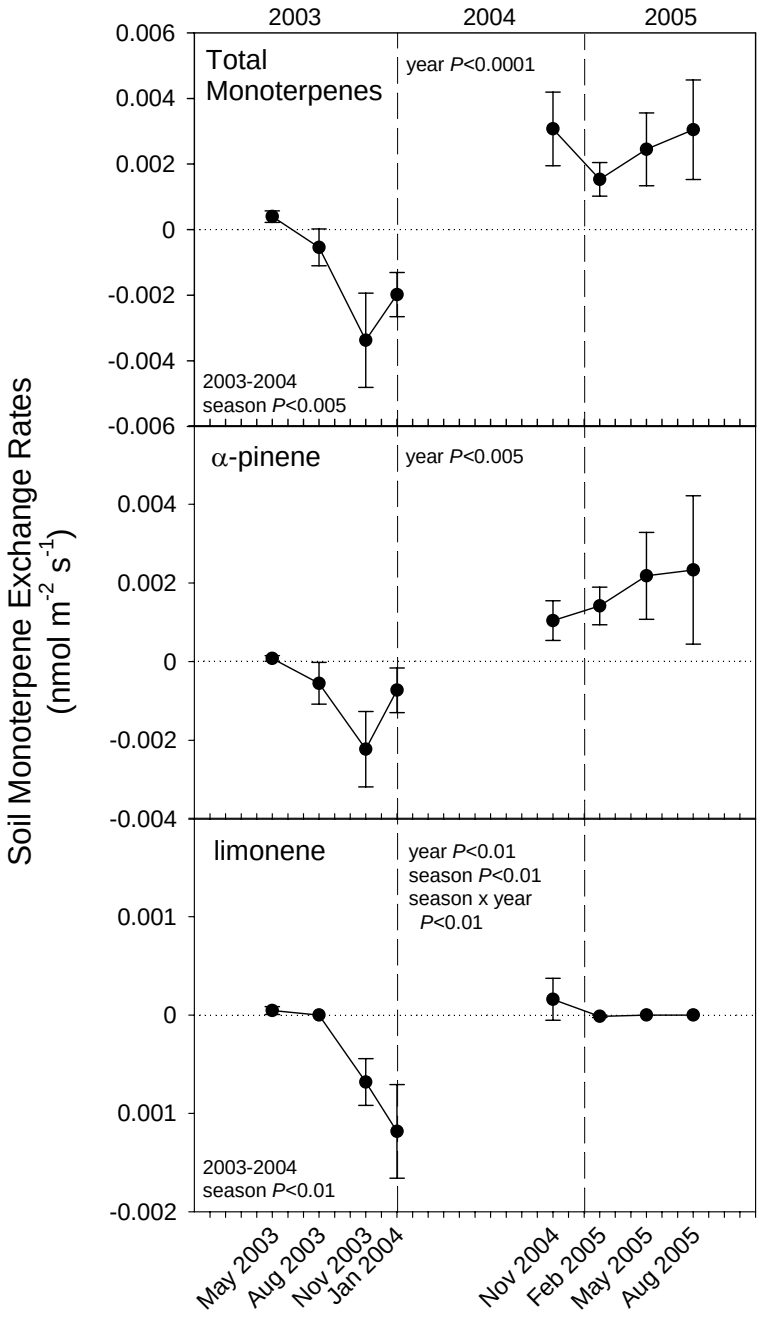

Figure 3.2. Seasonal course of soil exchange rates of total monoterpenes, α-pinene and limonene. Error bars indicate SEM (n=27). Significance for the overall global effect of the season or the year (repeated measurements ANOVA) is indicated inside the panels. Significance for the season effect in one sampling period (repeated measurements ANOVA) is also indicated inside the panels.

3.3.3. Other VOCs' exchange rates.

Background air concentration for all identified masses (analysed with the PTR-MS technique during the 2004-2005 sampling period) with a significant exchange rate response to seasonal changes are shown in Table 3.3. Among all the identified masses, maximum soil emission rates ranged from 0.23 nmol m^{-2} s^{-1} to 2.5 nmol m^{-2} s^{-1}. These maximum soil emission rates were measured in compounds such as M33 (methanol), M45 (acetaldehyde) and M43 (acetic acid) shown in figure 3.3 and M73 (C3 and C4 carboniles), M59 (acetone) and M61 (acetic acid) shown in figure 3.4. M137 (monoterpenes and sesquiterpenes), M57 ((E)-2-hexenal), M31 (formaldehyde) and M97 (heptanal) and M99 (hexenol) emission rates were lower, ranging from 0.01 to 0.04 nmol m^{-2} s^{-1} (Figs. 3.3-3.4). Even lower emissions rates were measured in other compounds like M123 (sesquiterpenes), M139 (C13 unsaturated alcohols) and M155 (linalool) (Fig. 3.3) or M127 (6-methyl-5-hepten-2-one), M199 (C13 unsaturated alcohols) and M143 (hexenyl acetates) (Fig. 3.4). Their maximum emission rates reached up to 0.005 nmol m^{-2} s^{-1}. Maximum soil VOC uptake rates measured, among the identified masses, ranged from -0.002 nmol m^{-2} s^{-1} to –0.1 nmol m^{-2} s^{-1} in VOCs such as M33 (methanol), M45 (acetaldehyde) and M43 (acetic acid) (Fig. 3.3) and M59 (acetone) (Fig. 3.4).

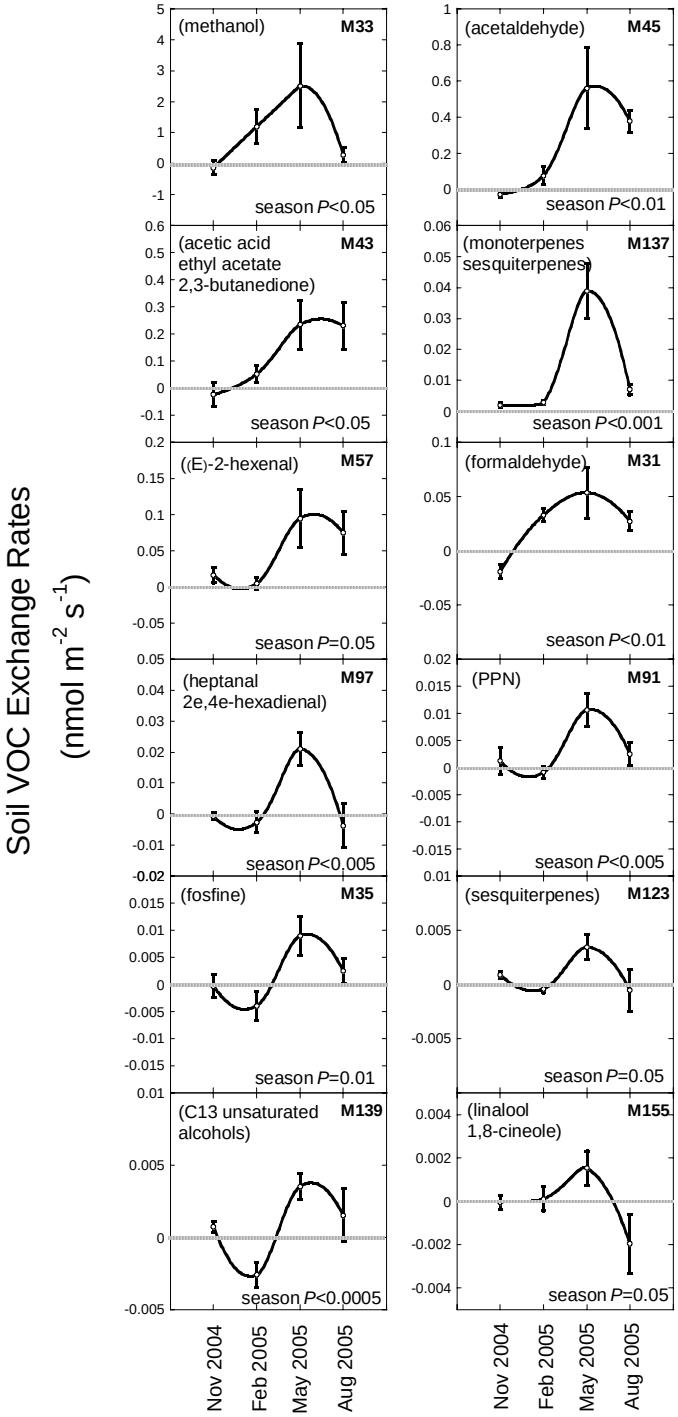

Figure 3.3. Soil VOC exchange rates detected during the 2004-2005 sampling period using PTR-MS technique that presented maximum exchange rates in spring.. Error bars indicate ± standard error of the mean (n=9). Significances for the overall season effect (repeated measurements ANOVA) are indicated inside the panels. The most likely VOC corresponding to each mass based on standard calibrations of the PTR-MS system and on the literature is indicated in brackets.

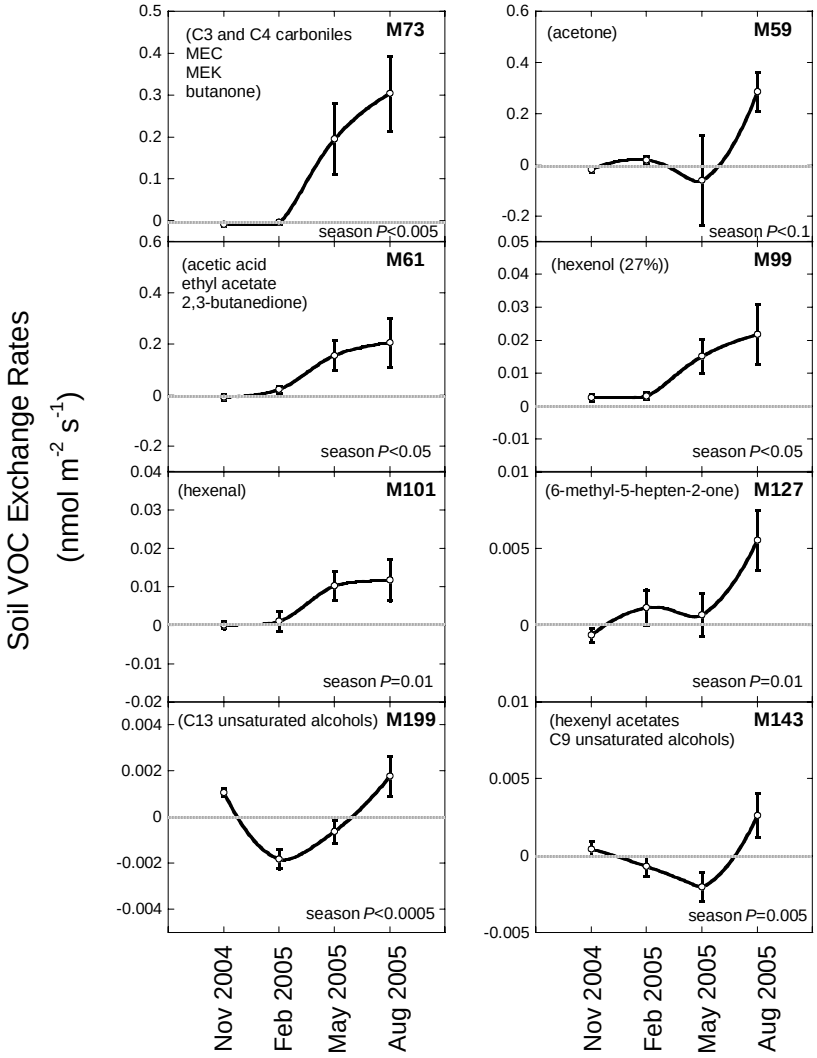

Figure 3.4. Soil VOC exchange rates detected during the 2004-2005 sampling period using PTR-MS technique that presented maximum exchange rates in summer. Error bars indicate ± standard error of the mean (n=9). Significances for the overall season effect (repeated measurements ANOVA) are indicated inside the panels. The most likely VOC corresponding to each mass based on standard calibrations of the PTR-MS system and on the literature is indicated in brackets.

3.3.4. Relationships between soil VOC exchange rates and soil temperature, moisture or soil CO_2 flux.

The relationships between total and individual monoterpene exchange rates as dependent variables and soil moisture, temperature and soil CO_2 flux as independent variables are plotted in Figure 3.5. Total monoterpene, α-pinene and limonene exchange rates were poorly or not correlated with soil moisture, temperature and CO_2 flux, as shown by the scatterplots (Fig 3.5).

Total monoterpene and α-pinene emission rates decreased significantly in response to increases in soil moisture, although the coefficients of determination were low (R^2=0.08; P<0.05 and R^2=0.13; P<0.005 respectively, Fig. 3.5). The scatterplots of total and individual monoterpenes' correlations with soil temperature showed hardly any significant correlation. The average of total monoterpene and α-pinene exchange rates in the first set, grouped from 5ºC to 18ºC, were significantly lower than the respective averages measured from the second set, from 20ºC to 33ºC (P<0.1 and P<0.05 for total monoterpene and α-pinene respectively, Fig. 3.5). In this study no significant correlations or clear trends were found in the relationships between monoterpene exchange rates and soil CO_2 flux (Fig. 3.5). Neither were they when the whole data was separated into the two different sampling periods, 2003-2004 and 2004-2005.

However, significant and positive correlations were found between other soil VOCs' exchange rates and soil moisture (correlations not shown). Compounds in figure 3.3 presented these relationships. All these VOCs' emission rates increased considerably in spring, when soil moisture was high (May 2005, Fig. 3.1). However, they did not increase in the other rainy season, autumn 2004. Thus the correlations found with soil moisture were quite low (R^2 up to 0.24). Other VOCs' exchange rates showed higher positive correlation with soil temperature than with soil moisture, with a common trend to increase their emission rates when soil temperature increased in summer (Figure 3.4). We found a better fit between VOC exchange rates and soil temperature than for soil moisture (R^2 up to 0.40). The majority of the masses which showed a significant correlation with soil CO_2 fluxes tended to increase their emission rates when soil CO_2 flux increased e. g. M45 (acetaldehyde), M137 (monoterpenes and sesquiterpenes) and other VOCs in figure 3.6. Yet the correlations found, when significant, again showed low R^2 values (up to 0.30).

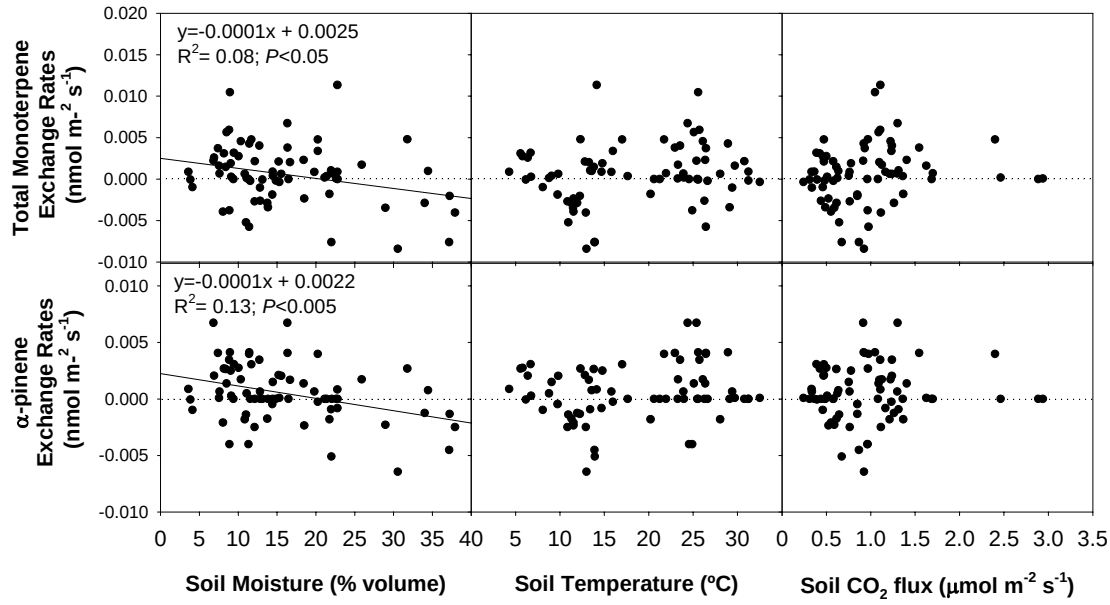

Figure 3.5. Relationships of total monoterpene and α-pinene exchange rates with soil moisture, temperature and CO_2 flux measured during the complete study period 2003-2005.

3.4. Discussion

3.4.1. Soil VOC exchange rates in a Mediterranean shrubland

The monoterpenes α-pinene and limonene, along with monoterpenes shown in Table 3.2, have also been detected in other studies of soil VOC exchange in a Mediterranean holm oak forest soil (Asensio *et al.*, 2007b) and in a non Mediterranean sitka spruce forest soil (Hayward *et al.*, 2001). As we removed the litter layer, the principal source of these terpenoids emitted by soil is likely to be the root system of plants. We were not able to directly identify the origin of the detected compounds since our VOC sampling method includes all living organisms in soil. However, *Pinus halepensis* is abundant at this site and the finding of high amounts of α-pinene, limonene, β-pinene and camphene in *Pinus* root emissions and content (Lin *et al.*, 2007) indicates that roots are the likely source of these monoterpenes.

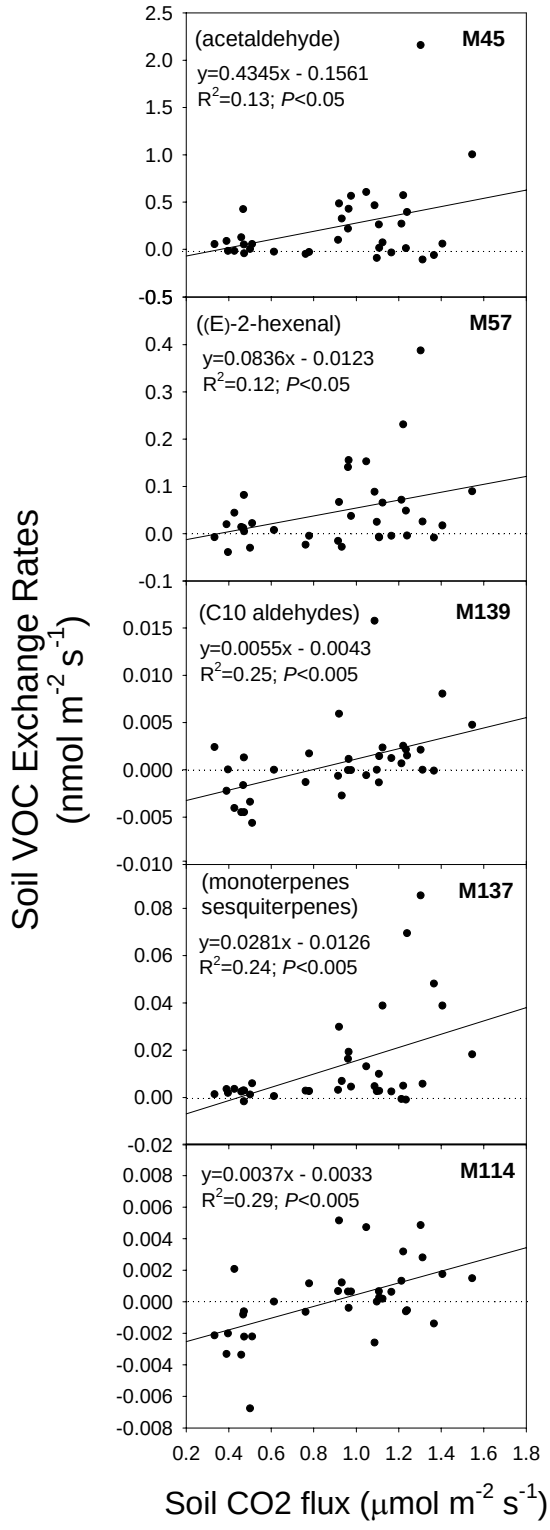

Figure 3.6. Examples of the relationships found between some identified VOC exchange rates and soil CO_2 flux during the 2004-2005 sampling period. The most likely VOC corresponding to each mass based on standard calibrations of the PTR-MS system and on the literature is indicated in brackets.

Since our sampling method did not damage the roots, belowground parts of the plants are probably affecting the total soil terpene emission rates through the production and release of terpenes to the rhizosphere by living roots or decomposing plant material.

In accordance with Asensio *et al.* (2007b), our results show that soil monoterpene emission rates in general are very low compared with monoterpene emissions from leaves. For example, Peñuelas and Llusià (1999) reported values of about 18 nmol m^{-2} s^{-1} from *Pinus halepensis* leaves in summer. So soil monoterpene emissions may represent a small part of the total monoterpene fluxes emitted to the atmosphere by a vegetated land surface. Maximum soil monoterpene emission rates reported in this study were similar to those maximum emission rates reported by Asensio *et al.* (2007b) in a Mediterranean holm oak soil forest (0.003 nmol m^{-2} s^{-1} and 0.004 nmol m^{-2} s^{-1}, respectively). We expected to find higher monoterpene emissions in this shrubland soil than in the holm oak forest soil, due to the previously mentioned presence of *Pinus halepensis*, a terpene storing tree with high monoterpene content in their roots and leaves (Llusià and Peñuelas, 1998, 2000; Lin *et al.* 2007). In contrast, the holm oak is a non terpene-storing species with lower monoterpene content in its leaves (Llusià and Peñuelas, 1998; 2000). As far as we know, there are no published works on holm oak monoterpene root content but, as with leaf content, it is likely to be lower than monoterpene content in *Pinus* roots. Therefore these results indicate that soil monoterpene emissions to the atmosphere are not one of the most important fates for soil monoterpenes. Monoterpenes might be stored in the soil mineral layers more than emitted to the atmosphere. However, so far there are few studies on soil's monoterpenes content (White, 1994; Smolander; 2006, Lin et al 2007) so research into terpene content in different soil layers is still needed.

Soil monoterpene uptake in this shrubland was lower than monoterpene uptake measured in the holm oak forest soil (–0.003 m^{-2} s^{-1} and -0.01 nmol, respectively). This difference is possibly due to differences in the physical soil features or in the soil microorganisms' biodegradation of terpenes, since soil VOC uptake activity is occurring by physical adsorption of VOCs by soil components (Pignatello and Xing, 1996) or by biodegradation by soil microorganisms (Van Roon *et al.*, 2005b). In fact, soil enzyme activity and soil CO_2 flux, which are good indicators of microorganisms' activity in soils (Hanson *et al.*, 2000; Baum *et al.*, 2003; Nannipieri *et al.*, 2003) were also lower in this shrubland soil than in the holm oak forest soil (Sardans and Peñuelas, 2005; Sardans et al, 2006; Asensio *et al.*, 2007b).

Except for mass 33 (methanol, Fig. 3.3), the other volatile compounds emission rates measured with the PTR-MS technique, were in the range or slightly higher than those reported in a holm oak forest soil by Asensio *et al.* (2007b) (maximum emission rates up to 0.6 nmol m^{-2} s^{-1} in this shrubland and up to 0.3 nmol m^{-2} s^{-1} in the holm oak forest). These results show again that soil VOC emission rates in general are very low, except for methanol. Methanol emission rates were much higher than other VOCs' emission rates measured in the holm oak soil forest and in this shrubland (maximum methanol emission rates 2.5 nmol m^{-2} s^{-1}, Fig. 3.3). Large methanol soil emissions (maximum about 4.6 nmol m^{-2} s^{-1}) have been reported above a bare agricultural field by Schade and Custer (2004). These authors suggest a methanol source at the soil surface and that its production is probably linked to a physico-chemical degradation process of soil organic matter. Since we removed the litter layer, the source of soil methanol emissions in this study should be in the soil.

Monoterpene and sesquiterpene (M137) emission rates measured in spring (May 2005; Fig. 3.3), using PTR-MS technique, were approximately ten times higher than the monoterpene emission rates measured using GC-MS technique (Fig. 3.2 and 3.3). The higher abundance of mass 137 might indicate the presence of high sesquiterpene amounts released by roots. In fact, sesquiterpenes are one of the most abundant compounds in *Pinus* roots (Lin *et al.*, 2007). The sum of all masses documented in literature as sesquiterpenes or sesquiterpene fragments (M109, M123, M149 and M205) clearly reflected the same emission pattern as mass 137, but the sum of these masses only doubled the mass 137 in spring 2005, indicating that there must be other sources contributing to the high mass 137 values of that spring sampling.

Our results showed maximum soil uptake rates ranging from –0.1 nmol m^{-2} s^{-1} to –0.5 nmol m^{-2} s^{-1} in some compounds like M33 (methanol, Fig. 3.3), M42 (acetonitrile), M46, M59 (acetone), M88 and M89 (ethyl acetate) (not shown). These values are comparable to foliar uptake rates reported by other authors (ranging from –0.4 nmol m^{-2} s^{-1} to –3 nmol m^{-2} s^{-1}) for some compounds like MIBK (methyl isobutyl ketone) (Tani *et al.*, 2007), monoterpenes (Copolovici *et al.*, 2005) or M31 (formaldehyde) (Filella *et al.*, 2006). However, there is still insufficient knowledge about the soil VOC sink strength to evaluate whether it could be small but non-negligible on a global scale. Soil VOC and other soil gas uptake fluxes have been described (Chang *et al.*, 2002; Pegoraro *et al.*, 2006; Asensio et al, 2007a, b; Chapuis-Lardy *et al.*, 2007) but they have received little attention due to the relatively low uptake rates and the quite high uncertainties

around soil VOC exchange processes. Soil VOC uptake rate depends on the air concentration and on the soil concentration resulting from the balance between soil VOC production and consumption (Pignatello and Xing, 1996), which may vary with the soil type and plants' species-specific variation of VOC content.

3.4.2 Interannual variation and seasonality of soil VOC exchange rates and relationships with soil temperature, moisture and CO_2 flux.

Results indicate that a severe drought period increases soil monoterpene emission rates in this Mediterranean shrubland (Fig. 3.2). Even though monoterpene exchange rates were very low, there were highly significant differences between the exchange rates measured in the two years of contrasting precipitation. This may be the result of lower uptake rates due to lower physiological activity during the drier sampling period 2004-2005. However, the fact that monoterpene exchange rates and soil CO_2 fluxes were not significantly correlated (Fig. 3.5) and that soil CO_2 fluxes did not decrease significantly during the dry year 2004-2005 (Fig. 3.1) suggest that the increase in soil monoterpene emission rates in response to lower soil water availability could be due to changes in soil physical properties induced by low soil moisture, rather than to a lower physiological activity of roots and microorganisms.

Seasonal changes in total monoterpene, α-pinene and limonene were smaller than interannual changes and the exchange rates measured were highly variable in some seasons (Fig. 3.2). Total and individual monoterpene exchange rates did not show overall significant seasonal effects in the second, dry sampling period, although there were significant seasonal changes in soil moisture and temperature (Fig. 3.1). These results, in addition to the very low, though significant, negative correlations found between monoterpene exchange rates and soil moisture (Fig. 3.5), suggest that monoterpene exchange rates are less affected by seasonal soil moisture changes than by long-term severe changes.

Results show that high soil temperatures increase soil monoterpene emission rates. This accords with Asensio *et al.* (2007b) results and it is possibly due to the higher volatilisation rates of volatile compounds in response to increases in temperature (Van Roon *et al.*, 2005a). Our results suggest that seasonal changes in soil temperature could have strong effects on monoterpene exchange rates, since monoterpene emissions were recorded mainly during the warm seasons (spring and summer) and most monoterpene uptake was recorded during the cold seasons (autumn and winter) as

shown in figure 3.5. The mean value of the monoterpene exchange rates measured from 20ºC to 33ºC, was positive and significantly higher than the exchange rate mean value calculated for the colder soil temperature range (Fig. 3.5).

There were significant seasonal variations in other VOCs' exchange rates (Fig. 3.3 and Fig. 3.4) together with changes in soil temperature, moisture and soil CO_2 flux variations (Fig. 3.1). Soil moisture effect on soil VOC exchange rates depends on the compound type, although results suggest that high soil moisture tended to increase soil VOC emissions, at least in those compounds which showed significant seasonal changes in their exchange rates (Figs. 3.3 and 3.4). The positive correlations found between several soil VOC exchange rates and soil moisture provides evidence of this trend. However, the correlations were not strong because soil moisture effects on VOC exchange rates were constrained by low soil temperatures; for example, in autumn (November 2004, Fig. 3.1). Similarly, we found positive correlations between soil VOC exchange rates and soil CO_2 flux (Fig. 3.6) suggesting that phenology of plants and soil microorganisms are also affecting VOC exchange rates. Soil CO_2 flux is considered to be a good indicator of the roots' and microorganisms' physiological activity (Hanson *et al.*, 2000). Thus, the increase of soil VOC emission rates in this Mediterranean shrubland in spring (Figs. 3.3 and 3.4) might be linked to the increase of root and microorganism activities in spring and autumn; for example the root-surface and soil phosphatase activities (Sardans *et al.*, 2006; Sardans *et al.*, 2007) in parallel with the increase of soil-available P in spring (Table 3.1). The decrease in available ammonium and nitrate in soil during spring and autumn (Table 3.1) corresponded to the plant growing seasons and it could be also related to the increase in soil VOC emission rates during spring.

Because maximum emission rates always occurred in spring or summer, when the soil temperature was high, we found significant positive correlations between several VOC exchange rates and soil temperature (not shown). Thus, results indicate again seasonal soil temperature controls on several soil VOCs' exchange, increasing soil VOC emission rates when soil temperature increased.

Table 3.3. Air concentrations of masses of volatile organic compounds in ppbv (1 ppbv = 1 part in 10^9 by volume) detected by PTR-MS during the 2004-2005 sampling period in Garraf. Only those masses that were identified and that were significantly affected by season or treatment are shown. Data are means ± SEM; n=27. The most likely VOC corresponding to each mass based on standard calculations of the PTR-MS system and literature is indicated in brackets. (MEK= Methyl Ethyl Ketone, PPN= Peroxy Propionic Nitrid anhydride)

	Nov 2004	Feb 2005	May 2005	Aug 2005
M31 (formaldehyde)	5.24±0.40	3.19±0.24	5.37±0.49	8.58±0.36
M33 (methanol)	222.55±4.34	179.92±0.31	231.80±7.32	77.85±9.83
M35 (phosphine))	0.52±0.06	0.52±0.07	0.81±0.05	0.18±0.05
M43 (acetic acid, ehtyl acetate, 2-3 butanedine)	12.24±1.18	13.68±0.24	18.24±0.30	27.03±2.88
M45 (acetaldehyde)	9.11±1.14	5.09±0.85	18.49±2.36	19.94±3.13
M57 ((E)-2-hexenal	2.98±0.16	2.86±0.18	13.08±1.22	9.44±0.99
M59 (acetone)	7.92±0.21	4.66±0.38	24.45±9.43	21.30±2.93
M61 (acetic acid)	4.59±0.30	2.96±0.41	10.08±0.13	23.58±2.54
M73 (methyl ehtyl cetona, butanona, MEK)	0.99±0.16	0.82±0.13	3.30±0.02	4.26±0.38
M91 (PPN)	1.37±0.03	0.09±0.02	0.74±0.10	0.45±0.06
M97 (heptanal, 2e-4e-hexadienal)	0.43±0.03	0.50±0.12	1.76±0.10	1.96±0.37
M99 (hexenol)	0.14±0.02	0.18±0.01	0.80±0.06	0.97±0.15
M101 (hexenal)	0.15±0.01	0.15±0.04	0.94±0.04	1.19±0.09
M114	0.01±0.09	0.09±0.03	0.10±0.02	0.13±0.01
M123 (sesquiterpenes)	0.04±0.00	0.06±0.02	0.25±0.02	0.19±0.05
M127 (C9 aldehydes)	0.07±0.02	0.03±0.03	0.38±0.04	0.31±0.07
M137 (monoterpenes, sesquiterpenes)	0.14±0.02	0.21±0.05	0.48±0.03	0.62±0.29
M139 (C10 aldehydes)	0.01±0.01	0.10±0.01	0.18±0.01	0.13±0.01
M143 (hexenyl acetates)	0.03±0.01	0.09±0.02	0.39±0.03	0.28±0.05
M155 (linalool, 1, 8-cineole)	0.02±0.01	0.02±0.01	0.12±0.02	0.16±0.06
M199 (C13 unsaturated alcohols)	0.00±0.00	0.05±0.02	0.13±0.00	0.07±0.06

3.5. Conclusions and final remarks

Soil VOC exchange rates measured in this Mediterranean shrubland are the result of the synthesis and uptake of VOCs which are dependent on temperature, water availability, phenology of plants and soil microorganisms, soil physical traits and concentration of the compounds in the atmosphere near the soil. Alpha-pinene and limonene were the most common identified soil exchanging monoterpenes. M33 (methanol), M43 (acetic acid, ethyl acetate), M45 (acetaldehyde), M59 (acetone) and M73 (C3 and C4 carboniles) showed the highest emission rates among all the identified VOCs. Exudates from rhizosphere activities, roots VOC production and organic matter decomposition by microorganisms in the soil organic or mineral horizons may be the most important sources of these VOCs.

Soil monoterpene and in general soil VOC emissions, except mass 33 (methanol) were very low compared with foliar VOC emission rates. However, some soil VOC uptake rates were comparable with those reported for leaves. These VOCs might be consumed by soil microorganisms or stored in the soil layers. Further studies are needed to corroborate these results and to investigate the possible importance of the soil VOC sink in chemistry-climate models.

Results suggest that severe long-term drought increases soil monoterpene emission rates in this Mediterranean shrubland. The increase in soil monoterpene emissions in response to lower soil water availability is probably linked to changes in soil physical properties induced by low soil moisture in this Mediterranean shrubland, since monoterpene exchange rates and soil CO_2 fluxes were not significantly correlated. Conversely, other soil VOCs increased their emission rates when soil moisture increased, although the effects were constrained by soil temperature, for example in the cold seasons. Most of these compounds' exchange rates showed significant correlations with CO_2 flux, suggesting that the increase of root and microbial activities during the growing seasons may increase these soil VOC exchange rates.

High soil temperatures increased emission rates of monoterpenes and other VOCs possibly due to higher volatilisation rates. Results suggest a strong seasonal control of soil temperature on monoterpene exchange rates.

The effect of soil moisture reduction on soil VOC exchange rates is still poorly understood because several factors are probably affecting in soil VOC exchange rates. Improved knowledge of the multiple interactions between rhizosphere components, and

of soil emission and uptake processes in general, will help to disentangle the effects on soil VOC exchange with the atmosphere resulting from the decrease in water availability projected for the next decades by climatic (IPCC, 2007) and ecophysiological models such as GOTILWA (Sabaté *et al.* 2002; Peñuelas *et al.* 2005a).

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Chapter 4

On-line screening of soil VOCs exchange responses to moisture, temperature and root presence

Abstract

The exchanges of volatile organic compounds (VOCs) between soils and the atmosphere are poorly known. We investigated VOC exchange rates and how they were influenced by soil moisture, temperature and the presence of plant roots in a Mediterranean forest soil. We measured VOC exchange rates along a soil moisture gradient (5%-12.5%-20%-27.5% v/v) and a temperature gradient (10ºC-15ºC-25ºC-35ºC) using PTR-MS. Monoterpenes were identified with GC-MS. Soils were a sink rather than a source of VOCs in both soil moisture and temperature treatments (-2.16 ± 0.35 nmol m^{-2} s^{-1} and -4.90 ± 1.24 nmol m^{-2} s^{-1} respectively). Most compounds observed were oxygenated VOCs like alcohols, aldehydes and ketones and aromatic hydrocarbons. Other volatiles such as acetic acid and ethyl acetate were also observed. All those compounds had very low exchange rates (maximum uptake rates from –0.8 nmol m^{-2} s^{-1} to –0.6 nmol m^{-2} s^{-1} for methanol and acetic acid). Monoterpene exchange ranged only from –0.004 nmol m^{-2} s^{-1} to 0.004 nmol m^{-2} s^{-1} and limonene and α-pinene were the most abundant compounds. Increasing soil moisture resulted in higher soil sink activity possibly due to increases in microbial VOCs uptake activity. No general pattern of response was found in the temperature gradient for total VOCs. Roots decreased the emission of many compounds under increasing soil moisture and under increasing soil temperature. While our results showed that emission of some soil VOCs might be enhanced by the increases in soil temperature and that the uptake of most soil VOCs uptake might be reduced by the decreases of soil water availability, the low exchange rates measured indicated that soil-atmosphere VOC exchange in this system are unlikely to play an important role in atmospheric chemistry.

Key words: Monoterpenes; PTR-MS; roots; soil moisture; soil VOC exchange; temperature.

4.1. Introduction

Soils are reported to be both source and sink of volatile organic compounds (VOCs). For example, acetone and methanol emissions have been described from agricultural soils (Schade and Custer, 2004), emissions of methanol, acetone and acetaldehyde in a ponderosa pine plantation were found to contribute significantly to the canopy level fluxes of such compounds (Schade and Goldstein, 2001), and monoterpene emissions from a Sitka spruce soil forest have been also characterised, although their contribution to the total monoterpene atmospheric budget was low (Hayward et al., 2001). Reports on the soil VOCs sink activity are more scarce. For example, VOC uptake in soil has been reported for atmospheric isoprene (Cleveland and Yavitt, 1998; Pegoraro et al., 2005) and for methane-derived compounds, such chloromethane (Keppler et al., 2005).

Roots and soil microorganisms are thought to be the principal sources of soil VOCs emission (Janson, 1993; Mackie and Wheatley, 1999; Chen et al., 2004; Steeghs et al., 2004; Ping and Boland, 2004). Likewise, roots can also uptake and accumulate or degrade some contaminant organic volatiles from the soil solution (Cho et al., 2005) and microorganisms can consume them (Cleveland and Yavitt, 1998; Yoo and Day, 2002). In addition, there could be interactions between these biological processes, for example, roots activity produces the enhancement of associated microbial populations by releasing carbon-rich exudates on the rhizosphere (Walker et al., 2003). Thus, constituents of soil VOCs dynamics, sources and sinks, are linked, resulting in complex interactions.

Both soil moisture and temperature are reported to affect physical VOC processes in soil (adsorption, dissolution, volatilisation) and also biological processes (biodegradation by microorganisms, uptake by plants) (Pignatello and Xing, 1996; Cho et al., 2005; Johnsen et al. 2005), which in turn affect the VOCs soil exchange rates.

Up to date, some studies have revealed interesting properties for volatile compounds that could play an important role in the soil e. g. bacterial volatiles such 2,3-butadienol and acetoin that promote growth in plants (Ryu et al., 2003), plant volatiles like sesquiterpenes that induce hyphal branching in AM fungi (Akiyama et al., 2005) or (z)-jasmone plant exudates which act as an aphid colonization signal for nearby plants (Chamberlain et al., 2001). Negative interactions between bacteria and fungi mediated by several VOCs, trimethylamine, benzaldehyde or N, N-dimethyloctylamine, have also been described (Mackie and Wheatley, 1999; Xu et al., 2004). Among VOCs, the

monoterpenes have been reported to be able to alter the rates of nitrogen cycle (White, 1994), act as potent biological inhibitors (Nishida et al., 2005) or as attractant to the larvae of some pine weevil species (Nordenhem and Nordlander, 1994) and to be used as growth substrate for bacteria (Misra et al., 1996).

All these examples make prominent the likely role of VOCs on soil ecology, and information on soil VOCs is still scarcely available (Asensio et al., 2007). Furthermore, it is likely that the soil warming and lower water availability predicted by GCM and ecophysiological models in the Mediterranean region (IPCC 2001; Peñuelas et al., 2005a) will affect soil VOCs exchange rates.

We aimed thus to obtain information about changes in real time on the soil VOCs source/sink activity, taking advantage of the recent availability of analytical instrumentation to conduct these measurements, and to obtain information on soil VOCs exchange rates responses to root presence, to the soil warming and to the lower water availability predicted by GCM and ecophysiological models in the Mediterranean region. With these aims, we monitored soil VOCs exchange rates in real time with the PTR-MS technique in a controlled 'soil+plant'-atmosphere and in a 'soil'-atmosphere systems, and submitted both 'soil + plant' and 'soil' systems to moisture and temperature gradients.

4.2. Material and methods

4.2.1. Plant Material

Quercus ilex seedlings were grown in a nursery (Forestal Catalana, S. A., Breda, Spain) under typical Mediterranean environmental conditions (mean annual average temperature 16ºC, mean annual precipitation 600mm). In November 2004, 2-year-old plants were transplanted with their intact roots to 2-L pots filled with soil from a natural holm oak forest in Prades mountains, South-Eastern Catalonia.

4.2.3. Soil Material

Soil samples were collected from the top soil layer (20 cm) in a natural holm oak forest growing in the Prades Mountain region, in South-Eastern Catalonia (41º 13'N, 0º55'E), on a south-facing slope (25% slope) at 930 m above sea level. All the soil mineral particles and organic matter were maintained to preserve natural soil structure. We

turned over the soil before putting it into the pots, in order to avoid compaction and therefore soil density in the pots was similar to soil density in the natural situation.

4.2.4. 'Soil + Plant' and 'Soil' treatments

Soil samples were used to fill six pots with *Quercus ilex* plants and further soil samples were used to fill six pots without plant. All the pots were maintained in winter Mediterranean-like conditions in the University experimental fields (average temperature and moisture for the three months: 7.9 ºC and 82.7%) from November 2004 to February 2005, when we started the experiment.

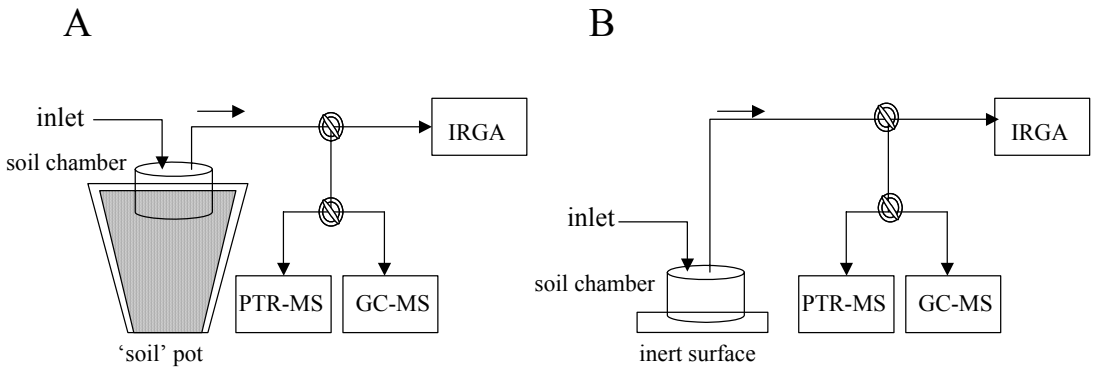

Figure 4.1. (A) Schematic overview of the system used for measurements of the soil VOCs exchange rate. The air enters into the system from the inlet, the air leaving the soil chamber is flowed by a T-system to an IRGA to measure soil CO_2 efflux or either to the PTR-MS and GC-MS to measure soil VOCs. (B) Schematic overview of the system used for measurements of the background VOCs (blank measurements). Air enters into the system from the inlet and is flowed to the PTR-MS or GC-MS as in the Fig. 4.1 A.

4.2.4. Moisture Gradient Treatment

Half of the pots (three 'soil + plant' and three 'soil' without plant) were maintained within a low irrigation regime (50 cm^3 each 3 days from November to February) and assigned for the soil moisture gradient treatment. These pots had 5% volume soil moisture when VOCs measurements started in February 2005. Soil temperature was constant at 20ºC during all the soil moisture treatment. A gradient from 5% to 27.5% (7.5% steps) was applied by uniformly spraying 200 cm^3 water over the pot surface in each moisture step. We waited 1 hour of stabilization after irrigation and then started soil and leaf measurements.

4.2.5. Temperature Gradient Treatment

The other six pots (three 'soil + plant' and three 'soil' without plant) were well watered (250 cm^3 each 3 days from November to February) and assigned for the temperature gradient treatment. These pots had 21% soil moisture when VOCs measurements started in February 2005. They were exposed to a temperature gradient: 10ºC, 15ºC, 25ºC and 35ºC. Soil temperature steps were reached by putting the pot into a thermostatic bath. The soil in the pot surface was open to the gas exchange and sampling. The soil same leaf temperatures were reached in the controlled environment cuvette used for leaf measurements.

4.2.6. Experimental System and Variables Measured

We measured soil CO_2 and VOC exchange, and leaf net CO_2 uptake and stomatal conductance at each soil moisture and temperature step. We measured one pot (one gradient) per day.

In every 'soil + plant' and 'soil' pot, a 97.5 cm^2 soil metal collar was inserted 10 cm into the soil 1 hour before starting the measurements. A soil hood chamber was placed hermetically onto the soil collar. This soil gas exchange system was prepared with one inlet for the ambient air and one outlet connected to an IRGA porometer (LCA-4, ADC; Hoddeson, Hertfordshire, UK). The air exiting from the enclosed soil flowed through a T-system to the PTR inlet for VOC analysis during 40 min and to the IRGA inlet for CO_2 analysis, as showed in the Figure 4.1 a.

For each soil PTR-MS VOC measurements, we also sampled the VOCs with carbon-trap adsorption tubes. We conducted GC-MS analyses to identify the different monoterpenes. The air exiting the soil chamber flowed through a T-system impelled by a pump towards a glass tube (11.5 cm long and 0.4 internal diameter) filled with VOC adsorbents Carbotrap C (300 mg), Carbotrap B (200 mg) and Carbosieve S-III (125 mg) separated by plugs of quartz wool and treated as described by Peñuelas et al. (2005b). After VOC sampling, the adsorbent tubes were stored at –30ºC until analysis (within 24-48 h). The VOC analyses were conducted in a GC-MS (HP59822B; Hewlett Packard, Palo Alto, CA, USA), as described by Peñuelas et al. (2005b).

Before each measurement of soil VOC exchange rate, we conducted system blank measurements by sampling air from an empty surface covered by teflon and enclosed by the soil hood, as it is shown in Figure 4.1 b. Ambient air was fluxed into the soil hood and the air coming out flowed through a T-system to either a PTR-MS system

or to an absorption glass tube for GC-MS analysis. Net soil VOCs fluxes were calculated by considering the stable difference in the VOCs concentration between the soil chamber air (soil VOCs) and the ambient air (background VOCs). Measuring replicate pots of the same treatment over different days could increase the variability of the measurements since they will vary with ambient air composition variations from day to day. In spite of this added daily variability, there were several masses (those presented here) which showed significant responses to the treatments or a clear trend, so we kept using ambient air to achieve maximum similarity with natural conditions. Soil and leaf CO_2 gas exchange measurements were also conducted with the empty soil hood and the empty leaf cuvette as additional control.

Soil moisture and temperature were also monitored at 10 cm depth, beside the collar to avoid mechanical disturbances to the enclosed soil. A HH2 soil moisture meter connected to a ML2x soil moisture sensor (Delta-T Devices Ltd, Cambridge, England) was used to measure soil moisture and a soil digital thermometer was used to measure temperature (TO 15, Jules Richard instruments, Argenteuil, France).

For leaf net assimilation and stomatal conductance measurements we used a portable photosynthesis system CIRAS-2 (PP Systems). An intact leaf was clamped in a Parkinson leaf cuvette (Std Broad 2.5, PP Systems, Hitchin, UK). At every soil moisture and temperature in each 'soil + plant' replicate we measured the same leaf to minimize leaf-to-leaf variability. Leaf cuvette temperature was controlled by CIRAS-2 and maintained at 20ºC during the soil moisture treatment and 10ºC, 15ºC, 25ºC and 35ºC during temperature treatment. All leaf measurements were conducted at 1000 μmol m^{-2} h^{-1} PPFD. After the soil moisture and temperature gradient treatment, the sampled leaf of each 'soil + plant' replicate was harvested to measure its surface area in a Li-Cor 3100 Area Meter (Li-Cor Inc., Lincoln, NE, USA).

4.2.7. Analysis of VOCs by PTR-MS and GC-MS

The PTR-MS system (PTR-MS-FTD hs; Ionicon Analitik, Innsbruck, Austria) provides real time highly sensitive detection of the mass selected ions that are characteristic of the molecules of interest. Both PTR-MS and its use in VOC analysis have been described in detail in Lindinger et al. (1998) and Fall et al. (1999). Detection of the protonated volatiles is produced in a quadrupole mass spectrometer. For many compounds, proton transfer from H_3O^+ was nondissociative; however, in the case of monoterpenes, dissociation occurred. Mass fragmentation and the fact that compounds

with the same molecular mass cannot be separately measured are the main limitations of using PTR-MS. We estimated the concentration of total monoterpenes as the concentration of ions with mass 137, 95, 81 and 67 because certain fragmentation of non-oxygenated monoterpenes in the drift tube occurred during ionization (checked with α-pinene standard). To solve the second limitation, we combined PTR-MS with GC-MS techniques to determine the different monoterpenes.

The PTR-MS instrument was calibrated using calibration standards for the most common compounds found in previous studies of this Mediterranean soil (Asensio et al., 2007). The quantification of VOCs was based on the use of 3 times replicated calibration standards (methanol, acetaldehyde, acetone, isoprene and α-pinene Abelló-Linde, Barcelona, Spain) for each day of measurements. The detection limit of he PTR model we used, a PTR-MS-FTD hs with a high sensitivity, was in the order of pptv and the reproducibility for quantitative analysis was better than 10%.

In this experiment the PTR-MS drift tube was operated at 2.1 mbar and 40ºC, with a drift field of 600 V cm^{-1}; the parent ion signal was maintained at ca. 3 x 10^6 counts per second during the measurements. We conducted scans of all masses between 22 and 205 and use the background values to calculate the exchange balance for each compound. The background air concentrations, one of the main factors driving such exchange, for all masses with significant exchange are shown in Table 4.1 and Table 4.2 (soil moisture and soil temperature treatments respectively). Screening soil VOCs concentrations in response to soil moisture and temperature changes with the PTR-MS technique allowed the measure of VOCs exchange rates in real time and therefore to wait until stabilization for sampling VOCs in each soil moisture or temperature set.

4.2.8. Statistical Analyses

Analyses of variance (ANOVA) were conducted in soil and leaf measurements for every mass with the VOC exchange rate as dependent variable and with treatment (soil moisture or temperature gradient) and the presence of living roots in soil as independent factors. When needed, Fisher's PLSD post hoc tests were conducted. All analyses (ANOVAs, Post Hoc Test and regression models) were performed with STATVIEW 5.01 software package (SAS Institute Inc., Cary, NC).

4.3. Results

4.3.1. Soil Moisture Treatment

Total VOCs and Monoterpenes

The overall exchange rate of VOCs resulted in soil uptake (Fig. 4.2). Mean values of the total soil VOC uptake rates were -2.16 ± 0.35 nmol m^{-2} s^{-1}. Soil monoterpene emissions prevailed over monoterpene uptake during the soil moisture treatment (Fig. 4.2), although the exchange rate values were very low. Maximum monoterpene emission rates were detected at 5% soil moisture and 21ºC soil temperature in the 'soil' measurements (0.009 ± 0.003 nmol m^{-2} s^{-1}, Fig. 4.2). Total monoterpene exchange rate values measured with the GC-MS technique were in agreement with PTR-MS values and trends (Figs. 4.2 and 4.3). The largest part of detected monoterpenes were α-pinene and limonene (Fig. 4.3).

Soil VOCs uptake increased with increases in soil moisture (soil moisture $p< 0.1$, Fig. 4.2). Similarly, soil monoterpene uptake increased at higher moistures (Fig. 4.2). The soil moisture treatment did not alter neither the composition nor the relative abundance of soil monoterpene exchange (Fig. 4.3).

Soil VOC uptake was larger in 'soil + plant' than in 'soil' measurements (roots $p< 0.1$, Fig. 4.2). Similar results were found for roots effect on soil monoterpene exchange ($p< 0.05$, Fig. 4.2). Limonene uptake was significantly larger when there were roots in soil (roots $p< 0.05$) (Fig. 4.3).

Other VOCs

The responses to soil moisture of individual compounds are available in the Supplementary Table 1. A great part of all masses which had a significant response to soil moisture treatment, such as formaldehyde, methanol, acetaldehyde, ethanol, hexanal and hexenol (Supplementary Table 1), followed the same pattern than total soil VOC exchange rate (Fig. 4.2). They were increasingly taken up from air to soil when soil moisture was increased. Only few masses which had a significant response to soil moisture treatment showed the inverse trend (M155, Supplementary Table 1).

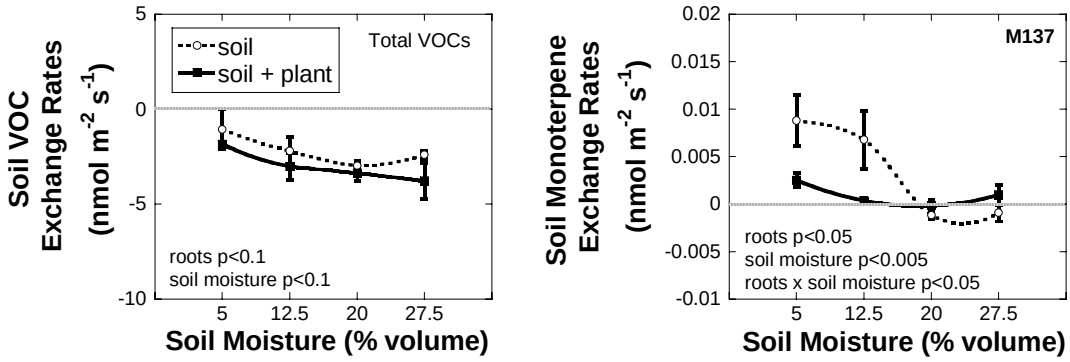

Figure 4.2. Total detected soil VOCs exchange rates and soil monoterpene exchange rates measured during the soil moisture treatment in the 'soil' and 'soil + plant' pots. Total VOCs are the sum of the exchange rate of scanned masses (22-205, except M37 and M39, water clusters and isotopes of water cluster). Error bars indicate ± standard error of the mean (n=3 'soil' pots and n=3 'soil + plant' pots, for both total VOCs and monoterpenes). Significances for the overall effect of the soil moisture treatment and the roots presence (ANOVA) are indicated inside the panels.

The major part of all masses which had a significant exchange rate response to the presence of roots effect showed a greater uptake in their presence than in their absence. Acetic acid, (E)-2-hexenal, benzene, xylene, M123 and p-cymene (Supplementary Table 1) followed this trend. The exchange rate response of a few masses such as M157 followed the contrary trend.

Apart from the masses shown in Supplementary Table 1, there were other masses with significant exchange rate responses to the soil moisture and to the roots presence which followed the main trends described in this Supplementary Table 1. For example, M32, M62, M84, M114, M120, M145 and M159 uptake increased with rising soil moistures and M90, M121, M122, M159, M164 and M194 uptake was larger in the 'soil + plant' measurements than in the 'soil' measurements.

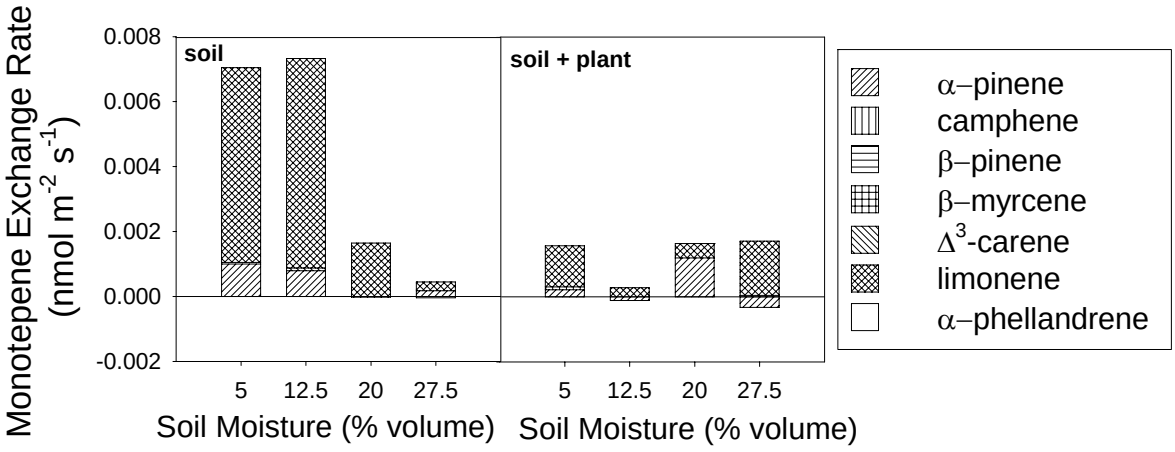

Figure 4.3. Relative composition of measured monoterpenes during the soil moisture treatment in the 'soil' and 'soil + plant' pots.

4.3.2. Soil Temperature Treatment

Total VOCs and Monoterpenes

During the soil temperature treatment there were also overall total soil VOCs and monoterpene uptake (mean values -4.90 ± 1.24 nmol m^{-2} s^{-1} and -0.0005 ± 0.001 nmol m^{-2} s^{-1} respectively, Fig. 4.4). Maximum monoterpene uptake rates were detected at 10ºC soil temperature and 21% soil moisture in the 'soil + plant' measurements (-0.004 ± 0.001 nmol m^{-2} s^{-1}, Fig. 4.4). The largest part of detected monoterpene exchanges were those of α-pinene and limonene, followed by those of camphene and 3-carene (Fig. 4.5). Beta-pinene, β-myrcene and α-phellandrene exchanges were detected occasionally.

Soil progressive warming did not cause a significant increase or decrease in total soil VOCs exchange neither did on the soil monoterpene exchange rates, but total soil VOCs uptake tended to be slightly greater at higher temperatures (25-35ºC) (Fig. 4.4). The soil temperature treatment did not alter neither the composition nor the relative abundance of soil monoterpene exchange (Fig. 4.5).

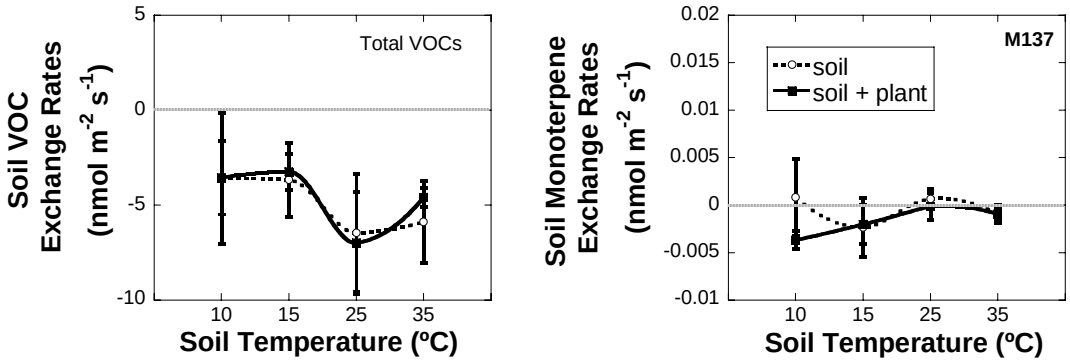

Figure 4.4. Total detected soil VOCs exchange rates and soil monoterpene exchange rates measured during the soil temperature treatment in the 'soil' and 'soil + plant' pots. Error bars indicate ± standard error of the mean (n=3 'soil' pots and n=3 'soil + plant' pots, for both total VOCs and monoterpenes).

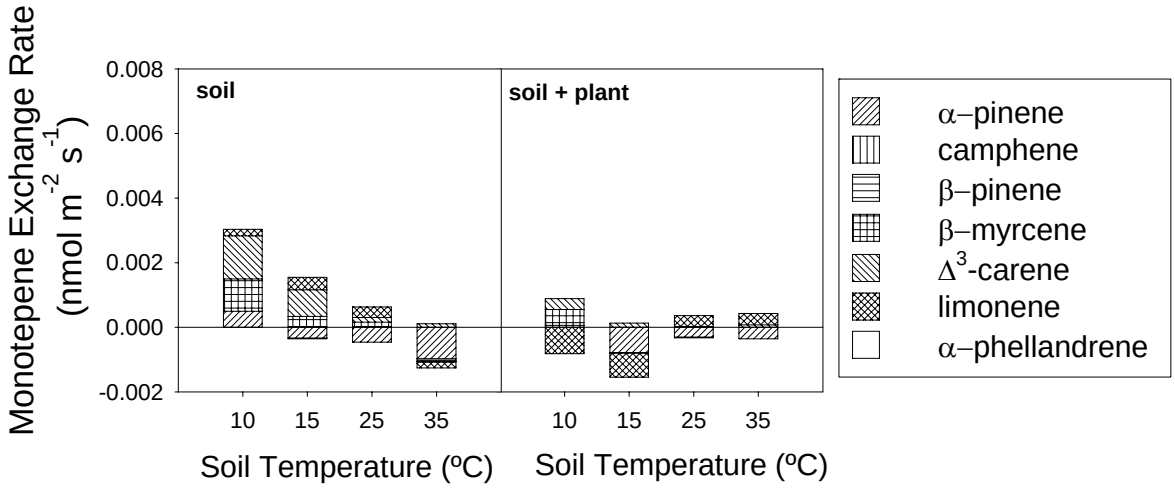

Figure 4.5. Relative composition of measured monoterpenes during the soil temperature treatment in the 'soil' and 'soil + plant' pots.

Other Compounds

The responses to soil temperature of individual compounds are available in the Supplementary Table 2. In the soil temperature treatment, an increased positive mass exchange rate was the common response to the increases in soil temperature. Most of the masses which presented a significant exchange rate response to soil temperature (such as M55, ethyl acetate, M91, M157, M167 and M195 in Supplementary Table 2) followed this trend.

The effect of the roots, when significant (Supplementary Table 2), was the same that in the soil moisture treatment. We found greater VOC uptake in the 'soil + plant' measurements than in the 'soil' measurements (Supplementary Table 2). Only some masses presented lower uptake in the 'soil + plant' than in the 'soil' measurements (M105, M116 and M190, not shown).

Apart from the masses given in Supplementary Table 2, there were other masses with significant exchange rate responses to the soil temperature and to the roots presence which followed the main trends described in this Supplementary Table 2. For example, M20, M30, M36, M38, M40, M111, M163, M169 and M192 exchange rate increased when rising soil temperatures. M104, M111, M124, M146, M151, M154, M158, M163, M174 and M186 uptake was also larger in the presence of roots.

4.3.3. Soil and leaf CO_2 exchange

Soil CO_2 emissions increased when soil moisture and temperature were increased (soil moisture $p< 0.005$; soil temperature $p< 0.0001$, Fig. 4.6). The highest CO_2 exchange rates were measured at 35ºC soil temperature in the 'soil + plant' measurements (27.88 ± 10.34 μmol m^{-2} s^{-1}). Minimum soil CO_2 production rates were found when soil moisture and temperature were the lowest (5% and 10ºC respectively, Fig. 4.6). These values ranged from 2.27 to 4.22 μmol m^{-2} s^{-1}. Soil CO_2 production was higher in 'soil + plant' than in 'soil' pots, although the differences were statistically significant only in the soil moisture treatment (roots $p< 0.0005$, Fig. 4.6).

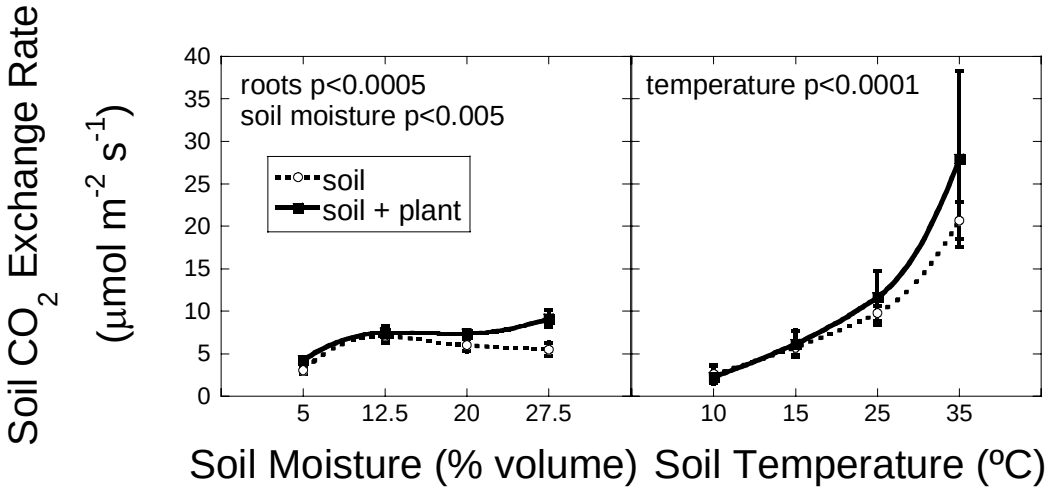

Figure 4.6. Soil CO_2 fluxes during the soil moisture and temperature treatments in the 'soil' and 'soil + plant' pots. Error bars indicate ± standard error of the mean (n=3 in 'soil' pots and n=3 in 'soil + plant' pots, for both the soil moisture and the temperature treatment). Significances for the overall effect of the soil moisture and temperature treatments and the roots presence (ANOVA) are indicated inside the panels.

Like for soil CO_2 exchange rates, net photosynthetic rates were higher in the soil temperature treatment than in the soil moisture treatment (maximum rates: 5.85 ± 0.31 μmol m^{-2} s^{-1} and 2.86 ± 0.23 μmol m^{-2} s^{-1} respectively, Fig. 4.7). Soil temperature and moisture conditions for these maximum rates were 10-15ºC in the soil temperature treatment and at 20% moisture in the soil moisture treatment. Stomatal conductance followed photosynthetic rate variations. They were maximum at 10ºC in the soil temperature treatment and 20% (v/v) in the soil moisture treatment (Fig. 4.7).

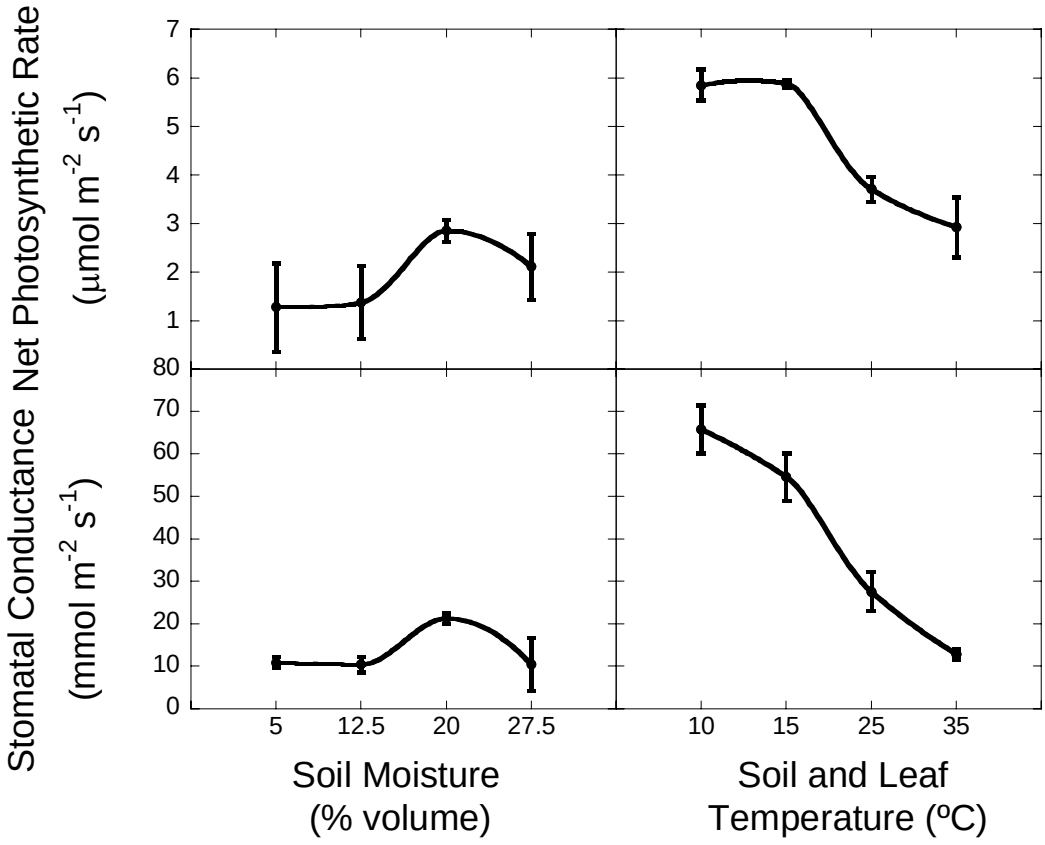

Figure 4.7. Net photosynthetic rates and stomatal conductances in *Quercus ilex* leaves during the soil moisture and temperature treatments. Error bars indicate ± standard error of the mean (n=3 leaves for both the soil moisture and the temperature treatment).

Table 4.1 Volatile compound concentrations (ppb) in air during the soil and soil + plant measurements in the soil moisture treatment. Data are means with SEM; n=3. Only those identified masses whose exchange rate responded significantly to soil moisture or/and roots are depicted.

	5%		12.5%		20%		27.5%	
	soil	soil+plant	soil	soil+plant	soil	soil+plant	soil	soil+plant
M31 (formaldehyde)	6.04±0.43	6.24±0.05	5.84±0.31	5.34±0.32	5.85±0.25	6.05±0.36	6.09±0.34	6.58±0.34
M33 (methanol)	88.95±16.73	78.56±6.28	93.35±20.67	77.48±10.75	78.45±14.98	69.12±12.29	73.23±9.56	93.67±19.69
M43 (acetic acid)	75.58±33.30	18.84±3.53	28.49±9.01	69.13±55.20	20.86±4.63	87.46±62.72	17.29±2.35	68.06±41.51
M45 (acetaldehyde)	7.40±1.34	7.05±0.89	6.09±0.16	5.55±0.49	7.16±0.73	6.40±0.49	6.92±0.90	7.05±0.40
M47 (etanol)	9.90±1.33	6.74±0.25	8.11±1.03	6.28±0.31	9.46±1.83	7.11±0.29	6.79±0.27	7.24±0.82
M57 ((E)-2-hexenal)	9.27±1.21	8.06±0.76	7.43±0.81	7.55±1.10	7.04±0.90	7.73±0.49	7.92±0.62	7.86±0.62
M59+M41 (acetone)	23.81±3.15	21.85±0.85	19.48±0.89	18.23±1.38	21.48±2.67	26.30±1.47	18.86±1.47	19.53±1.01
M79 (benzene)	0.48±0.05	0.40±0.05	0.47±0.06	0.51±0.11	0.39±0.06	0.50±0.10	0.36±0.02	0.34±0.12
M83 (hexanol + hexenol)	1.23±0.26	1.16±0.13	1.08±0.05	1.47±0.65	0.91±0.14	0.96±0.03	1.07±0.01	1.01±0.23
M93 (toluene)	1.22±0.35	0.50±0.02	1.02±0.11	0.99±0.26	0.83±0.06	1.21±0.59	0.54±0.07	0.86±0.27
M107 (xilene)	1.35±0.40	0.92±0.04	1.17±0.11	1.21±0.31	0.86±0.06	1.29±0.15	1.04±0.10	1.09±0.30
M123 (sesquiterpenes)	0.18±0.04	0.15±0.02	0.10±0.05	0.10±0.01	0.12±0.01	0.18±0.01	0.12±0.00	0.14±0.02
M135 (p-cymene)	0.24±0.06	0.29±0.01	0.21±0.06	0.17±0.06	0.21±0.08	0.22±0.02	0.26±0.06	0.29±0.02
M136 (p-cymene)	0.06±0.01	0.04±0.01	0.04±0.01	0.05±0.01	0.05±0.02	0.06±0.01	0.04±0.02	0.04±0.01
M137 (monoterpenes)	0.55±0.16	0.35±0.09	0.41±0.05	0.30±0.08	0.40±0.03	0.35±0.13	0.42±0.07	0.44±0.11
M155 (linalool)	0.07±0.01	0.06±0.02	0.05±0.01	0.03±0.00	0.04±0.01	0.02±0.02	0.02±0.00	0.03±0.01
M157 C10 (unsaturated alcohols)	0.08±0.01	0.05±0.01	0.07±0.03	0.07±0.03	0.10±0.02	0.08±0.02	0.11±0.01	0.07±0.02

Table 4.2 Volatile compound concentrations (ppb) in air during the soil and soil + plant measurements in the soil temperature treatment. Data are means with SEM; n=3. Only those identified masses whose exchange rate responded significantly to soil temperature or/and roots are depicted.

	10ºC		15ºC		25ºC		35ºC	
	soil	soil+plant	soil	soil+plant	soil	soil+plant	soil	soil+plant
M55 (1, 3 butadiene/water cluster)	198.73±82.14	188±45.06	149.85±25.81	130.21±24.99	197.80±28.81	189.83±67.74	186.81±60.65	131.05±22.94
M71 (ethyl acetate)	2.99±0.86	2.60±0.75	2.43±0.42	2.48±0.74	3.92±1.60	2.83±0.63	3.90±1.31	2.68±0.61
M91 (PPN)	0.52±0.16	0.54±0.18	0.53±0.21	0.56±0.20	0.73±0.30	0.55±0.11	0.93±0.39	0.49±0.21
M103 (hexenol)	0.17±0.04	0.24±0.05	0.05±0.01	0.13±0.03	0.15±0.03	0.07±0.02	0.15±0.04	0.14±0.02
M109 (sesquiterpenes)	0.69±0.28	0.86±0.19	0.77±0.04	0.89±0.09	0.95±0.09	0.88±0.05	1.16±0.05	1.32±0.16
M125 (C9 aldehydes)	0.15±0.05	0.25±0.06	0.21±0.02	0.27±0.03	0.19±0.09	0.22±0.04	0.35±0.06	0.16±0.00
M135 (p-cymene)	0.59±0.23	0.64±0.14	0.42±0.02	0.38±0.10	0.45±0.24	0.54±0.11	0.64±0.27	0.57±0.11
M137 (monoterpenes)	1.03±0.43	0.74±0.23	0.70±0.47	0.58±0.33	0.70±0.30	0.52±0.15	0.84±0.17	0.54±0.17
M157 (C10 unsaturated alcohols)	0.11±0.07	0.17±0.07	0.08±0.04	0.10±0.03	0.12±0.05	0.06±0.01	0.07±0.02	0.12±0.02
M167 (C12 aldehydes)	0.09±0.05	0.08±0.03	0.03±0.02	0.04±0.01	0.05±0.02	0.04±0.01	0.04±0.03	0.03±0.02
M171 C11 (unsaturated alcohols)	0.03±0.02	0.12±0.08	0.03±0.01	0.04±0.01	0.05±0.01	0.07±0.03	0.06±0.04	0.03±0.00
M195 (geranyl acetone)	0.05±0.04	0.03±0.02	0.01±0.01	0.02±0.01	0.01±0.01	0.01±0.01	0.02±0.01	0.04±0.02
M199 C13 (unsaturated alcohols)	0.04±0.01	0.03±0.02	0.00±0.00	0.02±0.00	0.02±0.01	0.03±0.02	0.03±0.02	0.03±0.02

4.4. Discussion

4.4.1. Mediterranean soil: sink more than source for VOCs.

Results showed the studied soil as behaved a sink rather than as a source of VOCs, since uptake overcame total soil VOC emissions in both soil moisture and temperature treatments (Fig. 4.2 and Fig. 4.4). However, the gradient in chemical potential between the soil and the atmosphere drives the soil-atmosphere gas exchange diffusive process, and the direction and magnitude of this gradient is determined by the concentrations in the soil and air and by the soil/air equilibrium partitioning coefficient K_{SA} (Hippelein and McLachlan, 1998). Thus, soil VOCs influx or efflux are concentration-dependent. In order to achieve maximum similarity with natural soil conditions, we have used atmospheric air regardless of the associated variability. The rates reported here depend on the concentrations of each VOC in the atmosphere during the soil VOCs sampling and these vary from day-to-day and between experiments (Table 1 and Table 2) introducing a large variability what makes conservative our conclusions on the treatments effects.

Monoterpene exchange rates measured in this experiment were concordant with exchange rates measured in the same soil type in natural conditions (Asensio et al., 2007) which ranged from –0.004 nmol m^{-2} s^{-1} to 0.004 nmol m^{-2} s^{-1}. Nonetheless, they were lower than those reported in previous studies of monoterpene emissions from soils i. e. 0.026 nmol m^{-2} s^{-1} (Hayward et al. 2001). This could be related to the environmental differences between a Mediterranean holm oak forest and a Sitka spruce plantation, e. g. plant species composition, lower biomass and drier conditions which all affect soil microbial activity. In any case, we also found limonene and α-pinene were the most abundant compounds (Hayward et al., 2001).

Most compounds observed in this study to be exchanged by this Mediterranean soil were oxygenated VOCs like alcohols, aldehydes and ketones and aromatic hydrocarbons. Other volatiles such as acetic acid or ethyl acetate were also observed. The compounds which presented the highest soil uptake rates were methanol and acetic acid (maximum uptake rates from –0.8 nmol m^{-2} s^{-1} to –0.6 nmol m^{-2} s^{-1}). Methanol is known to be degraded by different aerobic soil bacteria such methylotrophic bacteria, acidophilic bacteria or the ubiquitous saprophytic bacteria of the genus *Pseudomonas* (Boyd et al., 2000; Trotsenko et al., 2001). Aliphatic low molecular weight organic

acids, such acetic, constitute part of root exudates (Strobel, 2000) and they are known to be degraded by bacteria in soil forests (Evans, 1998).

Acetone was greatly emitted in 'soil' pots (0.4 nmol m^{-2} s^{-1}, Supplementary Table 1) when soil moisture was the lowest. Schade and Goldstein (2001) found significant soil and litter emissions of acetone in a ponderosa pine plantation. However little emissions of acetone were measured by Steeghs et al. (2004) from hairy roots cultures of *Arabidopsis* (0.02 nmol m^{-2} s^{-1}) when they are compared with our results in 'soil + plant' pots (0.11 nmol m^{-2} s^{-1}, Supplementary Table 1). Low exchange rate ranges from -0.04 nmol m^{-2} s^{-1} to 0.02 nmol m^{-2} s^{-1} were also observed for formaldehyde, acetaldehyde, ethanol, (E)-2-hexenal and ethyl acetate. More information about which compounds are exchanged and how biotic and abiotic factors can affect their exchange processes is thus needed.

4.4.2. Soil moisture and temperature effects on soil VOCs exchange

Increasing soil moisture resulted in higher soil sink activity. In parallel, we measured higher CO_2 exchange rates and net photosynthetic rates, corresponding to greater microbial and plant activity. Biodegradation of VOCs by soil microorganisms or roots could be responsible for the soil sink activity (Schade and Custer, 2004; Cho et al., 2005; Van Roon et al. 2005) as well as physical adsorption of VOCs to water, soil particles surface or organic matter and dissolution (partitioning) of VOCs in water (Pignatello and Xing, 1996).

The experimental increase of soil temperature enhanced the emission rates of some volatile compounds (Supplementary Table 2) but this trend was not appreciable in the total soil VOCs exchange (Fig. 4.4). The exponential increase of soil CO_2 exchange rate measured during the soil temperature experiment (Fig. 4.6) implies higher microbial activity and possibly higher microbial consumption activity, as occurred during the soil moisture treatment. Therefore, during the soil temperature treatment VOCs uptake is also likely to be enhanced but at high temperatures (25-35ºC) the rate of volatilisation might be higher than the uptake rate, resulting in a increasingly positive exchange rate balance in some compounds (Supplementary Table 2). As also for the soil moisture treatment, the experimental design does not enable us to discern the processes behind these changes. Further research is thus warranted.

4.4.3. Role of roots in soil VOCs exchange.

The results indicate that the presence of roots influences the flux. Roots bring down the emission rates of many compounds under increasing soil moisture and under increasing soil temperature (Supplementary Tables 1 and 2). The exudates released by roots can stimulate rhizospheric microbial population (Hamilton and Frank, 2001; Valé et al., 2005) and consequently VOCs consumption might be increased. The enhancement of microbial activity by root exudates is also supported by higher soil CO_2 emissions in the 'soil + plant' pots than in the 'soil' pots, during the soil moisture treatment (roots $p< 0.0005$, Fig. 4.6). This enhancement of microbial activity by roots could facilitate the uptake of limiting soil resources by plants (Hamilton and Frank, 2001). Root exudation is quickly assimilated by microbial population in the rhizosphere, thus increasing the rates of decomposition and thereby assimilable nutrients in soil. During the soil temperature treatment there was not a significant root effect on the soil CO_2 exchange measurements nor on the total soil VOCs exchange rates (Fig 4.6 and Fig. 4.4). These results suggest that roots play a key role in soil VOCs exchange increasing bacterial metabolism, especially when water is the limiting factor, which is a common situation in the studied Mediterranean ecosystem (Ogaya et al. 2003).

There were also a few cases in which roots increased the emission rates both in the soil moisture (M157, M160, M166 and M169) and the soil temperature treatment (M116 and M190). In all of them net emissions were very low, as it is shown for M157 in Supplementary Table 1 (range from 0 to 0.0005nmol m^{-2} s^{-1}). This confirms the potential ability of roots to emit volatile compounds, although in natural soils these emissions could be hidden by microbial consumption activity.

4.5. Conclusions and final remarks

The studied Mediterranean soil behaved more as a sink of VOCs than as a source. Under the most standard soil moisture and temperature conditions i. e. 20% v/v and 20ºC, there was overall VOC uptake. An increase in soil moisture produced an enhancement of the uptake activity of microorganisms or/ and an increase of physical passive storage of VOCs. Conversely, an increase in soil temperature increased VOCs emissions. Both biological sources (microorganisms or roots) or physical factors (volatilisation) seem involved in these responses.

Most VOCs increase their soil uptake at higher soil moistures. This was the response of formaldehyde, methanol, acetaldehyde, ethanol, acetone, hexanol + hexenol, monoterpenes, M93 and M155. Also greater uptake in presence of roots than in their absence was the common trend in VOCs such acetic acid, (E)-2-hexenal, benzene, xylene, p-cymene, C10 unsaturated alcohols, acetone, monoterpenes, M93 and M123 during the soil moisture treatment.

During the soil temperature treatment, an increasingly positive VOC exchange rate in response to increases in temperature was the principal trend detected in ethyl acetate, PPN, C10 unsaturated alcohols, C12 aldehydes, hexenol, p-cymene and M195, among other masses. Similarly to the soil moisture treatment, greater compound uptake in presence of roots than in their absence (e. g. in hexenol, C9 aldehydes, p-cymene, C11 unsaturated alcohols, C13 unsaturated alcohols and M109) was the principal trend when increasing soil temperature.

Results confirm the link between roots and microbial activity, especially when soil moisture was the limiting factor. We suggest that the role of roots on soil VOCs exchange was indirect by releasing exudates which enhanced microbial VOCs consumption activities.

VOCs showed low exchange rates. Methanol was the most uptaken compound because it presented the highest concentration in air, and also possibly due to the activity of aerobic methanotrophic bacteria, which are frequent in rhizosphere and phyllosphere.

Coupled GCM and ecophysiology models predict soil warming and lower water availability in the Mediterranean region (IPCC 2001; Sabaté et al., 2002; Peñuelas et al., 2005a) for the next decades. Our results suggest an enhancement of some soil VOCs emission in response to this increase in soil temperature and the reduction of soil VOCs uptake in response to low water availability. Although our results suggest a small significance of these soil VOCs exchange for the atmospheric chemistry, the climate change effects on soil VOCs exchange could significantly influence soil ecology. A wide variety of interactions might occur, so further research is warranted in this area.

Supplementary material.

Supplementary Table 1. Soil VOC exchange rates measured during the soil moisture treatment. Examples of compounds which showed a significant exchange rate response to soil moisture increases and to the roots presence. Data are means ± SEM; n=3 in 'soil' and 'soil + plant' pots. Significance for the overall effect of the soil moisture treatment and the roots presence (ANOVA) is indicated for each mass.

	significance		5%		12.5%		20%		27.5%	
	Moisture	Roots	soil	soil+plant	soil	soil+plant	soil	soil+plant	soil	soil+plant
M31 (formaldehyde)	p=0.05		-0.0322±0.0019	-0.0314±0.0026	-0.0307±0.0037	-0.0290±0.0021	-0.0337±0.0019	-0.0356±0.0028	-0.0352±0.0039	-0.0413±0.0034
M33 (methanol)	p<0.01		0.1240±0.1575	-0.2792±0.1561	-0.6283±0.2916	-0.6422±0.0917	-0.6384±0.1285	-0.5760±0.1061	-0.5918±0.0768	-0.8051±0.1686
M43 (acetic acid)		p<0.05	0.1851±0.0421	-0.0211±0.0351	-0.0793±0.0957	-0.0533±0.4414	-0.0850±0.0358	-0.5342±0.3924	-0.0680±0.0198	-0.4915±0.3303
M45 (acetaldehyde)	p<0.05		0.0169±0.0375	0.0113±0.0065	-0.0007±0.0134	-0.0012±0.0113	-0.0264±0.0087	-0.0204±0.0038	-0.0279±0.0061	-0.0328±0.0031
M47 (etanol)	p<0.05		-0.0135±0.0055	-0.0096±0.0052	-0.0277±0.0089	-0.0247±0.0046	-0.0546±0.0223	-0.0347±0.0010	-0.0240±0.0079	-0.0361±0.0087
M57 ((E)-2-hexenal)		p<0.1	0.0026±0.0125	-0.0030±0.0091	0.0158±0.0066	-0.0010±0.0084	0.0068±0.0044	-0.0058±0.0053	-0.0064±0.0036	-0.0123±0.0036
M59+M41 (acetone)	p<0.001	p<0.05	0.4365±0.1681	0.1060±0.0216	0.0516±0.0919	-0.0388±0.0263	-0.0880±0.0186	-0.1402±0.0089	-0.0718±0.0067	-0.1171±0.0086
M79 (benzene)		p<0.05	0.0010±0.0005	-0.0005±0.0007	0.0007±0.0013	-0.0016±0.0006	-0.0006±0.0002	-0.0021±0.0008	-0.0015±0.0003	-0.0012±0.0007
M83 (hexanol + hexenol)	p=0.1		0.0031±0.0027	0.0054±0.0044	0.0009±0.0005	0.0017±0.0017	0.0001±0.0016	-0.0005±0.0012	-0.0009±0.0008	-0.0018±0.0020
M93 (toluene)	p<0.05	p<0.05	0.0065±0.0044	0.0033±0.0019	0.0062±0.0034	-0.0009±0.0006	-0.0010±0.0014	-0.0036±0.0020	0.0007±0.0003	-0.0024±0.0013
M107 (xilene)		p<0.05	0.0045±0.0067	-0.0024±0.0009	0.0048±0.0029	-0.0037±0.0013	-0.0008±0.0009	-0.0048±0.0007	-0.0047±0.0011	-0.0049±0.0016
M123 (sesquiterpenes)		p<0.1	0.0000±0.0003	0.0001±0.0004	0.0005±0.0006	0.0003±0.0002	0.0005±0.0003	-0.0004±0.0001	0.0004±0.0002	-0.0003±0.0003
M135 (p-cymene)		p<0.01	0.0024±0.0019	-0.0006±0.0005	0.0016±0.0005	0.0007±0.0004	0.0017±0.0001	-0.0006±0.0005	-0.0001±0.0005	-0.0011±0.0002
M136 (p-cymene)		p<0.1	0.0004±0.0002	0.0001±0.0002	0.0002±0.0002	0.0000±0.0001	0.0002±0.0002	-0.0001±0.0004	0.0004±0.0004	-0.0001±0.0002
M155 (linalool)	p<0.05		0.0001±0.0002	-0.0003±0.0002	0.0005±0.0000	0.0003±0.0002	0.0003±0.0000	0.0006±0.0003	0.0002±0.0002	0.0003±0.0002
M157 C10 (unsaturated alcohols)		p<0.1	0.0000±0.0003	0.0003±0.0002	0.0000±0.0001	0.0004±0.0001	0.0002±0.0003	0.0003±0.0002	-0.0003±0.0001	0.0002±0.0003

Supplementary Table 2. Soil VOC exchange rates measured during the soil temperature treatment. Examples of compounds which showed a significant exchange rate response to soil temperature increases and to the roots presence. Data are means ± SEM; n=3 in 'soil' and 'soil + plant' pots. Significance for the overall effect of the soil temperature treatment and the roots presence (ANOVA) is indicated for each mass. (PPN: Peroxypropionic Nitric anhydride). T x R, interaction between temperature and root presence.

	significance			10ºC		15ºC		25ºC		35ºC	
	Temperature	Roots	T x R	soil	soil+plant	soil	soil+plant	soil	soil+plant	soil	soil+plant
M55 (1, 3 butadiene/water cluster)	p<0.05			-0.8929±0.3510	-0.6510±0.2609	-0.1959±0.2339	-0.3730±0.1702	-0.3375±0.2798	-0.5824±0.4038	0.0507±0.1176	0.1913±0.2963
M71 (ethyl acetate)	p<0.05			-0.0118±0.0034	-0.0105±0.0021	-0.0025±0.0028	-0.0064±0.0053	-0.0047±0.0048	-0.0035±0.0028	-0.0009±0.0030	-0.0002±0.0008
M91 (PPN)	p=0.05			-0.0010±0.0013	-0.0010±0.0013	0.0022±0.0015	-0.0012±0.0012	0.0065±0.0049	0.0021±0.0011	0.0051±0.0023	0.0032±0.0014
M103 (hexenol)	p<0.01	p<0.05	p<0.05	-0.0006±0.0005	-0.0021±0.0002	0.0004±0.0002	-0.0006±0.0003	-0.0009±0.0001	-0.0004±0.0003	-0.0007±0.0004	-0.0010±0.0002
M109 (sesquiterpenes)		p<0.05		-0.0018±0.0019	-0.0038±0.0007	-0.0006±0.0021	-0.0031±0.0003	-0.0006±0.0007	-0.0009±0.0008	-0.0006±0.0017	-0.0040±0.0007
M125 (C9 aldehydes)		p<0.05	p<0.05	0.0004±0.0004	-0.0016±0.0002	-0.0002±0.0010	-0.0009±0.0001	0.0011±0.0007	-0.0005±0.0005	-0.0005±0.0001	0.0006±0.0004
M135 (p-cymene)	p<0.01	p<0.05		-0.0022±0.0012	-0.0053±0.0005	0.0000±0.0008	-0.0002±0.0011	0.0000±0.0013	-0.0017±0.0007	-0.0020±0.0014	-0.0030±0.0008
M157 (C10 unsaturated alcohols)	.p<0.05			-0.0006±0.0005	-0.0011±0.0005	0.0002±0.0003	0.0000±0.0004	0.0002±0.0005	0.0010±0.0002	0.0005±0.0002	-0.0001±0.0003
M167 (C12 aldehydes)	p<0.05			-0.0005±0.0001	-0.0005±0.0002	-0.0002±0.0002	0.0001±0.0001	0.0002±0.0003	0.0003±0.0001	0.0001±0.0003	0.0000±0.0002
M171 C11 (unsaturated alcohols)		p<0.05	p<0.1	0.0003±0.0003	-0.0006±0.0003	0.0004±0.0002	-0.0001±0.0001	0.0004±0.0003	-0.0003±0.0001	-0.0004±0.0003	0.0001±0.0003
M195 (geranyl acetone)	p<0.05			-0.0005±0.0003	-0.0003±0.0001	0.0000±0.0001	-0.0001±0.0001	0.0003±0.0002	0.0002±0.0001	-0.0002±0.0001	0.0001±0.0001
M199 C13 (unsaturated alcohols)		p<0.05		-0.0002±0.0001	-0.0004±0.0001	0.0003±0.0001	-0.0002±0.0000	-0.0001±0.0002	-0.0002±0.0002	0.0001±0.0001	-0.0002±0.0001

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Chapter 5

The distribution of volatile isoprenoids in the top soil horizons around *Pinus halepensis* trees in field conditions

Abstract

A considerable proportion of rhizosphere sources of volatile isoprenoids may not diffuse through soil to the atmosphere. However, little is known about the quantitative and qualitative distribution of volatile isoprenoids in different soil systems. We measured the terpene concentration in pentane and water extracts from different soil layers (litter, organic, mineral top, mineral low) and from roots in top and low mineral layers on a distance gradient from three different *Pinus halepensis L.* trees.

The litter layer and top and low roots were identified as the largest sources of terpenes in soil. The most abundant terpenes in soil horizons were α-pinene, β-pinene, sabinene, β-caryophyllene and α-humulene. Alpha-pinene dominated the total terpene concentration in top (0.3 ± 0.1 mg g^{-1} or 38 ± 14 mg m^{-2}) and low roots (0.5 ± 0.2 mg g^{-1} or 14 ± 5 mg m^{-2}). However, β-caryophyllene was the most dominant compound in litter (3 ± 1 mg g^{-1} or 4336 ± 1002 mg m^{-2}). Alpha-pinene was also the dominant compound in the top and low mineral soil layers (4.1 ± 1.8 mg g^{-1} and 3.5 ± 1.7 mg g^{-1} respectively) but there were also relatively high concentrations of β-caryophyllene (2.3 ± 0.8 mg g^{-1} and 0.7 ± 0.3 mg g^{-1} in top and low mineral respectively). Terpene concentration in different soil layers decreased with increasing distance to the trunk, and this is related to changes in litter and roots type (from pine to grasses) on the distance gradient. Results suggest that litter could be a less variable source of terpenes for deeper soil layers than roots. There were differences between the α-pinene distribution and the distribution of other terpenes in the different soil layers, suggesting that litter of *P. halepensis* is probably the main source of the major terpene compounds, and in addition, long term emissions of α-pinene from *P. halepensis* roots also contribute to the α-pinene concentration of rhizosphere soils.

Key words: soil horizons, volatile isoprenoids, *Pinus halepensis*, *Pinus* root extracts, *Pinus* litter extracts, soil terpene distribution.

5.1. Introduction

Among the biogenic volatile organic compounds (BVOCs), volatile isoprenoids are the most abundant VOCs synthesised and emitted by plants. Isoprenoids, which consist of sequential combinations of a five-carbon structure units, isoprene (C_5H_8), have important biochemical, physiological and ecological functions which are essential for healthy function and survival in plant species (reviewed by Kesselmeier and Staudt, 1999; Owen and Peñuelas, 2005). In this study, we focus on monoterpenes and sesquiterpenes which are part of the large isoprenoid group and occur in almost all plants. Monoterpenes have two isoprene units ($C_{10}H_{16}$) and may be linear (acyclic) or contain a ring while sesquiterpenes have three isoprene units ($C_{15}H_{24}$) (Croteu, 1987; Kesselmeir and Staudt 1999, Peñuelas and Llusià, 2001). They have characteristic odours and they are only slightly soluble in water. Besides their physiological and ecological roles, they are emitted in significant amounts to the atmosphere (Guenther et al 1995; Peñuelas and Llusià, 2001) having significant roles in the chemistry and physics of the atmosphere (Fehsenfeld et al, 1992; Fuentes et al., 2000; Peñuelas and Llusià, 2003)

These volatile isoprenoids are also present in soil (White, 1991; 1994; Wheatley et al., 1996) where they have important ecological roles. They may be involved in nutrient cycling and may determine the rhizosphere community dynamics and structure. For example, White (1991, 1994) found certain monoterpenes could inhibit the net mineralization of nitrogen and net nitrification in soil. Misra et al. (1996) and other authors (Kleinheinz et al. 1999; Demyttenaere et al. 2000; Vokou et al., 2002; Yoo and Day 2002; Owen et al., 2007) found monoterpenes from forest-soil extracts and enriched cultures can be utilized by some soil microflora as growth substrates. These findings demonstrate the effect of terpenes on the catabolism kinetics of microbial populations. Volatiles in soil also are key compounds in the interactions between living soil organisms. Soil VOCs act as infochemicals in plant-insect relations when they are transmitted through the rhizosphere (Chamberlain et al., 2001; Nordenhem and Nordlander, 1994), and in plant-plant interactions, as for example when the monoterpenes produced by *Salvia leucophylla* have allelopathic effects by inhibiting cell proliferation and DNA synthesis in the root apical meristem of *Brassica campestris* seedlings (Nishida et al., 2005). Other VOC-mediated interaction in soils occur between bacteria and fungi (Mackie and Wheatley, 1999), in mycorrhiza (Raparinni et al., 2007),

and between bacteria and plants or fungi and plants (Ruy et al., 2003; Akiyama and Hayashi, 2006; Splivallo et al. 2007).

A considerable proportion of rhizosphere sources of volatile isoprenoids may not diffuse through soil to the atmosphere. It has been shown that soil terpene exchange rates with the atmosphere are low (Hayward et al., 2001; Asensio et al., 2007) and that observed rates of emission, when there is emission, are lower than those of uptake, when there is uptake (Asensio et al., 2007). However, little is known about the quantitative and qualitative distribution of volatile isoprenoids in different soil systems (White, 1991; 1994). The relative importance of different sources is also not known, but Janson et al. (1993) suggest roots as a possible source of monoterpenes in soil. Recently this was supported by measurements of monoterpene emissions in laboratory experiments from pine roots with qualitative evidence of the existence of monoterpenes in soils under pine trees (Lin et al., 2007). Previous studies have also explored the effect of the distance from a tree on the amount of monoterpenes found in the rhizosphere (Lin et al., 2007) and on microbial monoterpene consumption rates (Owen et al., 2007). Both studies found evidence of a gradient from high levels of monoterpenes and microbial consumption at the base of the trunk to lower levels of activity at the furthest distance. But not much is known about terpenes concentrations in different horizons, on whether there is a natural undisturbed root or litter source for soil content.

The aims of this work were: (i) to quantify the relative concentrations of terpenes in different soil layers (litter, organic layer, mineral layer directly underlying the organic layer, mineral layer 20 cm deep, and root material in the 2 mineral layers), and along a distance gradient from the trunk of a tree; (ii) to determine the pentane soluble fraction, i.e. the fraction likely to be colloid bound in the soils system or extractable from the air space; (iii) to quantify the water soluble fraction, i.e. the fraction more directly available in aqueous solution to soil microfauna; (iv) to investigate the effect of two variables that might affect the amount of volatile isoprenoid in the different soil horizons: namely (1) the fraction by weight of root material in the two mineral layers, and (2) the amount of litter per unit surface area; and (v) to discern between roots and litter as sources of soil BVOC concentrations.

5.2. Material and methods

5.2.1. Study site and experimental design

This study was performed at the campus of the Autonomous University of Barcelona (41º 30' N, 2º 6º E) in a 2.3 ha grassland. The vegetation consisted principally in Leguminosae herbs like *Trifolium repens L.*, *Psoralea bituminosa L.*, *Medicago minima (L.) Bartal.*, other herbs like *Plantago lanceolata L.* and grasses (*Lolium perenne L.*, *Brachypodium phoenicoides R. & S.* and *Bromus intermedius Guss.*). There were also isolated *Pinus halepensis L.* trees. The climate is Mediterranean, with wet springs and autumns and dry winters and summers, with mean annual temperature of 16ºC and mean annual rainfall of 575 mm. The soil texture type was silty-clay with high proportion of carbonates (pH 8) and high amounts of organic matter in the first soil horizons near the *Pinus* trees. The three pines showed similar ground surface covered by the crown vertical projection (mean large diameter = 18 m, mean small diameter = 10 m) and were of similar age (mean trunk diameter = 1.27 m). In each transect we marked 3 sampling points: point 1 which was nearest to the trunk, point 2 at 3 m from the trunk and point 3 which was furthest from the trunk, at 10 m. At each point, we collected soil material from the different horizons (litter, organic, mineral top, mineral low), and roots material from both top and low mineral horizons. All transects carried out were on the same direction E-W.

5.2.2. Field sampling

The field sampling was carried out during autumn, between the end of September 2006 and the middle of November 2006. Tree 2 soil samples were collected first on September 21, 26 and 29 (one transect per day), followed by transect replicates of tree 1 (October 23, 25 and 29) and finally tree 3 (November 9, 15 and 17). All transects were sampled in the morning of sunny or slightly cloudy days, between 9 am and 13 pm

At each point, a 0.0625 m^2 quadrat was placed on the soil surface and the total amount of litter and organic layers within the quadrat was collected. Below the organic layer, a sample of mineral soil material was collected at approximately 0-10 cm depth. Part of this top mineral soil sample was sieved (5 mm sieve) in the field immediately after collection for terpene extraction analyses in the laboratory. All the visible root material in the sieved subsample were removed immediately, to avoid terpene contamination of the mineral soil subsamples by broken roots. Then all the top mineral

roots with diameter > 0.5 mm present in the area enclosed by the quadrat were collected. The top 20 cm depth of soil was then removed and the same procedure was repeated at a level 20-30 cm below the surface ("low mineral"). Soil layers and root material collected for terpene extractions were placed in separate sealed glass jars, and stored in a cool chamber (4ºC) prior to terpene extraction (within the following 2 days) and analyses.

The total amounts of soil material collected in the field at each point and layer were gathered in trays and weighed in the laboratory. To determine the density of the soil litter and organic layers (total mass of the soil layer per area unit) we added the total mass in the trays and jars and divided by the area of the quadrat. After weighing the total, a fraction of fresh soil material from each layer was separated to determine water content of each layer. Water content was calculated as the difference between the fresh soil material weight and the dry soil material weight after 5 days in an oven at 60ºC. At each sample point, soil temperature was measured with a soil digital thermometer (TO 15, Jules Richard instruments, Argenteuil, France) at 10 cm depth, just beside the four corners of each quadrat to avoid mechanical disturbances to the enclosed soil. At the same time, soil moisture was measured with a HH2 soil moisture meter connected to a ML2x soil moisture sensor (Delta-T Devices Ltd, Cambridge, England) at 10 cm depth.

5.2.3. Pentane and water solutions for terpene extraction

For pentane extracts, we first prepared a concentrated standard with 50 ml of pure pentane and 160 µL of internal standard dodecane (99% Sigma-Aldrich). 2 ml of this pentane concentrated solution were made up to volume in a 200 ml flask with pure pentane to achieve a final concentration of $1.4 \cdot 10^{-4}$ mol L^{-1} for the internal standard. Distilled water was spiked with dodecane in a similar way for the water extracts. Both pentane and water spiked with internal standard were shaken manually and stored in the cool chamber (4ºC) until needed for terpene extraction. A new stock was made prior to extracting samples for each transect. The spiked solvents were shaken thoroughly before each sample extraction.

5.2.4. Terpene extraction method

Litter and organic layers and root material were placed in Teflon tubes with liquid nitrogen, and crushed mechanically with a Teflon embolus in order to facilitate extraction of terpenes. Sieved soil mineral layers did not need previous mechanical

extraction treatment. The amount of material extracted for litter, organic, mineral and root layers was 2, 3, 10 and 2 g. respectively. For pentane and water extracts, 5 ml of the spiked pentane (or deionised water) was added. Further 5 ml pure pentane (or deionised water) were added. After shaking manually, samples were placed in a mechanical shaking water-bath (WNB 7-45 Memmert, Schawabach, Germany) for 1 h at 25 ºC. Samples were then centrifuged for 15 min at 10000 rpm and 5-10ºC in order to separate the liquid and solid phases. The liquid phase of pentane extracts were then passed through a Pasteur pipette filled with anhydrous sodium sulphate in order to remove water remaining in the extract. The pentane extracts were put into opaque vials and stored at -30ºC until GC-MS analyses. After the centrifuging of water extracts, the decanted liquid phase was mixed with 3 ml of pure pentane in a second centrifuge tube which was sealed and manually shaken, then shaken again in a water bath at 25 ºC and centrifuged to transfer terpenes extracted from soil and roots material from the water to the pentane phase. After the final centrifuging of the water extracts, the top liquid phase (pentane) was pipetted out and passed through two anhydrous sodium sulphate columns to ensure that no water remained in the pentane phase. Finally, water extracts in pentane were decanted into opaque amber glass vials and stored at -30ºC until GC-MS analyses.

5.2.5. GC-MS analyses of terpenes

Soil terpene extract analyses were conducted using a GC-MS (Hewlett Packard HP59822B; Palo Alto, CA, USA). 3 μL of the extract were injected automatically by an automatic sample processor (FOCUS) (ATAS GL International BV 5500 AA Veldhoven, The Netherlands) in an OPTIC3 injector (ATAS GL International BV 5500 AA Veldhoven, The Netherlands) into a 30 m x 0.25 x 0.25 mm film thickness capillary column (HP-5, Crosslinked 5% pH Me Silicone; Supelco Inc.). The injector temperature (200ºC) was increased at 5 ºC s^{-1} to 300ºC. The injected sample was cryofocused at -20ºC for 2 min. After this time, the cryotrap was heated rapidly to 200 ºC. The column temperature (60 °C) was increased at 10 ºC min^{-1} to 150 ºC, and then at 40 ºC min^{-1} to 270 ºC. Helium flow was 0.7 mL min^{-1}. Total run time was 20 min and the solvent delay was 4 min. Identification of terpenes was conducted by GC-MS and by comparison with standards from Fluka (Buchs, Switzerland), literature spectra, using GCD Chemstation G1074A HP. Frequent calibration with standards of common terpenes (α-pinene, 3-carene, β-pinene, β-myrcene, p-cymene, limonene and sabinene) once every five

analyses was used for quantification and QA. Terpene calibration curves (N=4 different terpene concentrations) were always highly significant ($r^2 > 0.99$) in the relationship between signal and terpene concentration. The most abundant terpenes had very similar sensitivity (differences were less than 5%). In order to detect possible column contamination during the analyses, a blank sample of pure pentane was injected and analysed twice, prior to start the GC-MS analysis and at the end of the analyses for each tree. For determining the Method Detection Limit (MDL) we analysed 7 samples of concentration near the expected limit of detection. The standard deviation was then determined. The one-sided t distribution (six degrees of freedom t value = 3.14) was multiplied versus the determined standard deviation. This value resulted 5 ng on column and analyses of amounts in samples ≤ 5 g were considered as 0 g.

5.2.6. Statistical analyses

Analyses of variance (ANOVA) were conducted with terpenes concentration as the dependent variable and with soil layer and transect position as the independent factors. Correlation analyses were carried out between mean concentrations of compounds in the top and low mineral layers and total amounts of those compounds in a) litter b) roots in the low mineral layer and c) roots in the top mineral layer were carried out. These analyses were performed with the *Statview* software package (Abacus Concepts Inc., Cary, North Carolina, USA) and the *Statistica* software package (StatSoft, Inc. Tulsa, Oklahoma). Differences were considered significant at a probability level of $P < 0.05$. Cluster analysis of terpene concentration of pine soils was performed using non-standardized Euclidean distance for measuring multi-dimensional distance between points, and single linkage method was used for the clustering process (*Statistica* Statsoft, Inc. Tulsa, Oklahoma). The distance matrix between the different terpene compounds was calculated with mean concentration of each terpene in the top mineral layer, mean total concentration in the litter layer, mean total concentration in the top roots and mean total concentration in the low roots as input data. The data were transformed where necessary to appropriate units, so that the order of magnitude of the range of values of input data was minimised.

Table 5.1. Concentration of pentane and water extractable terpenes (μg g^{-1} of fresh material) within a horizon soil collections from 3 pine tree soil and in a distance gradient (0 m, 3 m and 10 m) from the trunk of the pine. Values are means ± SE (N=3 replicates per horizon in each sampling distance point). Blank spaces represent the absence of a soil horizon at one distance point. Within a column in each tree, values followed by different letters are significantly different (Post-hoc tests, $P<0.05$ according to the Fisher's test). Horizons in different distance positions followed by different letters in parentheses are significantly different (Post-hoc tests, $P<0.05$ according to the Fisher's test).

PENTANE EXTRACTS sample concentration μg g^{-1} of fresh material

	α-pinene			sabinene			β-pinene			β-caryophyllene			α-humulene		
Tree 1	0 m	3 m	10 m	0 m	3 m	10 m	0 m	3 m	10 m	0 m	3 m	10 m	0 m	3 m	10 m
Litter	1422.8±503.6 b (b)	288.9±32.2 a (a)	64.0±25.2 a (a)	366.1±79.2 b (b)	135.8±27.9 b (a)	15.6±8.0 b (a)	545.6±142.4 b (b)	90.6±16.7 b (a)	8.7±7.3 a (a)	429.4±55.8 b (a)	236.5±25.9 b (b)	34.3±13.5 b (c)	2161.9±465.9 b (b)	804.6±73.0 b (a)	211.8±94.7 b (a)
LM	2.7±1.6 a (a)	0.8±0.7 a (a)	0.0±0.0 a (a)	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
LR	674.3±133.5 ab (ab)	985.6±451.8 b (b)	2.2±2.2 a (a)	3.4±3.4 a (a)	0.0±0.0 a (a)	0.0±0.0 a (a)	45.5±14.9 a (a)	55.7±27.9 b (a)	0.0±0.0 a (a)	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	27.2±9.2 a (b)	10.2±8.9 a (ab)	0.0±0.0 a (a)
TM	4.7±1.0 a (b)	2.3±1.5 a (ab)	0.0±0.0 a (a)	0.1±0.1 a (a)	0.0±0.0 a (a)	0.0±0.0 a (a)	0.9±0.9 a (a)	2.2±2.2 a (a)	0.0±0.0 a (a)	0.0±0.0 a (a)	0.7±0.7 a (a)	0.0±0.0 a (a)	1.3±1.3 a (a)	1.4±1.4 a (a)	0.0±0.0 a (a)
TR	730.1±373.8 ab (a)	179.6±113.8 a (a)	69.4±69.4 a (a)	0.8±0.8 a (a)	0.0±0.0 a (a)	0.0±0.0 a (a)	29.0±15.2 a (a)	11.8±5.9 a (a)	3.2±3.2 a (a)	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	8.3±8.3 a (a)	0.4±0.4 a (a)	0.0±0.0 a (a)
Organic	162.2±142.0 a (a)	29.4±17.6 a (a)		40.1±11.7 a (a)	5.9±3.6 a (b)		1.6±0.8 a 8a)	1.0±1.0 a (a)		29.0±5.8 a (a)	7.7±1.5 a (b)		87.5±31.5 a (a)	8.0±2.4 a (a)	
Tree 2	0 m	3 m	10 m	0 m	3 m	10 m	0 m	3 m	10 m	0 m	3 m	10 m	0 m	3 m	10 m
Litter	841.2±197.9 b (b)	780.5±171.0 b (b)	153.0±13.8 b (a)	440.3±127.3 b (b)	357.6±140.4 b (ab)	59.3±13.5 b (a)	727.1±255.5 b (a)	604.4±210.1 b (a)	86.7±14.9 b (a)	6163.8±1483.4 b (b)	4544.0±990.6 b (ab)	1817.2±173.1 b (a)	1483.4±285.0 b (b)	1114.1±273.8 b (ab)	386.7±35.3 b (a)
LM	22.8±11.1 a (b)	3.8±1.9 a (ab)	0.0±0.0 a (a)	0.1±0.1 a (a)	0.0±0.0 a (a)	0.0±0.0 a (a)	0.5±0.5 a (a)	0.0±0.0 a (a)	0.0±0.0 a (a)	3.8±2.1 a (a)	0.2±0.1 a (a)	0.0±0.0 a (a)	0.3±0.3 a (a)	0.0±0.0 a (a)	0.0±0.0 a (a)
LR	815.7±406.7 b (a)	117.2±93.6 a (a)	0.0±0.0 a (a)	14.5±7.3 a (a)	0.6±0.6 a (a)	0.0±0.0 a (a)	64.6±20.1 a (b)	1.7±1.4 a (a)	0.0±0.0 a (a)	77.5±32.8 a (a)	13.7±12.2 a (a)	11.0±11.0 a (a)	13.1±6.3 a (b)	0.4±0.4 a (a)	0.0±0.0 a (a)
TM	23.8±11.9 a (a)	1.5±0.7 a (a)	0.0±0.0 a (a)	1.3±0.4 a (b)	0.1±0.1 a (a)	0.0±0.0 a (a)	1.0±0.5 a (b)	0.0±0.0 a (a)	0.0±0.0 a (a)	1.8±0.7 a (a)	1.3±0.3 a (a)	3.1±1.3 a (a)	0.1±0.1 a (a)	0.0±0.0 a (a)	0.2±0.2 a (a)
TR	910.4±106.6 b (b)	440.6±326.6 ab (ab)	0.0±0.0 a (a)	11.1±2.4 a (b)	4.9±4.9 a (ab)	0.0±0.0 a (a)	111.0±18.9 a (b)	50.3±44.3 a (ab)	0.0±0.0 a (a)	84.2±20.1 a (b)	18.1±11.8 a (a)	4.3±2.3 a (a)	15.5±3.6 a (b)	2.4±2.2 a (a)	0.0±0.0 a (a)
Organic	4.4±2.2 a (a)	24.3±15.6 a (a)		12.7±4.2 a (a)	10.4±2.6 a (a)		1.4±0.7 a (a)	3.0±1.5 a (a)		62.1±33.9 a (a)	49.4±4.2 a (a)		10.7±6.6 a (a)	7.9±0.7 a (a)	
Tree 3	0 m	3 m	10 m	0 m	3 m	10 m	0 m	3 m	10 m	0 m	3 m	10 m	0 m	3 m	10 m
Litter	807.7±79.7 ab (a)	416.4±114.1 b (b)		32.7±86.2 b (a)	242.9±62.3 b (a)		81.6±6.6 a (a)	43.9±11.3 b (b)		6136.2±2495.8 b	5466.4±1364.5 b		1590.2±715.3 b (a)	1393.3±362.7 b (a)	
LM	1.6±0.6 a (b)	0.1±0.1 a (a)	0.0±0.0 a (a)	0.2±0.2 a (a)	0.0±0.0 a (a)	0.0±0.0 (a)	0.0±0.0 a	0.0±0.0 a	0.0±0.0	1.7±1.7 a (a)	1.0±0.5 a (a)	0.0±0.0 (a)	0.2±0.2 a (a)	0.0±0.0 a (a)	0.0±0.0 (a)
LR	1763.0±878.9 bc (a)	163.1±80.3 a (a)	5.6±5.6 a (a)	41.0±27.8 a (a)	0.1±0.1 a (a)	0.0±0.0 (a)	77.2±45.4 a (a)	5.0±1.2 a (a)	0.0±0.0 (a)	242.6±137.2 a (a)	27.8±14.7 a (a)	0.0±0.0 (a)	41.5±26.6 a (a)	4.5±2.3 a (a)	0.0±0.0 (a)
TM	4.0±0.7 a (a)	0.2±0.2 a (a)	0.0±0.0 a (a)	1.9±1.0 a (a)	0.0±0.0 a (a)	0.0±0.0 (a)	0.0±0.0 a	0.0±0.0 a	0.0±0.0	8.8±5.9 a (a)	5.2±2.8 a (a)	0.0±0.0 (a)	7.2±7.2 a (a)	1.8±1.8 a (a)	0.0±0.0 (a)
TR	590.6±300.9 cd (a)	26.5±13.5 a (a)	5.0±5.0 a (a)	15.2±6.6 a (b)	0.0±0.0 a (a)	0.0±0.0 (a)	125.9±103.0 a (a)	0.5±0.5 a (a)	0.0±0.0 (a)	78.0±39.9 a (a)	4.9±2.5 a (a)	0.0±0.0 (a)	15.9±9.3 a (a)	0.6±0.6 a (a)	0.0±0.0 (a)
Organic	16.1±5.4 ad (a)	9.3±2.6 a (a)		62.7±41.2 a (a)	15.0±6.4 a (a)		0.1±0.1 a (a)	5.4±5.4 a (a)		62.4±31.9 a (a)	201.4±60.3 a (a)		13.6±7.9 a (a)	41.3±8.6 a (a)	

WATER EXTRACTS sample concentration μg g^{-1} of fresh material.

	α-pinene			sabinene			β-pinene			β-caryophyllene			α-humulene		
Tree 1	0 m	3 m	10 m	0 m	3 m	10 m	0 m	3 m	10 m	0 m	3 m	10 m	0 m	3 m	10 m
Litter	2.6±2.6 a (a)	0.0±0.0 (a)	0.3±0.3 a (a)	0.0±0.0	0.0±0.0	0.0±0.0	0.5±0.5 a (a)	0.0±0.0 (a)	0.0±0.0 (a)	53.5±23.9 b (b)	9.7±6.3 a (ab)	1.1±0.6 a (a)	5.0±3.4 a (a)	0.0±0.0 (a)	0.0±0.0 (a)
LM	0.0±0.0 a	0.0±0.0	0.0±0.0 a	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0 a	0.0±0.0	0.0±0.0	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0	0.0±0.0
TM	0.0±0.0 a	0.0±0.0	0.0±0.0 a	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0 a	0.0±0.0	0.0±0.0	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0	0.0±0.0
Organic	0.0±0.0 a	0.0±0.0		0.0±0.0	0.0±0.0		0.0±0.0 a	0.0±0.0		0.0±0.0 a	0.0±0.0 a		0.0±0.0 a	0.0±0.0	
Tree 2	0 m	3 m	10 m	0 m	3 m	10 m	0 m	3 m	10 m	0 m	3 m	10 m	0 m	3 m	10 m
Litter	1.0±0.5 b (a)	0.4±0.3 a (a)	0.0±0.0 (a)	0.2±0.2 a (a)	0.0±0.0 (a)	0.0±0.0 (a)	0.4±0.3 a (a)	0.0±0.0 (a)	0.0±0.0 (a)	2.8±1.5 a (a)	1.7±0.4 b (a)	3.7±3.4 a (a)	1.2±0.6 b (a)	0.5±0.2 b (a)	1.1±1.1 a (a)
LM	0.0±0.0 a	0.0±0.0 a	0.0±0.0	0.0±0.0 a	0.0±0.0	0.0±0.0	0.2±0.2 a	0.0±0.0 (a)	0.0±0.0 (a)	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
TM	0.0±0.0 a	0.0±0.0 a	0.0±0.0	0.0±0.0 a	0.0±0.0	0.0±0.0	0.0±0.0 a	0.0±0.0	0.0±0.0	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
Organic	0.0±0.0 a	0.0±0.0 a		0.0±0.0 a	0.0±0.0		0.0±0.0 a	0.0±0.0		0.0±0.0 a	0.0±0.0 a		0.0±0.0 a	0.0±0.0 a	
Tree 3	0 m	3 m	10 m	0 m	3 m	10 m	0 m	3 m	10 m	0 m	3 m	10 m	0 m	3 m	10 m
Litter	1.1±0.6 a (a)	2.0±1.7 a (a)		0.0±0.0	0.0±0.0 a		0.2±0.2 a (a)	0.0±0.0 (a)		22.5±4.3 b (a)	15.8±0.4 b (a)		3.1±1.2 b (a)	1.6±0.5 b (a)	
LM	0.3±0.3 a (a)	0.1±0.1 a (a)	0.2±0.1 a (a)	0.0±0.0	0.0±0.0 a	0.0±0.0	0.0±0.0 a	0.0±0.0	0.0±0.0	0.0±0.0 a	0.0±0.0 a	0.0±0.0	0.0±0.0 a	0.0±0.0 a	0.0±0.0
TM	0.0±0.0 a (a)	0.0±0.0 a (a)	0.4±0.3 a (a)	0.0±0.0 (a)	0.1±0.1 a (a)	0.0±0.0 (a)	0.0±0.0 a	0.0±0.0	0.0±0.0	0.0±0.0 a	0.0±0.0 a	0.0±0.0	0.0±0.0 a	0.0±0.0 a	0.0±0.0
Organic	0.0±0.0 a (a)	0.1±0.1 a (a)		0.0±0.0	0.0±0.0 a		0.0±0.0 a	0.0±0.0		0.0±0.0 a	0.0±0.0 a		0.0±0.0 a	0.0±0.0 a	

5.3. Results

5.3.1. Terpene concentration in soil layers and soil roots

Table 5.1 gives an overview of the concentrations of different compounds extracted from each soil layer or root sample. The organic layer at 10 m distance from the tree was absent for all trees. The litter was absent at 10 m distance only for tree 3.

The monoterpenes α-pinene, sabinene and β-pinene and the sesquiterpenes β-caryophyllene and α-humulene were the most abundant terpenes found in water and pentane extracts. As expected from their solubility, terpenes concentration in pentane extracts was higher than in water extracts (Table 5.1). We found relatively high concentrations of β-caryophyllene in water extracts from litter layers at 0 and 3 m distance, especially for trees 1 and 3.

5.3.2. Terpene concentration distribution in horizontal gradients.

Terpene concentrations measured in different soil layers and root extracts decreased from transect position 1 (closest point to the trunk) to transect position 3 (at 10 m distance, the most distant from the trunk; Fig. 5.1). Significant differences between transect positions were found for α-pinene concentration (0 m > 3 m, $P<0.05$; 0 m > 3 m; $P<0.005$; Fig. 5.1), but differences between transect positions for other compounds were not significant, probably because there was large variability in measured concentrations.

5.3.3. Terpene concentration distribution in vertical gradients.

Higher α-pinene concentrations were found in extracts of litter layers, roots and organic layers than in mineral layers (Fig. 5.1). Significant differences in the terpene concentration between layers, all trees and all points considered together, are shown in the first five columns of Table 5.2. As expected, we found significant differences in α-pinene concentration between the litter and mineral layers (comparisons 1 and 3, Table 5.2) and between the mineral layers and the low roots (comparisons 6 and 10, Table 5.2). We found only marginal significant differences between α-pinene concentration in top roots and both mineral layers (comparisons 8 and 13, $0.05<P<0.06$), indicated by an asterisk Table 5.2. We also expected to find significant differences between α-pinene concentration in the two mineral layers and the organic layer (comparisons 9 and 14,

Table 5.2) but measurements showed high variability (Table 5.1) resulting in non-significant differences between these layers. There were no differences in α-pinene concentration between both top and low mineral layers (comparison 7, Table 5.2). Comparisons between the layers with high α-pinene concentration showed that there were no differences between the litter layer, and top and low roots (comparisons 2 and 4, Table 5.2) nor between top and low roots (comparison 11, Table 5.2). Alpha-pinene concentration in the organic layer extracts was significantly lower than in litter and low roots extracts (comparisons 5 and 12, Table 5.2), but there were no differences between α-pinene concentrations in the organic layer and in the top roots (comparison 15, Table 5.2).

For all of the other compounds, sabinene, β-pinene, β-caryophyllene and α-humulene, we found significantly higher terpene concentration in the litter layer compared with the other layers and the soil roots extracts. However, there were no significant differences in concentrations of these compounds between organic layers, mineral layers, and roots (Fig. 5.1 and Table 5.2).

When each transect position was considered separately, significant differences in concentrations of all compounds except α-pinene were found for comparisons 1-5, similar to those found when considering all points together (data not shown). However, α-pinene concentration in soil layers and roots again showed a different vertical distribution compared with the other terpenes (comparisons at positions 0 m, 3 m and 10 m, Table 5.2). The significance of differences found in the α-pinene concentration between soil layers and roots increased when considering transect position at 0 m separately (column 6, Table 5.2).

5.3.4. Distribution of compounds between top and low mineral layers.

Alpha-pinene concentration in mineral layers showed a significant gradient for distance from the tree, (P=0.05) but there were no significant differences in concentration between top and low mineral layers (Table 5.2). Other compounds' concentration in mineral layers showed evidence of a distance gradient from the tree although it was significant only for sabinene (P<0.05). Higher terpene concentration was observed in the top mineral compared with the low mineral for all compounds (Fig. 5.3) but the differences were not significant (Table 5.2).

Table 5.2. Significant differences between concentrations of compounds in different soil layers and roots (all trees considered together).

		sabinene All points	β-pinene All points	β-caryophyllene All points	α-humulene All points	α-pinene All points	α-pinene 0 m	α-pinene 3 m	α-pinene 10 m
1	litter, mineral low	<0,0001	<0,0001	<0,0001	<0,0001	0,001	0,0011	0,0274	0,0021
2	litter, roots low	<0,0001	0.00001	<0,0001	<0,0001	ns	ns	ns	0,0024
3	litter, mineral top	<0,0001	<0,0001	<0,0001	<0,0001	0,0011	0,0011	0,0273	0,0021
4	litter, roots top	<0,0001	0.0002	<0,0001	<0,0001	ns	ns	ns	0,0093
5	litter, organic	<0,0001	0.0001	<0,0001	<0,0001	0,0052	0,0016	0,0328	ns
6	mineral low, roots low	ns	ns	ns	ns	0,0041	0,0007	ns*	-
7	mineral low, mineral top	ns	ns	ns	ns	ns	ns	ns	ns
8	mineral low, roots top	ns	ns	ns	ns	ns*	0,0091	ns	ns
9	mineral low, organic	ns	ns	ns	ns	ns	ns	ns	ns
10	roots low, mineral top	ns	ns	ns	ns	0,0041	0,0007	ns*	ns
11	roots low, roots top	ns	ns	ns	ns	ns	ns	ns	0,0021
12	roots low, organic	ns	ns	ns	ns	0,0160	0,0010	ns	0,0024
13	mineral top, roots top	ns	ns	ns	ns	ns*	0,0092	ns	0,0021
14	mineral top, organic	ns	ns	ns	ns	ns	ns	ns	0,0093
15	roots top, organic	ns	ns	ns	ns	ns	0,0137	ns	ns

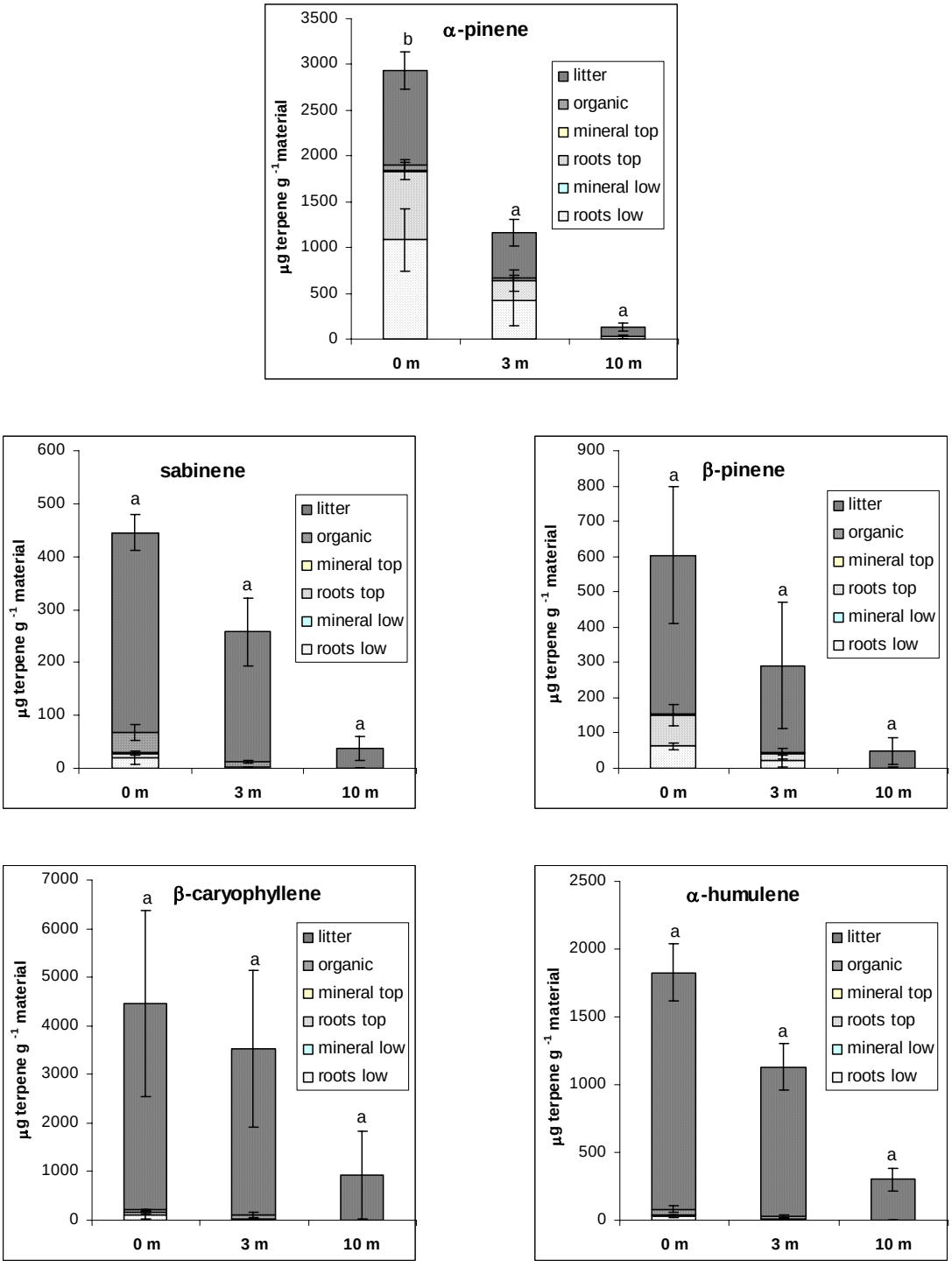

Figure 5.1. Horizontal and vertical gradients of compound concentrations in rhizosphere soils and roots of *Pinus halepensis*. Mean and SE of all trees. N=3 for all points, for all layers. N=2 for point 3 litter. For each compound, those transect points with the same letter are not significantly different according to the Fisher's test (P<0.05).

5.3.5. Source of compounds in soil.

To investigate the source of the compounds we performed a cluster analysis of terpenes mean concentration in top mineral layer, litter layer and top and low roots (Fig. 5.2). Alpha-pinene was the last compound to be incorporated into the cluster, being at a much greater distance than that separating the other four compounds (Fig. 5.2). To investigate the source of compounds we also represented mean concentrations of compounds in the top and low mineral layers as function of total amounts of those compounds in a) litter b) roots in the low mineral layer and c) roots in the top mineral layer (Fig. 5.3). The total concentration for litter, top mineral roots and low mineral roots are for the area enclosed by the quadrat which defined the sample (0.0625 m^2) and means are for all trees and all points. Left panels in figure 5.3 show a strong linear relationship between mean concentration for all trees, all points and all replicates of β-pinene, sabinene, β-caryophyllene and α-humulene in the top and low mineral layers plotted against the total amount of compound in the litter gathered from the quadrat (R^2 =0.96 and R^2 = 0.93 for top and low mineral respectively; Fig. 5.3). When α-pinene is included in the plots, the point for α-pinene lies outside and above the linear relationship defined by the other compounds. However, the relationships between terpenes concentrations in the top and low mineral layer with the total amounts of compounds in the roots gathered from the top and low mineral layers under the quadrat, show that without α-pinene, there is no relationship (central and right small panels; Fig. 5.3). Unlike the relationships with litter concentration, including α-pinene in the plots creates a relationship between greater compound concentration in the top and low mineral layers, and greater compound concentration in the top and low layer roots (central and right panels; Fig. 5.3).

Comparisons between terpenes concentration in the litter layer and top and low roots (comparisons 2 and 4, Table 5.2) showed significant higher concentration (expressed per unit mass of material) in litter than in roots extracts for all compounds, except for α-pinene. When the mean total terpene concentration (expressed per unit area of material) in litter, top roots and low roots harvested from the 0.0625 m^2 quadrats were compared (Fig. 5.4) we found that all individual compounds in litter, including α-pinene, were significantly higher than concentration in roots (two to four orders of magnitude greater, $P<0.05$; Fig. 5.4). The relative contribution of each compound to total concentration was similar in top and low roots, with α-pinene dominating the total terpene concentration (785 ± 159 mg m^{-2}, 38 ± 14 mg m^{-2} and 14 ± 5 mg m^{-2} in litter

and top and low roots respectively; Fig. 5.4). In litter, however, β-caryophyllene was the most dominant compound (4336 ± 1002 mg m^{-2}, 3.0 ± 1.6 mg m^{-2} and 0.7 ± 0.3 mg m^{-2} in litter and top and low roots respectively; Fig. 5.4). Similar to the profile of compounds in root extracts, α-pinene was the dominant compound in the top and low mineral soil layers (4.1 ± 1.8 mg g^{-1} and 3.5 ± 1.7 mg g^{-1} respectively; Fig. 5.4), and there were also relatively high concentrations of β-caryophyllene (2.3 ± 0.8 mg g^{-1} and 0.7 ± 0.3 mg g^{-1} in top and low mineral respectively; Fig. 5.4).

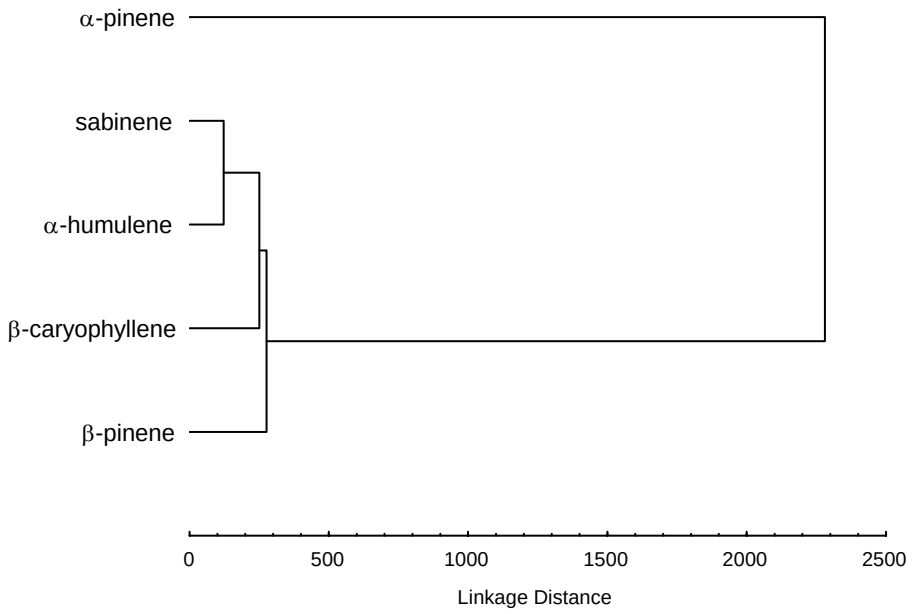

Figure 5.2. Dendrogram of the cluster analyses of the different terpene compounds described by their positions in the multi-dimensional space defined by their concentrations in different soil layers. Single linkage was used as amalgamation rule. Multi-dimensional distance between points is measured using non-standardized Euclidean distance.

5.4. Discussion

5.4.1. Terpenes concentration in pentane and water extracts.

The most abundant terpenes in soil horizons and roots extracts around *Pinus halepensis* trees reported here (α-pinene, β-pinene, sabinene, β-caryophyllene and α-humulene) were also noticeable in the composition of monoterpenes exchanged by soil to the atmosphere in coniferous and ever-green holm oak forests (Hayward et. al. 2001; Asensio et al. 2007), in the terpenoids composition of *Pinus* rhizosphere air (Smolander et al.; 2006; Lin et al., 2007) and in the terpene composition of *Pinus* roots (White 1991; Lin et al., 2007). It is noteworthy that we did not find limonene in large amounts in soil horizons and roots extracts, while it was one of the main contributing compounds to roots and soil emissions (Hayward et al., 2001; Asensio et al., 2007; Lin et al., 2007; Owen et al, 2007) and also to the total terpene concentration in *Pinus* roots, as reported by Lin et al. (2007). Inter specific differences in the terpenes relative composition between species studied (*P. halepensis*, *P. pinea*, *P. sylvestris, Picea sitchensis*, *Quercus ilex* or *Populus tremula)* are likely accounting for the different soil terpene composition.

Alpha-pinene, together with the sesquiterpenes β-caryophyllene and α-humulene, were particularly high in litter extracts. This is in agreement with the main terpenes found in *Pinus halepensis* leaves extracts (Llusià and Peñuelas, 2000; Llusià et al., 2006). The relative composition of terpenes in needle litter is related with green needle terpenes composition, although the concentration of different terpenes may change through time in decomposing needle litter (Kainulainen and Holopainen, 2002). Previous studies on needle litter and green needle terpenes concentration from *Pinus* trees showed significant inter and intra specific differences in the terpenes relative composition and type (Barnola et al., 1997; Kainulainen and Holopainen, 2002; Semiz et al., 2007; Thoss et al., 2007). The major differences found between terpenes concentration in *P. halepensis* litter here and other authors' reports for *P. sylvestris* and *P. caribaea* (Barnola et al., 1997; Kainulainen and Holopainen, 2002; Semiz et al., 2007) are the absence of significant amounts of Δ^3-carene and the higher concentration of sabinene and the sesquiterpenes Beta-caryophyllene and α-humulene in our *P. halepensis* litter extracts. β-caryophyllene concentration in litter from trees 2 and 3 was 14 times higher than β-caryophyllene concentration in litter from tree 1 (Table 5.1) which could be related with large amounts of fungal hyphae growing on litter in trees 2

and 3 and their absence on litter in tree 1. However, literature shows a range of both negative inhibitory and positive effects of sesquiterpenes on fungal activity (Cakir et al., 2004; Gao et al., 2005; Kucuk et al., 2006). Thus a relationship between high sesquiterpenes concentration in litter layer and extensive fungal hyphae development in litter should be tested. The present study further confirmed within-species variation in the constituents and relative composition of terpenes in *Pinus* needle litter and suggests that the chemical diversity of plant secondary metabolites may play an important role in soil microbial ecology through their effects on soil microbes, mycorrhizae and plant pathogens (Inderjit and Weiener, 2001).

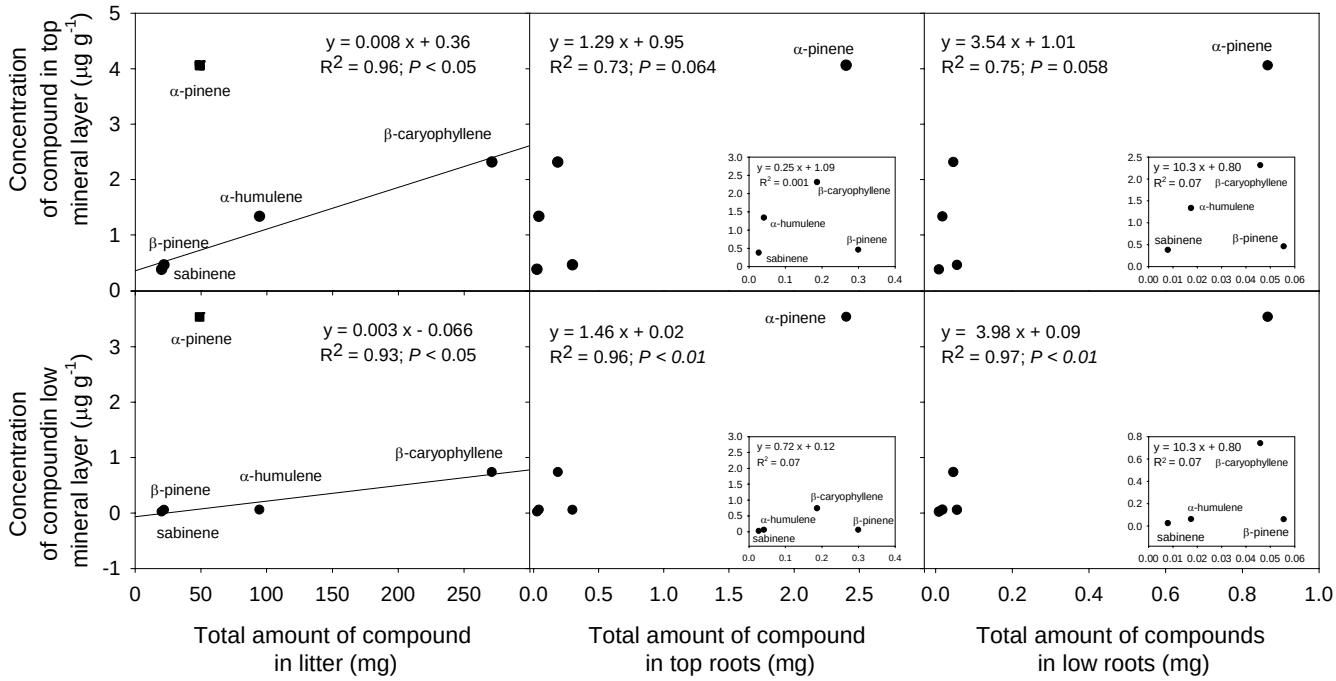

Figure 5.3. Left panels: Concentrations of individual terpene compounds: sabinene, β-pinene, α-humulene and β-caryophyllene (dots), without α-pinene (square), in the top and low mineral layers under a 0.0625 m^2 quadrat, plotted against the total amount of each compound in the litter gathered from the quadrat. Center and right panels: Concentrations of individual terpene compounds, included α-pinene, in the top and low mineral layers plotted against the total amount of each compound in the top and low roots. The small panels inside the graphs represent concentrations of individual terpene compounds, without α-pinene, plotted against the total amount of each compound in the top and low roots.

Not surprisingly, terpene concentrations in soil and roots were higher in pentane than in water extracts because non-oxygenated terpenes are only slightly soluble in water (Weidenhamer et al., 1993). The overall biodegradation rate of a sorbed organic chemical will be kinetically limited by the rate of diffusion (Liu et al., 2007). This means that in the soil system liquid phase (i.e. the aqueous medium), terpene availability is lower than for other water soluble and biodegradable compounds (e.g. ethanol and methanol). However, small amount of terpenes will be partitioned into the aqueous phase because of the slight water-solubility. The relatively high water solubility of β-caryophyllene in the litter layer shown by water extracts is an interesting finding because soil microorganisms are likely to obtain their carbon source in the aqueous phase. β-caryophyllene therefore may represent an accessible source of carbon for soil microbes, but further research is needed into soil microorganism metabolism to confirm this hypothesis. Miller and Allen (2005) proposed a model of biologically mediated transformation for water insoluble compounds, such as α-pinene, in biofilms. This model assumes that an enzymatic mediated first step is taking place, changing the initial insoluble compound into a more soluble compound. Other microorganisms are then capable of metabolising this more soluble by-product. These authors found that *cis*-2,8-*p*-menthandien-1-ol, the by-product of α-pinene degradation in biofilms, was also reported as one of several conversion products of myrcene by different strains of basidiomycetes, and it can also be produced from oxidation of other terpenes. The results of Miller and Allen (2005) suggest that soil enzyme activity may be linked with soil microbial terpene degradation and that, although water insoluble, terpenes occurring naturally in plant tissues and soil solution, may play an important role in soil ecology (Weidenhamer, 1993; Inderjit and Weiner, 2001).

5.4.2. Evidence of a soil terpene concentration gradient from the trunk of Pinus trees.

In agreement with Lin et al. (2007), our results showed a clear decrease in overall soil terpene concentration with increasing the distance to the trunk (Fig. 5.1). However, this finding does not indicate whether tree roots or litter is the main source of terpenes in the soil system, because terpene concentration of both roots and litter decreased with increasing distance from the trunk. The horizontal change in litter and roots type from the tree might explain the interaction found between the distance gradient and the terpenes concentration in different soil layers, especially for α-pinene (Table 5.2). Thus, near the tree (transect position 1) we found more *Pinus* litter and roots with higher α-

pinene concentration compared with the furthest position at 10 m from the tree (Fig. 5.1), where litter and roots were mostly from herbs, grasses and other non-woody plant types.

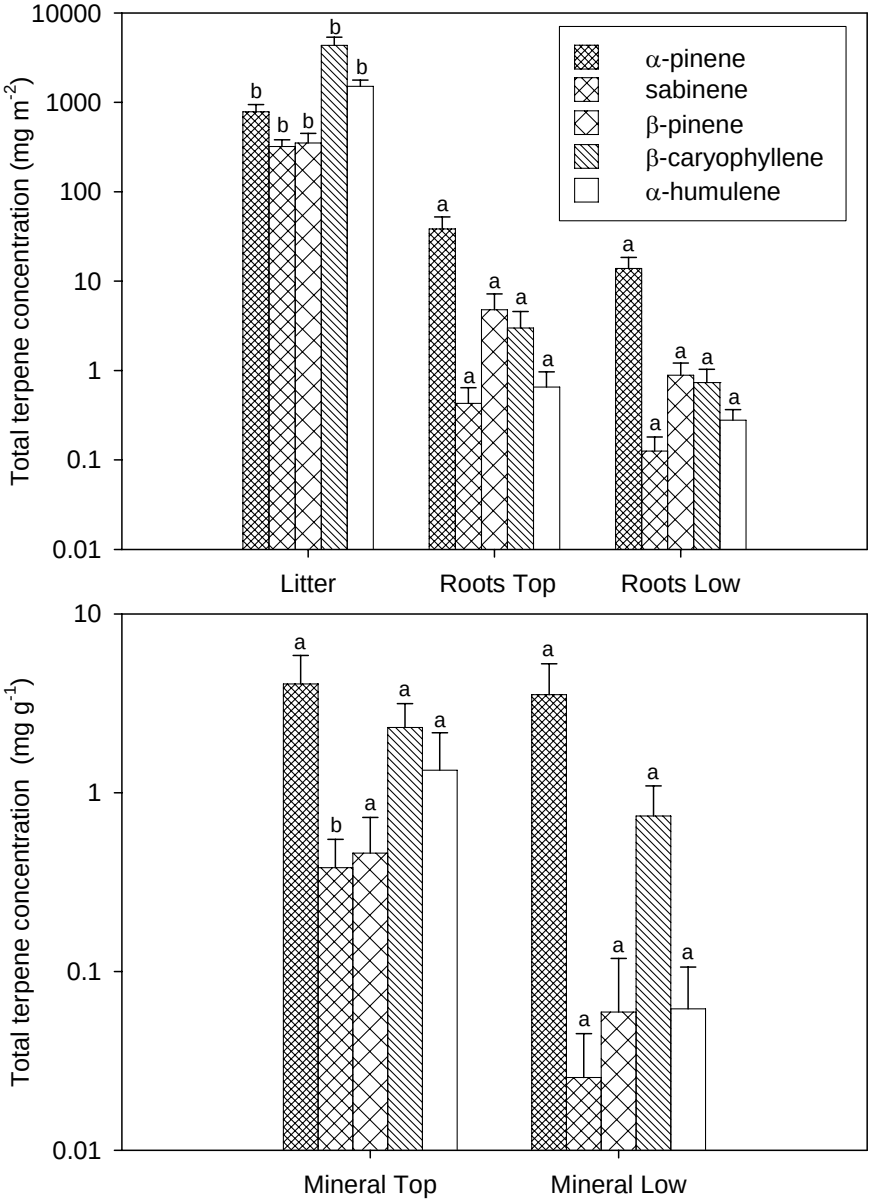

Figure 5.4. Total concentration of α-pinene, β-pinene, sabinene, β-caryophyllene and α-humulene in litter, top and low roots (upper panel) and in top and low mineral layers (lower panel) under 0.0625 m^2 quadrats. Values are total average ± SE (N=27). Comparisons between each compound's concentration in litter, top and low roots (upper panel) and top and between each compound's concentration in low mineral layers (lower panel) were carried out. Compounds with the same letter were not significantly different according to the Fisher's test (P<0.05).

Even though both litter and root terpene concentration decreased with increasing distance from the tree, the decrease was higher for roots than for litter (Fig. 5.1) suggesting that litter could be a less variable source of terpenes for deeper soil layers. The horizontal distribution gradient for terpenes in litter and roots might affect the terpene concentration in organic and mineral layers. In fact, these layers also showed a decreasing concentration gradient, although the differences were not significant for the organic layer (Fig. 5.1) and only significant for α-pinene and sabinene in the mineral layers.

Table 5.3. Ratio of total amount of compounds in litter:total amount of compounds in roots.

	litter:roots top	litter:roots low
α-pinene	14	46
β-pinene	58	385
sabinene	533	1428
β-caryophyllene	1150	2916
α-humulene	1865	4131

5.4.3. Terpenes concentration distribution in soil layers and soil roots.

As expected, the litter layer and top and low roots were identified as the largest sources of terpenes in soil, because both come from living plant material which is reported to be rich in terpenoids, among other compounds (Llusià et al. 2006; Lin et al., 2007). Terpene concentration in green needles reported by Llusià et al. (2006) was considerably higher than terpenes concentration measured here in soil litter layer suggesting a huge potential source for soil terpenes in fresh *Pinus* litter. Actually, except for α-pinene, the concentrations of all the other terpenes in litter were significantly higher than concentrations of terpenes measured in other soil layers and roots. This indicates that litter is the strongest source for the concentrations of sabinene, β-pinene, β-caryophyllene and α-humulene which are found in the other soil layers (organic and mineral). Terpenes concentration in litter is susceptible to seasonal changes due to changes in environmental conditions and decay status throughout the year (Kainulainen and Holopainen 2002). Therefore, concentration of terpenes in deeper soil

layers could be dependent on seasonal patterns of litter-fall inputs from the aboveground parts of the tree to soil surface.

The vertical distribution of α-pinene concentration in soil layers and roots was different to that found for other terpenes (Table 5.2 and Table 5.3) and provides evidence about the comparable α-pinene source strengths of litter and roots. At transect position 1 closest to the trees (0 m), and at the middle position (3 m) litter and roots were comparable sources of α-pinene, while at the furthest position from the tree (10 m), α-pinene concentration in roots was significantly lower than that of litter (Table 5.3). There were no significant differences between the α-pinene concentration in top and low roots. However, α-pinene concentration in low roots was higher than in top roots at 0 m distance and 3 m distance (Fig. 5.1), probably because at these transect positions the influence of the deep *Pinus* roots (compared with roots of non-tree species) was more important than at 10 m distance.

5.4.4. Source and distribution of compounds in top and low mineral layers: litter vs roots

Although not significant, sabinene, β-pinene, β-caryophyllene and α-humulene each tended to show higher concentrations in top mineral soils compared with the low mineral soils, whereas α-pinene showed no difference between top and low mineral layers (Fig. 5.3). This result demonstrates again the different behaviour of α-pinene concentration distribution, suggesting that the sources of α-pinene might differ from the source of other compounds in the mineral layers. The cluster analysis reinforced difference between the distribution of α-pinene and that of the other terpene compounds in the different soil layers. Figure 5.3 suggests that the concentration of each compound (except α-pinene) in the mineral layer is dependent upon its concentration in the litter above. Because α-pinene lies above the relationship line (left panels Fig. 5.3), it is likely that there is an extra source of this compound, possibly emissions from roots.

In spite of the extreme care taken during sampling, mechanical damage of roots while sampling could have affected the results, because disturbances of the root system cause elevated concentrations of terpenes in the soil atmosphere (Hayward et al., 2001; Smolander et al., 2006). If the source of α-pinene from root material were from broken roots, we would expect that β-pinene would also follow the patterns shown by α-pinene in Fig. 5.3, since both α-pinene and β-pinene concentration showed the same order of

magnitude in the ratio litter : top roots (Table 5.3). However, this was not the case, so we suggest that the different distribution of α-pinene in the mineral layers is due to long term root emission of this compound.

5.5. Concluding remarks

Needle litter of *Pinus halepensis* is probably the main source of the major terpene compounds (α-pinene, sabinene, β-pinene, α-humulene, β-caryophyllene) found in the mineral layers of rhizosohere soil near *P. halepensis* trees. However, there is evidence to suggest that long term emissions of α-pinene from *P. halepensis* roots also contribute to the α-pinene concentration in rhizosphere soils. We believe that this is the first experimental evidence in field conditions that natural emissions from roots might be an active source of monoterpenes in soils. There is a need for further investigations of the sources dynamics of terpenes in rhizosphere systems, particularly to investigate (1) the role of mycorrhizal fungi and needle-degrading microorganisms, and (2) conversions and use of terpenes as carbon sources.

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General Conclusions

General conclusions

This PhD thesis investigates the exchange rates and the content of volatile organic compounds (VOCs) and, especially, monoterpenes in the Mediterranean soils, and estimates possible changes in response to climate change.

The major conclusions are the following ones:

1) Total VOC and monoterpene exchange rates from soil surfaces are low. There is relatively low soil emissions contribution to total biogenic monoterpene emissions to the atmosphere on a land area basis. Soil VOC and monoterpene uptake rates were also low, though comparable with some foliar uptake rates. Further studies are needed to corroborate these results and the possible importance of the soil VOCs sink in chemistry-climate models.
2) Results suggest that the water availability reduction and the increase of temperature expected in the next decades might greatly affect soil VOC exchange rates to the atmosphere, but longer-term studies are needed to discern the response of soil VOC exchange rates to climate change. We found that drought and high temperatures tended to increase soil VOC emission rates. Results suggest the causes may be more the physical effects on soil features and VOCs volatility than the effects on soil roots and microorganisms activities. However, results also showed that drought reduced soil CO_2 fluxes, while high temperature increased them. Thus, drought and warming are affecting several biological below-ground and above-ground processes which could affect soil VOCs exchange. The final effect of climate change is not clear but uncoupling effects to different processes are thus likely to occur.

Specific conclusions

The specific conclusions of each one of the chapter-studies of this PhD thesis are the following ones:

Chapter 1. Seasonal Soil CO_2 and VOC exchange rates in a Mediterranean holm oak forest and the responses to drought.

Chapter 1.1. Seasonal soil and leaf CO_2 exchange rates:

1) In Mediterranean ecosystems, water is the principal factor controlling most of the aboveground and belowground processes resulting in a soil-moisture-dependent seasonal pattern for soil CO_2 emissions and leaf CO_2 uptake.
2) We have found a significant correlation between photosynthetic rates and soil respiration rates. However, this could be an indirect relationship resulting from seasonal variations of temperature, moisture and phenology or the effect of time lags between the assimilation of carbon in leaves and the carbon transport to the roots. Further studies considering autotrophic versus heterotrophic respiration separately are needed to gain knowledge on the relationships between above- and below-ground plant processes.
3) Prolonged low water availability in the drought treatment plots might have favoured root growth in those plots, resulting in enhanced root respiration. Thus, in summer, CO_2 emissions in drought plots could be mostly driven by autotrophic metabolism as a result of changes in the importance of root versus soil microbial activity.
4) The results suggested changes in drought conditions towards a decrease in the microorganisms/roots ratio of activities in the rhizosphere, especially in summer.

Chapter 1.2. Seasonal soil VOC exchange rates:

1) VOC and monoterpene emissions in the holm oak forest represent a very small percentage of the total C emitted as CO_2.
2) No significant direct relationships were found between the soil monoterpene exchange rates and the net photosynthetic rates in our study. The correlation between photosynthetic electron transport rates and emission rates does not necessarily gives mechanistic insight into the physiology of monoterpene leaf emission nor on the monoterpene emission from underground parts of plants. Further studies are needed to gain knowledge on these linkages between photosynthetic rates and soil emission rates.
3) Seasonal induction of VOC emissions by phenological processes must be considered as an additional factor which is likely to control VOC and monoterpene exchange between soil and atmosphere.
4) The factors determining the sink potential and the timescales for sink/source reversal are unknown but these results warrant further research to solve these questions.
5) Results also suggest changes towards a decrease in the ratio of microorganisms/roots activities in the rhizosphere, especially in summer. The ecological implications of these changes in soil VOC exchange rates need to be explored further.

Chapter 2. Internannual and interseasonal soil CO_2 efflux and VOC exchange rates in a Mediterranean holm oak forest in response to experimental drought:

1) Drought strongly reduces soil respiration. This was highlighted by an strong interannual variation in CO_2 exchange in this Mediterranean ecosystem. Soil moisture exerted a primary control seasonally and interannually on soil respiration.
2) Soil respiration responses to increases in soil temperature are constrained by soil moisture, due to the direct effects of soil moisture on microbial biomass and the indirect effects of moisture on the amount of photosynthate available as substrate for belowground root and rhizosphere respiration. As a result,

maximum rates of CO_2 efflux occurred in spring and autumn and the minimum values in summer.

3) Camphene, α-pinene and Δ^3-carene showed the highest exchange rates.
4) Soil monoterpenes exchange fluctuated randomly throughout the seasons. The interaction of multiple factors could have produced non-linear responses resulting in this random-like fluctuation.
5) The response of each individual monoterpene to drought treatment and to the seasonal variations seems to be monoterpene specific.
6) High soil temperatures in summer increased other VOC exchange rates. Drought treatment tended to increase the emission rates of several of these VOCs. Temperature seemed to affect the emission rates depending on the compound type.

Chapter 3. Interannual and seasonal changes in the soil exchange rates of monoterpenes and other VOCs in a Mediterranean shrubland:

1) Alpha-pinene and limonene monoterpenes were the most common identified soil exchanging monoterpenes. M33 (methanol), M43 (acetic acid, ethyl acetate), M45 (acetaldehyde), M59 (acetone) and M73 (C3 and C4 carboniles) showed the highest exchange rates among all the identified VOCs. Exudates from rhizosphere activities, roots VOCs production and organic matter decomposition by microorganisms in the soil organic or mineral horizons may be the most important sources of these VOCs.
2) Soil monoterpene exchange rates were very low so soil monoterpene emissions may represent a small part of the total monoterpene fluxes emitted to the atmosphere by a vegetated land surface. However, some soil VOC uptake rates were comparable with those reported for leaves. These VOCs might be consumed by soil microorganisms or stored in the soil layers.
3) Results suggest that severe long-term drought increases soil monoterpene emission rates in this Mediterranean shrubland. The increase in soil monoterpene emissions in response to lower soil water availability is probably linked to changes in soil physical properties induced by low soil moisture in this Mediterranean shrubland.

4) Conversely, other soil VOCs increased their emission rates when soil moisture increased, although the effects were constrained by soil temperature. These results showed that soil moisture effect on soil VOC exchange rates in this Mediterranean shrubland depends on the compound type.
5) High soil temperatures increase soil monoterpene and other VOC emission rates possibly due to higher volatilisation rates. Results suggest a strong seasonal control of soil temperature on monoterpene exchange rates.

Chapter 4. On-line screening of soil VOCs exchange responses to moisture, temperature and root presence:

1) The studied Mediterranean soil behaved more as a sink of VOCs than as a source. Under the most standard soil moisture and temperature conditions i.e. 20% v/v and 20°C, there was overall VOC uptake.
2) Increasing soil moisture resulted in higher soil sink activity. In parallel, we measured higher CO_2 exchange rates and net photosynthetic rates, corresponding to greater microbial and plant activity. Biodegradation of VOCs by soil microorganisms or roots could be responsible for the soil sink activity as also an increase of physical passive storage of VOCs.
3) Conversely, an increase in soil temperature increased VOC emission rates. Both biological sources (microorganisms or roots) or physical factors (volatilization) seem involved in these responses.
4) Results confirm the link between roots and microbial activity, especially when soil moisture was the limiting factor. We suggest that the role of roots on soil VOCs exchange was indirect by releasing exudates which enhanced microbial VOCs consumption activities.
5) Roots play a key role in soil VOCs exchange increasing bacterial metabolism, especially when water is the limiting factor, which is a common situation in the studied Mediterranean ecosystem.

Chapter 5. The distribution of volatile isoprenoids in the top soil horizons around *Pinus halepensis* trees in field conditions:

1) The most abundant terpenes in soil horizons and roots extracts around *Pinus halepensis* trees reported were α-pinene, β-pinene, sabinene, β-caryophyllene and α-humulene. Alpha-pinene, together with the sesquiterpenes β-caryophyllene and α-humulene, were particularly high in litter extracts.
2) The relatively high water solubility of β-caryophyllene in litter layer indicates soil microorganisms are likely to obtain their carbon source in the aqueous phase.
3) Litter layer and top and low roots of *Pinus halepensis* were identified as the largest sources of α -pinene, sabinene, β-pinene, β-caryophyllene and α-humulene which are found in the other soil layers (organic and mineral).
4) Soil terpene content decrease with increasing the distance to the trunk.
5) There is evidence to suggest that long term emissions of α-pinene from *P. halepensis* roots also contribute to the α-pinene content of rhizosphere soils.
6) There is a need for further investigations of the sources dynamics of terpenes in rhizosphere systems, particularly to investigate (1) the role of mycorrhizal fungi and needle-degrading microorganisms, and (2) conversions and use of terpenes as carbon sources.