SOIL AND VEGETATION CARBON DYNAMICS (STOCKS AND FLUXES) AND LITTER DECOMPOSITION IN A MEDITERRANEAN NON-TIDAL SALT MARSH

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Universitat
de Girona

Doctoral Thesis

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Lorena Carrasco Barea

2020

Doctoral Thesis

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2020

Doctoral Program in the Environment

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Thesis submitted in fulfilment of the requirements to obtain the Degree of Doctor at the
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Girona, 2 de juliol de 2020

Les Dres. Laura Llorens i Guasch i Dolors Verdaguer i Murlà, professores titulars del Departament de Ciències Ambientals de la Universitat de Girona, com a directores del treball de tesi doctoral titulat:

Soil and vegetation carbon dynamics (stocks and fluxes) and litter decomposition in a Mediterranean non-tidal salt marsh

realitzat per la doctoranda Lorena Carrasco Barea, donem el vistiplau a la seva presentació i defensa com a treball d'investigació original per a l'obtenció del títol de doctora per la Universitat de Girona.

Donem, així mateix, el nostre vistiplau al resum divulgatiu que es presenta en aquesta tesi i el consentiment per a que sigui publicat en els canals divulgatius de la Universitat i pels grups d'investigació que creguin convenients.

I per a que així consti i tingui els efectes oportuns, signem el present document.

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A mis padres

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Esta tesis ha sido realizada a través de un contrato predoctoral de la Universidad de Girona (IFUdG2015), y se enmarca dentro del proyecto Life Pletera (LIFE13 NAT/ES/001001).

CONTENT

LIST OF ABBREVIATIONS

AEA: Accumulated enzyme activities

AFDM: Ash free dry mass

AMC: 7-amino-4-methylcoumarin

ANOVA: Analysis of variance

BD: Soil bulk density

C: Carbon

CH_4: Methane

CO_2: Carbon dioxide

DIQC: 2,3-dihydroindole-5,6-quinone-2-carboxylate

DW: Dry weight

EC: Soil electrical conductivity

EEA: Extracellular enzyme activities

GLM: Generalized lineal model

GLMM: Generalized lineal mixed model

g_s: Stomatal conductance

HCI: Habitats of community interest

IRGA: Infrared gas analyser

iSR: Instantaneous soil respiration

iWUE: Intrinsic water-use efficiency

k: Litter decomposition rates

L-DOPA: L-3,4-dihydroxyphenylalanine

MUF: Methylumbelliferone

N: Nitrogen

NAPP: Plant net aboveground primary production

NBPP: Plant net belowground primary production

NER: Net exchange rate

OM: Organic matter

PCA: Principal component analysis

P: Phosphorous

PEIN: “Pla d’espais d’interès natural” (Plan of areas of natural interest)

Q_{min}: Soil carbon mineralization quotient

SMF: Daily soil methane fluxes

SOC: Soil organic carbon

SOM: Soil organic matter

SPA: Special Protected Area

SR: Daily soil respiration

$t_{0.5}$: Litter half-life

TA: Enzyme turnover activity

TN: Soil total nitrogen

Ts: Soil temperature

VWC: Soil volumetric water content

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ABSTRACT

Salt marshes are important ecosystems for global climate regulation because of their high plant primary production and low rates of organic matter decomposition, which would promote carbon accumulation into the soil. Literature concerning the carbon sequestration capacity of salt marshes has been mainly focused on the estimation of carbon stocks from vegetation and soil, and their changes over time (net primary production and soil carbon burial). However, few of these studies incorporate measurements of the structure and composition of plant communities, being also scarce the seasonal characterization of carbon fluxes from soil, and especially, from vegetation. Moreover, although litter decomposition rates in these ecosystems have been broadly estimated, other parameters related with the litter decomposition process, such as the litter chemical quality and microbial activity, remain poorly studied and thus understood. On the other hand, most studies on carbon sequestration capacity and litter decomposition have been performed in tidal salt marshes, especially from North America, being studies from non-tidal salt marshes, such as those of the Mediterranean Basin, really scarce.

In this context, the specific objectives of this thesis were: (1) to analyse the changes over one year in the structure and composition of plant communities, and also in the amount of carbon stored in vegetation and soil, of three non-tidal Mediterranean salt marsh habitats, as well as of a disturbed (and later restored) zone; (2) to assess daily and seasonally CO_2 fluxes from vegetation and soil, as well as soil CH_4 fluxes, in these three salt marsh habitats throughout one year; and (3) to study the litter decomposition of the dominant species of these salt marsh habitats considering the decomposition rate, the chemical quality and the microbial community dynamics of the litter.

The study was performed at La Pletera salt marsh (NE of the Iberian Peninsula), being the three well-preserved salt marsh habitats studied (halophilous scrub, salt meadow, and glasswort sward) habitats of community interest (HCI). The more mature plant communities were those of the halophilous scrub, dominated by *Sarcocornia fruticosa* and the salt meadow, dominated by *Elymus pycnanthus* and *Atriplex portulacoides*, which presented a higher amount of carbon stored in both vegetation and soil compared with the glasswort sward. This latter habitat was formed by pioneer

vegetation, concretely it was dominated by *Salicornia patula,* and results indicate that it will probably evolve towards a plant community dominated by late-successional species similar to that of the halophilous scrub. The disturbed zone, dominated by ruderal vegetation, regenerated naturally after the restoration, changing the plant community composition with the colonization by halophytic species, mainly *Suaeda maritima*, but also with some individuals of *Sarcocornia fruticosa*.

Standing biomass at La Pletera salt marsh was lower than that from tidal salt marshes, but it was similar to that from other Mediterranean non-tidal salt marshes. Conversely, soil organic carbon was generally lower than previous reported values for both types of salt marshes. The study of CO_2 and CH_4 fluxes revealed that *E. pycnanthus* was the species with the highest photosynthetic rates during the entire year, being also remarkable those of *S. patula* in summer*.* On the other hand, the four species studied (*S. fruticosa, E. pycnanthus, A. portulacoides* and *S. patula*) showed daily net CO_2 uptake from the green fraction during most of the year, except in December when *S. fruticosa* and *A. portulacoides* showed net CO_2 emission. The woody fraction of *S. fruticosa* and *A. portulacoides* showed net CO_2 uptake in winter and spring and in winter spring and summer, respectively. The halophilous scrub and the salt meadow showed higher soil CO_2 emissions than the glasswort sward, and, in general, these values were higher than those reported for other tidal salt marshes. At La Pletera salt marsh, CH_4 absorption and emission were also detected, being CH_4 emissions remarkably high, and, in general, higher than those of other salt marshes with high water table salinity, but similar to those of salt marshes with low salinity. The soils of the halophilous scrub and the salt meadow presented lower mineralization quotients than those of the glasswort sward, which would suggest a higher soil carbon sequestration potential.

The litter of *S. fruticosa* and *A. portulacoides*, likely due to their low lignin content, decomposed faster than that of *E. pycnanthus*. Regarding the microbial activity, bacteria dominated along all the litter decomposition process, although fungi became especially important in the later stages, when the relative lignin litter content increased. Litter decomposition of all the species was affected by the spatial heterogeneity, suggesting that flooding (in the halophilous scrub) or soil texture (in the salt meadow) could have greatly modified the process of decomposition.

Results from this thesis contribute to increase the knowledge on carbon dynamics and litter decomposition in Mediterranean Basin salt marshes. The conservation of these ecosystem and the recovery of their degraded areas, jointly with the reestablishment of their natural hydrologic fluxes, will contribute to increase the amount of carbon stored, avoiding its release to the atmosphere, which will guarantee the continuation of their valuable ecosystem services, including climate regulation.

RESUM

Les maresmes són ecosistemes importants per a la regulació global del clima a causa de la seva elevada producció primària vegetal i les baixes taxes de descomposició de matèria orgànica, promovent l'acumulació de carboni al sòl. La literatura sobre la capacitat de segrest de carboni de les maresmes s'ha centrat principalment en l'estimació dels estocs de carboni de la vegetació i del sòl i els seus canvis al llarg del temps (producció primària neta i segrest de carboni del sòl). No obstant això, pocs d'aquests estudis incorporen mesures de l'estructura i la composició de les comunitats vegetals, essent també escassa la caracterització estacional dels fluxos de carboni del sòl i, especialment, de la vegetació. A més, tot i que les taxes de descomposició de la fullaraca en aquests ecosistemes s'han estimat àmpliament, altres paràmetres relacionats amb el seu procés de descomposició, com ara la qualitat química i l'activitat microbiana, romanen pobrament estudiats, i per tant, poc entesos. D'altra banda, la majoria d'estudis sobre la capacitat de segrest de carboni i la descomposició de la fullaraca s'han realitzat en maresmes mareals, sobretot d'Amèrica del Nord, essent els estudis realitzats en maresmes no mareals, com ara les de la Conca Mediterrània, molt escassos.

En aquest context, els objectius específics d'aquesta tesi van ser: (1) analitzar els canvis durant un any en l'estructura i la composició de les comunitats vegetals, i també en la quantitat de carboni emmagatzemat a la vegetació i al sòl, de tres hàbitats de maresma mediterranis no mareals, així com d'una zona pertorbada (i posteriorment restaurada); (2) avaluar estacionalment els fluxos de CO_2 en diferents moments del dia, tant de la vegetació com del sòl, i també els fluxos de CH_4 del sòl, en aquests tres hàbitats de maresma al llarg d'un any; i (3) estudiar la descomposició de la fullaraca de les espècies dominants d'aquests hàbitats de maresma, tenint en compte la taxa de descomposició, la qualitat química i la dinàmica de la comunitat microbiana.

L'estudi s'ha realitzat a la maresma de la Pletera (NE de la Península Ibèrica); concretament, en tres tipus d'hàbitats (matollar halòfil, prat halòfil i salicornar herbaci) considerats d'interès comunitari (HCI). Les comunitats vegetals més madures van ser el matollar halòfil, dominat per *Sarcocornia fruticosa*, i el prat halòfil, dominat per *Elymus pycnanthus* i *Atriplex portulacoides*, els quals presentaven una quantitat més elevada de

carboni emmagatzemat, tant en la vegetació com en el sòl, en comparació amb el salicornar herbaci. Aquest últim hàbitat estava format per vegetació pionera, essent l'espècie dominant *Salicornia patula*, però els resultats indiquen que probablement evolucionarà cap a una comunitat vegetal dominada per espècies de successió tardana similars a les del matollar halòfil. La zona alterada, dominada per vegetació ruderal, es va deixar regenerar de forma natural després de la restauració, essent colonitzada per espècies halòfites, principalment *Suaeda maritima*, però també amb alguns individus de *Sarcocornia fruticosa*.

Els valors de biomassa a la maresma de La Pletera van ser inferiors als de les maresmes mareals, però similars als d'altres maresmes no mareals del Mediterrani. Per contra, el carboni orgànic del sòl va ser generalment inferior al descrit per a ambdós tipus de maresmes. L'estudi de fluxos de CO_2 i CH_4 va revelar que *E. pycnanthus* va ser l'espècie amb taxes fotosintètiques més elevades durant tot l'any, sent també notables les de *S. patula* a l'estiu. D'altra banda, les quatre espècies estudiades (*S. fruticosa, E. pycnanthus, A. portulacoides i S. patula*) van mostrar absorció neta diària de CO_2 per part de la fracció verda de les plantes durant la major part de l'any, excepte al desembre quan *S. fruticosa* i *A. portulacoides* van mostrar una emissió neta de CO_2. La fracció llenyosa de *S. fruticosa* i *A. portulacoides* va absorbir CO_2 de forma neta a l'hivern i a la primavera i a l'hivern, a la primavera i a l'estiu, respectivament. Les emissions de CO_2 del sòl van ser majors al matollar i al prat halòfil que al salicornar herbaci i, en general, aquests valors van ser superiors als descrits per a altres maresmes mareals. A la maresma de La Pletera es va detectar absorció i emissió de CH_4, essent les emissions de CH_4 notablement altes i, en general, superiors a les d'altres maresmes amb alta salinitat freàtica, però similars a les de les maresmes amb baixa salinitat. Els sòls del matollar i del prat halòfil van presentar coeficients de mineralització inferiors als del salicornar herbaci, cosa que suggereix un més elevat potencial de segrest de carboni del sòl en aquests habitats.

La fullaraca de *S. fruticosa* i *A. portulacoides*, probablement a causa del seu baix contingut en lignina, es va descompondre més ràpidament que la d'*E. pycnanthus*. Pel que fa a l'activitat microbiana, els bacteris van dominar al llarg de tot el procés de descomposició de la fullaraca, tot i que els fongs van esdevenir especialment importants en les etapes més tardanes, quan va augmentar el contingut relatiu de lignina. La descomposició de la fullaraca de totes les espècies es va veure afectada per l'heterogeneïtat espacial, cosa que suggereix que la inundació (en el matollar halòfil) i la textura del sòl (al prat halòfil) podrien haver influït en el procés de descomposició.

Els resultats d'aquesta tesi contribueixen a augmentar el coneixement sobre la dinàmica del carboni i la descomposició de la fullaraca a les maresmes de la Conca Mediterrània. La conservació d'aquests ecosistemes i la recuperació de les seves zones degradades, juntament amb el restabliment dels seus fluxos hidrològics naturals, contribuirà a augmentar la quantitat de carboni emmagatzemat, evitant el seu alliberament a l'atmosfera, garantint així el manteniment dels seus valuosos serveis ecosistèmics, entre ells la regulació climàtica.

RESUMEN

Las marismas son ecosistemas importantes para la regulación global del clima debido a su elevada producción primaria vegetal y a las bajas tasas de descomposición de la materia orgánica, promoviendo la acumulación de carbono en el suelo. La literatura sobre la capacidad de secuestro de carbono de las marismas se ha centrado principalmente en la estimación de los stocks de carbono de la vegetación y del suelo y en sus cambios a lo largo del tiempo (producción primaria neta y secuestro de carbono del suelo). Sin embargo, pocos de estos estudios incorporan medidas de la estructura y composición de las comunidades vegetales, siendo también escasa la caracterización estacional de los flujos de carbono del suelo y, especialmente, de la vegetación. Además, aunque las tasas de descomposición de la hojarasca en estos ecosistemas han sido ampliamente estimadas, otros parámetros relacionados con el proceso de descomposición de la hojarasca, como la calidad química del material y la actividad microbiana, permanecen pobremente estudiados, y, por tanto, poco entendidos. Por otra parte, la mayoría de los estudios sobre la capacidad de secuestro de carbono y la descomposición de la hojarasca se han realizado en marismas mareales, sobre todo de América del Norte, siendo realmente escasos los estudios realizados en marismas no mareales, como las de la Cuenca Mediterránea.

En este contexto, los objetivos específicos de esta tesis fueron: (1) analizar los cambios durante un año en la estructura y la composición de las comunidades vegetales, y también en la cantidad de carbono almacenado en la vegetación y el suelo, de tres hábitats de marisma mediterráneos no mareales, así como de una zona perturbada (y posteriormente restaurada); (2) evaluar estacionalmente los flujos de CO_2 de la vegetación y del suelo en distintos momentos del día, así como los flujos de CH_4 del suelo, en estos tres hábitats de marisma a lo largo de un año; y (3) estudiar la descomposición de la hojarasca de las especies dominantes en estos hábitats, teniendo en cuenta la tasa de descomposición, la calidad química y la dinámica de la comunidad microbiana.

El estudio se ha realizado en la marisma de la Pletera (NE de la Península Ibérica), siendo los hábitats de marisma bien conservados estudiados (matorral halófilo, pastizal salino y herbazal halófilo) hábitats de interés comunitario (HCI). Las comunidades

vegetales más maduras fueron la del matorral halófilo, dominada por *Sarcocornia fruticosa*, y la del pastizal salino, dominada por *Elymus pycnanthus* y *Atriplex portulacoides*, las cuales presentaron una mayor cantidad de carbono almacenado, tanto en la vegetación como en el suelo, en comparación con el herbazal halófilo. Este último hábitat estaba formado por vegetación pionera, siendo *Salicornia patula* la especie dominante, aunque los resultados indican que probablemente evolucionará hacia una comunidad vegetal dominada por especies de sucesión tardía similares a las del matorral halófilo. La zona alterada, dominada por vegetación ruderal, se dejó regenerar de forma natural después de la restauración, siendo colonizada por especies halófilas, principalmente *Suaeda maritima*, pero también con algunos individuos de *Sarcocornia fruticosa*.

Los valores de biomasa en la marisma de La Pletera fueron inferiores a los de las marismas mareales, pero similares a los de otras marismas no mareales del Mediterráneo. Por el contrario, el carbono orgánico del suelo fue generalmente inferior al de ambos tipos de marismas. El estudio de los flujos de CO_2 y CH_4 reveló que *E. pycnanthus* fue la especie con las tasas fotosintéticas más elevadas durante todo el año, siendo también notables las de *S. patula* en verano. Por otro lado, las cuatro especies estudiadas (*S. fruticosa, E. pycnanthus, A. portulacoides y S. patula*) mostraron absorción neta diaria de CO_2 por parte de la fracción verde de las plantas durante la mayor parte del año, excepto en diciembre cuando *S. fruticosa* y *A. portulacoides* mostraron una emisión neta de CO_2. La fracción leñosa de *S. fruticosa* y *A. portulacoides* mostró absorción neta de CO_2 en invierno y primavera y en invierno, primavera y verano, respectivamente. Los suelos del matorral halófilo y del pastizal salino mostraron mayores emisiones de CO_2 en comparación con el suelo del herbazal halófilo, y, en general, estos valores fueron superiores a los descritos en otras marismas mareales. En la marisma de La Pletera, se detectó absorción y emisión de CH_4, siendo las emisiones de CH_4 notablemente altas y, en general, superiores a las de otras marismas con alta salinidad en la capa freática, pero similares a las de marismas con baja salinidad. Los suelos del matorral halófilo y del pastizal salino presentaron coeficientes de mineralización inferiores a los del herbazal halófilo, cosa que sugiere que estos hábitats presentan un potencial de secuestro de carbono del suelo más elevado.

La hojarasca de *S. fruticosa* y *A. portulacoides* se descompuso más rápidamente que la de *E. pycnanthus,* probablemente debido a su bajo contenido en lignina. En cuanto a la actividad microbiana, las bacterias dominaron a lo largo de todo el proceso de descomposición de la hojarasca, aunque los hongos fueron especialmente importantes en las etapas más tardías, cuando el contenido relativo de lignina aumentó. La descomposición de la hojarasca de todas las especies se vio afectada por la heterogeneidad espacial, lo que sugiere que la inundación (en el matorral halófilo) y la textura del suelo (en el pastizal salino) podrían haber influido en el proceso de descomposición.

Los resultados de esta tesis contribuyen a aumentar el conocimiento sobre la dinámica de carbono y la descomposición de la hojarasca en las marismas de la Cuenca Mediterránea. La conservación de estos ecosistemas y la recuperación de sus zonas degradadas, juntamente con el restablecimiento de sus flujos hidrológicos naturales, contribuirán a aumentar la cantidad de carbono almacenado, evitando su liberación a la atmósfera, garantizando así que puedan seguir prestando sus valiosos servicios ecosistémicos, entre ellos la regulación climática.

1. INTRODUCTION

1.1. The role of salt marshes in the global carbon balance

The increase of anthropogenic greenhouse gas emissions since the pre-industrial era has driven the global climate change, which is one of the main challenges that humans will face in the 21st century (IPCC, 2014). In particular, there has been a continuous increase of carbon dioxide (CO_2) and methane (CH_4) concentrations in the atmosphere in the last decades, being the global average atmospheric CO_2 and CH_4 in 2019 409.9 ± 0.9 ppm and 1867.1 ± 1.0 ppb, respectively (Dlugokencky, 2020; ESRL/NOAA, 2020), representing the 147% and 258%, respectively, of the atmospheric concentration of these gases in 1750 (Blunden & Arndt, 2019). Concretely, the increment of atmospheric CO_2 during the last decades has been considered the main cause of global climate change (IPCC, 2014).

In this context of continuous global warming associated to the increase of the amount of atmospheric greenhouse gases, ecosystems are important for global climate regulation since they have direct and indirect influence on local and global climate. At local scale, land use changes can affect temperature and humidity conditions, while at global scale, ecosystems play a key role in climate either sequestering or emitting greenhouse gases. In fact, land-use changes, especially deforestation and degradation of ecosystems, have been considered the second most important cause of atmospheric CO_2 rise, behind the burning of fossil fuels (Canadell et al., 2007). Therefore, strategies to reduce CO_2 emissions include the conservation of ecosystems with high carbon sequestration rates and high amounts of carbon stored (Canadell & Raupach, 2008).

Salt marshes, as well as wetlands in general, are among the ecosystems that play an important role in global climate regulation. According to the Millenium Ecosystem Assessment (2005), climate regulation through the capture and emission of carbon is one of the ecosystem services of these ecosystems strongly associated to the human well-being. This is because salt marshes are considered one of the most powerful carbon sinks on Earth, together with other vegetated coastal systems as mangroves and seagrasses (Laffoley & Grimsditch, 2009). Salt marshes, for instance, sequester carbon in a rate approximately 55 times higher than tropical rainforests and their global carbon burial (87.2 Tg C y^{-1}) is comparable, and even higher, than those of terrestrial forests (53.0 Tg C y^{-1} for temperate, 78.5 Tg C y^{-1} for tropical and 49.3 Tg C y^{-1} for boreal forests) (McLeod et al., 2011). These values of global carbon burial in salt marshes are remarkably important given that the global surface area of salt marshes is around 0.2-

4%, 0.1-2% and 0.2-3% of the total land area of temperate, tropical and boreal forests, respectively (McLeod et al., 2011).

In addition to climate change regulation, salt marshes provide numerous ecosystem services, such as water regulation and purification, erosion prevention along shorelines, control of flooding and storm protection and food supply for wildlife support, among others (Costanza et al., 1997). However, despite all these ecosystem services that salt marshes provide, they have suffered historically, but still nowadays, large area losses due to anthropic activities, such as drainage and conversion to agriculture, grazing, water storage, fisheries, mineral exploitation, hunting, harvest of wetland vegetation, urban development, tourism, and water sports (Airoldi & Beck, 2007). Some estimates suggest that, globally, over the last centuries (since around 1800s), 25% of the salt marshes have been lost (McLeod et al., 2011). Moreover, because of their coastal location, salt marshes present a great risk of disappearing due to the sea level rise caused by climate change.

1.2. Main reservoirs and processes of carbon cycle in salt marshes

The high capacity to sequester carbon of the salt marshes is due to a high plant primary production (Mitsch & Gosselink, 2015) and slow rates of soil organic matter decomposition caused by anaerobic soil conditions during flooding periods that avoid the return of carbon dioxide to the atmosphere promoting its accumulation into the soil (Chmura et al., 2003; Kayranli et al., 2010). In these ecosystems, carbon is sequestered over the short term (decennial) in vegetation biomass and over longer time scales (millennial) in the soil (Duarte et al., 2005). Fluxes of carbon between these two carbon stocks and the atmosphere are mediated by processes such as plant photosynthesis and respiration, as well as biomass decomposition, which is related with humification and soil respiration (Figure 1.1). When both aerial and subterranean vegetal tissues die, the decomposition process begins. As a consequence, a part of the carbon that comes from vegetal tissues is mineralized to inorganic forms and emitted to the atmosphere, while another part ends up stored into the soil as humic substances (Berg & McClaugherty, 2014). Depending on the velocity of the decomposition, one of these processes (mineralization or humification) will predominate over the other, being the humification dominant when low decomposition rates occur. However, although soil anoxic conditions can promote carbon burial in salt marshes, and wetlands in general, it is important to consider that one of the final products of the organic matter decomposition under anoxic conditions is methane, which presents a warming potential higher than CO_2. In fact, it has been estimated that 20-25% of the global methane emissions are produced by wetlands (Whiting & Chanton, 2001).

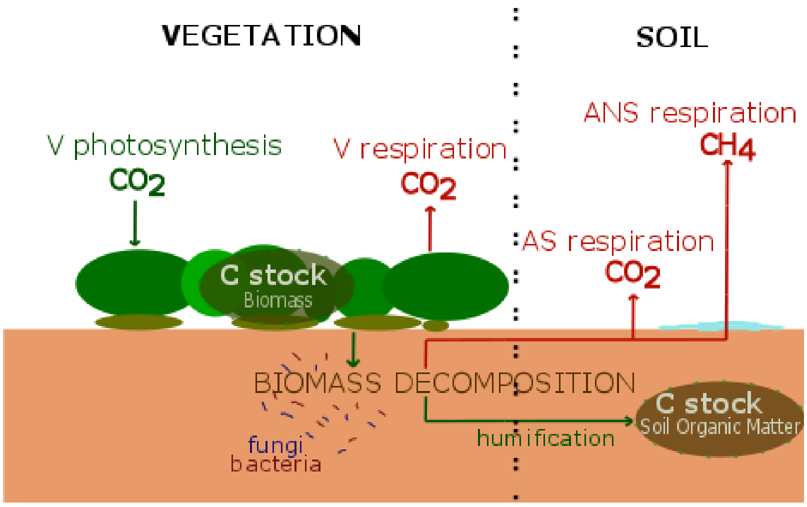


Figure 1.1. Scheme of the main processes related with the carbon balance in salt marshes. V photosynthesis: photosynthesis of salt marsh vegetation; V respiration: respiration of salt marsh vegetation; AS respiration: soil aerobic respiration; ANS respiration: soil anaerobic respiration. Source: prepared by the author.

1.2.1. Net primary production and carbon stored in soil and vegetation in salt marshes

The high primary production that salt marshes usually have (Mitsch & Gosselink, 2015) is greatly explained by their closeness to hydrological fluxes (watersheds including surface water (rivers) and groundwater) which acts as important nutrient sources to salt marsh vegetation (Hopkinson & Giblin, 2008; Mitsch & Gosselink, 2015). Studies of net primary production and/or standing biomass have been broadly performed in tidal salt marshes especially in North America (Cameron, 1972; Cruz, 1974; Mahall & Park, 1976; Linthurst & Reimold, 1978; White et al., 1978; Hopkinson et al., 1980; Zedler et al., 1980; Connor, 1995; Darby & Turner, 2008) but also in Europe (Benito & Onaindia, 1991; Bouchard et al., 1998; Bouchard & Lefeuvre, 2000; Palomo & Niell, 2009; Neves et al., 2010). However, literature of non-tidal salt marshes, as those of the Mediterranean Basin, is scarcer (Berger et al., 1978; Ibañez et al., 1999; Curcó et al., 2002; Scarton et al., 2002). Moreover, there is high variability in net primary production within salt marshes, since plant growth is directly affected by several abiotic factors (and their combination), among them flooding frequency and duration, soil salinity, temperature, rainfall, nutrient availability, oxygen levels and soil properties (Montemayor et al., 2014). For instance, net aboveground primary production (NAPP) of salt marshes along the Atlantic coast of United States and Canada is usually lower at high latitudes because of the shorter duration of the growing season and the lower temperatures (Turner, 1976; Kirwan et al., 2009). Besides that, when temperate and Mediterranean climate in salt marshes are compared, NAPP is usually lower in the latter because summer low rainfall and high temperatures in Mediterranean climates cause soil hypersalinity and, thus, limit plant growth (Ibañez et al., 2000). In the same line, within Mediterranean salt marshes, plant growth could be more disfavoured in non-tidal than in tidal salt marshes, since the absence of daily tides promotes higher conditions of soil hypersalinity (Ibañez et al., 2000).

Most of the studies performed in salt marshes regarding plant production have focused on aboveground primary production, without considering the root system, although, in some cases, the production of belowground biomass can be even higher than that of the aboveground part (Chmura, 2011). Roots can also play a key role in the increase of soil organic carbon, since aboveground biomass is susceptible to be exported outside the salt marsh, while belowground biomass remains stored in the soil, ready to be decomposed *in situ*. In this sense, Palomo and Niell (2009) found a higher contribution of belowground biomass compared to aboveground biomass to soil organic carbon. On the other hand, belowground biomass plays a key role in soil vertical accretion, since it contributes to maintain soil elevation with respect to the sea level, helping consequently to preserve salt marshes against sea level rise (Chmura, 2011). Nevertheless, despite the importance of the root system in the carbon and soil dynamics, only in two of the studies performed in the salt marshes of the Mediterranean Basin, the plant belowground biomass and primary production have been measured (Curcó et al., 2002; Scarton et al., 2002).

Besides vegetation, the other important reservoir of carbon in salt marshes (and in terrestrial ecosystems in general) is the soil, which usually stores higher amounts of carbon compared to vegetation (Dalal & Allen, 2008). Soil organic carbon comes mainly from decomposition of both aerial and subterranean vegetal tissues (Chmura, 2011). In salt marshes, the relatively small herbivore consumption favours that most of the primary production becomes litter, being decomposed and finally incorporated into the soil (Mann, 1988). Depending on its duration, flooding can create anoxic conditions in the soil, reducing the efficiency of organic matter decomposition and favouring soil carbon sequestration (Chmura et al., 2003; Megonigal et al., 2004). Moreover, unlike other ecosystems, soils of salt marshes do not become saturated with carbon, since they can accrete vertically allowing a good maintenance of carbon burial over time (McLeod et al., 2011), as long as the natural ecosystem functioning is maintained (Mckee et al., 2007). Consequently, salt marshes are among the ecosystems with higher soil carbon sequestration capacity. The great capacity of salt marsh soils to sequester carbon was highlighted by Duarte et al. (2005), who compared values from several coastal ecosystems all over the world and found maximum values for salt marshes (151 g C m^{-2} y^{-1}), followed by mangroves (139 g C m^{-2} y^{-1}), seagrasses (83 g C m^{-2} y^{-1}), estuaries (45 g C m^{-2} y^{-1}) and continental shelf (17 g C m^{-2} y^{-1}). Carbon stored in the soil of salt marshes has been broadly studied in temperate tidal salt marshes of the Atlantic coast of Europe and especially in the Atlantic coast of U.S.A., being the Mediterranean non-tidal salt marshes less studied. In fact, from 96 studies reviewed by Chmura et al. (2003), only one included data from the Mediterranean Basin (Hensel et al., 1999). Since then, a few more studies on soil carbon sequestration rates and/or soil organic carbon content have been performed in Mediterranean Basin salt marshes (Curcó et al., 2002; Scarton et al., 2002; Palomo & Niell, 2009) giving similar values than those obtained for temperate tidal salt marshes. However, because of the high spatial variability (between and within) of the salt marshes in the Mediterranean basin (Ibañez et al., 2000), more studies are needed to confirm it.

In addition, salt marshes have suffered high degradation and spatial restriction/constraint over the last decades (McLeod et al., 2011) Anyhow, because of their high ecological value and ecosystem services they provide, including the global climate regulation through their high capacity of storing high amounts of carbon (Costanza et al., 1997; McLeod et al., 2011), salt marshes have been subject to restoration efforts (Craft et al., 1999; Warren et al., 2002). The return of native halophytic vegetation to restored zones is the most common indicator of restoration success/achievement (Morgan & Short, 2002; Mossman et al., 2012a). However, when restoration has been implemented, it may last decades to reach a mature plant community with recovered ecosystem functionality, such as carbon burial, and be as efficient in carbon dynamics as the plant communities of the unaltered salt marsh (Craft et al., 1999; Moreno-Mateos et al., 2012, 2015; Mossman et al., 2012b). The structure and composition of plant communities are also relevant in carbon stocks studies, since different plant communities in salt marshes can differently contribute to the carbon stored in both vegetation and soil (Elsey-Quirk et al., 2011; Ford et al., 2019). Concretely, the composition of the plant community, the nature of dominant species, and the plant diversity largely determine the biomass, litter production and decomposition, root exudates and root distribution, influencing therefore vegetation and soil organic carbon stocks (De Deyn et al., 2008; Ford et al., 2016). Moreover, the age and maturity of a plant community may also affect the capacity of increasing their carbon stocks since in older and more mature stands the vegetation growth use to be lower (Gower et al., 1996; Yu et al., 2017). Nevertheless, in few studies the relationships among plant community features and carbon stored in vegetation and soil (Elsey-Quirk et al., 2011; Ford et al., 2019) or among plant community features and primary production and standing biomass (García et al., 1993; Lefeuvre et al., 2002) have been considered. Moreover, to our knowledge, studies on natural plant communities colonization capacity to assess the achievement of non-tidal Mediterranean salt marshes restoration are lacking.

1.2.2. Carbon fluxes in salt marshes

In salt marsh ecosystems the high net primary productivity and thus, the high carbon inputs by plant photosynthesis, mainly contributes to the decrease of atmospheric CO_2 concentrations (McLeod et al., 2011; Mitsch & Gosselink, 2015). Although a part of this CO_2 is released by autotrophic respiration, the net balance is generally positive, thus being atmospheric carbon eventually incorporated into the ecosystem (Duarte et al., 2005). Nevertheless, previous studies on the photosynthetic capacity of salt marshes halophytic species have mainly focused on the effect that salinity has in the photosynthesis rates, being studies mostly performed under controlled conditions

(Kuramoto & Brest, 1979; Kemp & Cunningham, 1981; Pearcy & Ustin, 1984; Pezeshki et al., 1987; Nieva et al., 1999; Hester et al., 2001; Castillo et al., 2005; Redondo-Gómez et al., 2007, 2006; Mateos-Naranjo et al., 2013; Duarte et al., 2014) and less frequently under field conditions (Drake, 1989; Warren & Brockelman, 1989; Maricle & Maricle, 2018). Moreover, even less studies have been performed to characterize the CO_2 exchange from salt marshes plant species considering the diurnal and seasonal temporal patterns under natural conditions (Antlfinger & Dunn, 1979; Nieva et al., 2003; Das Neves et al., 2008). Moreover, previous research has been carried out in tidal salt marshes, but, as far as we know, none has been realized in Mediterranean non-tidal salt marshes.

Photosynthetic rates in salt marshes plants depend on several factors, which affect consequently the atmospheric CO_2 removal. First, different species with different photosynthetic carbon metabolism (such as C_3 and C_4 species) and/or belonging to different plant classes (such as monocotyledonous and dicotyledonous species) share the same habitats contributing differently to the photosynthetic removal of atmospheric CO_2. For instance, C_4 or monocotyledonous plants use to have higher photosynthetic rates than C_3 or dicotyledonous plants (Pearcy & Ustin, 1984; Nieva et al., 1999). Besides that, within the same species, photosynthetic rates can vary depending on abiotic factors, such as light, temperature, flooding regime, salinity or nutrient availability (Drake, 1989; Huckle et al., 2000), being in general assumed that day hours with high solar radiation (midday) favour plant photosynthetic activity (Antlfinger & Dunn, 1979; Nieva et al., 2003). However, other factors such as temperature and soil moisture can also down- or up-modulate plant photosynthesis, daily or seasonally (Das Neves et al., 2008). In salt marshes subjected to Mediterranean climate, high temperature and low soil moisture can become noticeable limiting factors for photosynthesis at midday especially during summer (Das Neves et al., 2008). Moreover, the seasonal pattern can also vary among species, and within the same species depending on the tidal regime (Nieva et al., 2003; Das Neves et al., 2008). Soil salinity may also negatively affect the photosynthesis of salt marsh plant species, although soil salinity values at which CO_2 uptake is reduced may differ among species (Pearcy & Ustin, 1984; Nieva et al., 1999; Redondo-Gómez et al., 2006, 2007).

Carbon fluxes from soil are also important in the role that salt marshes play in climate regulation (Chmura et al., 2003; Bridgham et al., 2006). Previous studies to characterize the soil CO_2 and CH_4 fluxes have been performed in salt marshes subjected to temperate (Bartlett et al., 1987; Magenheimer et al., 1996; Hirota et al., 2007; Chmura et al., 2011; Khan, 2016) and also subtropical climate (DeLaune & Pezeshki, 2003; Chen et al., 2013, 2018; Sun et al., 2013; Wang et al., 2016; Hu et al., 2017), but studies in Mediterranean salt marshes are scarce (Wang, 2018). Indeed, to our knowledge, there are no studies on soil C fluxes in non-tidal salt marshes from the Mediterranean basin.

Atmospheric carbon emissions (CO_2 and CH_4) from soil are related with the organic matter decomposition by microorganisms (Chmura, 2011). Because of that, CO_2 and CH_4 emissions use to be positively correlated with soil organic carbon content (Wang et al., 2016; Li et al., 2019). In salt marshes, flooding also has a major effect on CO_2 and CH_4 emissions, since determines which process, aerobic respiration or anaerobic metabolism, prevails. Under aerobic conditions, organic matter can be oxidized completely by heterotrophic microorganisms to CO_2, whereas during flooding periods, in which soil presents anoxic conditions, aerobic respiration is replaced by fermentation and methanogenesis (Mitsch & Gosselink, 2015). Indeed, a negative relationship among tidal range (which affects soil aeration and oxygen availability) and soil CO_2 emission has been reported, being CO_2 emissions higher in the upper zone of tidal salt marshes that are not subjected to the daily tidal influence compared to the lower zone (Khan, 2016; Wang, 2018). Moreover, ecosystems that usually, or at least during some periods of the year, have submerged soils, as occurs in salt marshes, are among the major sources of CH_4 (Dalal & Allen, 2008). However, soil CH_4 emissions varied greatly with salinity, being, in general, negatively affected by increases in the electrical conductivity of the soil or the water table (Bartlett & Harriss, 1993; Poffenbarger et al., 2011; Livesley & Andrusiak, 2012). This is because, in saline environments, the presence of sulphate (SO_4^{2-}) is common, thus allowing sulphate-reducing bacteria to outcompete with methanogens for energy sources such as acetate or hydrogen, which, consequently, disfavour and even inhibit methane production (DeLaune et al., 1983; Bartlett et al., 1987). However, despite the inhibitory effects of sulphate, methane production can persist in saline marshes (Megonigal et al., 2004;

Weston et al., 2011). Probably, if less preferred substrates for sulphate-reducing bacteria, such as methylated amines, were available, simultaneous activity of sulphate reducing bacteria and methanogens might occur, as well as, if substrates for both microbial functional groups were abundant, their competition could be relieved (Oremland & Polcin, 1982; Lee et al., 2008). Temperature also affects soil carbon emissions in salt marshes, with the highest CO_2 and CH_4 emissions being mostly recorded in the warmest season, since high temperatures enhance metabolic activity of soil microorganisms (Wang et al., 2016; Hu et al., 2017; Chen et al., 2018).

1.2.3. Litter decomposition in salt marshes

Litter decomposition is one of the key processes of transformation of organic matter (OM) into its inorganic constituents, allowing the recycle of chemical elements in the Earth and the sequestration of organic carbon in soils (Chmura, 2011). The decomposition and thus the mineralization of plant material is an important part of the biogeochemical cycles and thus, of ecosystems functioning. Specifically in salt marshes, our study subject, a deep knowledge of litter decomposition is crucial as being ecosystems expected to play a key role in the global C cycle (Laffoley & Grimsditch, 2009). Studies up to now on litter decomposition in salt marshes have been mainly focused on the measurement of decomposition rates, i. e. the velocity at which plant material is degraded. Litter decomposition rates have been broadly documented from salt marsh species around the world, for instance in tidal Atlantic salt marshes of North America (Mckee & Seneca, 1982; White & Trapani, 1982; Valiela et al., 1984, 1985; Foote & Reynolds, 1997; Windham et al., 2004), Central America (Rejmánková & Houdková, 2006; Rejmánková & Sirová, 2007), South America (Negrin et al., 2012, 2014) and Europe (Bouchard et al., 1998, 2003; Bouchard & Lefeuvre, 2000; Castro & Freitas, 2000; Caçador et al., 2007; Pereira et al., 2007; Palomo & Niell, 2009; Simões et al., 2011); in tidal Pacific salt marshes of Asia (Liao et al., 2008; Li et al., 2016) and Australia (Van der Valk & Attiwill, 1983; Dick et al., 2002); as well as in non-tidal salt marshes of the Mediterranean Basin (Ibañez et al., 1999; Curcó et al., 2002; Scarton et al., 2002; Menéndez & Sanmartí, 2007; Sanmartí & Menéndez, 2007; Menéndez, 2008). Considering all these studies, one of the conclusions is that litter decomposition rates vary greatly, especially, among species, but also among the different parts of the plant (leaves, herbaceous or woody stems and roots). For instance, leaves of Poaceae salt marsh species usually present slower decomposition rates in comparison with leaves or herbaceous stems of succulent Chenopodiaceae plants (Bouchard & Lefeuvre, 2000; Simões et al., 2011). In general, within the same species, leaves and herbaceous stems generally have higher decomposition rates than woody stems and roots (Ibañez et al., 1999; Curcó et al., 2002; Scarton et al., 2002; Windham et al., 2004). However, despite the importance of litter decomposition in soil C sequestration in these ecosystems, other relevant parameters, such as litter quality or the composition of the heterotrophic microbial community and their extracellular enzyme capabilities, as well as the relationship among all these parameters over the litter decomposition process, have been poorly studied and thus, they are not well understood.

Litter quality has been considered as one of the main factors that affect decomposition rates (Valiela et al., 1985; Duan et al., 2018). In general, it is considered that tissues with a lower C/N ratio decompose faster (Enríquez et al., 1993). Besides, the specific plant chemical composition may affect their decomposition. The main components of plant tissues are polysaccharidic compounds, including cellulose, hemicellulose, and phenolic compounds (such as lignin), which represent about 35-50%, 20-35% and 5-30%, respectively, of the plant dry weight (Behera et al., 2017); the concentration of these compounds in the litter determines its hardness, affecting its decomposability (Schwarz, 2001; Duan et al., 2018). However, there are few studies that estimate the concentration of these compounds in salt marsh plants, being these studies mainly performed in monocotyledonous species from Poaceae and Cyperaceae families (Valiela et al., 1984; Rejmánková & Houdková, 2006; Liao et al., 2008; Duan et al., 2018). To our knowledge, there are no studies performed in succulent Chenopodiaceae salt marsh species, which are abundant in Mediterranean Basin salt marshes.

Soil microbial communities, especially fungi and bacteria, play a key role in the plant material decomposition process, since they use OM as the main source of energy for growing (Buscot & Varma, 2005). Specifically, decomposer microorganisms produce extracellular enzymes (hydrolases and oxidoreductases) that convert litter macromolecules into smaller, readily utilizable compounds which can be transported into cells and metabolized as energy sources or used as building blocks (Moorhead &

Sinsabaugh, 2006). The most common enzymes related with the degradation of plant carbon-rich structural compounds are cellulases, hemicellulases (glucosidases, xylanases, mannanases), pectinases, phenol oxidases and peroxidases (Sinsabaugh et al., 2002). During the litter decomposition process there is a close relationship among the litter chemical quality, the microbial community and the extracellular enzyme activity (Mora-Gómez et al., 2016). Moorhead & Sinsabaugh, (2006) proposed that, at the beginning of the decomposition process, a functional group, or guild, of opportunistic microorganisms rapidly colonize litter, consuming intermediate metabolites and soluble polymers. Then, a guild of decomposers starts the degradation of cellulose and hemicellulose through the action of hydrolytic and oxidative enzymes and finally, a guild of miners, which grow very slowly, degrade lignin through the action of oxidative enzymes. Therefore, the enzymatic profile changes throughout the litter decomposition process. For instance, β-glucosidase would dominate the first stages of decomposition because more labile compounds, such as soluble carbohydrates, are abundant (Sinsabaugh et al., 2002), although it would also be active during the final step of cellulose depolymerization, specifically, throughout the hydrolysis of cellobiose to glucose (Behera et al., 2017) (Figure 1.2). Oxidative enzymes, such as the phenol oxidase, are usually more active at the last stages of decomposition, where mainly the most recalcitrant compounds, such as lignin, remain (Mora-Gómez et al., 2016). However, in these stages, other C-degrading enzymes could also be present since lignin decomposition does not provide energy directly, and thus more labile organic carbon compounds are needed for microorganisms to function (Haider, 1992). Moreover, as the main litter components do not contain nutrients, microorganisms also produce extracellular enzymes, including peptidases, ureases and phosphatases (Sinsabaugh et al., 2002; Romaní et al., 2006) to acquire these nutrients from other sources, such as amino acids and amino sugars for N (Nannipieri & Eldor, 2009) and phospholipids and phosphosaccharides for P (Sinsabaugh & Shah, 2011).

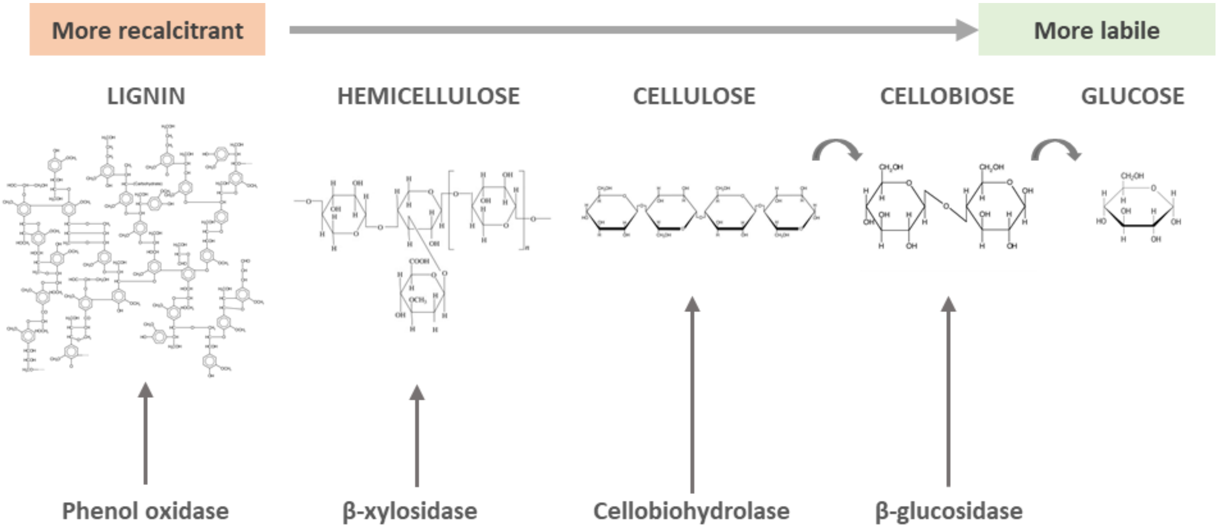


Figure 1.2. Relationship between litter compounds and the action of extracellular C-degrading enzymes according to (Sinsabaugh et al., 2002).

In salt marshes, studies evaluating extracellular enzyme activities (EEA) during the litter decomposition process are almost inexistent. To our knowledge, only Rejmánková & Sirová (2007) studied EEA during the litter decomposition process and they did in a slightly saline tidal wetland (soil electrical conductivity from 0.2-5 dS m^{-1}). They found that the decomposition rate of *Eleocharis cellulosa* litter was positively correlated with the microbial biomass and the EEA. Nevertheless, more data is needed to better understand the relationship between litter EEA and decomposition rates in salt marshes, since it will probably vary depending on the species and/or the salinity level. In fact, several studies have reported that EEA differed between bare and vegetated soils of salt marshes, varying also according to the dominant plant species of the vegetated soils (Costa et al., 2007; Oliveira et al., 2010; Kim et al., 2018). Other studies have shown that the EEA decreased with increasing soil salinity (Caravaca et al., 2005; Huang & Morris, 2005; Hu et al., 2012).

Litter degrading enzymes are synthesized by microorganisms; therefore, the estimation of the relative biomass of bacteria and fungi, the main groups of decomposers, as well as the study of their temporal dynamics, is important in order to improve the knowledge on the litter decomposition process. Fungi tend to be more sensitive to soil salinity than bacteria (Sardinha et al., 2003; Wichern et al., 2006). However, in salt marshes, few studies have attempted to assess the biomass of fungi or bacteria in the litter decomposition process and results about their relative dominance are controversial. For instance, Benner et al. (1984) and Benner et al. (1986) found that bacteria dominate the degradation of radiolabelled lignocellulosic compounds from *Spartina alterniflora* leaves. However, in other studies, also from *S. alterniflora,* it was observed a fungal dominance in the decomposition of leaves (Samiaji & Bärlocher, 1996; Newell & Porter, 2000). However in these studies, dominance of fungi was likely due to the fungal colonization of the senescent or dead leaves when they were still attached to the plant, while, after leaves fell down, the importance of bacteria in the process increased (Newell et al., 1996; Samiaji & Bärlocher, 1996). These results are in accordance with some studies using monocotyledonous salt marsh species of the Poaceae, Cyperaceae and Juncaceae families, where a sharp and fast decrease in the fungal biomass of their litter was observed when senescent or dead leaves were placed on the soil surface (Castro & Freitas, 2000; Menéndez & Sanmartí, 2007; Sanmartí & Menéndez, 2007; Menéndez, 2008). However, in these studies, bacteria were not estimated. Hence, to better understand the litter decomposition process in salt marshes, more studies on the role of microorganisms (fungi and bacteria) in this important process are needed.

Besides litter quality and the decomposer community, environmental factors such as temperature, humidity, oxygen supply, and pH also affect litter decomposition (Buscot & Varma, 2005). Temperature positively affects litter decomposition, since metabolic activity of soil microorganisms is enhanced under high temperatures (Buscot & Varma, 2005; Álvarez & Bécares, 2006). Soil water also affects decomposition because microorganisms depend on it for mobility and survival. In salt marshes and other ecosystems that can be flooded and consequently have to cope with periods of low oxygen availability or anoxia, such as wetlands and mangroves, litter decomposition is slowed down (Gingerich et al., 2014). The slowdown in litter decomposition under anoxic conditions could be due to changes in the metabolism of microorganisms towards fermentation, nitrate and sulphate reduction or methane production, which are less energy-efficient (Megonigal et al., 2004). Under these conditions, degradation of aromatic and phenolic substances, such as lignin, which requires oxygen, is not possible and, consequently, these compounds accumulate (Haider, 1992). The negative effect that flooding periods (especially their duration) have on organic matter mineralization in salt marshes have been previously observed (Mckee & Seneca, 1982; Lewis et al., 2014). On the other hand, the spatial heterogeneity can also be important in the litter decomposition process, since some of the environmental conditions described above, such as oxygen or nutrient, organic matter and water content, can vary at the microhabitat scale. In this sense, Menéndez & Sanmartí (2007) reported differences in the remaining ash free dry mass of litter located in two different microhabitats at the Ebro delta and Rejmánková & Houdková (2006), working in a Central America salt marsh, found that the spatial heterogeneity of the site had similar or even higher effect in litter decomposition than litter quality. Hence, experimental designs of litter decomposition studies need to take into account the high natural spatial variability found in the field (Bradford et al., 2016), and this would be especially relevant in the case of Mediterranean Basin salt marshes (Ibañez et al., 2000).

1.3. La Pletera salt marsh

The three studies of this thesis have been performed at La Pletera salt marsh, a coastal Mediterranean non-tidal salt marsh located in the north of the river Ter mouth in the municipality of Torroella de Montgrí (Girona, NE of the Iberian Peninsula, 42°1'51"N 3°11'33"E; Figure 1.3). Like other coastal ecosystems, La Pletera salt marsh has suffered a high anthropogenic pressure, mainly due to the tourism. In this sense, in 1986, an urbanization project started at La Pletera, which drained the salt marsh dumping piles of rubble to avoid the natural flooding. In 1988, the first phase of the urbanization was completed, with several streets being built (Figure 1.4a). Meanwhile, the Catalan Government approved the Natural Areas Law (Llei 12/1985 d’espais naturals) and the Natural Areas Plan (Decret 328/1992 Pla d’espais d’interès natural, PEIN) even though La Pletera was not protected by any legal entity. However, in 1998, the Spanish Government changed the limits of the maritime-terrestrial public domain and the whole area of La Pletera salt marsh became protected under the Coastal Law 22/1988. As a consequence, La Pletera salt marsh was declared non-urbanizable zone and was included in the PEIN. In 2008, it was also included in the Natura 2000 network. In 2010, the creation of the Natural Park of the Montgrí, Medes Islands and Baix Ter allowed La Pletera salt marsh to be part of a Special Protected Area (SPA).

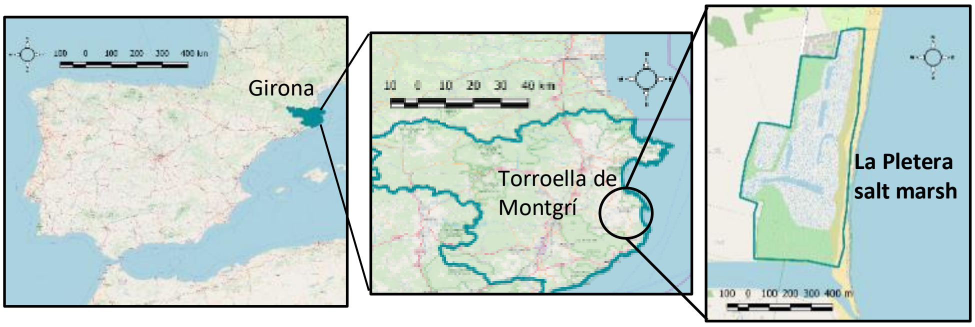


Figure 1.3. Geographical location of La Pletera salt marsh. In the map on the left, Girona province (at the NE of the Iberian Peninsula) is highlighted. The central map shows the municipality of Torroella de Montgrí and the map on the right depicts the salt marsh called La Pletera.

Over the last decades, three Life Projects have been carried out in the Baix Ter wetlands in order to recover La Pletera salt marsh and some surrounding areas. The first Life Project called "Life Ter Vell-Pletera: Restoration and arrangement of ponds and coastal systems in the Baix Ter (1999-2003)" had the objective of improving the conservation of dune and salt marsh vegetation communities, but specially, of the populations of the Spanish toothcarp (*Aphanius iberus*), an endangered endemic fish from the Iberian Peninsula. The objective of the second Life Project "Life Emys Ter: Amphibian and aquatic reptiles, habitat recovering in Baix Ter wetlands (2005-2008)" was to recuperate the habitat for the European pond turtle (*Emys orbicularis*) and other amphibians by means of establishing some temporary or perennial freshwater lagoons. The third and last Life project "Life Pletera: Deurbanization and restoration of La Pletera salt marsh (2014-2018)" (http://lifepletera.com/es/life-pletera/), had the objective to restore La Pletera in order to recover its ecological functionality. To accomplish this, the remaining urban infrastructures (promenade and access) were dismantled and replaced by a coastal lagoon system and the piles of debris were removed with the aim of establish the original ground level and, consequently, the original hydrological movement, favoring the natural recolonization of the restored bare zone with typical halophytic salt marsh vegetation from the surrounding areas (Figure 1.4). The thesis that I present was conducted in the context of this third Life Project.

Figure 1.4. La Pletera salt marsh before restoration in 2015 (a) and after restoration in January 2017 (b).

La Pletera salt marsh is composed by several coastal confined lagoons, dunes with psamophilic vegetation and dense banks of halophytic vegetation. It is also a non-tidal salt marsh where astronomical tides are almost imperceptible (generally 0.2-0.3 m). Meteorological events, like strong easterly winds and rainfall, can cause the flooding of this salt marsh, either by direct water input from the sea or by the rainfall itself and the concomitant rise of the aquifer water table (Menció et al., 2017). Direct sea inputs only occur when the waves of the storms exceed approximately three meters in height and sea water can overpass the dunes located in parallel to the coastline. Hence, rainfall is usually the main cause of flooding, reaching high water level especially along the days when 100 mm of total accumulated rainfall occur. The greatest floods are reached when rainfall and sea storms occur at the same time, which mostly happens at autumn (Pascual & Martinoy, 2017).

When considering the plant communities of La Pletera, two zones can be clearly differentiated: the disturbed zone, which had ruderal vegetation at the beginning of the study and was subjected to the restoration actions of the Life Pletera Project in 2016, where the piles of debris (and thus the vegetation that was established there) were removed, and the well-preserved zone, formed by natural halophytic vegetation. Regarding the plant communities of La Pletera, on the one hand the disturbed zone was considered as a single habitat, but being different before and after the restoration (Bou et al., 2018). On the other hand, in the well-preserved zone three habitats of Community Interest were differentiated according to the dominant plant species and taking into account the Habitat Directive (Council Directive 92/43/EEC; European Commision, 1992) (Table 1.1), which will be referred along this text as salt marsh habitats.

Table 1.1. Study zones and habitats considered (taking into account the type of vegetation), with their corresponding habitat codes. HCI codes are from the Habitat Directive (Council Directive 92/43/EEC; European Commision, 1992) and CORINE codes from European CORINE (Coordination of Information on the Environment) Biotope project, being revision of correspondences among HCI and CORINE codes as well as revision and modification of the restored zone code performed by Bou et al. (2018). The areas that each habitat occupy at La Pletera salt marsh and a brief description of the dominant vegetation are also shown.

Zone	Habitat	Area (ha)	HCI code	CORINE code	Habitat description
Salt marsh habitats	Mediterranean halophilous scrub	15.0	HCI 1420	15.612; 15.616	Perennial succulent halophilic vegetation, dominated by *Sarcocornia fruticosa* with presence of *Atriplex portulacoides*
	Mediterranean salt meadow	10.8	HCI 1410	15.51; 15.53; 15.572+;	Mediterranean halophilic meadows, dominated by *Elymus pycnanthus* and *Atriplex portulacoides*, with the presence of *Juncus acutus* and *Phragmites australis*
	Glasswort sward	4.1	HCI 1310	15.1133	Annual pioneer vegetation of saline soils, dominated by *Salicornia patula,* with the presence of *Suaeda maritima* and *Sarcocornia fruticosa*
Disturbed/ Restored zone		10.0	-	CORINE 87.21^{+}	Disturbed: Ruderal vegetation dominated by *Foeniculum vulgare* and *Inula viscosa*
			-	87a	Restored: Annual pioneer vegetation of saline soils dominated by *Suaeda maritima*

All the dominant species (Figure 1.5) of the salt marsh habitats (*Sarcocornia fruticosa*, *Atriplex portulacoides, Elymus pycnanthus* and *Salicornia patula*) are C_3 species. *Sarcocornia fruticosa* (L.) A.J. Scott is a shrub perennial halophyte of the

Chenopodiaceae family with highly reduced leaves and in which the photosynthetic tissue corresponds to the succulent articulated green stems situated at the most external part of the canopy, being the basal part usually dominated by woody branches. *Atriplex portulacoides* (L.) Aellen is a subshrub perennial halophytic species, also of the Chenopodiaceae family, that mainly growths in a creeping form. It is woody at the base and herbaceous at the upper part, which is formed by slightly fleshy leaves (Castroviejo, 1990). *Salicornia patula* Duval-Jouve belongs to the same family as the previous ones, being an annual succulent herb with also highly reduced leaves and succulent articulated green stems (Davy et al., 2001). Finally, *Elymus pycnanthus* (Godr.) Melderis is perennial, herbaceous and the only dominant monocotyledonous species of La Pletera, belonging to the Poaceae family.

(a) ***Sarcocornia fruticosa*** **(b)** ***Atriplex portulacoides***

(c) ***Elymus pycnanthus*** **(d)** ***Salicornia patula***

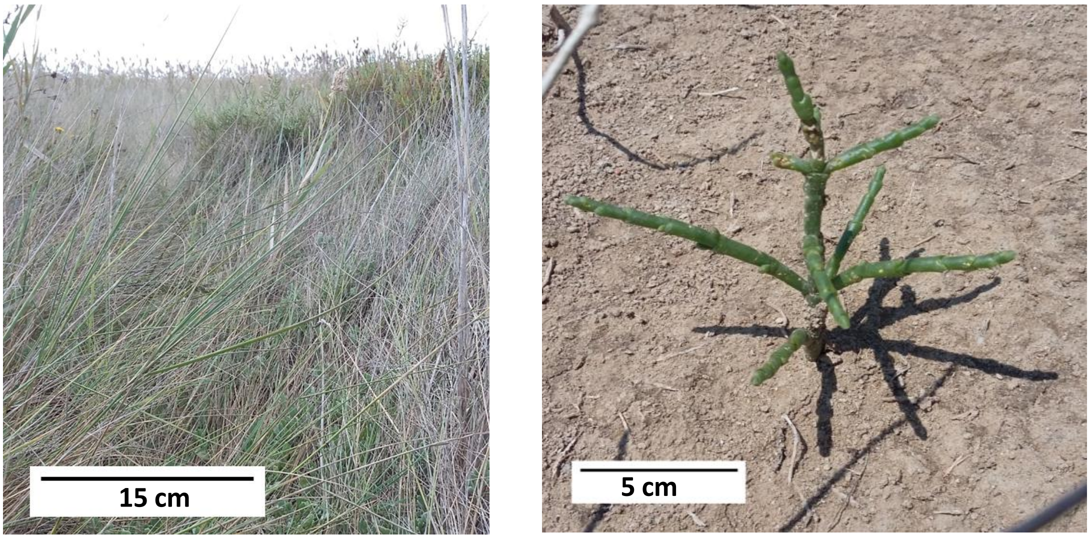


Figure 1.5. Dominant species of the salt marsh habitats of La Pletera salt marsh.

The Study 1 of this thesis was conducted in the well-preserved and the disturbed, and lately restored, zones, while the other two studies (Study 2 and 3 of this thesis) were performed at the three salt marsh habitats of the well-preserved zone of La Pletera salt marsh.

2. OBJECTIVES

The global aim of this thesis was to study the soil and vegetation carbon dynamics (stocks and seasonal fluxes) and litter decomposition in the non-tidal Mediterranean salt marsh La Pletera (NE Iberian Peninsula), taking into account the main habitats that conform this salt marsh.

The specific objectives of this thesis were:

1. To analyse the changes in the structure and composition of plant communities, as well as in the amount of carbon stored in vegetation and soil, of the three salt marsh habitats in one year, as well as of the disturbed (and later restored) zone, of La Pletera salt marsh **(Study 1)**.
2. To assess the daily and seasonal CO_2 fluxes of vegetation, as well as the CO_2 and CH_4 fluxes from soil, in the salt marsh habitats of La Pletera salt marsh throughout one year **(Study 2)**.
3. To analyse the litter decomposition process of three of the most abundant species of La Pletera (*Sarcocornia fruticosa*, *Elymus pycnanthus* and *Atriplex portulacoides*) in their natural habitats **(Study 3)**.

To achieve the first specific objective, several parameters, such as the percentage of plant cover (total and living), species richness, relative abundance of dominant species, Shannon-Wiener index of biodiversity, species evenness and maximum height of the vegetation (total and living), were analysed in the selected salt marsh habitats along two consecutive years and in the disturbed zone before and after the restoration. Besides, the amount of carbon stored in aboveground plant biomass (green, woody and standing dead tissues), belowground plant biomass, litter and soil was estimated for the different habitats of the well-conserved zone (along two consecutive years) and for the disturbed zone before the restoration. One year after the restoration of this latter zone, the amount of carbon was also determined in the aboveground and belowground plant biomass.

To achieve the second objective, instantaneous net CO_2 exchange rates from the dominant plant species of La Pletera were seasonally measured after sunrise, at midday, before sunset and at night, in green and woody (if were present) tissues. CO_2 and CH_4 fluxes from soils (or from water when soils were flooded) were also seasonally measured.

To achieve the third objective, the litter decomposition rate, together with litter quality parameters (carbon, nitrogen, cellulose and lignin content), fungal and bacterial biomass and the potential activity of extracellular enzymes involved in plant decomposition (ß-D-glucosidase, ß-D-xylosidase, leucine-aminopeptidase and phenol oxidase) were measured throughout the study period for each species. In order to embrace the spatial variability, two zones were considered for each species within each habitat.

3. METHODOLOGY

3.1. Study area

The well-preserved zone of La Pletera salt marsh, shows a clear zonation of vegetation according to the period of flooding (which affects soil aeration and salinity), and coinciding with the typical zonation found in salt marshes (Adam, 1990; Mitsch & Gosselink, 2015). The Mediterranean halophilous scrub was flooded at different times of the year, but the process was slightly different in the two areas in which this habitat was divided. The eastern area is mainly flooded by direct entrance of the sea during sea storms, being the period of flooding usually long (even several months), while the western area is mostly flooded by rainfall, (as in the case of Mediterranean salt meadow) being the duration of flooding usually short (several days or some weeks), although always longer than in the salt meadow. In the western area, sea water entrance can occur, especially with strong sea storms, but always the amount of sea water reaching this area is much lower than in the eastern part. The Mediterranean salt meadow, located in the area furthest from the sea (Figure 3.1), is rarely flooded, being mostly puddled rather than submerged during the flooding periods. Because of the distance to the sea, flooding of these salt meadow was mainly due to rainfall, either by direct water input or by the rise of the water table. The glasswort sward, which are dominated by annual pioneer vegetation, had the longest flooded periods, which could last several months, and both rainfall and sea storms seemed to have similar contribution to the flooding. Within this habitat, the area further to south is where the flooding is longer, probably because the soils of this area have low permeability due to a higher content of clay (Pascual & Martinoy, 2017). From the study of soil profiles performed by Amorós (2018), soil of the three salt marsh habitats have been classified, according to the Soil Survey Staff (2014), as Fluvaquent typic.

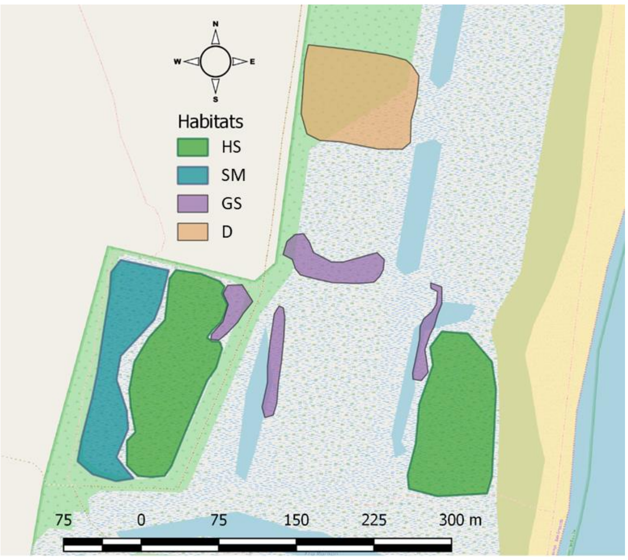


Figure 3.1. Distribution of the selected areas of each salt marsh habitat studied at La Pletera salt marsh: HS: Halophilous scrub dominated by *Sarcocornia fruticosa*; SM: Salt meadow dominated by *Elymus pycnanthus* and *Atriplex portulacoides*; GS: Glasswort sward dominated by *Salicornia patula*; D: Disturbed zone dominated by ruderal vegetation before restoration and by annual pioneer halophytic vegetation after the restoration (from Study 1).

The area presents a coastal Mediterranean-type of climate with the lowest temperatures found in winter and the highest in summer (Figure 3.2a). The highest monthly mean rainfall was recorded in autumn for the period 2008-2017 (Figure 3.2b). In particular, the total rainfall of the years 2015, 2016 and 2017, when the samplings of this thesis were conducted, was 316.6, 487.4 and 544.7 mm year^{-1} respectively (Figure 3.2b).

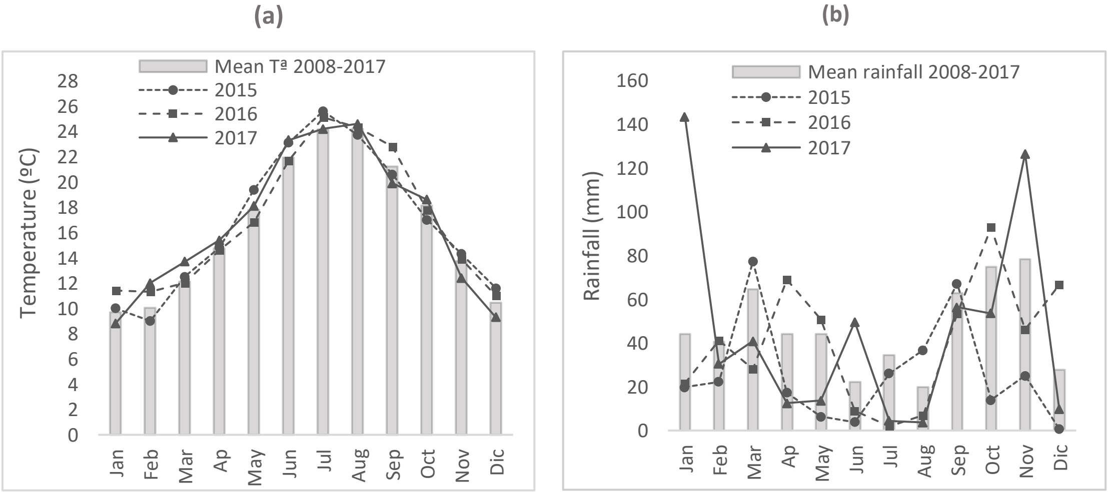


Figure 3.2. Monthly mean daily temperature (a) and monthly total rainfall (b) for each one of the three years in which samplings were performed (2015, 2016, 2017) and 10-year average (2008–2017) for both variables (a, b). Climate data was obtained from l'Estartit meteorological station (Pascual, 2017), located at 2.5 km from La Pletera salt marsh.

3.2. Study 1: Structure and composition of plant communities and carbon stored in vegetation and soil in the salt marsh habitats and in the disturbed and later restored zone

This study was performed in the three well-preserved salt marsh habitats and in the disturbed, and later restored, zone. Several parameters related with the structure and composition of plant communities were estimated in the salt marsh habitats in two consecutive years and in the disturbed zone before and after the restoration. Carbon stored in aboveground plant biomass, belowground plant biomass, litter and soil was estimated for the salt marsh habitats along two consecutive years and for the disturbed zone before the restoration, being also estimated the carbon in the aboveground and belowground plant biomass one year after the restoration.

3.2.1. Study of the plant community structure and composition

The study of the structure and composition of plant communities of the salt marsh habitats (halophilous scrub, salt meadow and glasswort sward) and the disturbed zone was performed using the point quadrat method (Heslehurst, 1971). The three salt marsh habitats were sampled in summer of 2015 and 2016, whereas the disturbed zone was sampled in summer of 2015 (before the restoration) and in summer of 2017 (after the restoration performed in 2016). In summer 2017, we also sampled a zone restored in 2015 that had not been previously sampled. Point quadrat data were collected using a grid frame of 80 x 80 cm with 64 points separated 10 cm. A vertical metal pin with diameter of 7 mm was used to record, for each point of the grid, the maximum height of each plant species touching the pin, distinguishing the following parts: living green biomass, living woody biomass, or standing dead tissues. For each of the three salt marsh habitats, five randomly selected plots (80 x 80 cm) were sampled each year (2015 and 2016). In the disturbed zone, ten randomly selected plots of the same size were sampled in 2015 and the same plots were also sampled in 2017, one year after restoration. In 2017, seven other randomly selected plots (80 x 80 cm) belonging to an area of the disturbed zone that was restored in 2015 were also sampled. This allowed us to compare plant colonization one and two years after the restoration.

Different parameters related with the structure and composition of the plant communities were calculated for each plot: percentage of plant cover, percentage of living plant cover, species richness (*S*), relative abundance of the dominant species (p_i), Shannon-Wiener index of biodiversity (*H*), species evenness (*J*) (Pielou, 1969), maximum height of the vegetation and maximum height of the living parts of the vegetation. Calculations of *H* and *J* were performed using the following equations:

$$H = -\sum_{i=1}^{S} p_i \, log_2 p_i \;\; ; \quad J = \frac{H}{H_{max}} = \frac{-\sum_{i=1}^{S} p_i log_2 p_i}{log_2 S}$$

were p_i is the relative abundance of the species and *S* is the species richness.

3.2.2. Plant biomass and carbon stored in the different plant fractions

In each one of the three salt marsh habitats, all the aboveground plant biomass of five randomly selected plots (80 x 80 cm) was harvested in summer (July) 2015 and 2016. After removing the aerial plant biomass, superficial litter was collected in a quarter of each one of the plots, and plant roots were sampled at 0-20 and 20-40 cm depth in 2015 and at 0-20 cm in 2016 by the extraction of three soil cores (Eijkelkamp auger set; 8 cm diameter, 20 cm long) per plot. In 2016, root sampling was only performed at 0-20 cm depth because data from 2015 demonstrated that 94%, 83% and 75% of the belowground biomass of the halophilous scrub, salt meadow and glasswort sward habitats, respectively, was located at the first 20 cm.

In the disturbed zone, all the aboveground plant biomass of each one of the ten plots (80 x 80 cm) used to study plant community composition and structure was harvested in summer 2015 (before the restoration) and 2017 (one year after the restoration). In this zone, sampling of the belowground biomass in summer 2015 was performed at 0-20 and 20-40 cm by removing all the soil from one fourth of the area of the plot since the accumulation of stones complicated the extraction of cores. After the restoration, in summer 2017, due to the scarcity of vegetation and the small size of the individuals, belowground biomass was determined by collecting the entire radicular part of each individual (roots were never deeper than 20 cm).

Aboveground plant biomass from each plot was taken to the laboratory and sorted by species and living green, living woody, and standing dead biomass. Once in the laboratory, roots were also separated from soil samples. Litter and roots were rinsed with tap water to eliminate residual soil particles. All the plant material was dried in an oven at 70 ºC to constant weight.

To estimate the carbon stored in the vegetation, for each plot, dried plant material of each fraction (living green and woody biomass and standing dead tissues of the dominant species, as well as roots and litter) was homogenized with a grinder (Taurus, Spain). The amount of dried plant material used was from 25 to 100% depending on the total amount of each fraction in each plot, i.e. 25-50% when the total weight of the fraction was higher than 80 g; >50% when the weight was 15-80 g and 100% when the weight was lower than 15 g. After that, a part of the homogenized material (two vials of 2 ml capacity) was ground to fine powder with a ball mill (Mixer Mill MM 400, Retsch GmbH, Haan, Germany) for elemental composition analyses. To perform these analyses, for each plot, two subsamples of each fraction of around 4 mg were weighted (microbalance Sartorius 2MP, Germany) and placed in tin capsules for C and N analyses. Total C and N estimations were performed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the Stable Isotope Facility of the University of California (Davis; USA). For each plot, carbon stored in the vegetation (g C m^{-2}) was estimated multiplying the carbon concentration (mg C g DW^{-1}) of each plant fraction by its total weight (g m^{-2}).

3.2.3. Soil characterization and carbon stored in the soil

In the three salt marsh habitats, one composite soil sample per plot was obtained at 0-20 cm depth from the five selected plots where the plant biomass was harvested in summer 2015 and 2016. In summer 2015, we also took soil samples from three of the selected plots at 20-40 cm depth, which allowed us to determine that, as for the roots, most of the carbon stored in the soil was located at the first 20 cm (72%, 70% and 65% for the halophilous scrub, salt meadow and glasswort sward habitats, respectively). The same three cores (Eijkelkamp auger set; 8 cm diameter, 20 cm long) extracted for each plot for plant belowground biomass sampling was used to obtain the soil samples. For each plot, the three soil subsamples were bulked to give one composite soil sample per depth. In the disturbed zone, soil samples were also collected in summer 2015 from samples taken to obtain plant roots, i.e. from soil removed at 0-20 and 20-40 cm from one fourth of the area of the plot.

To characterise the soil of the three salt marsh habitats some physical and biochemical parameters were analysed: pH, electrical conductivity (EC), the soil textural classes, soil bulk density (BD), soil organic carbon (SOC) and soil total nitrogen (TN). Before the analysis, samples were air-dried and sieved to 2 mm. Soil pH was determined potentiometrically in distilled water and with a soil:water ratio 1:2.5 using a Crison 20 pH meter (Crison Instruments S.A., Barcelona, Spain). Electrical conductivity was measured with a soil:water ratio 1:5 using a Crison micro CM 2200 conductivity meter (Crison Instruments S.A., Barcelona, Spain). Regarding soil textural classes, the percentage of sand was determined using sieves with different mesh sizes (200 and 20 µm), whereas the percentage of silt and clay was estimated using a calibrated volumetric pipette and considering the principle of sedimentation of soil particles. The dimensional classes estimated were 2000-20 µm for sand fraction, 20-2 µm for silt, and <2 µm for clay fraction according to the International Society of Soil Science (ISSS). Soil bulk density (BD) was estimated by weighting a known volume of soil after being dried at 105 ºC to constant weight. Soil organic carbon (SOC) was quantified by the dichromate wet oxidation method (Walkley-Black method) in presence of concentrated sulfuric acid while soil total nitrogen (TN) was estimated by means of the Kjeldahl method (Van Reeuwijk, 2002). pH, EC and TN were estimated in three composite soil samples per habitat at each depth in 2015 and in five composite soil samples per habitat at 0-20 cm depth in 2016. Soil textural classes were determined only in the samples of 2016. SOC (%) and BD were analysed at 0-20 cm depth in all the five soil composite samples of each year (2015 and 2016), being SOC also estimated at 20-40 cm depth in the three soil samples of 2015. The amount of organic carbon stored at 0-20 cm, expressed in g C m^{-2}, was estimated from SOC and BD values.

3.2.4. Data analysis and statistics

Two-way ANOVAs were performed to evaluate the differences between the three salt marsh habitats in the parameters studied, using habitat and year as fixed factors. When the interaction between the two factors was significant, differences between years (for each habitat) and among habitats (within each year) were evaluated by means of one-way-ANOVAs, using year or habitat, respectively, as fixed factor. When there were no significant differences between years and the interaction between year and habitat was not significant, differences among habitats were tested (by means of one-way ANOVAs or Kruskall-Wallis tests) pooling the data for the two years. In the case of the disturbed zone, the plant structure and composition and the estimated carbon stored in the vegetation, litter and soil of the ruderal community (before the restoration) was compared to the one of the halophilous scrub by one-way ANOVAs. The reason for this comparison is that it is expected that this zone once restored will become similar to the halophilous scrub habitat over the years. Differences in the structure and composition of plant communities one and two years after the restoration were also analysed by one-way ANOVAs. When the assumptions of normality and/or homoscedasticity were not reached for the variables analysed (which happened for the percentage of plant cover, percentage of living plant cover, species richness and Shannon-Wiener index of biodiversity for both the salt marsh habitats and the disturbed zone, and for the evenness when comparing the halophilous scrub with the ruderal community), Mann-Whitney U-tests or Kruskall-Wallis tests were applied.

For each one of the salt marsh habitats, two-way ANOVAs were performed using year and species (taking into account the dominant ones) as fixed factors to evaluate the differences in: 1) the relative abundance and the percentage of the total aboveground biomass of each species, and 2) the carbon concentration of the different fractions of the aboveground biomass. For those variables in which the assumption of normality and/or homoscedasticity was not met (this was the case of the relative abundance of *A. portulacoides* in the halophilous scrub and the relative abundance of all the species of the glasswort sward), Mann-Whitney U-tests were performed. On the one hand, in the case of the disturbed and later restored zone, differences among species in the percentage of the total aboveground biomass they represent were analysed by means of one-way ANOVAs for the ruderal community (disturbed) and Mann-Whitney U-tests for the plant community present one year after the restoration (since the assumption of normality was not reached). On the other hand, to evaluate which fraction of the aboveground biomass (living green, living woody or standing dead biomass) of the most abundant species of each habitat (*Sarcocornia fruticosa*, *Elymus pycnanthus* and *Salicornia patula* for the halophilous scrub, the salt meadow and the glasswort sward, respectively) had the highest amount of carbon, two-way ANOVAs were performed for each species, using year and fraction as fixed factors. When the interaction between year and species (for the parameters commented above) or year and fraction was significant, differences among species or fractions were assessed within each year. Conversely, when the interaction and the year factor were not significant, one-way ANOVAs were performed pooling the data for the two years and using species or fraction as fixed factor. Besides, to analyse which compartment (vegetation or soil) stored more carbon in the three salt marsh habitats, one-way ANOVAs were performed using compartment as fixed factor for each habitat.

The Shapiro-Wilk test was used to test normality, while the homogeneity of variances was analyzed with the Levene's test. Tukey's HSD *post-hoc* tests were applied when there were significant differences among habitats, species or fractions. Pairwise comparisons were used to identify differences between habitats when non-parametric tests were performed. For all the statistical tests, the significance level considered was p-value < 0.05. Statistical analyses were done using SPSS software (IBM SPSS statistics, Corporation, Chicago, USA).

3.3. Study 2: Seasonal carbon fluxes from vegetation (CO_2) and soil (CO_2 and CH_4)

This study was performed in the three habitats of the well-preserved zone of La Pletera salt marsh. Instantaneous net CO_2 exchange rates were seasonally measured at different times of the day, in green and woody (if present) tissues of the dominant plant species of each habitat. Daily CO_2 and CH_4 fluxes from soils (or from water when soils were flooded) were also seasonally measured.

3.3.1. Seasonal CO_2 fluxes from vegetation

During 2017, carbon dioxide (CO_2) fluxes, measured as instantaneous net CO_2 exchange rates (NER), were monitored for the dominant species of the salt marsh habitats (*Sarcocornia fruticosa*, *Atriplex portulacoides*, *Elymus pycnanthus* and *Salicornia patula*). Measurements were performed in green and woody plant tissues (except for *E. pycnanthus* and *S. patula*, which only had green tissues) using a PLC3 conifer leaf chamber (80 x 40 mm) connected to an infrared gas analyser (IRGA; CIRAS-II, PPsystems

USA) (Figure 3.3). To measure the CO_2 fluxes from the woody tissues of *S. fruticosa* and *A. portulacoides*, stems of maximum 3 mm of diameter were used, since thicker stems did not allow closing the PLC3 leaf cuvette chamber. In a parallel study (Study 1), it was estimated that stems with a diameter ≤ 3 mm represented 35% and 100% of the total woody live biomass of *S. fruticosa* and *A. portulacoides*, respectively.

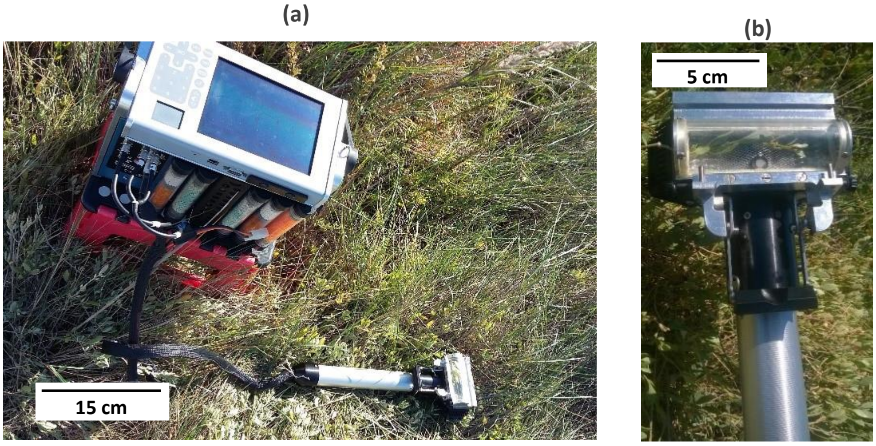


Figure 3.3. Infrared gas analyser (IRGA) (a) connected to a PLC3 conifer leaf chamber (b) to measure net CO_2 fluxes from vegetation.

Measurements of daily CO_2 fluxes were always carried out on sunny days after sunrise, at midday, before sunset and at night, being measurements performed in sun-exposed vegetal tissues when measuring in sunlight time. Night measurements were performed one hour after the complete absence of light, usually 2 hours after the sunset. To assess seasonal CO_2 fluxes, measurements were made in different days throughout the year as indicated in Table 3.1.

Plant fractions used to measure CO_2 fluxes were collected and stored in a fridge until the sampled tissue area was determined in the laboratory. To analyse the sampled stem areas of *S. fruticosa* (green and woody), *S. patula* (green) and *A. portulacoides* (woody), it was assumed that the stems were cylinders and that during daytime measurements only half of each stem received direct sunlight, according to the previous studies of Redondo-Gómez et al. (2006) and Pérez-Romero et al. (2019). Thus, in the daytime measurements, the cylinder area was divided by 2 to obtain the illuminated area. The sampled leaves of *A. portulacoides* were scanned (Epson Perfection 1250, USA) and from these images the area was calculated using ImageJ software (Schneider et al., 2012). For night measurements the area of the scanned leaves of *A. portulacoides* was multiplied by 2. The sampled areas of *E. pycnanthus* leaves were estimated assuming they were rectangles when they were fully expanded (multiplying in these cases the obtained values by 2 for nigh measurements) or cylinders when they were folded (dividing in these cases the obtained values by 2 for daytime measurements). Instantaneous NER was expressed as µmol CO_2 m^{-2} s^{-1} where m^{-2} refers to tissue area. Stomatal conductance values (g_s) were also obtained with the IRGA when NER was measured. Intrinsic water-use efficiency (iWUE) for midday measurements of green tissues was calculated as the ratio between photosynthetic rates and g_s.

Table 3.1. Summary of the samplings performed to determine CO_2 fluxes from green and thin woody plant tissues (Ø ≤ 3mm) throughout one year (2017). The frequency of samplings per season and the number of plants used per species (n) are shown for the different times of the day when measurements were taken.

Time of the day	**n**	**Frequency of samplings**	
		Green tissues	**Thin woody tissues**
After sunrise	4	Every month and a half approximately (twice/season): 9 February, 10-16 March, 19-20 April, 8-13 June, 10-12 July, 4-11 September, 23 October, 6 December	Every three months approximately (once/season): 10 March, 15-16 May, 24-28 August, 7-15 November
Midday	6		
Before sunset	4		
Night	4	Every three months approximately (once/season): 10 February, 13 June, 28 August, 15 November	

To have a rough estimation of the role that each species might play in sequestering or emitting CO_2 over an entire day (integrated daily CO_2 flux), CO_2 fluxes measured 4 times per day (after sunrise, at midday, before sunset and at night) were integrated for each species considering the instantaneous NER data and the duration of each time period (Table 3.2). NER after sunrise, at midday and before sunset was measured twice per season, while NER at night was measured once per season. For this reason, to estimate daily NER of green tissues, we used the same NER values obtained at night (once per season) for the two sampling days of each season.

Table 3.2. Duration (hours and minutes) of the periods in which the day was divided for each month in 2017.

	Duration day periods (h)			
Month	**After sunrise**	**Midday**	**Before sunset**	**Night**
January	3:46	1:57	3:46	14:31
February	3:51	2:51	3:51	13:27
March	3:53	4:09	3:53	12:05
April	4:07	5:05	4:07	10:41
May	4:22	5:48	4:22	9:28
June	4:19	6:30	4:19	8:52
July	3:39	7:31	3:39	9:11
August	3:25	6:55	3:25	10:15
September	3:42	5:01	3:42	11:35
October	3:53	3:15	3:53	12:59
November	3:44	2:19	3:44	14:13
December	3:32	2:05	3:32	14:51

The estimation of the duration of each period of the day (after sunrise, midday, before sunset and night) for the different sampling days was performed as follows: the peak sun hours, defined as the number of hours per day in which solar irradiance is up to 1000 Wm^{-2} (Sen, 2008), were obtained for La Pletera salt marsh for every month of 2016 from the database of (Huld, 2017). These hours were considered as the midday period (Figure 3.4). Then, the daytime length was estimated taking into account the monthly average time in which the sun appears and goes down (http://www.sunrise-and-sunset.com/es/sun/espana/torroella-de-montgri). Finally, the peak sun hours (midday period) were subtracted to the total daytime hours for each month and the result was divided by two to have the duration of the period after sunrise and before sunset. Thus, the period after sunrise comprised the hours between the appearance of the sun and the start of the midday period, while the period before sunset were the hours between the end of the midday period and the sunset (Figure 3.4).

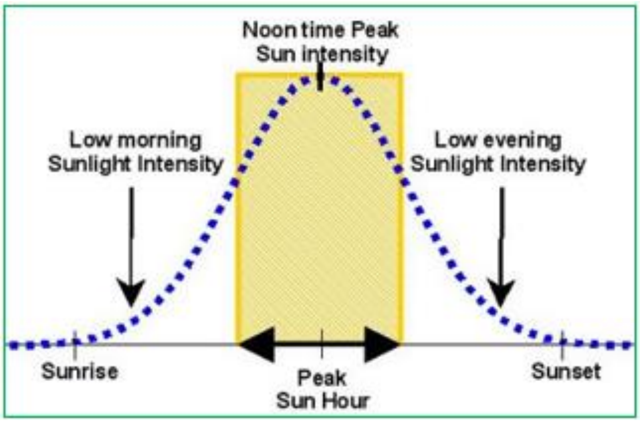


Figure 3.4. Scheme showing the peak sun hours in relation to the daytime duration obtained from Sameti et al. (2014).

3.3.1.1. Seasonal changes in plant biomass

To estimate changes in green and woody aerial plant biomass throughout the year, all the aboveground vegetation of three plots (0.5 m x 0.5 m) per habitat and season was harvested. In the case of the glasswort sward, samplings were only performed in spring and summer since the dominant species of this habitat, *Salicornia patula*, is annual and it was not present in autumn and winter. Sampling plots were randomly selected and, once harvested, the plant material of each plot was separated by species and living (distinguishing between green and woody tissues) and dead biomass. Biomass from each fraction was weighed after being oven-dried at 75°C for 48 hours.

3.3.2. Seasonal carbon (CO_2 and CH_4) fluxes from soil

3.3.2.1. Soil CO_2 measurements

Measurements of soil CO_2 fluxes were performed every month and a half during 2017, usually one or two days before or after the measurements of CO_2 fluxes from vegetation (green tissues). Soil CO_2 measurements were carried out by the soda lime method (Edwards, 1982) which is based on the capacity of the soda lime to absorb CO_2, using a cover box system (explained below) (Alef & Nannipieri, 1995). Soda lime is a chemical composed mainly by calcium hydroxide [$Ca(OH)_2$] (around 75%) and water (around 20%), having also a small fraction of sodium hydroxide [NaOH] (around 3%) and potassium hydroxide [KOH] (around 1%). When CO_2 reacts with the hydroxides of this chemical, the formation of calcium carbonates, and at less degree sodium and potassium carbonates, plus water occurs, increasing, in consequence, the weight of soda lime. Thus, soil respiration is estimated from the gain in soda lime dry weight after the chemical is oven dried to retain only the CO_2.

In the field, static opaque chambers called “cover boxes” (PVC cylinders of 11 cm of diameter and 13 cm of height) were inserted 5 cm into the soil, remaining 8 cm of the cover box above soil surface (Figure 3.5a, b). In each habitat, a cover box was placed in five of the plots used to estimate the carbon stored in vegetation and soil (see Study 1). At midday of each sampling day, an open glass vessel containing soda lime, previously oven dried at 105 °C and weighed, was placed inside each cover box, which was immediately closed with a hermetic cover (Figure 3.5b). After approximately 24 h, the cover box was opened and the glass vessel with soda lime was hermetically sealed and collected. In the laboratory, the soda lime was again oven dried at 105 °C and weighted. The CO_2 absorbed by the soda lime was calculated by multiplying the weight gain by 1.69 as a water correction factor (Grogan, 1998; Emran et al., 2012). Daily soil respiration rates (SR; g CO_2 m^{-2} d^{-1}) were calculated as follow:

$$SR = \frac{\left(SL_f - SL_i\right) * 1.69}{A * t}$$

where SL_f is the soda lime dry weight (in g) after being placed in the field; SL_i is the initial soda lime dry weight (in g) before being placed in the field; *A* is the soil surface area (m^2), i.e. the area of the cover box (0.0095 m^2); and *t* is the time (in days) that soda lime remained in the field.

Figure 3.5. Cover boxes used to take the measurements of net CO_2 and CH_4 fluxes from soil when it was not flooded (a and b) and in the case that the soil could be flooded (c and d). When soil was not flooded, soda lime was placed inside the cover box (a) and then the cover box was hermetically closed (b).

When the soil was flooded (Table 3.3), measurements of soil CO_2 fluxes could not be performed using the soda lime method, and instead air samples from the same points previously measured were collected and CO_2 analysed by gas chromatography. To do this, cover boxes high enough to allow that a part of the cylinder remained outside of the water (i.e., 40 cm height) were used (Figure 3.5b, c). For each cover box, the height of the part of the cylinder remaining outside the water was measured to know the air volume inside the cylinder. After 24h of being hermetically closed, an air sample from the cylinder was taken inserting a syringe through the rubber septum on the lid of the cylinder, being stored in a 5-ml vacuum vial. Control air samples were also taken just before closing the cylinders to know the initial value of CO_2. Samples were kept at low temperatures and then analysed by gas chromatography at the Laboratory of Chemical and Environmental Engineering (LEQUIA) of the Research Technical Services of the University of Girona, where an Agilent 7890A gas chromatograph (Agilent Technologies, USA) was used. Daily soil respiration rates measured by gas chromatography (SR_C; g CO_2 m^{-2} d^{-1}) were calculated as follow:

$$SR_C = \frac{(W_f - W_i)}{A * t}$$

where W_f is the amount of CO_2 (grams) in the air inside the cover-box after the time t; W_i is the initial amount of CO_2 (grams) in the air inside the cover-box before being closed; A is the soil surface area (m^2), i.e. the area of the cover box (0.0095 m^2); and t is the time that the cover box remained closed (in days). W_f *and* W_i were estimated from volumetric concentration (%) considering the air volume inside the cover box in each sampling date and the density of CO_2 (0.001842 g cm^{-3}). The soda-lime method was chosen to measure SR when soil was not flooded instead of doing always the measurements with the gas chromatography method because this is a more reliable method since gas chromatography have been observed that can underestimate the CO_2 emission rate in comparison to other methods by up to 45 % (Lou & Zhou, 2006).

In addition to daily measurements, instantaneous soil respiration rates (iSR) (from measures of one-minute duration) were also determined at midday during the same days in which samplings with soda lime were performed. To take these measurements, an opaque cylindrical chamber (area: 0.007 m^2, volume: 1.2 l) connected to the infrared gas analyser (IRGA; CIRAS-II, PPsystems USA) was used.

Table 3.3. Summary of the samplings performed to determine CO_2 fluxes from the soil throughout year 2017 indicating the number of flooded and non-flooded plots in every sampling day and the method used in each situation.

Habitat	**Flood state**	**Method**	**Winter**		**Spring**		**Summer**		**Autumn**	
			Feb	**Mar**	**Apr**	**Jun**	**Jul**	**Sep**	**Oct**	**Dec**
Halophilous scrub	Non-flooded	Soda-lime and IRGA		5	5	5	5	5	5	5
	Flooded	Gas chromatography	5							
Salt meadow	Non-flooded	Soda-lime and IRGA		5	5	5	5	5	5	5
	Flooded	Gas chromatography	5							
Glasswort sward	Non-flooded	Soda-lime and IRGA		2	5	5	5	5	5	2
	Flooded	Gas chromatography	5	3						3

3.3.2.2. Soil CH_4 measurements

Methane fluxes between the atmosphere and the soil surface (or the water surface when the soil was flooded) were estimated using cover boxes and taking air samples after 24 hours, coinciding with the same sampling days in which soil CO_2 measurements were performed. Cover boxes were hermetically closed and after 24 h an air sample was taken and immediately transferred to a 5-ml vacuum vial. Air samples were also taken before closing the cylinders to know the initial value of CH_4 (control). The samples were kept at low temperatures and then analysed by gas chromatography (Agilent 7890A gas chromatograph, Agilent Technologies, USA) at the LEQUIA laboratory of the Research Technical Services of the University of Girona. When the soil was flooded the same air samples taken to estimate the CO_2 flux were used to determine the CH_4 flux. Daily soil methane fluxes measured by gas chromatography (SMF; g CH_4 m^{-2} d^{-1}) were calculated as follow:

$$SMF = \frac{\left(Wm_f - Wm_i\right)}{A * t}$$

where Wm_f were the grams of CH_4 in the air inside the cover-box after the time t; Wm_i were the initial grams of CH_4 in the air near the cover-box at the beginning of the measurements; A was the soil surface area (m^2), i.e. the area of the cover box (0.0095 m^2); and t was the time that the cover box remained closed (in days). Wm_f *and* Wm_i were estimated from volumetric concentration (%), considering the air volume inside the cover box in each sampling date and the density of CH_4 (0.000656 g cm^{-3}).

3.3.2.3. Carbon mineralization quotient

The carbon mineralization quotient (Q_{min}) represents the fraction of soil organic carbon (SOC) mineralized in a given period of time (Pinzari et al., 1999). Specifically, it represents the carbon that is emitted to the atmosphere under inorganic forms (CO_2 and CH_4), considering that it has been produced in a superficial soil layer, in relation to the carbon that is stored in the soil (SOC) at the same soil layer. Concretely, in our case, the depth considered was the first 20 cm since the majority of the SOC was stored there (Study 1). To estimate Q_{min}, soil emissions of CO_2 (i.e. soil respiration) and CH_4 (as product of SOC mineralization under anaerobic conditions) were considered. Hence, daily carbon mineralization quotients (Q_{min}) were calculated for each sampling date following this equation:

$$Q_{min} = \frac{C\_CO2 + C\_CH4}{SOC}$$

were *C_CO2* was the carbon emitted as CO_2 (mg C g soil^{-1} d^{-1}); *C_CH4* is the carbon emitted as CH_4 (mg C g soil^{-1} d^{-1}) and *SOC* is the soil organic carbon of the first 20 cm depth (mg SOC g soil^{-1}).

3.3.2.4. Soil environmental measurements

At each sampling date and measurement location, soil temperature (Ts) was monitored with a portable thermometer at the first 12 cm depth (Digital Portable Thermometer AI 368, Acez; Singapore), as well as the soil volumetric water content (VWC) with a 20 cm rod (FieldScout TDR 300 soil moisture meter, Spectrum technologies Inc; USA), and the soil electrical conductivity (EC) (conductivity meter 254, CRISON instruments; Spain). Since the high EC values of these soils could affect the measurements of VWC, a correction of VWC values was done by obtaining calibration curves from TDR readings and moisture content measured directly by soil weight loss over time (taking into account the soil bulk density) in undisturbed soil samples.

3.3.3. Data analysis and statistics

To evaluate whether the studied plant species differed in their instantaneous net CO_2 exchange rates (NER) from green and woody tissues, and in their stomatal conductance and intrinsic water-use efficiency from green tissues, two-way ANOVAs were performed for each time of the day separately (after sunrise, midday, before sunset and night for NER and midday for g_s and iWUE) using species and sampling day as fixed factors. When the interaction between species and sampling day was significant, one-way-ANOVAs were performed for each sampling day using species as fixed factor and for each species using sampling day as fixed factor.

To evaluate changes in biomass of green and woody tissues among seasons and species, two-way-ANOVAs were performed using species and season as fixed factors. When the interaction between both factors was significant, changes in biomass among seasons were evaluated for each species separately by means of one-way-ANOVAs, using season as fixed factor.

To evaluate differences among habitats in soil respiration (SR), instantaneous respiration rates (iSR), methane fluxes (SMF) and carbon mineralization quotient (Q_{min}), as well as in soil temperature (Ts), electrical conductivity (EC) and volumetric water content (VWC), mixed models were performed. Repeated measures ANOVAs were not used because of the presence of missing values in two sampling days at the glasswort sward (in March and December this habitat had only two non-flooded plots). Mixed models were performed using habitat as fixed factor, sampling date as repeated factor (repeated measures) and plots (subjects) as random factor. The interaction between the habitat and sampling date factors was also included. Data were analysed using the diagonal repeated covariance type, which was appropriate considering the type of data and was also confirmed by comparing the different options in the models using the Akaike Information Criteria. When the interaction between habitat and sampling date was significant, one-way-ANOVAs were performed for each sampling day using habitat as fixed factor, except for March and December. In these two months, the glasswort sward had only two non-flooded plots, and, as a consequence, data did not accomplish homoscedasticity and normality; therefore, non-parametric Kruskall-Wallis tests were used to detect significant differences among habitats for these two months.

Correlations were performed between: a) midday NER of the perennial species (*S. fruticosa*, *A. portulacoides* and *E. pycnanthus*) and edaphic (VWC and EC) and climatic (maximum air temperature, relative humidity and vapor pressure deficit) parameters, b) night NER and minimum air temperature, c) soil carbon fluxes (SR, iSR, SMF) and edaphic parameters (VWC, EC and Ts) measured during the entire year, and d) soil carbon fluxes of July (SR, iSR, SMF) and soil organic carbon (SOC) and total nitrogen content (TN) measured in July of 2015 and 2016 (data from Study 1). Pearson’s correlation tests were usually applied, although the Spearman rank correlation coefficient was used when data did not follow a normal distribution. Besides, two principal component analyses (PCA) were performed, one of them using SR, SMF, VWC, EC and Ts of the entire year and the other using SR, SMF, SOC and TN of July. Mean values of these variables for each sampling day and habitat were ordinated in the PCA plots.

The Shapiro-Wilk test was used to check the normality of data, while the Levene's test was applied to evaluate the homogeneity of variances. Tukey's HDS *post-hoc* tests were applied when the factors (species, sampling day and/or season) were significant. When data did not accomplish the assumptions of the ANOVA non-parametric Kruskall-Wallis tests were performed, and differences among the levels of each factor were tested by means of pairwise comparisons. For all the statistical tests, the significance level considered was $p < 0.05$. Statistical analyses were performed using SPSS software (IBM SPSS statistics, Corporation, Chicago, USA) except in the case of the PCA, which was performed using R software version 3.5.2. (R Development Core Team, 2018) and the FactoMineR package (Lê et al., 2008).

3.4. Study 3: Litter decomposition of the dominant species of the halophilous scrub and the salt meadow

In this study, the litter decomposition rate, several litter quality parameters (carbon, nitrogen, cellulose and lignin content), fungal and bacterial biomass and the potential activity of extracellular enzymes involved in plant decomposition were measured in the litter of the dominant species of the halophilous scrub (*Sarcocornia fruticosa*) and the salt meadow (*Atriplex portulacoides* and *Elymus pycnanthus*).

3.4.1. Experimental design

Litter decomposition analyses were carried out in two habitats: the halophilous scrub and the salt meadow (Figure 3.6). The glasswort sward (dominated by annual pioneer vegetation) was not considered in this study since the amount of litter was insignificant. To account for the spatial variability, 2 zones, characterized by different edaphic parameters, were considered in each habitat (Table 3.4).

To assess the litter decomposition process the litterbag technique was applied (Graça et al., 2005). Senescent or recently dead plant material of the dominant species of these two habitats was collected (from both zones of each habitat) at early November 2016, coinciding with the peak of autumn senescence of these plants. Specifically, senescent stems of *Sarcocornia fruticosa* (fertile branches after fruiting, formed by a central woody stem from which herbaceous stems emerge) were collected in the halophilous scrub, while senescent leaves of *Atriplex portulacoides* and recently dead leaves of *Elymus pycnanthus* were sampled in the salt meadow (Figure SM 6). Once collected, all the plant material was taken to the laboratory, where it was mixed to provide a uniform initial sample for each species. The plant material was then air-dried for 9 days at room temperature, and, after this period, a fraction was separated for each species (specifically 3 subsamples corresponding to around 450 mg of dry weight each one) to estimate initial litter quality parameters (see below 3.4.2) and ash content. Fractions of approximately 4 g of air-dried plant material for each species were also separated and placed into nylon litterbags of 15 cm x 15 cm and 1 mm of mesh size. The specific litterbag mesh size was selected to allow the entry of edaphic mesofauna and avoid plant material loss. To estimate the initial oven-dried weight of plant material from each litterbag, two extra samples of about 4 g of air-dried material for each species were oven-dried (70 °C) until constant weight and a moisture correction factor was calculated. In mid-November 2016, litterbags were placed in the field. As commented above, to take into account the spatial variability of the habitats, litterbags of each species were placed at three sites in each one of the two zones of the corresponding habitat (Figure 3.6).

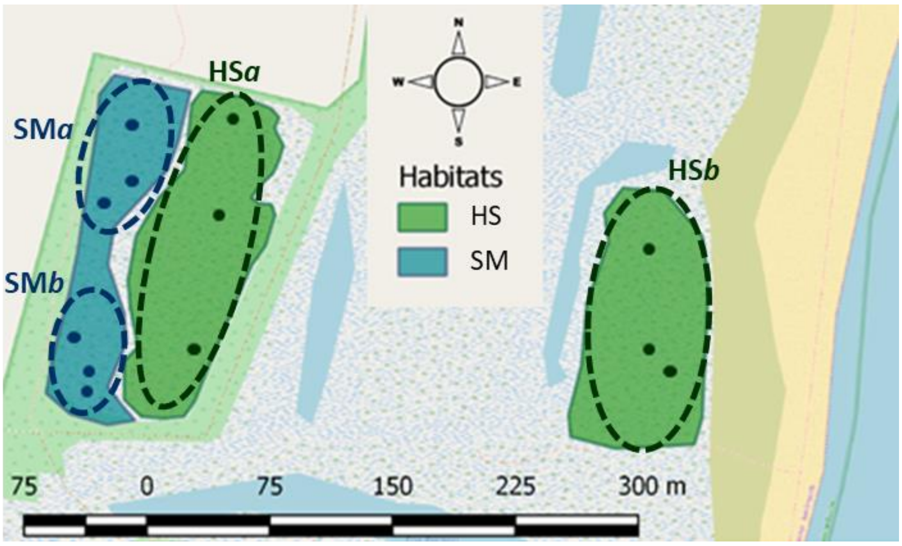


Figure 3.6. Diagram showing the two salt marsh habitats where the litter decomposition study was performed. HS: Halophilous scrub (dominated by *Sarcocornia fruticosa*); SM: Salt meadow (dominated by *Atriplex portucaloides* and *Elymus pycnanthus*). Two zones were considered within each habitat according to its spatial variability (HS*a* and HS*b* for the halophilous scrub and SM*a* and SM*b* for the salt meadow). The dots indicate the sites where litterbags were placed in the field.

Table 3.4. Mean ± SE (n=3 for zone *a* of both habitats; n=2 for zone *b* of both habitats) for soil parameters from the two zones within each habitat. HS: Halophilous scrub; SM: Salt meadow. Data of soil organic carbon (SOC), total nitrogen (TN), C/N ratio, pH and sand, silt and clay proportions are from soil samples (0-20 cm depth) from Study 1 of this thesis. Electrical conductivity (EC), soil volumetric water content (VWC) and soil respiration (SR) are instantaneous measurements (for VWC, the first 20 cm were considered) performed in July and taken for the Study 2 of this thesis.

Hab	**Zone**	**SOC** (%)	**TN** (%)	**C/N**	**pH**	**Sand** (%)	**Silt** (%)	**Clay** (%)	**EC** (dS m^{-1})	**VWC** (%)	**SR** (g CO_2 m^{-2} d^{-1})
HS	a	3.9	0.182	20.8	7.48	36.1	30.6	33.3	3.7	33.3	0.82
		± 1.3	± 0.054	± 1.7	± 0.26	± 3.0	± 3.9	± 1.1	± 1.6	± 2.8	± 0.03
	b	2.2	0.086	25.8	7.81	20.0	36.9	43.1	12.6	22.4	0.79
		± 0.1	± 0.001	± 1.6	± 0.04	± 8.6	± 3.8	± 12.3	± 0.7	± 0.5	± 0.10
SM	a	2.6	0.116	23.2	7.63	30.1	37.9	32.0	1.2	18.9	0.67
		± 0.3	± 0.011	± 4.7	± 0.02	± 12.6	± 5.1	± 7.7	± 0.8	± 2.4	± 0.06
	b	1.3	0.069	18.5	8.10	50.3	24.4	25.3	0.6	6.7	0.79
		± 0.1	± 0.009	± 1.0	± 0.10	± 1.06	± 1.3	± 0.3	± 0.3	± 0.1	± 0.08

Litterbags were attached using nylon string in 6 groups of 6 litterbags for *S. fruticosa* and *A. portulacoides* (36 litterbags in total for each species) and in 6 groups of 8 litterbags for *E. pycnanthus* (48 litterbags in total). One group of *S. fruticosa* litterbags per site (3 sites per zone) were placed in the halophilous scrub and two groups per site, one corresponding to *A. portulacoides* and the other to the *E. pycnanthus*, were placed in the salt meadow. Litterbags were situated on the soil surface under the canopy of each respective species, and tied to an iron stick with nylon string to allow the movement of the bags during the flooding periods. Six litterbags per species (one per site, and thus three per zone and habitat) were periodically collected for chemical and biological analyses (Table 3.5). At the beginning of the decomposition process, litterbags collections were more frequent in order to record the rapid weight loss that usually occurs during the first weeks, as observed in other Mediterranean salt marsh (Simões et al., 2011). The remaining ash free dry mass (to estimate decomposition rates) and the potential extracellular enzyme activities of the litter were measured every time litterbags were collected. The rest of the parameters (carbon, nitrogen, cellulose and lignin content and bacterial and fungal biomass) were measured three times throughout the litter decomposition period specifically on days 19, 64 and 92 for *S. fruticosa* and *A. portulacoides* and on days 19, 92 and 357 for *E. pycnanthus.*

Table 3.5. Days in which litterbags for the three species studied were collected from the field after being placed in their respective habitats.

Habitat	Species	Days of litterbag collection
Halophilous scrub	*Sarcocornia fruticosa*	5, 10, 19, 38, 64, 92
Salt meadow	*Elymus pycnanthus*	5, 10, 19, 38, 92, 164, 240, 357
	Atriplex portulacoides	5, 10, 19, 38, 64, 92

3.4.2 Litter ash free dry mass, decomposition rate and half-life

Plant material from field sampled litterbags was rinsed with distilled water to remove soil particles and mesofauna, oven-dried (70 °C) and weighed to assess mass loss from the start of the experiment. A fraction (about 200 mg) of the oven-dried plant material and a fraction of the initial litter after being oven-dried (70°C) were combusted at 450 °C for 4h and weighed to estimate the ash free dry mass (AFDM, %) by dividing the weight lost after combustion by the oven-dry weight and multiplying by 100.

Litter decomposition rates were estimated using the single exponential decay model regression: $X_t = X_0 * e^{-kt}$, where X_t is the litter AFDM at time t (days), X_0 is the estimated initial AFDM and k is the decomposition rate coefficient. The litter half-life was estimated using the equation $t_{0.5} = 0.693/k$, where $t_{0.5}$ is the half-life and k is the decomposition rate (Olson, 1963). Both parameters were calculated for each species from litter collected at each site (three per zone and habitat).

3.4.3. Litter quality: C, N, cellulose and lignin content

A fraction of the litter initially harvested and a fraction of litter from the sampled litterbags were oven-dried and ground to a fine powder with a ball mill (Mixer Mill MM 400, Retsch GmbH, Haan, Germany) for chemical analyses. For total C and N measurements, 4 mg of fine powder were weighted (microbalance Sartorius 2MP, Germany) and placed in tin capsules that were sent to the Stable Isotope Facility of the University of California (Davis, USA) for their analyses. The analyses were conducted using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

Cellulose and lignin content were measured gravimetrically from 240 mg of fine powder dry plant material according to Gessner (2005). Briefly, to remove protein and other acid-soluble material that would have interfered with the cellulose and lignin determination, samples were heated for 1 hour in an acid detergent solution (hexadecyltrimethylammonium bromide at 20 g l^{-1} in sulphuric acid 0.5M). After that, the solution was filtrated using glass gooch type crucibles and the crucibles containing the residue of the filtrated samples were overnight oven-dried (105ºC) and weighted. Then, the residues were treated three times with 72% sulphuric acid and the solution resultant, containing the dissolved cellulose, filtered. After that, the crucibles containing the residues without the cellulose were overnight oven-dried (105ºC) and weighted again. The cellulose content (%) was estimated as the loss weight due to 72% H_2SO_4 treatment divided by the initial oven-dry weight of the sample and multiplied by 100. Finally, the residues that remained in the crucible were combusted at 500 °C for 4.30 h and weighed again. Lignin content (%) was estimated as the loss weight after combustion divided by the initial oven-dry weight of the sample and multiplied by 100.

All the litter quality parameters analyzed were expressed in percentage, considering the units of compound in 100 units of litter biomass ash free dry mass. Molar C/N and lignin/N ratios were also calculated from C, N and lignin content.

3.4.4. Litter extracellular enzyme activity and enzyme efficiency

Potential extracellular enzyme activity of four enzymes related to litter decomposition was measured according to the methods described in Romaní et al. (2006). The four enzymes were: ß-D-glucosidase and ß-D-xylosidase involved in cellulose and hemicellulose hydrolytic degradation, respectively; leucine-aminopeptidase related with peptide hydrolytic degradation and phenol oxidase involved in lignin oxidation. Hydrolytic enzymes were measured by fluorimetric assays using fluorescent methylumbelliferone (MUF) linked to the artificial substrates for ß-D-glucosidase and ß-D-xylosidase (MUF-ß-D-glucopyranoside and MUF-ß-D-xylopiranoside, respectively) and fluorescent 7-amino-4-methylcoumarin (AMC) linked to the artificial substrate L-leucine-4-methyl-7-coumarinylamide hydrochloride (L-leucin-AMC) for leucine-aminopeptidase. When a specific enzyme breaks the link between the fluorescent molecule and its artificial substrate, the fluorescent molecule releases and the fluorescence detected at the end of the incubation period can be directly related to the number of links broken by the enzyme (Hoppe, 1993). Activity of oxidative enzyme phenol oxidase was measured by spectrophotometry using L-3,4-dihydroxyphenylalanine (L-DOPA) as oxidizable substrate for detection of phenol oxidase activity. One of the products of L-DOPA oxidation is a red coloured compound, the 2,3-dihydroindole-5,6-quinone-2-carboxylate (DIQC) which can be quantified by measuring its absorbance at 460 nm and can be directly related to the activity of phenol oxidase. All the artificial substrates were from Sigma-Aldrich (Missouri, USA).

Enzyme assays were conducted under substrate saturated conditions previously determined from saturation curves. These curves were made by plotting extracellular enzyme activity versus substrate rising concentrations for each species. Then, for each enzyme and according to the Michaelis-Menten kinetics, the concentration at which enzyme activity started to be constant was considered the substrate saturate concentration. A summary of the different enzymes, artificial substrates and final saturating concentrations is given in Table 3.6.

Table 3.6. Summary of extracellular enzymes measured specifying the EC (Enzyme Comission number), the artificial substrates, and the final substrate saturating concentration. MUF: methylumbelliferone; AMC: 7-amino-4-methylcoumarin.

Enzyme	EC number	Artificial substrate	Concentration
ß-D-glucosidase	EC 3.2.1.21	MUF-ß-D-glucopyranoside	0.5 mM
ß-D-xylosidase	EC 3.2.1.37	MUF-ß-D-xylopiranoside	0.5 mM
Leucine-aminopeptidase	EC 3.4.11.1	L-leucine-4-methyl-7-coumarinylamide hidrocloride (L-leucine-AMC)	0.3 mM
Phenol oxidase	EC 1.10.3.2 and 1.14.18.1	L-3,4-dihydroxyphenylalanine (L-DOPA).	5 mM

Potential extracellular enzyme activities were analysed on the same day in which litterbags were harvested from the field. To do this, a fraction of material from litterbags was rinsed with distilled water to remove soil particles and mesofauna, and immediately used for analyses. All the enzyme activity assays and the preparation of the MUF and

AMC standards (from 0 to 100 µM) were performed using water from a lagoon located at La Pletera salt marsh area and near to the sampling site that was previously filtered with a 0.2 µm pore size nylon filter to avoid the presence of microorganisms. Thus, for the analyses of the hydrolytic enzyme activity, material from litterbags were placed in 15 ml vials with 4 ml of filtered water together with the corresponding volume of MUF- or AMC-linked artificial substrate to reach the specific substrate saturate concentration. Control of filtered water activity (without litter) was performed for each enzyme and sampling date, using the same substrate concentration used for the samples. Besides, to convert the fluorescence values in concentrations of MUF and AMC, standards from 0 to 100 µM of both fluorescent molecules were also performed for each sampling date, and regression equations were calculated. Samples, controls and standards were kept to react for 1 h at 20 °C under agitation and darkness. After that, the reactions were stopped by adding 4 ml of glycine buffer (0.05 M, pH 10.4) to each sample, control and standards. Fluorescence was measured at 365/455 nm excitation/emission wavelength for ß-D-glucosidase and ß-D-xylosidase, and at 364/445 nm for leucine-aminopeptidase, using a multifunctional microplate reader Infinite 200 PRO (Tecan, Switzerland). Extracellular enzyme activities, expressed as µmol MUF or µmol AMC (depending on the enzyme analysed) per unit of time and ash free dry mass (AFDM) of plant material were quantified from fluorescence values, using the regression equation estimated from standards of MUF and AMC and subtracting the control values. After the activity enzyme analyses, samples were oven-dried (70^{o}C) to constant weight and weighed, being the fraction used from each litterbag about 0.04 g of oven-dry material. For the analysis of phenol oxidase activity, plant material was also placed in 15 ml vials with 2 ml of filtered water and 2 ml of L-DOPA (10 mM) in acetate buffer (pH 5) to reach the final substrate saturation concentration of 5 mM. A blank was analyzed for each sample (plant material in acetate buffer without L-DOPA). Besides, a control of filtered water activity (without plant material) was also analyzed. Samples, blanks and control were incubated for 2 h at 20^{o}C under agitation and darkness. After that, absorbance at 460 mm was immediately measured using a multifunctional microplate reader Infinite 200 PRO (Tecan, Switzerland). Phenol oxidase activity was estimated by dividing the absorbance by the extinction coefficient 1.66 mM (Sinsabaugh & Linkins, 1990) and subtracting for each sample the values obtained for the blank. Activity values were expressed as µmol

DIQC (2,3-dihydroindole-5,6-quinone-2-carboxylate, product of the L-DOPA degradation) per unit of time and ash free dry mass (AFDM) of plant material.

Enzyme efficiencies were calculated as turnover activities (TA), which are the inverse of the regression slope between ln % remaining AFDM regressed linearly to the accumulated enzyme activity (AEA, mmol g AFDM^{-1}) (Simon et al., 2009). AEA was calculated using the formula

$$AEA=\sum_{i=0}^{n} E_i T_i$$

where n is the number of samplings, E_i is the mean enzyme activity of two successive measurements, and T_i is the time between the two measurements. TA is expressed as mmol of enzyme produced to decompose one gram of litter, and higher values mean low enzyme efficiencies.

3.4.5. Bacterial carbon biomass

Bacterial carbon biomass was estimated from bacteria cell counts obtained with the flow cytometry technique. Plant material directly collected from each litterbag (which corresponded to about 0.05 g of dry material) were preserved in 2% formaldehyde solution and stored until analyses. To conduct the analyses, samples were sonicated (40 W, 40KHz, Selecta, Spain) for two 1 min cycles to detach bacteria from plant material, and cells were dislodged by vortexing (IKA® VORTEX, Genius 3, Sigma-Aldrich, Missouri, USA). After that, solid particles were left to sediment from the solution (1-2 min), and, to favour cell separation, 200 µl of the solution was dissolved in 1800 µl of 0.05 M pyrophosphate (sodium pyrophosphate decahydrate) solution (1:10 sample: solution) and posteriorly, 20 µl of the 1:10 dilution were dissolved again in 1980 µl of 0.05 M pyrophosphate to reach the final concentration of 1:1000 sample: solution. Then, 400 µl of the sample-pyrophosphate solution were stained with 4 µl of the fluorescent Syto13 solution (5 µM, Fisher, Pennsylvania, USA) and incubated in dark conditions for 15-30 min. After that, 10 µl of bead solution (10^6 beads ml^{-1}, Fisher, 0.1 µm) was added to the samples to normalize fluorescent data from Syto 13. Bacteria cells were counted using a flow cytometer (FACSCalibur, Becton and Dickinson, New Jersey, USA), and bacterial biomass in terms of carbon was estimated as 2.2 x 10^{-13} gC µm^{-3} (Bratbak & Dundas, 1984), considering bacteria cell biovolume as 0.1 µm^3 (Theil-Nielsen & Sondergaard, 1998).

3.4.6. Fungal carbon biomass

Fungal carbon biomass was estimated from ergosterol concentration in the litter according to (Gessner, 2005b), since this lipid is found almost exclusively in fungi and it has been demonstrated to be a useful indicator of fungal biomass (Lee et al., 1980). Plant material directly collected from each litterbag (which corresponded to about 0.1 g of dry material) was stored in the freezer at -24 °C and lyophilized just before the analyses. The lyophilized samples were weighed and lipids extracted with KOH-methanol solution heated at 80 °C for 30 min. The extracts were filtered using solid-phase extraction cartridges (Waters Sep-Pak®, Vac RC, tC18, 500 mg sorbent), and the ergosterol retained in the cartridges was eluted with isopropanol. Ergosterol was quantified by using high-performance liquid chromatography (HPLC analyser Waters corporation, USA), equipped with a Waters Nova-Pak®C18 4µm chromatographic column (3.9 × 300 mm). The peaks of the samples in the chromatograph were then compared with the absorption peaks of external ergosterol standards at 282 nm. Ergosterol concentration was converted to fungal biomass considering that there are 5.5 µg of ergosterol in one gram of fungal biomass (Gessner & Chauvet, 1993) and converted to carbon content considering that fungal dry mass has a 43% of carbon (Baldy & Gessner, 1997).

3.4.7. Data analysis and statistics

Differences among species

Differences among species in the initial values of the litter quality parameters (C, N, C/N ratio, cellulose, lignin and lignin/N ratio) were analysed by means of one-way-ANOVAs using species as fixed factor.

Differences among species in the litter quality parameters, the extracellular enzyme activity and the microbial carbon biomass, considering the entire decomposition period for each species, were analysed by means of generalized linear mixed models (GLMMs) (GLMs with a random factor), using species as fixed factor, zone (which was nested within habitat) as a random factor and time as a covariate. GLMs are statistical linear models constituted by three main components: the response variable with a distribution in the natural exponential family; the explanatory variables of the model and a link function that specifies the relationship between the response and the explanatory variables (P.McCullagh and J.A.Nelder, 1989). These models are a proper option for ecological studies where normal distribution and homoscedasticity assumptions of the variables are not reached even after appropriated transformation (Bolker et al., 2009) and thus general lineal models (as ANOVAs) cannot be applied, as it was the case of the variables analysed when testing differences among species considering the entire decomposition period studied. Moreover, GLMs can handle more complicated scenarios, as it is the case of the present study, since the litterbags of the different species were not sampled at the same dates and the period of time they were in the field also varied among the species.

Since exponential regressions between the % of remaining AFDM and time were significant using the data obtained from the 6 replicates per species, differences among species in litter decomposition rates (k) and in litter half-life ($t_{0.5}$) were tested by means of GLMMs using the estimated k and $t_{0.5}$ values as dependent variables (in which the different times were already integrated), species as fixed factor and zone (which was nested within habitat) as a random factor. For turnover activity (TA), lineal regressions among ln % remaining AFDM and accumulated enzyme activity (AEA) were not significant, thus, differences among species were also tested by means of GLMMs but the model was performed using ln % remaining AFDM as dependent variable, species as fixed factor, zone (nested within habitat) as random factor and AEA as covariate.

Within species analysis: time and zone effects

Repeated measures ANOVAs were applied for each species separately to analyse differences in the litter quality parameters, the extracellular enzyme activity and the microbial carbon biomass between zones within each habitat throughout the decomposition process (time). When the interaction between time and zone was significant, one-way-ANOVAs were performed to analyse differences between zones within each sampling day. When the time factor (or the interaction among time and zone) was significant, differences among sampling dates were analysed by means of pairwise comparison for each species, considering the zones separately if the interaction among time and zone was significant. Differences in the decomposition rates (k) and the litter half-life ($t_{0.5}$) between zones within each habitat were tested using one-way ANOVAs for each species, using zone as fixed factor. For the turnover activity (TA), since lineal regressions among ln % remaining AFDM and accumulated enzyme activity (AEA) were not significant, regression slopes were not homogeneous and variables were not normally distributed and homoscedastic, differences among zones within each species were tested my means of GLMs. These models were constructed using ln % remaining AFDM as dependent variable, AEA as covariate and zone as fixed factor.

A principal component analysis (PCA) was performed using the following variables: % remaining AFDM, litter quality, microbial carbon biomass and extracellular enzymes activities. Mean values of these variables for each sampling day and species were ordinated in the PCA plot.

The Shapiro-Wilk test was used to test normality, while the homogeneity of variances was analyzed with the Levene's test. For all the statistical tests, the significance level considered was p-value < 0.05. *P*-values of the fixed factors species and zone were estimated comparing the GLMMs or GLMs performed with and without the fixed factor by means of one-way ANOVAs. All the GLMs and GLMMs models were chosen following the Akaike Information Criteria (AIC). R software version 3.5.2 (R Development Core Team, 2018) has been used to perform the GLMMs and the GLMs, by means of the glmmADMB package (Skaug et al., 2018), and the PCA, through the FactoMineR package (Lê et al., 2008). Repeated measures ANOVAs and one-way ANOVAs were performed with SPSS software (IBM SPSS statistics, Corporation, Chicago, USA).

4. RESULTS

4.1. Study 1: Structure and composition of plant communities and carbon stored in vegetation and soil in the salt marsh habitats and in the disturbed and later restored zone

4.1.1. Structure and composition of the studied plant communities

4.1.1.1. Salt marsh habitats

The point quadrat method allowed us to describe the floristic composition of the three habitats of community interest (HCI) studied in the well-preserved zone of La Pletera salt marsh. In these habitats, the number of species identified varied between 7 and 15, being only 1 or 2 species dominant in each one of them (Table 4.1 and Table 4.2). No significant differences were observed in the relative abundance of these dominant species between the two years of sampling (Table 4.2). However, in the glasswort sward habitat, *Salicornia patula* tended to decrease from one year to the other (marginally significant differences, p=0.095).

Table 4.1. List of species found in the three salt marsh habitats of the La Pletera salt marsh. Species dominant within each habitat are marked in bold (they had the highest relative abundance, see Table 4.2) in each habitat.

	Salt marsh habitats	
Halophilous scrub	**Salt meadow**	**Glasswort sward**
Aster tripolium	*Aetheorhiza bulbosa*	*Atriplex portulacoides*
Atriplex portulacoides	*Artemisia gallica*	*Juncus acutus*
Elymus elongatus	***Atriplex portulacoides***	*Parapholis filiformis*
Elymus pycnanthus	*Avena barbata*	***Salicornia patula***
Inula crithmoides	*Bromus sterilis*	*Sarcocornia fruticosa*
Juncus acutus	*Dactylis glomerata*	*Spergularia marina*
Limonium narbonense	*Dorycnium pentaphyllum* subsp *gracile*	*Suaeda maritima*
Parapholis filiformis	***Elymus pycnanthus***	
Phragmites australis	*Geranium dissectum*	
Sarcocornia fruticosa	*Inula crithmoides*	
Spergularia marina	*Juncus acutus*	
	Limonium virgatum	
	Phragmites australis	
	Trifolium scabrum	
	Vicia hirsuta	

Table 4.2. Relative abundance (mean ± SE, n= 5) of the most abundant species of each one of the salt marsh habitats in the two sampling years (2015 and 2016). *P*-values have been obtained by means of one-way ANOVAs or Mann-Whitney U-tests (indicated with asterisks) using year as fixed factor.

Habitat	Species	Year	Relative abundance (%)	*p*-value
Halophilous scrub	*Sarcocornia fruticosa*	2015	75.0 ± 13.5	0.768
		2016	69.3 ± 12.9	
	Atriplex portulacoides	2015	2.9 ± 1.3	0.841*
		2016	12.4 ± 8.8	
Salt meadow	*Elymus pycnanthus*	2015	60.4 ± 8.5	0.981
		2016	60.7 ± 10.0	
	Atriplex portulacoides	2015	20.5 ± 7.0	0.947
		2016	19.7 ± 9.0	
Glasswort sward	*Salicornia patula*	2015	98.4 ± 1.2	0.095*
		2016	73.2 ± 18.6	
	Suaeda maritima	2015	1.6 ± 1.2	0.690*
		2016	5.2 ± 3.4	
	Sarcocornia fruticosa	2015	0 ± 0	-
		2016	20.6 ± 19.9	

Both, the halophilous scrub and the salt meadow showed higher plant cover (98-100%) than the glasswort sward (78-80%) (Table 4.3). The highest maximum height of vegetation was found in the halophilous scrub and the salt meadow, with the glasswort sward showing the lowest vegetation (Table 4.3). The salt meadow had the highest plant species richness and diversity, although differences with the halophilous scrub were not significant. The Shannon index was always below 3 for the three habitats, indicating low plant species diversity, especially in the case of the glasswort sward (Table 4.3). There were no significant differences between years in the studied parameters in the case of the halophilous scrub and the salt meadow (Table 4.3). Conversely, the glasswort sward, which is characterized by having mostly annual vegetation, experienced an increase in plant maximum height between 2015 and 2016 (Table 4.3).

Table 4.3. Parameters related to the structure of the plant community (mean ± SE, n=5) of the three salt marsh habitats. Different letters indicate significant differences among habitats. Asterisks and values marked in bold indicate significant differences between years within each habitat (*p*-value < 0.05).

Habitat	Year	Plant cover (%)	Living plant cover (%)	Maximum height of vegetation (cm)	Maximum height of living vegetation (cm)	Richness	Shannon Index	Evenness
Halophilous scrub	2015	98.1 ± 1.5	87.2 ± 7.6	49.5 ± 6.6	46.7 ± 5.5	2.8 ± 0.6	0.73 ± 0.33	0.44 ± 0.13
	2016	99.4 ± 0.6	92.2 ± 7.4	46.8 ± 7.2	46.0 ± 7.0	2.4 ± 0.7	0.79 ± 0.34	0.82 ± 0.08
		a	a	a	a	ab	ab	ab
Salt meadow	2015	100.0 ± 0.0	93.1 ± 3.7	42.5 ± 5.6	40.1 ± 5.2	3.4 ± 0.5	1.29 ± 0.21	0.76 ± 0.08
	2016	100.0 ± 0.0	99.4 ± 0.4	43.7 ± 4.0	41.2 ± 4.1	4.2 ± 0.9	1.28 ± 0.27	0.67 ± 0.09
		a	a	a	a	a	a	a
Glasswort sward	2015	79.7 ± 3.7	66.3 ± 5.9	**5.8 ± 0.5 ***	**3.6 ± 0.5 ***	1.4 ± 0.3	0.09 ± 0.06	0.23 ± 0.06
	2016	78.4± 5.7	49.4 ± 11.5	**10.6 ± 1.6 ***	**9.8 ± 1.2 ***	1.8 ± 0.4	0.31 ± 0.16	0.43 ± 0.10
		b	b	b	b	b	b	b

4.1.1.2. Disturbed zone

Before the restoration, the plant community of the disturbed area was constituted by a large number of ruderal species, which are typical of environments altered by human action, being particularly abundant *Foeniculum vulgare* (relative abundance: 40.3 ± 7.4%) and *Inula viscosa* (relative abundance: 13.5 ± 7.1%) (Table 4.4).

Table 4.4. List of species found in the disturbed zone before restoration and one and two years after being restored. Dominant species of each plant community in the different years are marked in bold. Relative abundance (RA) of these species, considering only plots with vegetation is also given (before restoration n=10; one year after restoration n=4; two years after restoration n=7).

Disturbed zone		
Before the restoration (2015)		**After the restoration**
Alyssum maritimun	***Inula viscosa*** **(RA: 13.5 ± 7.1 %)**	**One year after the restoration**
Asphodelus fistulosus	*Oryzopsis miliacea*	*Atriplex prostrata*
Asteriscus spinosus	*Plantago coronopus*	*Cynodon dactylon*
Asterolinon linum-stellatum	*Plantago lanceolata*	***Suaeda maritima*** **(RA: 95.8 ± 4.2 %)**
Avena barbata	*Plantago viva*	
Campanula erinus	*Reichardia picroides*	**Two years after the restoration**
Dactylis glomerata	*Sanguisorba minor*	*Atriplex prostata*
Daucus carota	*Scabiosa atropurpurea*	***Sarcocornia fruticosa*** **(RA: 13.9 ± 10.7 %)**
Euphorbia segetalis	*Sedum sediforme*	*Spergularia marina*
Foeniculum vulgare **(RA: 40.3 ± 7.4 %)**	*Sonchus tenerrimus*	***Suaeda maritima*** **(RA: 72.5 ± 13.8 %)**
Helichrysum stoechas	*Trifolium scabrum*	
Hypochoeris radicata	*Urospermum dalechampii*	

When the disturbed area was restored, a drastic change in species composition was observed. After one year, recolonization by pioneer species of saline soils, especially *Suaeda maritima*, was observed (Table 4.4). Two years after the restoration, *S. maritima* was still the dominant species with a relative abundance of 72.4 ± 13.7%, but the appearance of *Sarcocornia fruticosa*, the dominant species of the halophilous scrub, was recorded (Table 4.4).

Table 4.5. Parameters related to the structure of the plant community (mean ± SE, n=10) for the Mediterranean halophilous scrub and the disturbed zone before restoration. In the case of halophilous scrub, data from 2015 and 2016 were pooled since no significant differences between years were found. *P*-values have been obtained by means of one-way ANOVAs or Mann-Whitney U-tests (indicated with asterisks) with habitat as fixed factor. Significant *p*-values are indicated in bold.

Habitat	Plant cover (%)	Living plant cover (%)	Maximum height of vegetation (cm)	Maximum height of living vegetation (cm)	Richness	Shannon Index	Evenness
Halophilous scrub	98.8 ± 0.8	89.7 ± 5.1	48.2 ± 4.6	46.4 ± 4.2	2.6 ± 0.4	0.76 ± 0.32	0.59 ± 0.14
Disturbed zone (Ruderal vegetation)	81.9 ± 4.2	36.7 ± 5.8	32.8 ± 2.8	37.6 ± 3.8	5.5 ± 0.5	1.77 ± 0.12	0.73 ± 0.04
***p*-value**	**<0.001***	**<0.001***	**0.011**	0.137	**<0.001***	**0.004***	0.460*

Before the restoration, the ruderal plant community showed a lower plant cover and maximum height, but a higher species richness and diversity, than the halophilous scrub (Table 4.5). After the restoration, a significant increase in living plant cover and species richness was detected from the first to the second year (Table 4.6).

Table 4.6. Parameters related to the structure of the plant community (mean ± SE) for the disturbed zone, one (n=10) and two years (n=7) after restoration. To determine the Shannon index, evenness and height variables, only plots with vegetation were considered and therefore n=4 (instead of 10) for these parameters one year after the restoration. *P*-values have been obtained by means of one-way ANOVAs or Mann-Whitney U-tests (indicated with asterisks) with year as fixed factor. Significant *p*-values are indicated in bold.

Disturbed zone	Plant cover (%)	Living plant cover (%)	Maximum height of vegetation (cm)	Maximum height of living vegetation (cm)	Richness	Shannon Index	Evenness
One year after restoration	3.9 ± 2.3	2.5 ± 1.8	2.6 ± 0.8	3.2 ± 0.5	0.5 ± 0.2	0.16 ± 0.16	0.16 ± 0.16
Two years after restoration	9.6 ± 1.6	8.9 ± 1.6	3.6 ± 0.6	3.5 ± 0.6	1.7 ± 0.4	0.52 ± 0.27	0.90 ± 0.05
***p*-value**	0.079*	**0.005***	0.366	0.802	**0.019***	0.164*	0.057

4.1.2. Changes in plant biomass and net primary production, as well as in the amount of litter, of the salt marsh habitats

In the studied salt marsh habitats, living, dead and total aboveground biomass as well as the litter amount did not differ significantly between 2015 and 2016 (Figure 4.1 and Figure 4.2b). As a consequence, and because at La Pletera there are no signs of herbivory (personal observations) and biomass exportation is expected to be low (see section 5.1.2), net aboveground primary production (NAPP) from one year to the other was considered zero for the three habitats. However, there was a tendency ($p = 0.056$) to increase total aboveground biomass in the glasswort sward (Figure 4.1). Regardless of the year, the halophilous scrub was the habitat with the highest living and total aboveground biomass, while no significant differences in the standing dead biomass were found between this habitat and the salt meadow.

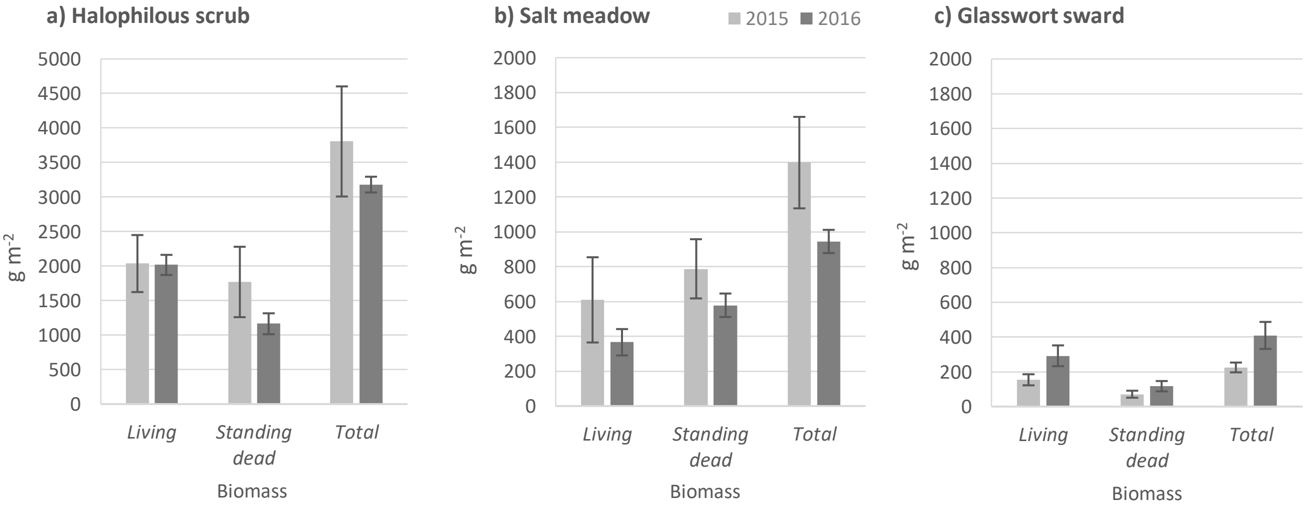


Figure 4.1. Aboveground living, standing dead and total biomass for the halophilous scrub (a), the salt meadow (b), and the glasswort sward (c) salt marsh habitats in 2015 and 2016. Bars represent standard errors (n=5). Note the different scale on the Y-axis of the halophilous scrub graph compared to the graphs for the other two habitats.

Differences between years in plant belowground biomass were neither significant for any of the salt marsh habitats (Figure 4.2a). Therefore, no net belowground primary production (NBPP) was detected from one year to the other. Regardless of the year, the halophilous scrub and the salt meadow showed always a higher plant belowground biomass and litter amount than the glasswort sward (Figure 4.2).

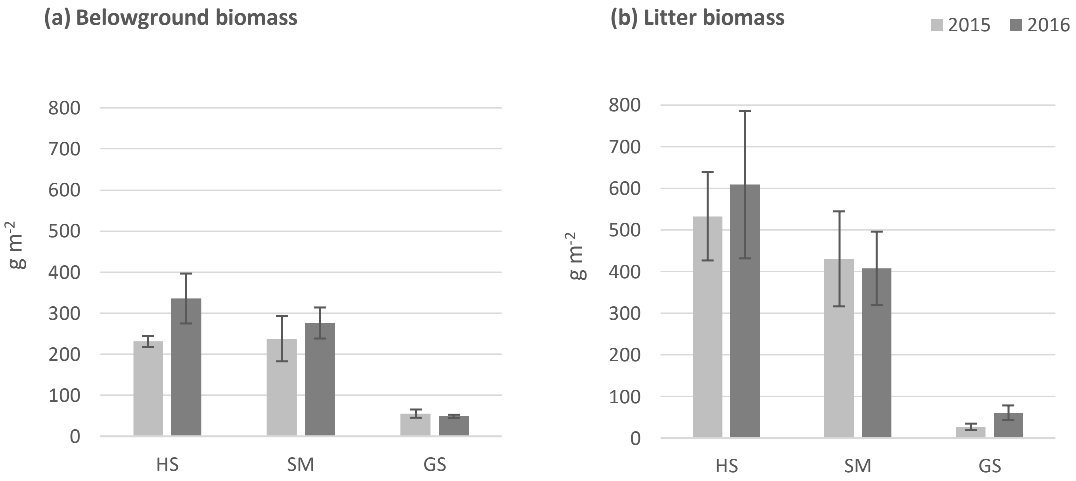


Figure 4.2. Plant belowground biomass at 0-20 cm depth (a) and litter biomass on the soil surface (b) of the three salt marsh habitats in 2015 and 2016. HS: Halophilous scrub; SM: Salt meadow; GS: Glasswort sward. Bars represent standard errors (n=5).

4.1.3. Carbon stored in vegetation, litter and soil

4.1.3.1. Salt marsh habitats

We did not detect significant differences between years in the carbon (C) concentration of the green and woody living plant aboveground fractions (µg mg DW^{-1}) of any of the dominant species of each habitat. Since the interaction between year and species was neither significant, data for both years were pooled for each species (Table 4.7). Among species, *E. pycnanthus* had the highest values of C concentration in green tissues, while *S. patula* had the lowest. Among habitats, values of C concentration for *S. fruticosa* were higher in the halophilous scrub than in the glasswort sward, although differences were only significant for the woody fraction. For the standing dead fraction, the C concentration of *E. pycnanthus* and *S. patula* differed between years, with values being significantly lower in 2015. When comparing species, *S. patula* showed the lowest C concentration in 2015 and 2016, although in 2016 values did not differ of that of *A. portulacoides* (Table 4.7).

Table 4.7. Carbon concentration (mean ± SE) in the plant aboveground fractions (green living, woody living and standing dead) of the dominant species of each habitat. Mean values are given for each year (n=5) when differences between years were significant. If not, mean values were obtained by pooling the data for the two sampling years (n=10). No SE and statistical results are given for the standing dead fraction of *S. fruticosa* in glasswort sward because this fraction was present only in one plot. Asterisks indicate significant differences between years for each species. When the interaction between species and year was significant differences among species were tested for each year, and they were indicated with different lowercase letters for 2015 and with capital letters for 2016. *P*-values < 0.05 for consider significant differences. ND: No data.

			Carbon concentration (μg mg DW^{-1})		
Habitat	**Species**	**Year**	**Plant aboveground fractions**		
			Green living	**Woody living**	**Standing dead**
Halophilous scrub	*S. fruticosa*	2015	298.7 ± 5.3 c	435.4 ± 9.8 a	445.2 ± 9.4 a
		2016			463 ± 3.1 A
Salt meadow	*E. pycnanthus*	2015	443.8 ± 1.9 a	ND	427.8 ± 2.4* a
		2016			440.8 ± 1.6* AB
	A. portulacoides	2015	338.9 ± 3.3 b	436.5 ± 2.8 a	421.1 ± 7.9 a
		2016			376.6 ± 24.7 C
Glasswort sward	*S. patula*	2015	269.1 ± 6.5 d	ND	357 ± 16.2* b
		2016			400.7 ± 7.6* BC
	S. fruticosa	2016	278.6 ± 7.1 cd	392.2 ± 18.3 b	449.5

In the salt marsh habitats, C concentration of the belowground biomass was lower in 2016 than in 2015 (Table 4.8). Regardless of the year, the salt meadow showed a higher C concentration in the belowground fraction than the halophilous scrub and the glasswort sward. Differences between years in the litter C concentration were only observed in the halophilous scrub and in the glasswort sward, with higher values in 2016 in both cases. The glasswort sward had the lowest litter C concentration regardless of the year (Table 4.8).

Table 4.8. Carbon concentration (mean ± SE, n=5) in the plant belowground fraction and litter of the three-well preserved habitats. Asterisks indicate significant differences between years for each habitat. Different letters indicate significant differences among habitats. *P*-values < 0.05 for consider significant differences.

		Carbon concentration (µg mg DW^{-1})	
Habitat	**Year**	**Belowground fraction**	**Litter**
Halophilous scrub	2015	390.5 ± 9.1*	436.3 ± 2.4*
	2016	359.5 ± 16.9* b	461.7 ± 5.8* a
Salt meadow	2015	417.6 ± 7.1*	437.9 ± 1.7
	2016	396.8 ± 10.4* a	440.8 ± 1.7 a
Glasswort sward	2015	362.7 ± 5.4*	284.1 ± 25.3*
	2016	339.6 ± 7.6* b	372.2 ± 8.2* b

Regarding the estimated overall carbon stored (g C m^{-2}) in the different plant fractions, in the litter and in the soil of the different habitats, we did not find significant differences between years, and the interaction between habitat and year was neither significant. For this reason, data for the two sampling years (2015 and 2016) were pooled (Table 4.9). The halophilous scrub had the highest amount of carbon stored in plant living, standing dead and total aboveground fraction, although differences with the salt meadow were not significant for the standing dead fraction (Table 4.9). The halophilous scrub and salt meadow habitats also showed similar values of carbon stored in the plant belowground fraction, the litter and the soil (Table 4.9).

Table 4.9. Carbon stored in plant living, dead, total aboveground and belowground fraction and in the litter and soil (0-20 cm depth) in the three salt marsh habitats considering the two sampling years together (mean ± SE, n=10). Different letters indicate significant differences among habitats in the carbon content of each fraction (*p*-value < 0.05).

	Carbon amount (g C m^{-2})					
	Plant fractions					
Habitat	**Living aboveground**	**Standing dead aboveground**	**Total aboveground**	**Total belowground**	**Litter**	**Soil**
Halophilous scrub	808.1 ± 88.0 a	654.7 ± 120.2 a	1462.8 ± 178.8 a	103.4 ± 9.0 a	258.2 ± 45.5 a	2248.4 ± 387.6 a
Salt meadow	206.8 ± 51.4 b	297.4 ± 40.2 a	504.3 ± 60.7 b	103.3 ± 12.3 a	184.1 ± 29.8 a	1774.5 ± 233.9 a
Glasswort sward	59.3 ± 9.9 c	36.4 ± 7.4 b	95.7 ± 14.3 c	18.2 ± 1.8 b	15.1 ± 4.2 b	896.6 ± 126.8 b

Sarcocornia fruticosa accounted for ≈ 94% of the total biomass of the halophilous scrub (Table 4.10), with the highest amount of carbon being stored in both woody and standing dead biomass of this species (Table 4.11). In the salt meadow, *Elymus pycnanthus* represented ≈ 68% of the total biomass, showing the highest amount of carbon stored in the standing dead fraction (Table 4.10 and Table 4.11). In the glasswort sward, *Salicornia patula* accounted for ≈ 99% in 2015 and ≈ 72% in 2016 of the total biomass, with a similar amount of carbon being stored in living and dead tissues (Table 4.10 and Table 4.11).

Table 4.10. Percentage of the total aboveground biomass accounted for each one of the most abundant species of each habitat (mean ± SE). When differences between years were significant, mean values are given for each year (n=5). If not, mean values are estimated pooling the data for the two sampling years (n=10). Different letters indicate significant differences (*p*-value < 0.05) among species within the same habitat (and year in the case of the glasswort sward). ND: no data.

Habitat	Species	Percentage of total aboveground biomass	
Halophilous scrub	*Sarcocornia fruticosa*	93.5 ± 2.9	
Salt meadow	*Elymus pycnanthus*	68.4 ± 7.6 a	
	Atriplex portulacoides	17.2 ± 6.7 b	
	Other sp	14.4 ± 4.6 b	
Glasswort sward		**2015**	**2016**
	Salicornia patula	98.9 ± 0.5	71.8 ± 15.3 a
	Sarcocornia fruticosa	ND	21.3 ± 15.7 b

Table 4.11. Biomass and carbon stored in green, woody (in the case of woody species) and standing dead tissues of the dominant species of each habitat (mean ± SE, n=10). Different letters indicate significant differences between fractions within the same species (*p*-value < 0.05).

		Biomass (g m^{-2})	Carbon (g C m^{-2})
Halophilous scrub ***Sarcocornia fruticosa***	Green	404.2 ± 59.8 a	120.1 ± 17.4 a
	Woody	1512.4 ± 180.6 b	663.9 ± 78.1 b
	Standing dead	1423.8 ± 265.9 b	652.2 ± 121.0 b
Salt meadow ***Elymus pycnanthus***	Green	174.2 ± 17.9 a	77.2 ± 7.8 a
	Standing dead	598.3 ± 105.5 b	260.0 ± 45.9 b
Glasswort sward ***Salicornia patula***	Green	186.5 ± 45.5 a	49.2 ± 11.4 a
	Standing dead	87.1 ± 16.5 a	33.2 ± 6.2 a

All the soils showed slightly alkaline pH values, with differences among habitats being significant only at 0-20 cm. At this depth, the glasswort sward had the highest pH values, although differences with the salt meadow were not significant. Electrical conductivity values indicated that the halophilous scrub and the glasswort sward were much more saline than the salt meadow at the two studied depths. The proportions of the different textural classes at 0-20 cm presented a remarkably high variation, which could mask possible differences among habitats (Table 4.12). No differences among habitats were observed either for the soil bulk density. The halophilous scrub and the salt meadow had significantly higher values of soil organic carbon (SOC) at 0-20 cm depth, either expressed per unit area (g C m^{-2}; Table 4.9) or as a percentage (%; Table 4.12), than the glasswort sward. However, at 20-40 cm, the salt meadow showed as low SOC (%) values as the glasswort sward (Table 4.12). Regarding soil total nitrogen content (TN), differences among habitats followed the same pattern observed for SOC at 0-20 cm, with the highest values of TN found in the halophilous scrub and the salt meadow, while differences among habitats were not significant at 20-40 cm. Nevertheless, regardless of the depth, the C/N ratio did not vary among the habitats.

Table 4.12. Mean ± SE for the soil physical and biochemical parameters studied: soil organic carbon (SOC), total nitrogen (TN), C/N ratio, electrical conductivity (EC) and bulk density (BD), sand, silt and clay proportions (n=10 for SOC and BD, n=8 for TN, C/N, pH, EC, n=5 for textural classes at 0-20 cm; n=3 for all the parameters measured at 20-40 cm). HS: Halophilous scrub; SM: Salt meadow; GS: Glasswort sward. Different letters indicate significant differences (*p*-value < 0.05) among habitats at 0-20 cm (lowercase letters) and 20-40 cm (capital letters) depth.

Depth (cm)	**Hab**	**pH**	**EC** (dS m^{-1})	**Sand** (%)	**Silt** (%)	**Clay** (%)	**BD** (g cm^{-3})	**SOC** (%)	**TN** (%)	**C/N**
0-20	**HS**	8.2 ± 0.1 a	5.6 ± 0.8 a	29.4 ± 7.7 a	33.7 ± 4.0 a	36.9 ± 3.9 a	1.09 ± 0.06 a	1.04 ± 0.14 a	0.045 ± 0.005 a	26.5 ± 3.8 a
	SM	8.4 ± 0.1 ab	1.3 ± 0.4 b	45.6 ± 8.3 a	25.8 ± 5.5 a	28.6 ± 2.9 a	1.03 ± 0.02 a	0.88 ± 0.13 a	0.046 ± 0.005 a	18.2 ± 1.4 a
	GS	8.6 ± 0.1 b	5.3 ± 0.8 a	38.4 ± 7.0 a	28.0 ± 4.9 a	33.6 ± 2.3 a	1.15±0.01 a	0.39 ± 0.06 b	0.023 ± 0.003 b	18.8 ± 1.8 a
20-40	**HS**	8.5 ± 0.1 A	2.9 ± 0.3 A	ND	ND	ND	ND	0.60 ± 0.09 A	0.024 ± 0.002 A	25.0 ± 3.3 A
	SM	8.7 ± 0.2 A	0.7 ± 0.3 B	ND	ND	ND	ND	0.23 ± 0.03 B	0.013 ± 0.004 A	28.0 ± 14.2 A
	GS	8.7 ± 0.1 A	2.5 ± 0.4 A	ND	ND	ND	ND	0.20 ± 0.08 B	0.012 ± 0.006 A	20.7 ± 5.5 A

In the halophilous scrub, no significant differences (*p* = 0.199) were found between the amount of carbon stored in the soil (0-20 cm depth) and in the vegetation (above and belowground) (Figure 4.3). Conversely, the salt meadow and the glasswort sward habitats stored a significantly (*p* < 0.001 for both habitats) higher amount of carbon in the soil than in the vegetation (Figure 4.3).

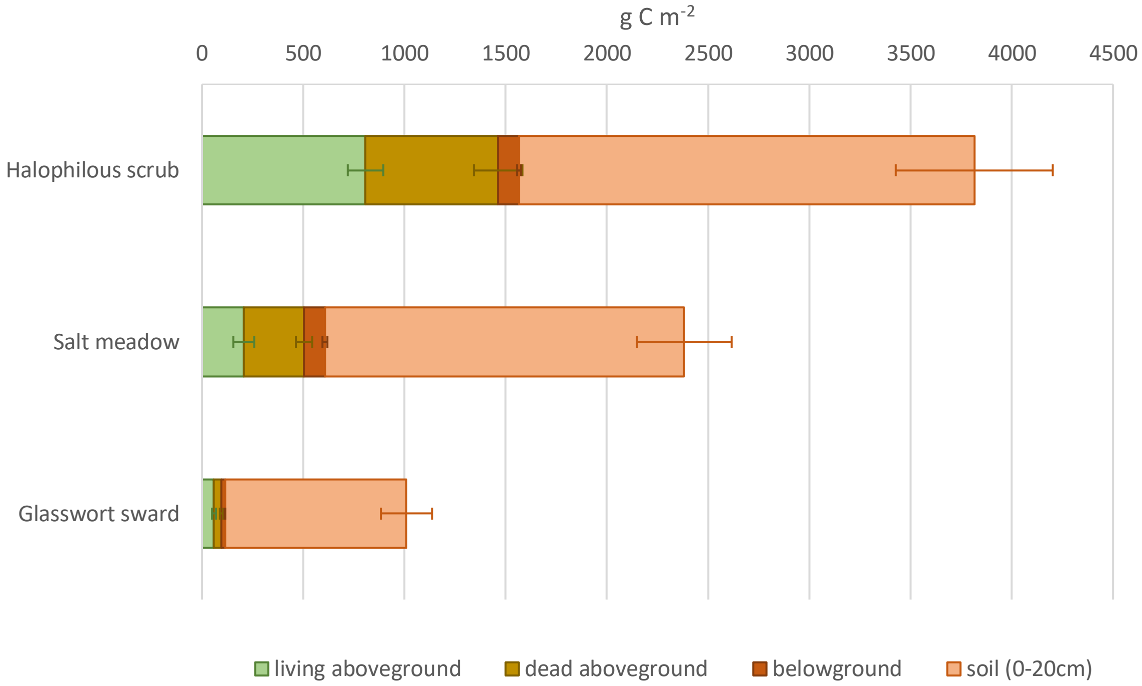


Figure 4.3. Carbon content in the vegetation (living and dead aboveground and belowground biomass) and soil (0-20 cm depth) per square meter (g C m^{-2}). Bars represent ± standard errors (n=10).

4.1.3.2. Disturbed zone

In the disturbed zone, the dominant species in 2015 (before the restoration) were *Foeniculum vulgare* and *Inula viscosa*, which did not show significant differences in their relative biomass (Table 4.13).

Table 4.13. Percentage of total aboveground biomass accounted for the dominant species of the disturbed zone (mean ± SE) before restoration (2015) and in 2017, i.e., one year after the restauration performed in 2016. Different letters would indicate significant differences between species within the same year (*p*-value < 0.05). Other species are those listed in Table 4.4.

	Percentage of total aboveground biomass	
Disturbed zone	***2015*** *(n=10)*	***2017*** *(n=4)*
Foeniculum vulgare	32.9 ± 9.6 a	
Inula viscosa	19.1 ± 9.9 a	
Other sp	47.9 ± 10.2 a	
Suaeda maritima		73.6 ± 21.7 a
Other sp		22.7 ± 22.7 a

When the carbon stored in the different plant fractions of the dominant species of the disturbed zone (before the restoration) was compared to those of the dominant species of the halophilous scrub, higher values of carbon stored in the aboveground (living, dead and total) and belowground fraction, as well as in the litter, were found in the halophilous scrub (Table 4.14). However, the amount of carbon stored in the soil was higher in the disturbed zone (Table 4.14).

Table 4.14. Carbon stored in plant living, dead and total aboveground and belowground fractions, litter and soil (0-20 cm depth) in the halophilous scrub and in the disturbed zone before the restoration (ruderal community) (mean ± SE, n=10). *P*-values obtained from one-way ANOVAs, with habitat as fixed factor, are shown. Significant p-values are indicated in bold.

Carbon amount (g C m^{-2})	**Plant fractions**				**Litter**	**Soil**
	Living aboveground	**Dead aboveground**	**Total aboveground**	**Total belowground**		
Halophilous scrub	808.1 ± 88.0	654.7 ± 120.2	1462.8 ± 178.8	103.4 ± 9.0	258.2 ± 45.5	2248.4 ± 387.6
Disturbed zone (Ruderal vegetation)	54.5 ± 13.6	99.9 ± 14.8	154.4 ± 26.6	49.5 ± 17.9	44.2 ± 5.7	4158.4 ± 695.4
***p*-value**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**<0.001**	**0.037**

One year after the restoration, the amount of carbon stored in the disturbed zone was very low for all the fractions because the vegetation was still very scarce (Table 4.15). Indeed, only four of the ten plots sampled had vegetation. At this moment, the dominant species was *Suaeda maritima*, accounting for ≈ 74% of the total biomass (Table 4.13).

Table 4.15. Carbon stored in plant living, dead and total aboveground and belowground fractions, one year after the restoration of the disturbed zone (mean ± SE, n=4). Plots had no litter.

Carbon amount (g C m^{-2})	**Plant fractions**			
	Living aboveground	**Dead aboveground**	**Total aboveground**	**Total belowground**
Disturbed zone (Restored)	0.83 ± 1.97	0.24 ± 0.73	1.07 ± 2.03	0.17 ± 0.31

4.2. Study 2: Seasonal carbon fluxes from vegetation (CO_2) and soil (CO_2 and CH_4)

4.2.1. Seasonal instantaneous CO_2 fluxes from vegetation at different time periods of the day

Differences among species in instantaneous net CO_2 exchange rate (NER) from green tissues depended on the time of the day and the sampling day (Figure 4.4). After sunrise, differences in NER were only observed in the spring and summer months. Specifically, *E. pycnanthus* had the highest photosynthetic rates (negative NER) in March and April (with no significant differences with *S. fruticosa* in April) and *S. patula* in June and July, which would correspond to its growth period (Figure 4.4a). At midday, *E. pycnanthus* showed the highest photosynthetic rates during all the year, except in June and July, when *S. patula* showed the highest values (Figure 4.4c). Before sunset, the highest photosynthetic rates were recorded for *E. pycnanthus* and *S. fruticosa* in February and March, for *S. patula* In June, and the rest of the year also for *E. pycnanthus* (Figure 4.4e). At night, the highest respiration values (positive NER) were found in August and November for *E. pycnanthus* and *S. fruticosa* (Figure 4.4g). Overall, green tissues of the three perennial halophytic species studied (*S. fruticosa*, *E. pycnanthus* and *A. portulacoides*) presented the highest photosynthetic activity (i.e. more negative values of NER) in spring (March or April). Moreover, the highest photosynthetic rates were recorded at midday for the four species, although temporal patterns of photosynthesis for each species at the three times of the day (after sunrise, at midday and before sunset) slightly varied (Figure SM 3). For *S. fruticosa* and *S. patula*, photosynthesis showed similar seasonal patterns for the three periods of the day. Conversely, seasonal changes in photosynthetic rates of *E. pycnanthus* and *A. portulacoides* were similar after sunrise and before sunset, but they were different from the temporal pattern observed at midday (Figure SM 3). NER values from the thin woody stems of *S. fruticosa* and *A. portulacoides* did not differ after sunrise and at midday, but before sunset *A. portulacoides* generally presented lower NER values (Figure 4.4 b, d, f). At night, significantly higher respiration values were found for *A. portulacoides* in June, and for *S. fruticosa* in November (Figure 4.4h). Interestingly, thin woody stems of both species showed net CO_2 uptake during most of the sampling days and daylight periods considered, except in autumn (November).

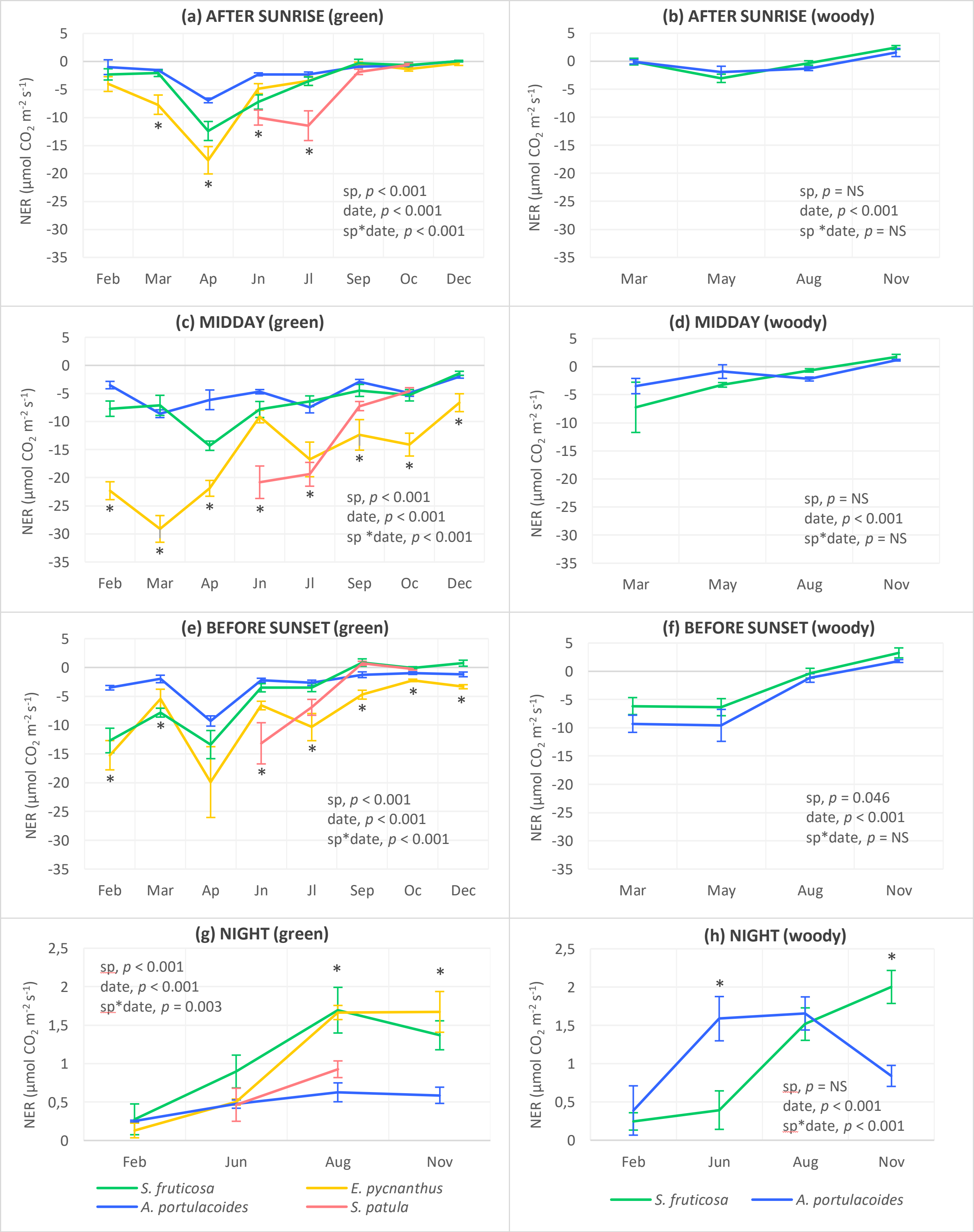


Figure 4.4. Instantaneous net CO_2 exchange rate (NER) of the four studied species after sunrise (a, b), at midday (c, d), before sunset (e, f) and at night (g, h), from green and woody tissues, respectively. Negative values indicate net photosynthetic activity, while positive values indicate net respiration. Bars represent standard errors (n = 4 for the period after sunrise, before sunset and night, and n = 6 for midday). Significant *p*-values of the species and date factors and their interaction according to the two-way-ANOVAs results are also shown. Asterisks indicate significant differences among species in each sampling date ($p < 0.05$), being depicted only when the interaction between habitat and sampling date was significant. NS: Not significant.

No significant correlations were found between midday NER and soil VWC, soil EC, maximum air temperature, air relative humidity or air vapor pressure deficit as well as between night NER and minimum air temperature for any of the three perennial species studied (results not shown).

4.2.1.1. Seasonal stomatal conductance and intrinsic water-use efficiency of green tissues at midday

During most of the year, *E. pycnanthus* showed the highest values of stomatal conductance (g_s) at midday, while *A. portulacoides* had the lowest (Figure 4.5a). In July and October, no significant differences in g_s were found among the four species studied. Regarding intrinsic water-use efficiency, differences among species were significant only in September, when *S. fruticosa* showed the lowest values and no significant differences were found among the other three species (Figure 4.5b).

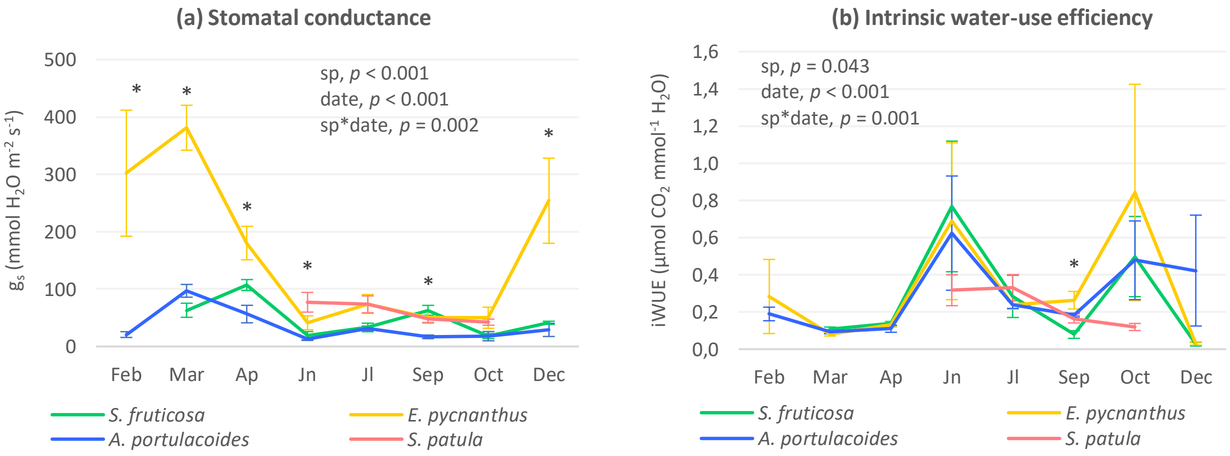


Figure 4.5. Stomatal conductance (a) and intrinsic water-use efficiency (photosynthesis/stomatal conductance) (b) of green tissues of *S. fruticosa*, *E. pycnanthus*, *A. portulacoides* and *S. patula* at midday (mean ± SE, n = 6). February values of *S. fruticosa* are missing because g_s values were too low to be properly determined. Significant *p*-values of the species and date factors and their interaction according to the two-way-ANOVAs results are also shown. Different letters indicate significant differences among species within each sampling day ($p < 0.05$).

4.2.1.2. Integrated daily CO_2 fluxes from vegetation

Integrated daily fluxes of CO_2 for each species were estimated from the instantaneous NER data taken 4 times per day (every 1.5 months) and revealed that green tissues of *S. fruticosa*, *E. pycnanthus* and *A. portulacoides* sequestered CO_2 during most of the sampling dates, except in late autumn (December) when *S. fruticosa* and

A. portulacoides showed net CO_2 emissions (Table 4.16). *S. patula* always sequestered CO_2. In turn, the thin woody stems of *S. fruticosa* and *A. portulacoides* presented a negative daily CO_2 balance (CO_2 sequestration) in winter and spring, and in winter, spring and summer, respectively. Overall, these results suggest that the vegetation of La Pletera salt mash would be a sink of CO_2 during most of the year.

Table 4.16. Estimated daily net CO_2 exchange rates per unit of tissue area for the day sampled in the different months throughout 2017. Negative values indicate net CO_2 uptake while positive values indicate net CO_2 emissions.

Daily NER ($mmol\ CO_2\ m^{-2}\ d^{-1}$)	**Winter**		**Spring**		**Summer**		**Autumn**	
Green tissues	**Feb**	**Mar**	**Apr**	**Jun**	**Jul**	**Sep**	**Oct**	**Dec**
S. fruticosa	-273.5	-233.4	-610.2	-322.6	-208.7	-2.8	-8.6	72.8
E. pycnanthus	-489.7	-612.7	-938.5	-375.2	-580.2	-221.9	-136.5	-6.8
A. portulacoides	-86.6	-167.4	-335.0	-165.2	-247.8	-55.4	-53.6	2.2
S. patula				-832.5	-736.2	-107.5		
Woody tissues	**March**		**May**		**August**		**November**	
S. fruticosa	-184.2		-201.9		42.9		193.5	
A. portulacoides	-166.0		-144.9		-38.0		97.8	

4.2.1.3. Seasonal changes in the aboveground biomass of the studied species

The living aboveground biomass (green and woody tissues) of the perennial species studied did not change throughout the year, except in the case of *S. fruticosa* that showed about 2.5-fold higher green biomass in summer than in winter (Figure 4.6a). *E. pycnanthus* had also the maximum green biomass in summer, although differences with the other seasons were not significant. *S. fruticosa* was the species with the highest green and woody biomass (Figure 4.6), although differences in green biomass with *A. portulacoides* were only significant in summer. The woody fraction of *S. fruticosa* was higher than the green fraction in all the seasons.

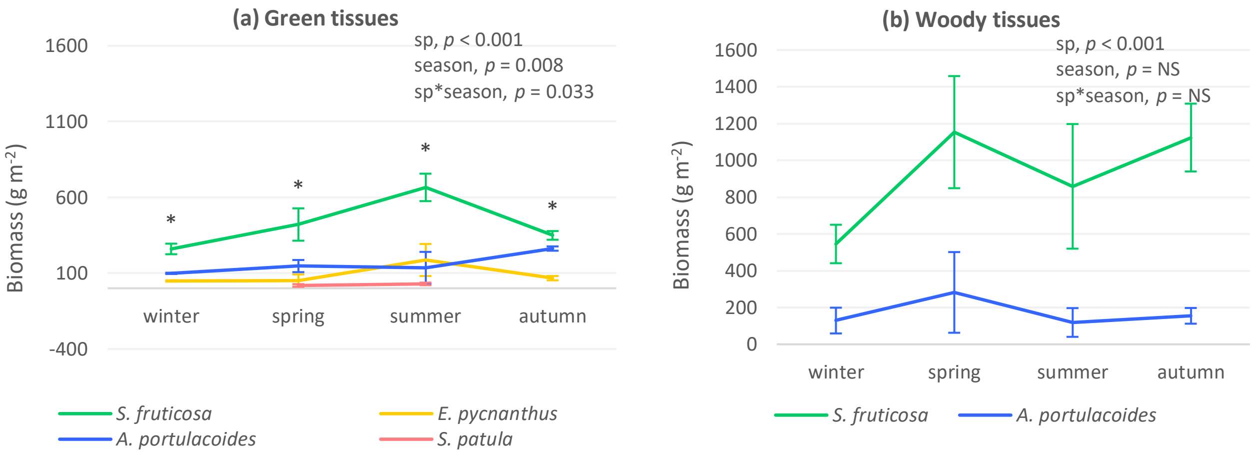


Figure 4.6. Seasonal aboveground biomass of green (a) and woody (b) tissues for the studied species. Bars represent standard errors. Significant *p*-values of the species and season factors and their interaction according to the two-way-ANOVAs results are also shown. Asterisks indicate significant differences among species within each season ($p < 0.05$), being depicted only when the interaction between habitat and sampling date was significant. NS: Not significant.

4.2.2. Seasonal carbon fluxes from soil

4.2.2.1. Soil environmental measurements

The highest soil temperature (Ts) was registered during late spring and summer (Figure 4.7a), in agreement with the higher air temperatures recorded during these months (Figure 3.2). The volumetric water content (VWC) also significantly differed according to the sampling date but only for the halophilous scrub in which the highest values were found in October (Figure 4.7b). Despite the glasswort sward showed the highest soil temperature during most of the year, there were no significant overall differences among habitats in VWC. Nevertheless, soil electrical conductivity (EC) was significantly higher in the glasswort sward, followed by the halophilous scrub and the salt meadow, which was the least saline (Figure 4.7c). The highest values of EC were recorded in June, although these values did not differ significantly from those obtained in July and September.

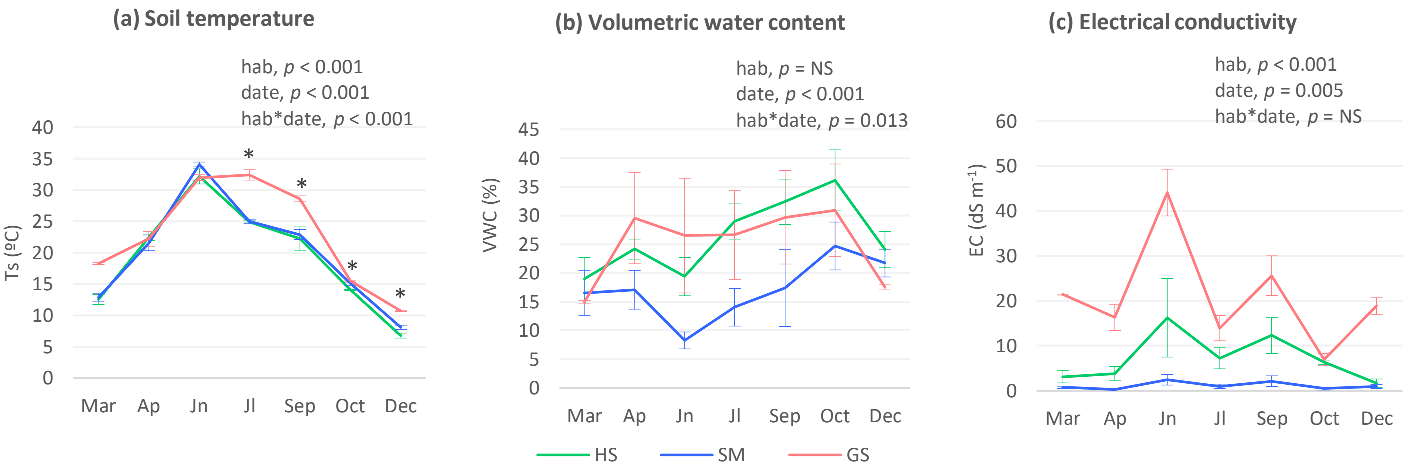


Figure 4.7. Soil temperature (a), volumetric water content (b) and electrical conductivity (c) of the plots where soil carbon fluxes were measured for each sampling date and habitat. Only non-flooded plots are considered. HS: Halophilous scrub; SM: Salt meadow; GS: Glasswort sward. Bars represent ± standard errors (n=5, except in the glasswort sward in March and December in which n=2). Significant *p*-values of the habitat and date factors and their interaction according to the two-way-ANOVAs results are also shown. Asterisks indicate significant differences among habitats within a sampling date ($p < 0.05$), being depicted only when the interaction between habitat and sampling date was significant. For VWC, the interaction between habitat and sampling date was significant, but after analysing the differences among habitats within each sampling date no significant results were obtained. NS: Not significant.

4.2.2.2. Soil CO_2 fluxes

Daily soil respiration (SR) for non-flooded soils of the three salt marsh habitats ranged from 4.0 ± 0.03 to 19.4 ± 0.9 g CO_2 m^{-2} d^{-1}, with significant differences among habitats and sampling dates. Indeed, in the three habitats, the highest values of SR occurred in July and the lowest in October and December (Figure 4.8). When soils were flooded, emissions were remarkably lower (Figure 4.8). Regarding the differences among habitats, the halophilous scrub and the salt marsh meadow showed significantly higher SR values than the glasswort sward (Figure 4.8).

When considering the instantaneous respiration of non-flooded soils (iSR) at midday, differences among habitats were less evident (Figure 4.9), since significant differences were only found in April and July, when the salt meadow had higher iSR than the glasswort sward. The seasonal trend of iSR was different depending on the habitat. Indeed, while the maximum iSR values were observed in March in the three habitats, the lowest ones were recorded in September for the halophilous scrub and the salt meadow and in December for the glasswort sward.

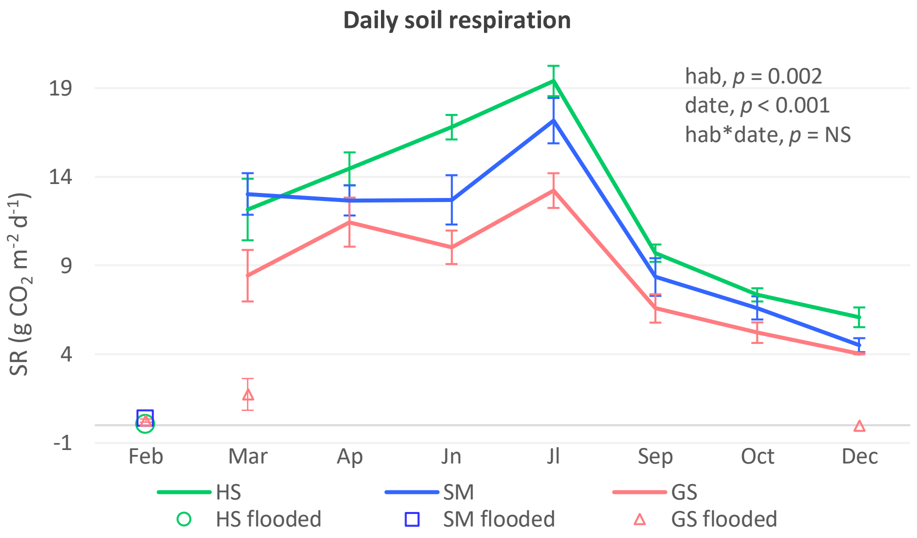


Figure 4.8. Daily soil CO_2 flux (respiration) measured with the soda-lime method (when the soil was not flooded) or by gas chromatography (when the soil was flooded). Lines represent the CO_2 flux measured in non-flooded soils, whereas symbols represent the CO_2 flux measured in flooded soils. HS: Halophilous scrub; SM: Salt meadow; GS: Glasswort sward. Bars represent ± standard errors (n=5). In February, plots from the three habitats were flooded. In March and December, the glasswort sward had flooded (n=3) and non-flooded plots (n=2). Significant *p*-values of the habitat and date factors and their interaction according to the two-way-ANOVAs results are also shown. NS: Not significant.

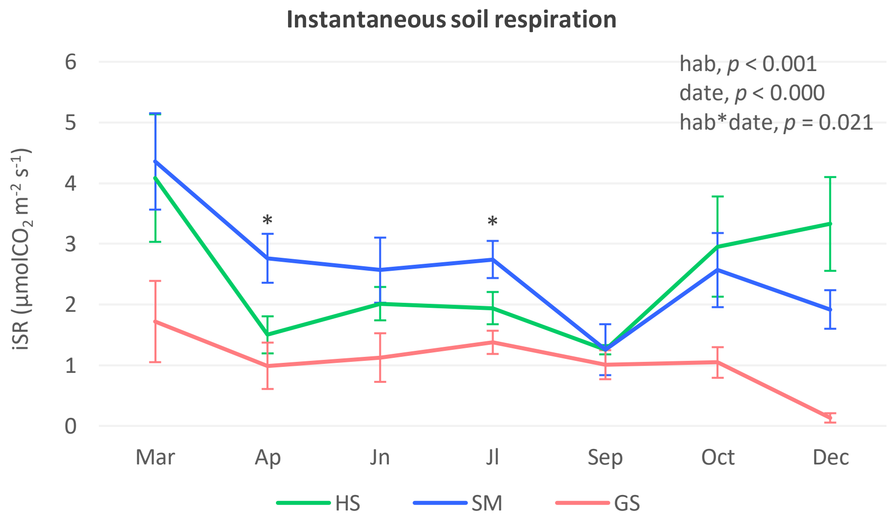


Figure 4.9. Instantaneous soil CO_2 flux (respiration) measured at midday with an IRGA in non-flooded soils. HS: Halophilous scrub; SM: Salt meadow; GS: Glasswort sward. Bars represent ± standard errors (n=5, except in the glasswort sward in March and December in which n=2). Significant *p*-values of the habitat and date factors and their interaction according to the two-way-ANOVAs results are also shown. Asterisks indicate significant differences in soil respiration among habitats within a sampling date ($p < 0.05$).

4.2.2.3. Soil CH_4 fluxes

Regarding soil methane flux (SMF) some negative values (indicating net CH_4 consumption) were found in the three habitats, but peaks of emissions with absolute values remarkably higher than those of CH_4 absorption were also observed (Figure 4.10). In the halophilous scrub CH_4 emissions were only detected in three samplings, in June, with high values, and in April and September, with lower values (Figure 4.10). The highest CH_4 absorption in this habitat was observed in February, when the soil was flooded. In the salt meadow and the glasswort sward CH_4 absorption was only observed in October (Figure 4.10).The maximum CH_4 emissions in the salt meadow was in July, and, in the glasswort sward in March in flooded soils and in June in non-flooded ones (Figure 4.10).

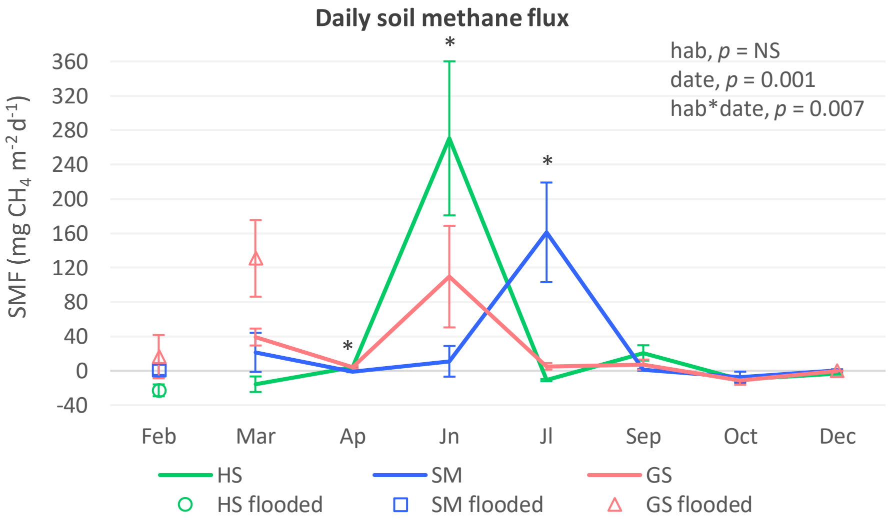


Figure 4.10. Daily soil methane (CH_4) flux (SMF). Lines represent CH_4 flux measured in non-flooded soils and symbols represent CH_4 flux measured in flooded soils. HS: Halophilous scrub; SM: Salt meadow; GS: Glasswort sward. Bars represent ± standard errors (n=5). In February, plots from the three habitats were flooded. In March and December, the glasswort sward had flooded (n=3) and non-flooded plots (n=2). Significant *p*-values of the habitat and date factors and their interaction according to the two-way-ANOVAs results are also shown. Asterisks indicate significant differences in CH_4 flux among habitats in non-flooded soils within a sampling date ($p < 0.05$). NS: Not significant.

4.2.2.4. Carbon mineralization quotient

The sampling date and the type of habitat had a significant effect on the carbon mineralization quotient (Q_{min}), although the interaction between these two factors was not significant. For all the habitats, the highest Q_{min} values were found in July and the lowest in October and December. The glasswort sward showed the highest mineralization quotients, while values for the halophilous scrub and the salt meadow were similar (Figure 4.11).

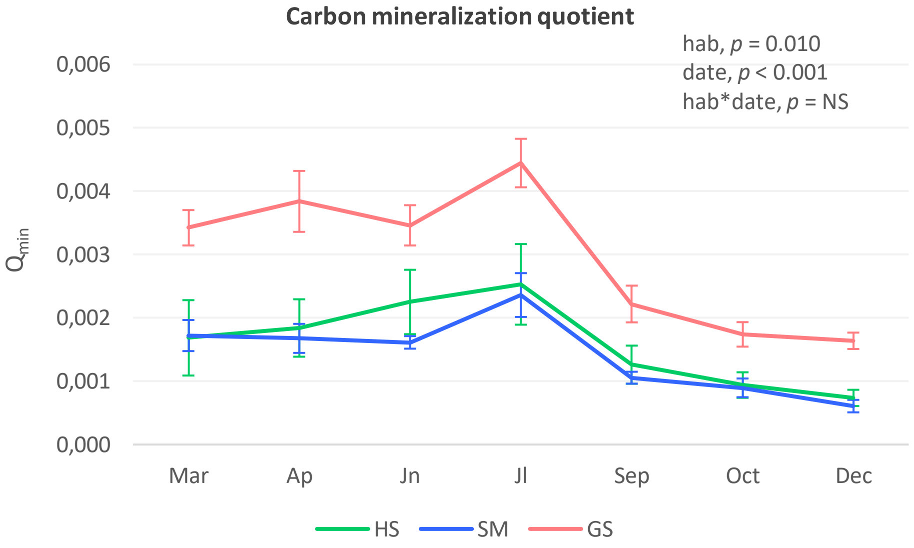


Figure 4.11. Daily soil carbon mineralization quotients. HS: Halophilous scrub; SM: Salt meadow; GS: Glasswort sward. Bars represent ± standard errors (n=5, except in the glassword sward in March and December in which n=2). Significant *p*-values of the habitat and date factors and their interaction according to the two-way-ANOVAs results are also shown. NS: Not significant.

4.2.2.5. Relationship among soil environmental parameters and soil carbon fluxes

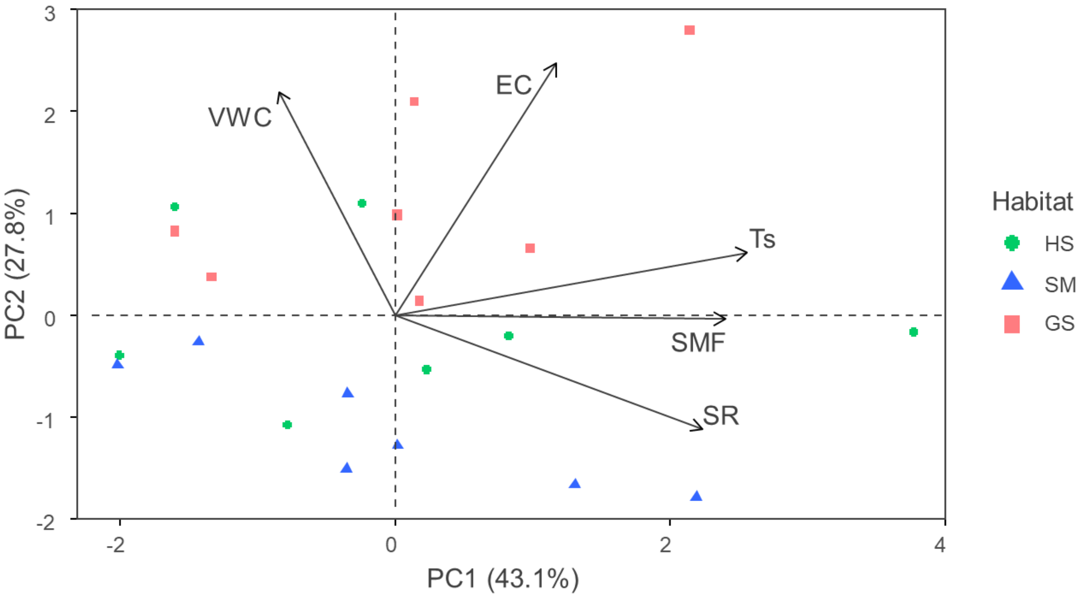
Daily soil CO_2 and CH_4 fluxes (SR and SMF) were positively correlated with soil temperature (Ts), while instantaneous soil CO_2 fluxes (iSR) at midday were negatively correlated with soil electrical conductivity (EC) (Table 4.17).

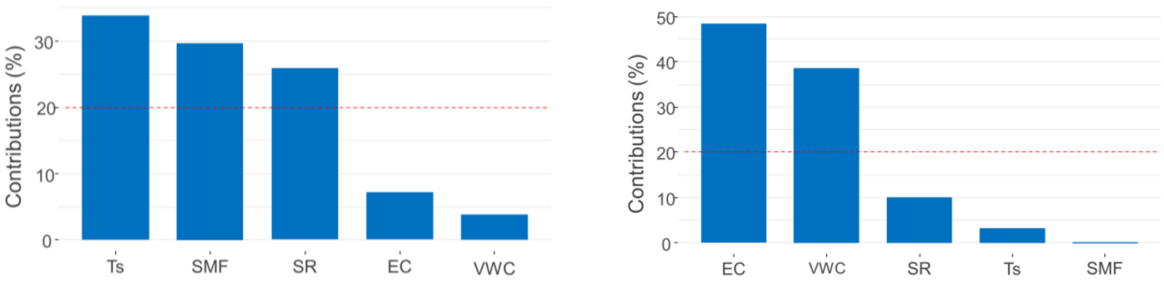
Table 4.17. Pearson's correlation coefficients (r) between daily soil CO_2 flux, midday soil instantaneous CO_2 flux or daily soil CH_4 flux and the following edaphic parameters measured at midday: soil volumetric water content (VWC), electrical conductivity (EC) and soil temperature (Ts). Significant correlations ($p < 0.01$) are indicated with asterisks (**) and highlighted in bold.

	VWC	**EC**	**Ts**
Daily soil CO_2 flux	-0.218	-0.117	**0.568****
Soil CO_2 flux at midday	-0.296	**-0.573****	-0.318
Daily soil CH_4 flux	-0.332	0.310	**0.557****

In the PCA two principal components were identified, which together explained 70.9% of the total variance, with PC1 and PC2 explaining 43.1% and 27.8% of the total variance respectively (Figure 4.12). Ts, SMF and SR were the most important parameters related with PC1 (all with positive contributions) whereas soil EC and VWC showed the highest contribution (both positive) to PC2. There was a clear segregation produced by PC2 between the salt meadow and the glasswort sward supported by evident differences in their soil EC and VWC (Figure 4.12c).

(a)





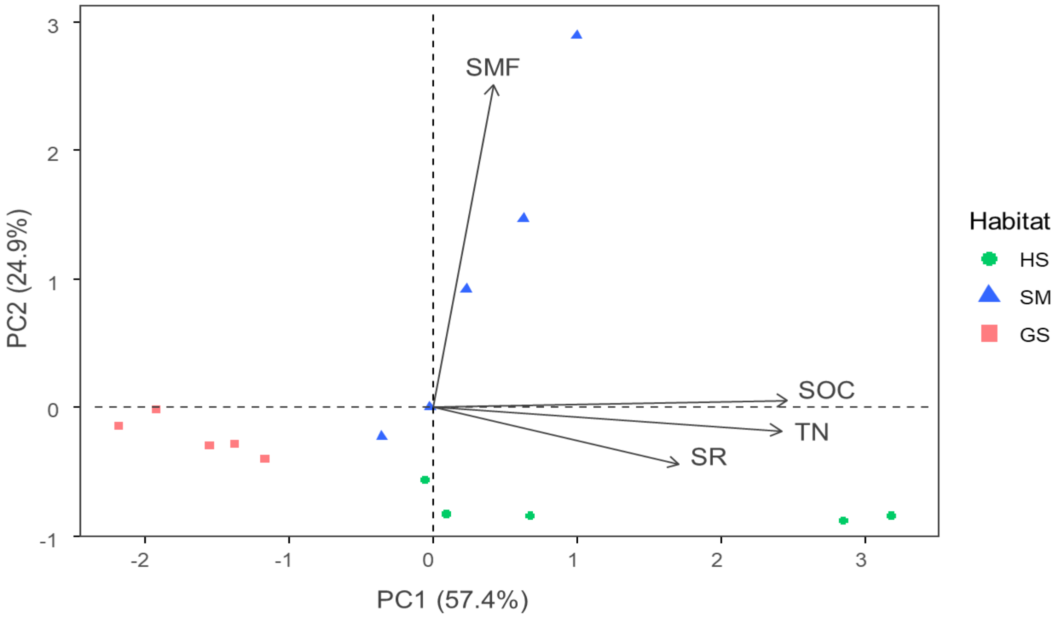
(b) Contribution of variables to PC1

(b) Contribution of variables to PC2

Figure 4.12. Ordination plot obtained from principal components analysis considering mean values for each one of the 7 sampling days of soil daily CO_2 emissions (SR), soil daily CH_4 fluxes (SMF), soil temperature (Ts), soil volumetric water content (VWC) and soil electric conductivity (EC) (a); contribution of the different variables to the principal component 1 (b) and 2 (c). Only non-flooded plots are considered (n=5 except in the glasswort sward in March and December in which n=2). HS: Halophilous scrub; SM: Salt meadow; GS: Glasswort sward. In graph b and c the red dashed line indicates the expected average contribution according to the model. Those variables with a contribution larger than this percentage are considered variables with an important contribution to the component

When data of soil carbon fluxes (SR and SMF) measured in July 2017 and soil organic carbon (SOC) and total nitrogen (TN) measured in July 2015-2016 were ordinated in a PCA, two principal components were identified, explaining together 82.3% of the total variance (PC1 explained 57.4% and PC2 24.9% of the variance) (Figure 4.13). SOC and TN were the most important parameters related with PC1, being both contributions positive, while SMF was the only parameter which contributes to PC2. There was a clear segregation produced by PC1 between the halophilous scrub and the salt meadow on one hand and the glasswort sward on the other hand, highlighting the differences in SOC and TN among these habitats (Figure 4.13).

(a)



(b) Contribution of variables to PC1 **(b) Contribution of variables to PC2**

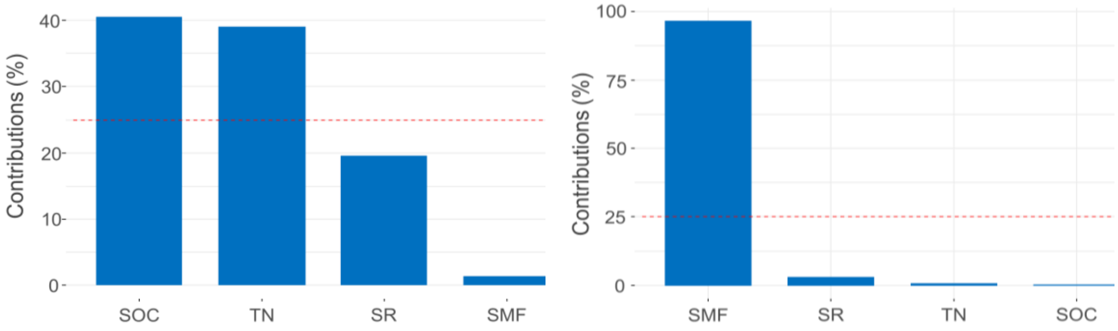


Figure 4.13. Ordination plot obtained from principal components analysis considering values of July of soil daily CO_2 emissions (SR), soil daily CH_4 fluxes (SMF), soil organic carbon (SOC) and soil total nitrogen (TN); contribution of the different variables to the principal component 1 (b) and 2 (c). HS: Halophilous scrub; SM: Salt meadow; GS: Glasswort sward. In graphs b and c, the red dashed line indicates the expected average contribution according to the model. Those variables with a contribution larger than this percentage are considered variables with an important contribution to the component.

4.3. Study 3: Litter decomposition of the dominant species of the halophilous scrub and the salt meadow

4.3.1. Litter quality and decomposition rates

Differences among species

Initial litter quality parameters differed among species (Table 4.18). Thus, whereas *S. fruticosa* litter had the initial lowest C and highest N, and thus, lowest C/N ratio, no significant differences were observed for these parameters among *A. portulacoides* and *E. pycnanthus*. Indeed, the C/N ratio for *S. fruticosa* litter was half of that of the other two species. Moreover, *S. fruticosa* litter had the lowest initial cellulose content and the lowest lignin/N ratio, while *E. pycnanthus* litter showed the highest values for these parameters. Both *S. fruticosa* and *A. portulacoides* litter had lower lignin content than *E. pycnanthus* (Table 4.18).

Table 4.18. Mean ± SE (n=3) of initial litter quality parameters. % refers to units of compound in 100 units of litter biomass ash free dry mass. Significance (*p*-value) for the species factor is given, being highlighted in bold when significant. Different letters indicate significant differences among species ($p < 0.05$).

Initial litter quality	**C** (%)	**N** (%)	**C/N**	**Cellulose** (%)	**Lignin** (%)	**Lignin/N**
S. fruticosa	45.0 ± 0.2 b	1.79 ± 0.02 a	25.1 ± 0.2 b	18.0 ± 0.4 c	8.5 ± 0.1 b	4.73 ± 0.02 c
A. portulacoides	46.5 ± 0.3 a	0.84 ± 0.01 b	55.3 ± 0.5 a	20.6 ± 0.4 b	7.4 ± 0.2 b	8.81 ± 0.46 b
E. pycnanthus	46.5 ± 0.1 a	0.87 ± 0.03 b	53.3 ± 1.8 a	45.2 ± 0.6 a	10.5 ± 0.2 a	12.08 ± 0.08 a
Species (*p*-value)	**0.003**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**<0.001**

Considering the whole studied period (Table 4.19), no significant differences were observed in the C content of the litter among the three species, while *S. fruticosa* litter maintained the highest N content and the lowest C/N and lignin/N ratios. *E. pycnanthus* litter showed the highest cellulose content throughout the study, while no significant differences were observed in the lignin content among species (Table 4.19).

Table 4.19. Mean ± SE of litter quality parameters, considering the values of the entire decomposition period (n=24). % refers to units of compound in 100 units of litter biomass ash free dry mass. Significance (*p*-value) for the species factor is given, being highlighted in bold when significant. Different letters indicate significant differences among species ($p < 0.05$).

Overall litter quality	**C** (%)	**N** (%)	**C/N**	**Cellulose** (%)	**Lignin** (%)	**Lignin/N**
S. fruticosa	47.6 ± 0.4 a	2.00 ± 0.04 a	24.0 ± 0.4 b	26.9 ± 1.3 b	13.4 ± 1.0 a	6.6 ± 0.4 b
A. portulacoides	47.0 ± 0.4 a	0.93 ± 0.04 b	52.1 ± 1.6 a	24.3 ± 0.7 b	11.8 ± 0.8 a	12.7 ± 0.6 a
E. pycnanthus	48.1 ± 0.4 a	1.08 ± 0.05 b	46.0 ± 1.4 a	44.4 ± 0.4 a	15.0 ± 0.9 a	13.8 ± 0.5 a
Species (*p*-value)	0.356	**<0.001**	**<0.001**	**<0.001**	0.265	**0.005**

Regarding litter decomposition rate (k) and litter half-life, *E. pycnanthus* had the lowest and the highest values, respectively (Table 4.20). Concretely, k of *E. pycnanthus* was around 7-fold lower than that of *S. fruticosa* and 13-fold lower than that of *A. portulacoides*.

Table 4.20. Mean ± SE of the litter decomposition rate and half-life for the three studied species (n=6). Significance (*p*-value) for the species factor is given, being highlighted in bold when significant. Different letters indicate significant differences among species ($p < 0.05$).

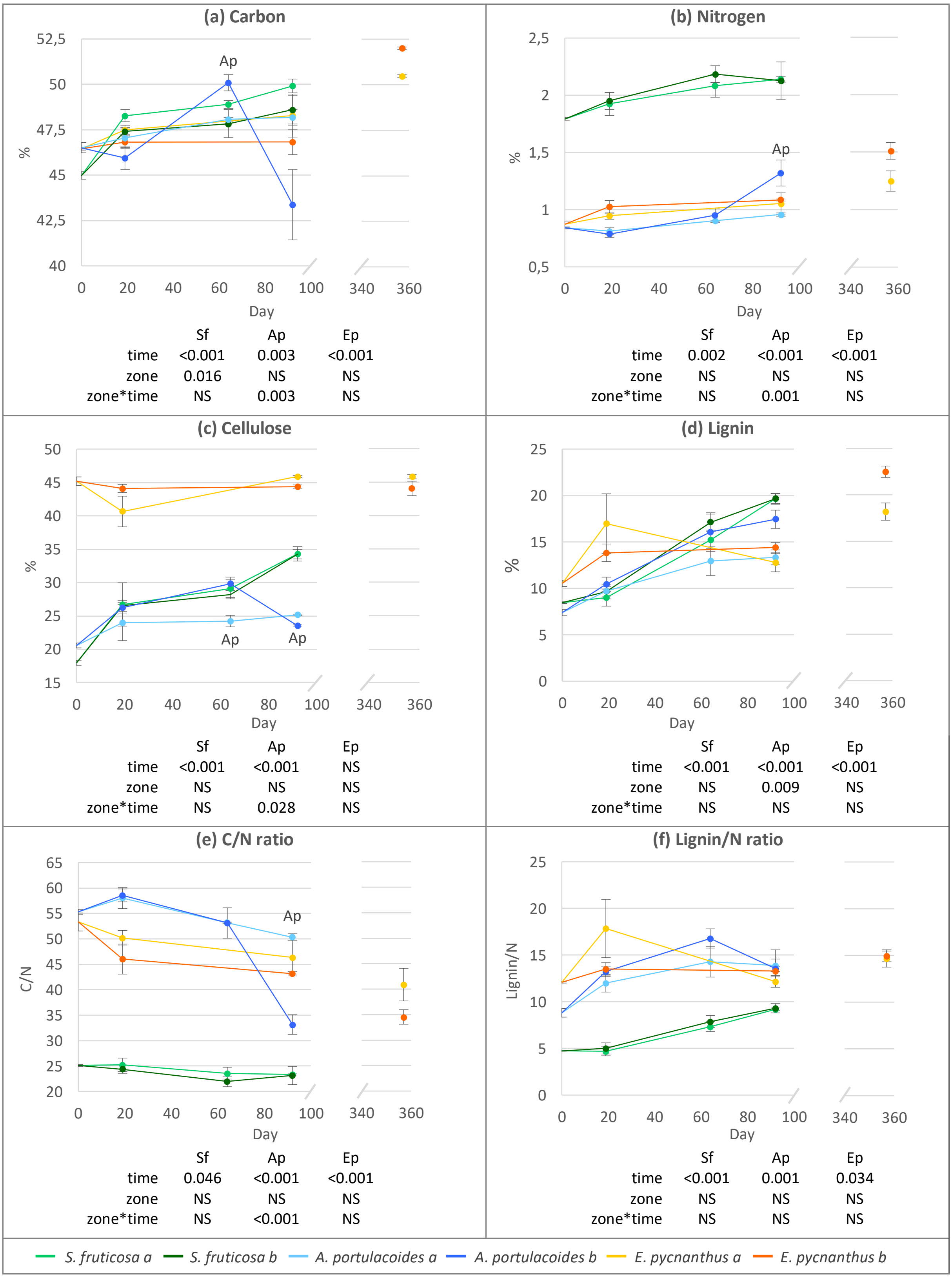
	k (day^{-1})	**$t_{0.5}$** (days)
S. fruticosa	0.0102 ± 0.0002 a	68.3 ± 1.5 a
A. portulacoides	0.0193 ± 0.0068 a	70.8 ± 21.7 a
E. pycnanthus	0.0015 ± 0.0001 b	477.8 ± 29.1 b
Species (*p*-value)	**<0.001**	**<0.001**

Within species analyses: time and zone *effects*

In the halophilous *S. fruticosa* shrub, the litter C content increased over time, but especially at the beginning of the decomposition process, with the highest differences in C content being observed from initial values to those of day 19. Moreover, overall the study period, the C content was significantly higher in the litter placed in zone *a* compared to that of zone *b* (Figure 4.14a). In contrast, all the other litter quality parameters measured (N, cellulose, lignin, C/N and lignin/N) were only affected by time, increasing throughout the decomposition process, except in the case of the C/N ratio, which decreased over time (Figure 4.14b-f).

Regarding salt meadow *A. portulacoides*, changes in the litter content in C, N, and cellulose, as well as in the C/N ratio, differed depending on the zone and the date (there was a significant interaction between these two factors). Specifically, differences between zones appeared at the end of the decomposition process, when values were significantly higher in zone *b* on day 64 for C and cellulose and on day 92 for N. As a consequence, the C/N ratio was lowest in zone *b* on day 92 (Figure 4.14a, b, c, e). On the other hand, the lignin content of *A. portulacoides* litter significantly increased over time in both zones, with overall values being higher at zone *b* (Figure 4.14d). The lignin/N ratio of *A. portulacoides* litter increased through the decomposition process independently of the zone (Figure 4.14e, f).

E. pycnanthus litter quality did not differ between zones, but significantly changed over time. Indeed, increases in litter C, N, lignin and lignin/N ratio, as well as decreases in the C/N ratio, were observed throughout the decomposition period (Figure 4.14a, b, d, e, f). The cellulose content was the only parameter that did not change over time (Figure 4.14c).



(a) Carbon

	Sf	Ap	Ep
time	<0.001	0.003	<0.001
zone	0.016	NS	NS
zone*time	NS	0.003	NS

(b) Nitrogen

	Sf	Ap	Ep
time	0.002	<0.001	<0.001
zone	NS	NS	NS
zone*time	NS	0.001	NS

(c) Cellulose

	Sf	Ap	Ep
time	<0.001	<0.001	NS
zone	NS	NS	NS
zone*time	NS	0.028	NS

(d) Lignin

	Sf	Ap	Ep
time	<0.001	<0.001	<0.001
zone	NS	0.009	NS
zone*time	NS	NS	NS

(e) C/N ratio

	Sf	Ap	Ep
time	0.046	<0.001	<0.001
zone	NS	NS	NS
zone*time	NS	<0.001	NS

(f) Lignin/N ratio

	Sf	Ap	Ep
time	<0.001	0.001	0.034
zone	NS	NS	NS
zone*time	NS	NS	NS

Figure 4.14. Litter content of carbon (a), nitrogen (b), cellulose (c) and lignin (d), carbon/nitrogen ratio (e) and lignin/nitrogen ratio (f). % refers to units of compound in 100 units of litter biomass ash free dry mass. Bars represent standard errors (n=3). Significant *p*-values for the factors time and zone and for their interaction according to the repeated measures ANOVAs are also given. Initial letters of the corresponding species (Sf: *S. fruticosa;* Ap: *A. portulacoides;* Ep: *E. pycnanthus*) indicate significant differences between zones (a and b) for each species and sampling day ($p < 0.05$), being depicted only when the interaction between zone and time was significant. NS: Not significant.

The litter decomposition rate (k) and half-life ($t_{0.5}$) of *S. fructicosa* differed between zones, being significantly higher and lower, respectively, in zone *a* (Table 4.21). However, the % of remaining AFDM was similar in both zones on the last sampling date (Figure 4.15). For *A. portulacoides,* the litter decomposition rate and half-life also differed between zones, being these differences very pronounced (Table 4.21) and resulting in a % of remaining AFDM of about 68% and 10% in zone *a* and *b*, respectively, on the last sampling day (Figure 4.15). Conversely, the decomposition rate and half-life of *E. pycnanthus* litter did not differ between zones (Table 4.21), resulting in similar % of remaining AFDM on the last sampling day (Figure 4.15).

Table 4.21. Mean ± SE (n=3) of the decomposition rates (k) and half-life ($t_{0.5}$) of the litter of each species placed in the two zones with each habitat. HS: Halophilous scrub; SM: Salt meadow. *P*-values for the zone factor are given for each species, being highlighted in bold when significant.

		k (day^{-1})	**$t_{0.5}$** (days)
S. fruticosa	zone *a* (HS)	0.0106 ± 0.0002	65.4 ± 1.2
	zone *b* (HS)	0.0097 ± 0.0001	71.3 ± 1.0
	p-value	**0.023**	**0.022**
A. portulacoides	zone *a* (SM)	0.0059 ± 0.0005	118.0 ± 9.5
	zone *b* (SM)	0.0326 ± 0.0074	23.6 ± 5.4
	p-value	**0.023**	**0.001**
E. pycnanthus	zone *a* (SM)	0.0014 ± 0.0001	514.9 ± 49.0
	zone *b* (SM)	0.0016 ± 0.0001	440.8 ± 21.3
	p-value	0.278	0.238

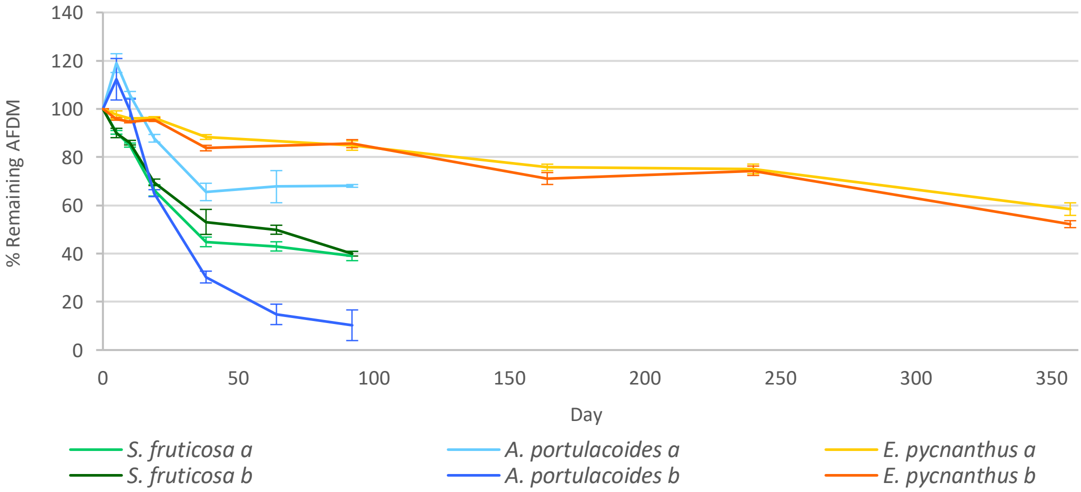


Figure 4.15. Litter decomposition process expressed as remaining ash free dry mass (AFDM, % of the initial) for each sampling date and for the three species, considering separately the litterbags placed in the two zones within each habitat (for each species, the pale colour indicates data from zone *a* and the dark colour from zone *b*). Bars represent standard errors (n=3).

4.3.2. Extracellular enzyme activity and enzyme efficiency

Differences among species

Considering the overall data obtained from the entire decomposition period, potential extracellular enzyme activities (EEA) differed among species for the four studied enzymes (Table 4.22). Specifically, the highest extracellular β-glucosidase and β-xylosidase activities were found for *E. pycnanthus* litter, although values did not differ from those of *A. portulacoides.* For the leucine-aminopeptidase, *A. portulacoides* showed the highest values of EEA, despite they were not statistically different from those of *S. fruticosa* (Table 4.22). Regarding the phenol oxidase activity, *E. pycnanthus* had significantly lower values than the other two species.

Table 4.22. Mean ± SE of the potential extracellular activity of the four enzymes studied, considering the entire decomposition period (n=36 for *S. fruticosa* and *A. portulacoides*; n=48 for *E. pycnanthus*). Significance (*p*-value) for the species factor is given, being highlighted in bold when significant. Different letters indicate significant differences among species ($p < 0.05$)

	β-glucosidase (µmol MUF g AFDM^{-1} h^{-1})	**β-xylosidase** (µmol MUF g AFDM^{-1} h^{-1})	**Leucine-aminopeptidase** (µmol AMC g AFDM^{-1} h^{-1})	**Phenol oxidase** (µmol DIQC g AFDM^{-1} h^{-1})
S. fruticosa	2.28 ± 0.31 b	1.45 ± 0.44 b	12.76 ± 1.59 ab	0.93 ± 0.14 a
A. portulacoides	1.80 ± 0.25 ab	0.69 ± 0.12 ab	17.25 ± 2.39 a	1.72 ± 0.58 a
E. pycnanthus	3.11 ± 0.44 a	1.95 ± 0.30 a	14.31 ± 3.03 b	0.64 ± 0.12 b
Species (*p*-value)	**0.002**	0.085	**0.004**	**<0.001**

For the four enzymes, litter of *E. pycnanthus* showed the highest turnover activity (TA), i.e., the lowest enzyme efficiency, being values from the other species more than one order of magnitude higher than those of *E. pycnanthus* (Table 4.23). In contrast, no significant differences were found between *S. fruticosa* and *A. portulacoides* litter for this variable (Table 4.23).

Table 4.23. Mean ± SE of enzyme efficiencies, expressed as turnover activity (TA; mmol g AFDM $^{-1}$), for each one of the four studied enzymes and for each species (n=6). TA = 1/k2 (k2 is the slope of ln % remaining AFDM regressed linearly to AEA). Higher values of TA mean less enzyme efficiency. Significance (*p*-values) for the accumulated enzyme activity (AEA) covariate and for the species factor are also given, being highlighted in bold when significant. Different letters indicate significant differences among species ($p < 0.05$).

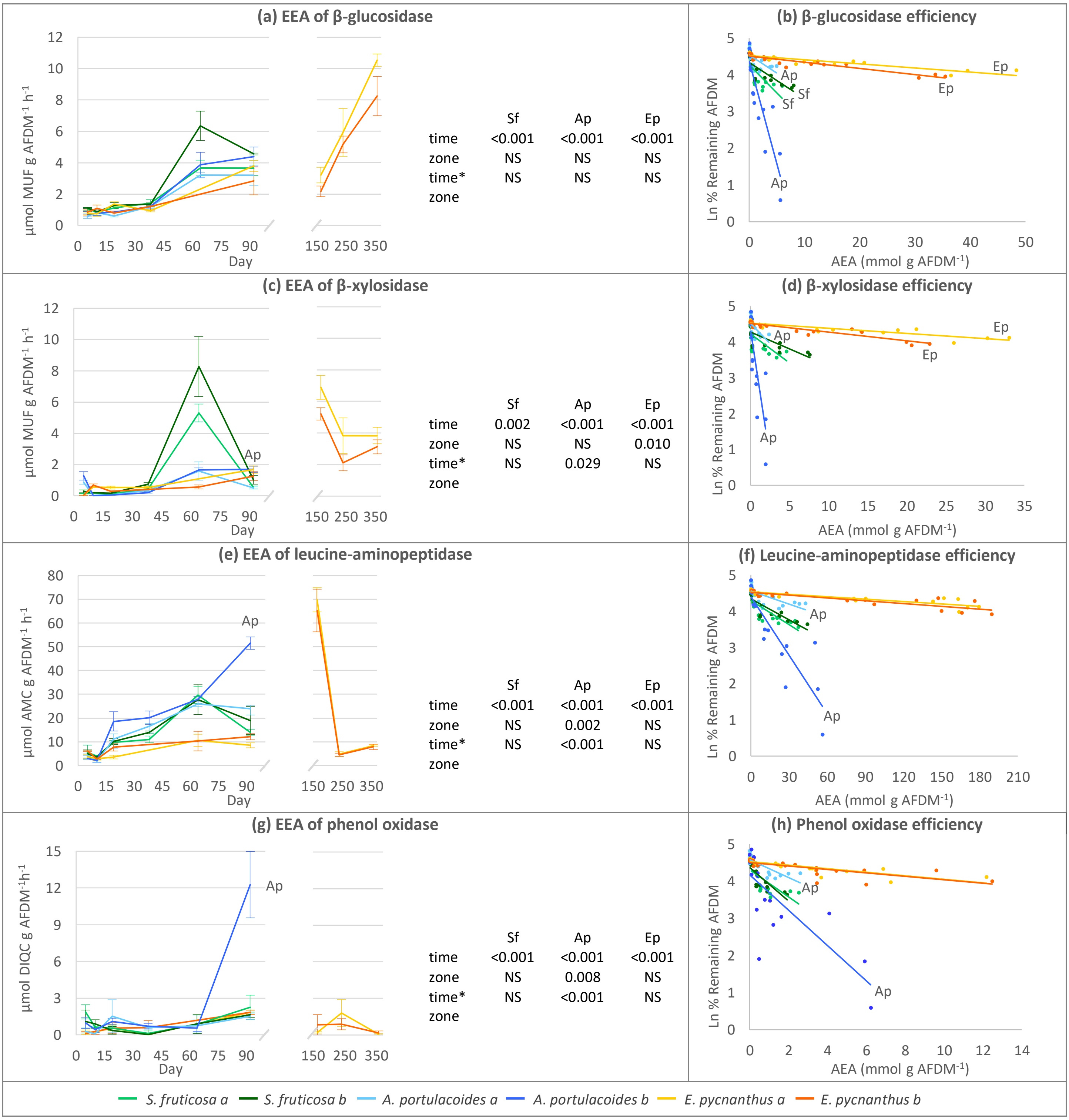
	TA β-glucosidase	**TA β-xylosidase**	**TA Leucine-aminopeptidase**	**TA Phenol oxidase**
S. fruticosa	9.30 ± 1.50 b	9.27 ± 1.68 b	48.64 ± 5.61 b	2.50 ± 0.39 b
A. portulacoides	2.57 ± 0.45 b	1.07 ± 0.23 b	24.06 ± 3.67 b	2.06 ± 0.29 b
E. pycnanthus	75.75 ± 5.61 a	57.84 ± 4.84 a	417.01 ± 32.52 a	21.28 ± 2.36 a
AEA (*p*-value)	**<0.001**	**0.001**	**<0.001**	**<0.001**
Species (*p*-value)	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Within species analyses: time and zone *effects*

For *S. fruticosa* litter, the extracellular enzyme activity of the four enzymes analysed changed over time, but not between zones. Indeed, the activity of β-glucosidase was higher in the two last sampling dates (days 64 and 92) (Figure 4.16a), while the activity of β-xylosidase and leucine-aminopeptidase peaked on day 64 and then declined (Figure 4.16c, e). The phenol oxidase activity in *S. fruticosa* litter was low and quite constant throughout the decomposition period, being slightly higher on the last sampling date (Figure 4.16g).

Regarding *A. portulacoides* litter, changes over time in the extracellular enzyme activities differed depending on the zone (except for β-glucosidase), showing significantly higher values in zone *b* on the last sampling date (day 92) (Figure 4.16c, e, g). The β-glucosidase activity increased over time in both zones (Figure 4.16a).

The activity of the four enzymes in *E. pycnanthus* litter was affected by time but not by zone, except in the case of β-xylosidase, since the activity of this enzyme was higher in zone *a* throughout the whole study period (Figure 4.16a, c, e, g). Regarding the changes over time, there was a steep increase in the β-glucosidase activity over the entire study (Figure 4.16a), while the activities of β-xylosidase and leucine-aminopeptidase peaked on day 164 (Figure 4.16c, e). The phenol oxidase activity was, in general, low and slightly increased with time (Figure 4.16g).



(a)

	Sf	Ap	Ep
time	<0.001	<0.001	<0.001
zone	NS	NS	NS
time* zone	NS	NS	NS

(c)

	Sf	Ap	Ep
time	0.002	<0.001	<0.001
zone	NS	NS	0.010
time* zone	NS	0.029	NS

(e)

	Sf	Ap	Ep
time	<0.001	<0.001	<0.001
zone	NS	0.002	NS
time* zone	NS	<0.001	NS

(g)

	Sf	Ap	Ep
time	<0.001	<0.001	<0.001
zone	NS	0.008	NS
time* zone	NS	<0.001	NS

Figure 4.16. Potential extracellular enzyme activities (EEA) and enzyme efficiencies, represented by means of linear regressions of ln of remaining ash free dry mass (Ln % remaining AFDM) against accumulated enzyme activity (AEA), for β-glucosidase (a, b), β-xylosidase (c, d), leucine-aminopeptidase (e, f) and phenol oxidase (g, h). In the EEA graphs (a, c, e, g), bars represent standard errors (n=3). Significant *p*-values for the factors time and zone and for their interaction according to the repeated measures ANOVAs for EEA are given. Initial letters of the corresponding species (Sf: *S. fruticosa*; Ap: *A. portulacoides*; Ep: *E. pycnanthus*) indicate significant differences between zones (a and b) for each species (at each sampling day in the case of EEA graphs) (p < 0.05), being depicted only (in EEA graphs) when the interaction between zone and time was significant. In the enzyme efficiency graphs (b, d, f, h) steeper slopes represent higher enzyme efficiencies, i.e., less enzyme produced to decompose a gram of leaf litter. Note the different scale graduation of axis x in EEA graphs after the discontinuity. NS: Not significant.

Regarding the turnover activity (TA) of the studied enzymes for *S. fruticosa* litter, TA differed between zones only in the case of β-glucosidase, with zone *a* showing lower values (i.e. higher enzyme efficiency) (Figure 4.16b, Table 4.24). In the case of *A. portulacoides* litter, TA of the four enzymes was significantly higher in zone *a* (Figure 4.16, Table 4.24), while, in *E. pycnanthus* litter, differences between zones were observed for β-glucosidase and β-xylosidase TA, with values being also slightly higher in zone *a* (Figure 4.16, Table 4.24).

Table 4.24. Mean ± SE (n=3) of enzyme efficiencies, expressed as turnover activity (TA; mmol g AFDM^{-1}), for each species and zone within each habitat. TA = 1/k_2 (k_2 is the slope of ln % remaining AFDM regressed linearly to AEA). Higher values of TA mean less enzyme efficiency. HS: Halophilous scrub; SM: Salt meadow. *P*-values of GLM results are also given, being highlighted in bold when significant.

		TA β-glucosidase	**TA β-xylosidase**	**TA Leucine-aminopeptidase**	**TA Phenol oxidase**
S. fruticosa	zone *a* (HS)	6.6 ± 1.5	6.2 ± 1.5	45.1 ± 7.9	2.8 ± 0.7
	zone *b* (HS)	10.2 ± 1.7	10.6 ± 2.2	51.9 ± 7.6	2.2 ± 0.4
	p-value	**0.039**	0.078	0.200	0.881
A. portulacoides	zone *a* (SM)	10.1 ± 3.3	4.4 ± 1.7	82.8 ± 21.4	4.2 ± 0.9
	zone *b* (SM)	1.8 ± 0.2	0.7 ± 0.1	18.6 ± 2.4	2.1 ± 0.4
	p-value	**<0.001**	**<0.001**	**<0.001**	**0.002**
E. pycnanthus	zone *a* (SM)	89.3 ± 8.5	69.4 ± 6.8	451.1 ± 47.2	21.0 ± 2.8
	zone *b* (SM)	59.5 ± 4.9	41.3 ± 3.6	387.7 ± 43.6	21.6 ± 3.9
	p-value	**0.014**	**0.008**	0.203	0.477

4.3.3. Microbial biomass

Differences among species

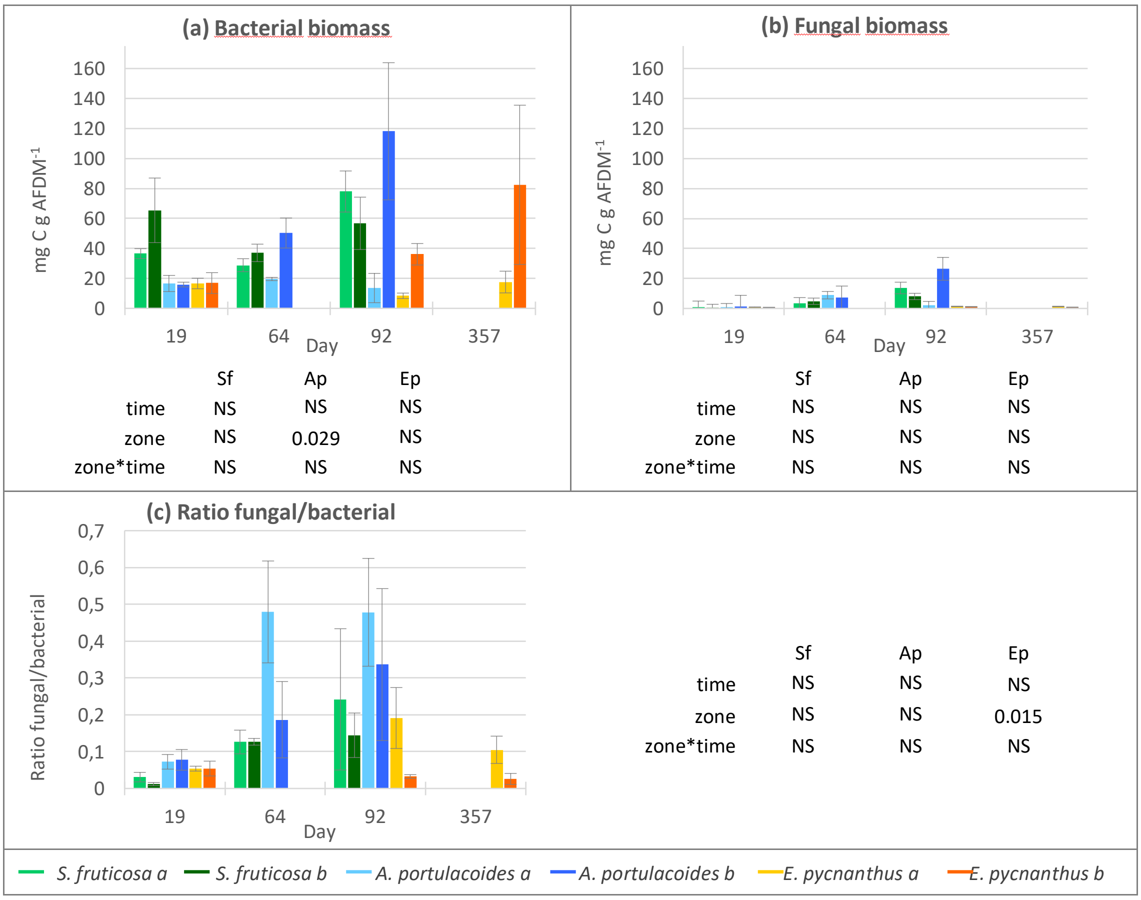
E. pycnanthus litter had lower values of bacterial and fungal biomass and lower fungal/bacterial biomass ratio than the other two species, but differences with *A. portulacoides* were not significant for bacterial biomass. Bacterial and fungal biomass was similar for *A. portulacoides* and *S. fruticosa* litter, although the fungal/bacterial biomass ratio was nearly 2.5-fold higher in the former (Table 4.25).

Table 4.25. Mean ± SE of bacterial biomass, fungal biomass and fungal/bacterial biomass ratio considering the values of the entire decomposition period for each species (n=18). Significance (*p*-value) for the species factor is given, being highlighted in bold when significant. Different letters indicate significant differences among species ($p < 0.05$).

	Bacterial biomass (mg C g AFDM^{-1})	**Fungal biomass** (mg C g AFDM^{-1})	**Fungal/bacterial biomass ratio**
S. fruticosa	50.4 ± 6.4 a	5.3 ± 1.8 a	0.11 ± 0.03 b
A. portulacoides	39.0 ± 11.0 ab	7.8 ± 2.9 a	0.27 ± 0.07 a
E. pycnanthus	29.6 ± 9.8 b	1.0 ± 0.1 b	0.08 ± 0.02 c
Species (*p*-value)	**0.025**	**<0.001**	**<0.001**

Within species analyses: time and zone effects

The bacterial and fungal biomass, and the fungal/bacterial biomass ratio, did not vary significantly over time for any of the three species studied, despite they tended to increase throughout the decomposition period (Figure 4.17).


(a) Bacterial biomass

(b) Fungal biomass

(c) Ratio fungal/bacterial

(a)

	Sf	Ap	Ep
time	NS	NS	NS
zone	NS	0.029	NS
zone*time	NS	NS	NS

(b)

	Sf	Ap	Ep
time	NS	NS	NS
zone	NS	NS	NS
zone*time	NS	NS	NS

(c)

	Sf	Ap	Ep
time	NS	NS	NS
zone	NS	NS	0.015
zone*time	NS	NS	NS

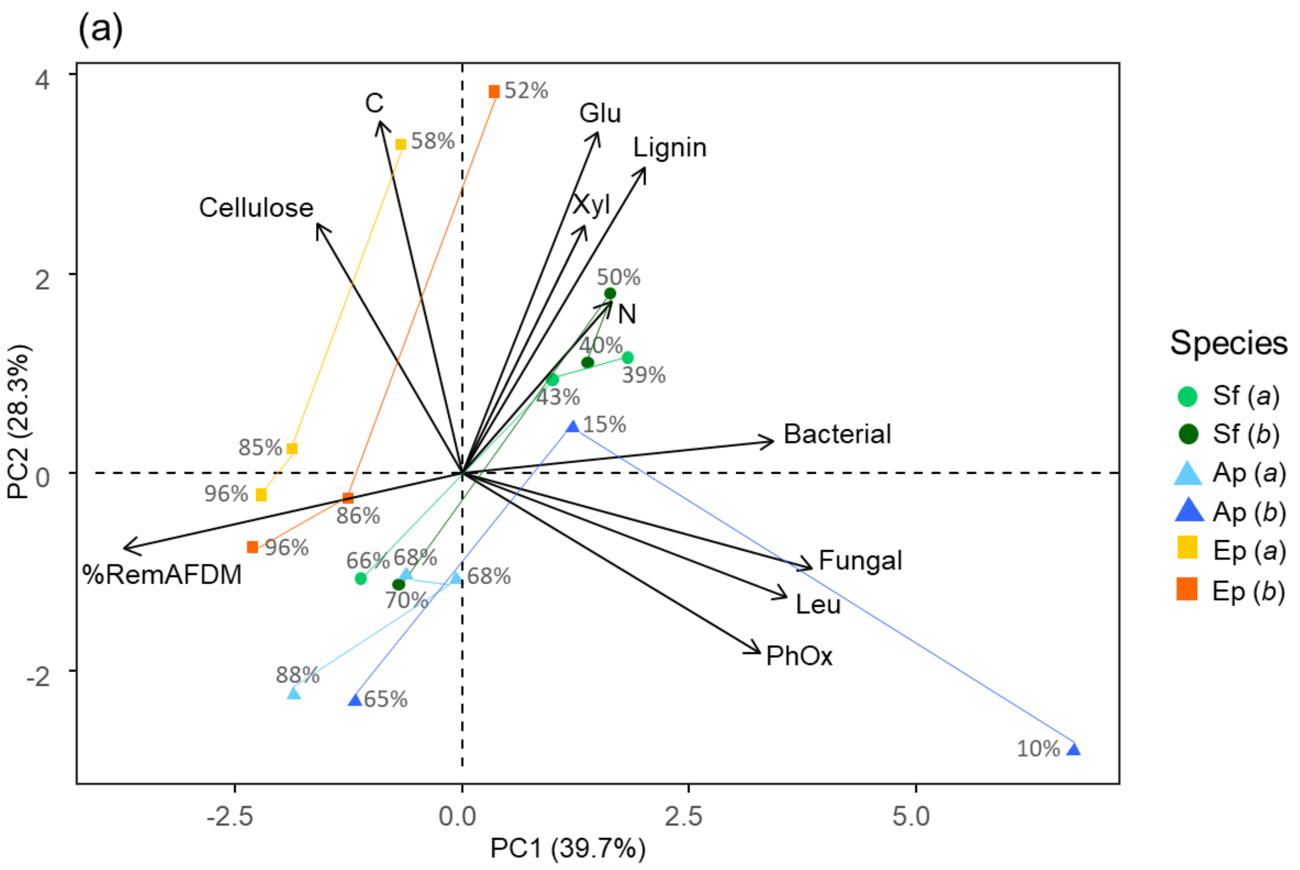
Figure 4.17. Bacterial (a) and fungal (b) biomass and fungal/bacterial biomass ratio (c) for the litter of each species, sampling date and zone. Sf: *S. fruticosa*; Ap: *A. portulacoides*; Ep: *E. pycnanthus*. Bars represent standard errors (n=3). Significant *p*-values for time and zone factors and for their interaction (according to the repeated measures ANOVAs) are given. NS: Not significant.

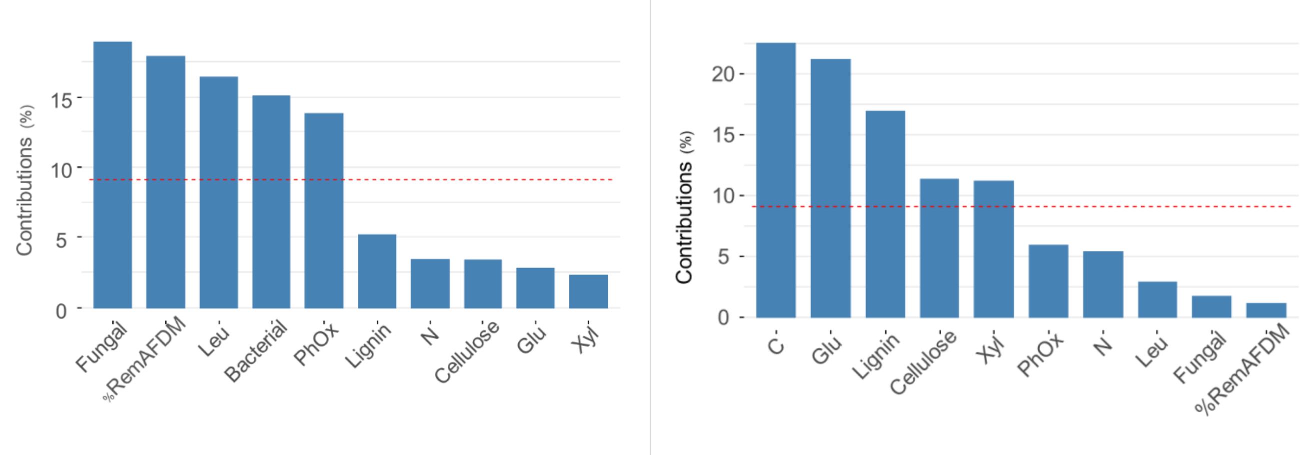
Regarding the differences between zones, *A. portulacoides* litter had, in general, significantly higher bacterial biomass in zone *b* (Figure 4.17a), while *E. pycnanthus* litter showed lower fungal/bacterial biomass ratio in this zone (Figure 4.17c).

4.3.4. Principal components analysis using the decomposition parameters.

In the PCA, two principal components were identified, PC1 and PC2, which together explained 68% of the total variance (39.7% and 28.3%, respectively) (Figure 4.18). Fungal and bacterial biomass, leucine-aminopeptidase and phenol oxidase activities, and the remaining AFDM were the most important parameters related with PC1 (all with positive contributions, except the remaining AFDM). The litter content in lignin, cellulose, and C, as well as the β-glucosidase and β-xylosidase activities, showed the highest contribution (all positive) to PC2. The ordination plot showed a clear segregation of the three species, especially between *A. portulacoides* and *E. pycnanthus*

(a)





(b) Contribution of variables to PC1

(c) Contribution of variables to PC2

Figure 4.18. Ordination plot obtained from principal component analysis considering the following variables measured in the litter of the three studied species: % remaining AFDM (%RemAFDM), quality parameters (C, N, cellulose and lignin content), extracellular enzyme activities (β-glucosidase (Glu), β-xylosidase (Xyl), leucine-aminopeptidase (Leu) and phenol oxidase (PhOX)), and bacterial and fungal carbon biomass (a); contribution of the different variables to the principal component 1 (PC1) (b) and 2 (PC2) (c). The symbol labels in graph (a) indicate the % of litter mass remaining for each species at three time points (beginning, middle and end of the studied decomposition period) and for each one of the two zones within each habitat. The coloured lines are connecting the values for the three sampling times for each species and zone from the beginning to the end of the studied period. The red dashed lines of graph (b) and (c) indicate the expected average contribution. Those variables with a contribution larger than this percentage are considered variables with an important contribution to the component.

5. DISCUSSION

5.1. Study 1: Structure and composition of plant communities and carbon stored in vegetation and soil in the salt marsh habitats and in the disturbed and later restored zone

5.1.1. Plant community structure and composition

The halophilous scrub and the salt meadow presented no changes from 2015 to 2016 in the plant community structure parameters, including the relative abundance of the dominant species (Table 4.2 and Table 4.3). These results, together with the high percentage of plant cover, indicate that these habitats have a mature plant community (Valiela et al., 2000). Conversely, the glasswort sward has a lower plant cover in comparison to the other habitats, and its vegetation increased in height from one year to the other, which would be at least partially explained by the appearance of *Sarcocornia fruticosa* in 2016 with a relative abundance of 21% (which also contributes to explain the decrease of the relative abundance of *Salicornia patula* from one year to the other). These results are in accordance with the fact that this is a plant community dominated by pioneer species, which will probably evolve towards a plant community dominated by late-successional species similar to that of the halophilous scrub.

The three salt marsh habitats have low values of species richness and Shannon index. In fact, when comparing the halophilous scrub with the ruderal plant community (i.e. the community found in the disturbed zone before being restored) higher species richness and biodiversity were found in the latter. Accordingly, salt marshes are usually species-poor ecosystems, since high salinity and flooding during long periods would limit the establishment of more sensitive species, allowing only the survival of highly specialized species (Adam, 1990).

The disturbed zone experienced a drastic change in species composition after restoration (Table 4.4). The ruderal plant community (dominated mainly by *Foeniculum vulgare* and *Inula viscosa*) was replaced by pioneer species of saline soils, first for *Suaeda maritima*, and, two years after the restoration, the appearance of *Sarcocornia fruticosa* was relevant, suggesting that this area will probably become a halophilous scrub in the future. These results are in accordance with those of Mossman et al. (2012a) who observed the dominance of annual pioneer salt marsh species such as *Salicornia europaea* and *Suaeda maritima* and also the presence of other typical perennial halophytic species of the zone just one year after the restoration of an European Atlantic tidal salt marsh. In this sense, Teutli-Hernández et al. (2019) demonstrated in a mangrove ecosystem how the pioneer species that colonize the bare saline soils can acts as nurse species facilitating the colonization and growing of the more late-successional species in a relative short period of time (less than 3 years). However, for other plant community parameters, to reach similar values of those of well-preserved salt marsh sites would require more time. In this sense, Morgan & Short (2002) found that vegetation cover or plant biodiversity of restored tidal salt marshes in the Northwestern Atlantic became similar to those found in mature reference sites 10 years after restoration.

5.1.2. Changes in plant biomass, plant net primary production and litter biomass

No significant changes between years in plant above (total, living or dead) or belowground biomass were found in any of the three salt marsh habitats (Figure 4.1 Figure 4.2). The amount of litter did not change significantly either. Besides, at La Pletera there are no signs of herbivory (personal observations) and biomass exportation is expected to be low because of the absence of high tides and the presence of dense banks of vegetation (especially in the halophilous scrub and the salt meadow), hindering the movement of vegetal material to the boundary areas. Thus, no net aboveground (NAPP) or belowground (NBPP) primary production was detected for any of the three salt marsh habitats. These results were not expected, since salt marshes have been largely recognized as one of the most productive ecosystems in the world (Gattuso et al., 1998). However, there is high variability among salt marshes in terms of productivity (Ibañez et al., 2000). In this sense, climate is among the most important parameters that can affect salt marsh primary production, since it affects water availability and soil salinity. Related with this, tidal salt marshes located at the Atlantic coast of USA and Europe, subjected to mild temperatures and rainfall distributed throughout the year, usually have higher values of NAPP than tidal salt marshes located at the Pacific coast of USA (California) and non-tidal salt marshes of the Mediterranean Basin, which are subjected to dry summer periods with high temperatures (Ibañez et al., 2000). The reason is that low rainfall and high temperatures, along with the lack of flooding during summer in the Mediterranean Basin salt marshes, causes high soil hypersalinity, with plants subjected to salt and water stress (Cameron, 1972; Berger et al., 1978). In fact, in

Mediterranean ecosystems (Figueroa & Davy, 1991) and in tidal European salt marshes (Leeuw et al., 1990) variations in plant productivity are strongly related to variations in rainfall. At La Pletera salt marsh, 2015 and 2016 were particularly dry years (317 and 487 mm y^{-1}, respectively; being 574 mm y^{-1} the mean of the last 10 years). Moreover, accumulated rainfall between the two biomass sampling dates was 370 mm y^{-1} (from July 2015 to July 2016), being the lowest value in the last 10 years (mean from July to July for the last 10 years: 555 mm y^{-1}). Hence, the low rainfall occurred between the two sampling dates might explain, at least partially, the absence of NAPP and NBPP in the three salt marsh habitats of La Pletera. For the halophilous scrub and the salt meadow, another possible explanation for not having found significant primary production from one year to the other is that these habitats have mature plant communities, since it is known that NAPP is lower in the older and more mature stands (Gower et al., 1996; Yu et al., 2017). In the case of the glasswort sward, the youngest habitat, there was a tendency (despite it was not significant) to increase the aboveground biomass from 2015 to 2016, which reinforces the previous idea.

The studied Mediterranean halophilous scrub showed values of aboveground biomass, considering the two sampling years (3492.4 ± 393.9 g m^{-2}), at the upper part of the range obtained for similar Mediterranean Basin salt marshes also dominated by *S. fruticosa* (588 to 3515 g m^{-2}) (Curcó et al., 2002). Conversely, the estimated aboveground biomass of the Mediterranean salt meadow and the glasswort sward (1171.3 ± 148.3 g m^{-2} and 317.3 ± 49.6 g m^{-2}, respectively) is slightly lower than the values reported for other salt marshes dominated by the same or similar species. For instance, studies in tidal salt marshes of Cantabria (North Spain) (Benito & Onaindia, 1991), Britain and Normandy (North France) (Bouchard & Lefeuvre, 2000) and Portugal (Caçador et al., 2003) reported values of total aboveground biomass of 1267-2500 g m^{-2} in areas dominated by *A. portulacoides* and of 2600 g m^{-2} in areas dominated by *Elymus pugens.* Values of total aboveground biomass obtained in the studies of Benito & Onaindia (1991) and Bouchard & Lefeuvre (2000) for areas dominated by pioneer succulent annual vegetation of the Chenopodiaceae family, such as *Suaeda maritima* and *Salicornia ramocissima*, were 480 and 550 g m^{-2}, respectively. One factor that might contribute to explain these differences in total aboveground biomass between the salt marshes of these previous studies, especially those of Cantabria and Normandy, and La

Pletera salt marsh is the climate. While rainfall is well distributed throughout the year in the former, drier and warmer summer periods at La Pletera salt marsh, along with soil hypersalinity (which is also increased because the absence on flooding during summer) would disfavour primary production. In the case of the salt marshes of Portugal that also have a Mediterranean climate, the tidal movement might contribute to avoid soil hypersalinity benefiting, consequently, a higher primary production compared to Mediterranean non-tidal salt marshes (Carrasco-Barea et al., 2018).

Regarding plant belowground biomass, summer mean values in areas dominated by *S. fruticosa* in the Po (Scarton et al., 2002) and Ebro deltas (Curcó et al., 2002) were around 4200 g m^{-2} (at 0-30 cm depth) and 1050 g m^{-2} (at 0-50 cm depth), respectively, while in the halophilous scrub of La Pletera the estimated values were 283.4 ± 34.2 g m^{-2} (at 0-20 cm depth), having been sampled, in all these cases, almost all the root biomass (around 95%). Remarkably high values obtained in the Po delta, unlike other Mediterranean salt marshes, were justified by the high tidal movement, which would contribute to avoid water stress and hypersalinity during summer, favouring vegetation growth (Scarton et al., 2002). In accordance, mean plant belowground biomass in the salt meadow of La Pletera (257.0 ± 32.2 g m^{-2} at 0-20 cm depth) was notably lower compared to the values reported for summer months in a tidal salt marsh of the Northeastern Atlantic (Netherlands) in two sites dominated by *E. pycnanthus* and *A. portulacoides* (mean belowground biomass at 0-20 cm depth was around 4000 and 5800 g m^{-2}, respectively; Groenendijk & Vink-Lievaart, 1987).

Considering the amount of litter, studies performed in areas dominated by *S. fruticosa* from other Mediterranean salt marshes, such as the Rhône and Ebro deltas, found similar mean values than those obtained in the halophilous scrub of La Pletera (571.1 ± 98.1 g m^{-2}), ranging from 383 to 761 g m^{-2} (Berger et al., 1978; Curcó et al., 2002). Conversely, the amount of litter estimated in the salt meadow of La Pletera (419.3 ± 68.1 g m^{-2}) were higher than those of two tidal salt marshes in Portugal dominated by *A. portulacoides*, in which annual mean values ranged from 21 to 120 g m^{-2} (Caçador et al., 2003). These results probably highlight the importance that the absence of tides has on the litter accumulation in non-tidal salt marshes, as La Pletera. In the study of Caçador et al. (2003), despite the higher aboveground biomass of *A. portulacoides* reported, a lower litter amount was found, since they estimated that around 86% of the carbon produced in the salt marsh was exported out due to the daily tides.

5.1.3. Carbon stored in vegetation and soil

5.1.3.1. The case of the three salt marsh habitats

The salt marsh habitat with the highest amount of carbon stored in the aboveground biomass was the halophilous scrub, due to the elevated biomass of *Sarcocornia fruticosa*, which represented 93.5 ± 2.9 % of the total biomass of this habitat. This carbon was basically stored in the woody and the standing dead fractions of plants, which showed the highest values of biomass but also the higher carbon concentration compared to green tissues (Table 4.11 and Table 4.7). The fact that the aboveground woody tissues of *S. fruticosa* in the glasswort sward showed lower carbon concentration than in the halophilous scrub (Table 4.7) would be in accordance with the shorter age of plants in the glasswort sward, since younger individuals usually have lower C concentrations than older ones (Vogt et al., 1993; Uri et al., 2012).

Carbon concentration of belowground biomass decreased from 2015 to 2016 in all the salt marsh habitats, which could be related with the low rainfall occurred between these two sampling dates, and is in accordance with the absence of net primary production. In this sense, after a stressful period, there is a reduction in the concentration of non-structural carbohydrates in the roots of trees (Launay et al., 2009; Klein et al., 2014), which could be related with the remobilization of stored carbon from roots to other organs to support the main plant functions during or after the stress period (Villar-Salvador et al., 2015).

The amount of carbon stored in the soil of the halophilous scrub and the salt meadow did not differ significantly, being higher than that found in the soil of the glasswort sward. This would be in accordance with the higher values of carbon stored in the below and aboveground plant fractions, as well as in the litter, found in the halophilous scrub and in the salt meadow, compared to the glasswort sward. Values of SOC at 0-20 cm depth in La Pletera salt marsh were 0.012 ± 0.002, 0.009 ± 0.001 and 0.004 ± 0.001 g C cm^{-3} for the halophilous scrub, salt meadow and the glasswort sward, respectively. These values are lower than those found, also for the upper 20 cm of soil, in the Rhône (Hensel et al., 1999), Ebro (Curcó et al., 2002), and Po (Scarton et al., 2002) deltas with values of 0.079, 0.060, and 0.068 g C cm^{-3}, respectively, which would be in accordance with the higher plant belowground biomass in these salt marshes compared to La Pletera, as discussed before. On the other hand, SOC in the salt marshes of Po, Ebro and Rhône deltas was estimated from soil organic matter (SOM) obtained by the loss-on-ignition method, which could have led to an overestimation of SOM, since a part of inorganic carbon and especially the structural water can be lost during the ignition, especially in soils containing large amounts of clay (Mook & Hoskin, 1982; Craft et al., 1991). This overestimation in our study is avoided since SOC is quantified by the dichromate wet oxidation method.

In salt marshes and wetlands, soils usually store more carbon than vegetation (Dalal & Allen, 2008). Indeed, in well-preserved salt marshes, vegetation usually maintains carbon steady stocks, while sequestered carbon is mostly buried in the soil (Murray et al., 2010). Besides, carbon remains stored in vegetation for a short-term period (decennial), whereas soil carbon can remain stored during long-term periods (millennial) (Duarte et al., 2005). In the salt marsh habitats of La Pletera, carbon stored in the soils (at 0-20 cm depth) is higher than that stored in the vegetation (considering above and belowground parts), although differences between the two compartments were not significant in the case of the halophilous scrub (Figure 4.3). This would highlight the importance of the vegetation of this habitat (dominated by *S. fruticosa*) in the maintenance of the carbon stored in La Pletera salt marsh.

5.1.3.2. The case of the disturbed zone

Before the restoration, the disturbed zone had a lower and a higher amount of carbon stored in the vegetation and in the soil, respectively, compared to the halophilous scrub (Table 4.14). However, the higher amount of carbon stored in the soil of the disturbed zone might be misleading, since these soils were basically made up of rubble (it was used to raise the soil level) coming from other places. Moreover, the fact that the carbon stored in vegetation in the halophilous scrub was almost 8-fold times higher than in the ruderal community would support the idea that salt marshes need to be preserved since they are important carbon reservoirs.

One year after the restoration (2017), the vegetation was still very scarce in the restored zone and remarkably low values of carbon stored in plant biomass was obtained (Table 4.15). In this sense, although some parameters related with the composition and structure of the plant community can reach similar levels of well-preserved salt marshes sites in a relatively short period of time (< 10 years) (Morgan & Short, 2002; Mossman et al., 2012a), a totally similar vegetation structure and ecosystem functions, such as carbon burial, may take decades to reach equivalence (Craft et al., 1999; Moreno-Mateos et al., 2012, 2015; Mossman et al., 2012b). Hence, although an increment in plant cover and in the entrance of species typical of halophilous scrub was detected in the restored area of La Pletera, it will probably take several decades to fully reach the structure and carbon storage level found in the mature halophilous scrub habitat.

5.2. Study 2: Seasonal carbon fluxes from vegetation (CO_2) and soil (CO_2 and CH_4)

5.2.1. Carbon fluxes from vegetation

The comparison of the instantaneous net CO_2 exchange rates (NER) from the four dominant species of the three studied habitats of La Pletera salt marsh revealed that *Elymus pycnanthus,* the dominant species of the salt meadow, presented the highest photosynthetic rates during most of the year, reaching 29 µmol CO_2 m^{-2} s^{-1} in March. Photosynthesis rates of *E. pycnanthus* were only exceeded by those of *Salicornia patula* in June/July, which could be explained by the high photosynthetic activity that annual species usually show after germination in order to create new tissues relatively fast. Higher net photosynthetic rates in monocotyledonous grasses (Poaceae and Cyperaceae families) compared to succulent Chenopodiaceae species have been previously reported in salt marshes species (Kuramoto & Brest, 1979; Pearcy & Ustin, 1984; Nieva et al., 1999). In our study, *E. pycnanthus*, as a Poaceae species, has dumbbell-shaped stomata, while the other two perennial species studied, *Sarcocornia fruticosa* and *Atriplex portulacoides*, have kidney-shaped stomata. Dumbbell-shaped guard cells are surrounded by subsidiary cells that participate in the pore movements through physical interaction with guard cells, allowing a faster response and a wide pore aperture (Grantz & Zeiger, 1986; Franks & Farquhar, 2007). These conditions result in higher photosynthetic rate capacity, especially in fluctuating environments such as salt marshes, when a fast stomatal response is an advantage for the photosynthetic process (Franks & Farquhar, 2007; Chen et al., 2017). In accordance with its higher photosynthetic rates, *E. pycnanthus* also showed greater stomatal conductance values compared to the other two perennial species during almost the entire study period. The higher carbon concentration found in the leaves of *E. pycnanthus* (444 ± 6 µg C mg^{-1} DW) compared to those found in the green tissues of *A. portulacoides* and *S. fruticosa* (339 ± 10 and 299 ± 17 µg C mg^{-1} DW, respectively) (Study 1 on this thesis) would also be in accordance with its higher photosynthetic rates. It is known that a salt tolerance mechanism in monocotyledonous is the maintenance of high intracellular levels of soluble carbohydrates (such as sucrose, fructose and glucose) as compatible solutes for osmotic adjustment, while dicotyledonous generally transport and store Na^+ and Cl^- ions in the aerial parts of plant and accumulate in the cytoplasm mainly amino acids and quaternary amines (Albert & Popp, 1977; Gorham et al., 1980; Briens & Larher, 1982; Gil et al., 2013). The accumulation of Na^+ and Cl^- in dicotyledonous would be in accordance with their low percentage of ash free dry mass (AFDM) observed in senescent herbaceous stems and leaves of *S. fruticosa* and *A. portulacoides* (80% and 56% of AFDM, respectively) compared to recently dead leaves of *E. pycnanthus* (95% of AFDM) (Study 3 of this thesis). Nevertheless, the high C content in *E. pynctantus* leaves in comparison to the other species could also be related to its high cellulose and lignin content, as the litter analysis indicate (Study 3 of this thesis). To have high lignin levels to harden leaves is also a strategy related with plant tolerance to salt, drought and other stresses (Moura et al., 2010). Nevertheless, whether improved photosynthesis rates in *E. pycnanthus* in comparison to *S. fruticosa* and *A. portulacoides* are likely related to its distinct stomata morphology, the fate of photoassimilates in *E. pycnanthus* require further research to elucidate mechanisms underlying salt stress tolerance in this species For instance, it would be interesting to perform an extensive analysis of cell osmolytes.

In the case of *S. fruticosa*, the maximum mean value of photosynthetic rate recorded at La Pletera salt marsh was 14.3 ± 0.8 µmol CO_2 m^{-2} s^{-1}, being higher than those obtained in other studies in which maximum values found were between 3-6 µmol CO_2 m^{-2} s$^{-1,}$ (Abdulrahman & Williams, 1981; Nieva et al., 1999; Redondo-Gómez et al., 2006; Redondo-Gómez & Mateos-Naranjo, 2010). Nevertheless, these previous studies with *S. fruticosa* were performed with potted plants, collected from field sites and then cultivated under controlled conditions of light, temperature and soil salinity. The same occurred with *E. pycnanthus* and *S. patula,* being its maximum photosynthetic rates

(29.1 ± 2.4 and 20.8 ± 2.9 µmol CO_2 m^{-2} s^{-1}, respectively) higher than that of *E. pycnanthus* (18 µmol CO_2 m^{-2} s^{-1}) reported by Rozema & Diggelen (1991) and the annual species *Salicornia ramosissima* (14 µmol CO_2 m^{-2} s^{-1}) reported by Pérez-Romero et al. (2018) growing under controlled conditions and subjected to an increasing range of soil salinity. On the contrary, for *A. portulacoides* from La Pletera salt marsh, the maximum mean photosynthetic rate (9.3 ± 0.8 µmol CO_2 m^{-2} s^{-1}) was lower than the obtained for the same species (15 to 18 µmol CO_2 m^{-2} s^{-1}), in plants under controlled conditions and subjected an increasing range of soil salinity (Redondo-Gómez et al., 2007), or under field conditions (Das Neves et al., 2008). Overall, previous results highlight the scarcity of studies available on photosynthetic rates for dominant salt marsh plant species under natural field conditions and evidence the consistent differences between data depending on the conditions at which the plants are subjected, natural or controlled. This aspect might be of great relevance if data of plant photosynthetic rates were required in predictive carbon balance studies.

In general, the perennial halophytic species studied (*S. fruticosa*, *E. pycnanthus* and *A. portulacoides*) presented the highest photosynthetic activity in March and/or April coinciding with their growing season and favourable environmental conditions (maximum temperatures around 18°C), being the annual temporal pattern very similar of that observed by Nieva et al. (2003) in a non-tidal population of *Spartina alterniflora* located at the upper part of a tidal salt marsh in Portugal. In autumn, the photosynthetic rates of La Pletera plants were much lower than in spring despite environmental parameters, such as temperature and soil moisture, were also favourable to photosynthesis (especially in October, where maximum temperature was 21°C and soil VWC was even higher than in March and April). One possible explanation might be related with the high accumulation of ions and soluble carbohydrates that these species would present after a salt stress period, such as the one occurring in the Mediterranean salt marshes during summer (Redondo-Gómez et al., 2007; Gil et al., 2011, 2014). On the one hand, in dicotyledonous species, high salinity conditions can induce the accumulation of Na^+ and Cl^- in the cytoplasm (Munns, 1993) affecting the photosynthesis process (Chaves et al., 2009; Almeida et al., 2017). On the other hand, the accumulation of high intracellular levels of soluble carbohydrates as a salt tolerant mechanism in salt marsh monocotyledonous species (Gil et al., 2013) can promote a feedback inhibition of photosynthesis (Munns, 1993). According to the higher photosynthetic rates in spring, the highest values of green biomass of *S. fruticosa* were found in summer. Differences among seasons in green biomass of the other two species were not significant, despite other studies found peaks of green biomass for these species in summer (Wolff et al., 1979; Ibañez et al., 2000; Scarton et al., 2002).

Previous studies have reported a reduction of photosynthesis when soil salinity was around 50 dS m^{-1} in *S. fruticosa* (Nieva et al., 1999; Redondo-Gómez et al., 2006) 40 dS m^{-1} in *A. portulacoides* (Redondo-Gómez et al., 2007), and 15.9 dS m^{-1} in *E. pycnanthus* (Rozema & Diggelen, 1991), being thus the latter the most sensitive species to salinity according to bibliography. In our study, soil electrical conductivity was not correlated with NER values for any of these three species (p=0.976 for *S. fruticosa*; p=0.389 for *E. pycnanthus*; p=0.240 for *A. portulacoides*), likely due to the low soil electrical conductivity values found in the halophilous scrub and in the salt meadow (maximum mean values were 16.2 ± 8.8 and 2.4 ± 1.2 dS m^{-1}, respectively).

Intrinsic water use efficiency (iWUE) did no differ among species during practically the entire year (except in September when *S. fruticosa* had lower values than the other three species), despite higher iWUE values in monocotyledons than in dicotyledons were previously found (Nieva et al., 1999). Differences among species of the two groups were not detected in the present study, since *E. pycnanthus* had higher photosynthetic rates but also higher values of stomatal conductance compared to the other species. Mean maximum values of iWUE obtained for the four species (0.77, 0.62, 0.84 and 0.33 µmol CO_2 $mmol^{-1}$ H_2O for *S. fruticosa, A. portulacoides, E. pycnanthus* and *S. patula,* respectively) are slightly higher than those obtained under field natural conditions in other salt marshes for *Spartina densiflora* (0.10 µmol CO_2 $mmol^{-1}$ H_2O; Nieva et al., 2003), *Atriplex portulacoides* and *Limoniastrum monopetalum* (0.4 µmol CO_2 $mmol^{-1}$ H_2O; Das Neves et al., 2008) and under controlled conditions for *Salicornia ramosissima* (0.10 µmol CO_2 $mmol^{-1}$ H_2O Pérez-Romero et al., 2018). Moreover, maximum values reported in this study in comparison to others with species from similar environments (for instance, Alessio et al., 2004) or different (Soh et al., 2019) highlight the great efficiency that La Pletera salt marsh dominant species can reach. Besides that, the high iWUE values found in these species suggest a different physiological response of plants. While the three perennial species would reach high iWUE by lowering stomatal conductance, *S. patula* would increase the photosynthesis rates, mainly in June and July, highlighting also the efficient photosynthetic metabolism that *S. patula* has during the unfavorable Mediterranean summer conditions.

Thin woody tissues (stem diameter < 3 mm) of *S. fruticosa* and *A. portulacoides* showed considerably high photosynthetic rates, especially before sunset in March and May, with values of photosynthesis that could reach 12 µmol CO_2 m^{-2} s^{-1}. These results would be in agreement with data reported for photosynthesis in woody tissues of shrubs and trees from other ecosystems, such as the savannah (Levy & Jarvis, 1998; Cernusak et al., 2006) or from Californian evergreen species (Saveyn et al., 2010). As occurs with the woody stems of previous studied species, at La Pletera salt marsh, the thin brown woody stems of *S. fruticosa* and *A. portulacoides* presented a thin layer of green cells under the periderm (Figure SM 4), which would likely be responsible for the recorded photosynthesis by using the light that passes through the thin bark surface layer and the CO_2 that penetrates through lenticels. Woody stems have not usually been considered in studies aimed to characterize the CO_2 exchange from vegetation. However, our results highlight its importance and claim that woody stems analysis should be included in these studies, especially in ecosystems like the Mediterranean salt marshes dominated by succulent Chenopodiaceae species, as those of the genera *Sarcocornia,* in which the woody fraction represent a significant fraction of the plant aerial biomass.

The four studied species showed the highest night respiration rates in summer (August) and/or autumn (November), being especially elevated the values found for the green tissues of *S. fruticosa* and *E. pycnanthus* during these two months. Night respiration on summer seems not to be affected directly by air temperature, since during the night samplings of June and August it did not greatly differ (19.6 and 22.2 °C, respectively), while values of night respiration were much higher in August. In fact, night NER was not correlated with minimum air temperature for any of these three species ($p = 0.759$ for *S. fruticosa*; $p = 0.920$ for *E. pycnanthus*; $p = 0.777$ for *A. portulacoides*). In *E. pycnanthus* and *S. fruticosa,* the high values of night respiration registered in August coincide with their flowering period and, thus, with a high energetic demand (Bustan & Goldschmidt, 1998; Lambers et al., 2008). Besides, in the case of *S. fruticosa,* flowers, which are very abundant and small (Figure SM 5), remained inside the chamber during the measurements, and this could have contributed to increase respiration rates in

August (Bustan & Goldschmidt, 1998). On autumn, the temperature during the sampling of November was colder (4.6 °C) similar to that of February (5.9°C), but, despite this, respiration rates were also very high. These high night respiration values might be explained, at least partially, by the accumulation due to low temperatures of soluble carbohydrates and/or the increase in the chloroplast redox status (Koch, 1996; Atkin et al., 2005), although more research is needed to clarify this.

5.2.2. Carbon fluxes from soil

The halophilous scrub and the salt meadow showed higher soil respiration (SR) values than the glasswort sward. These differences might be related to the C and/or the N content of the soil, which can affect the microorganism-mediated soil organic matter decomposition (Gougoulias et al., 2014; Oertel et al., 2016). Positive correlations between CO_2 emissions and soil organic carbon (SOC) or total nitrogen (TN) have been found for soils of either coastal saline (Wang et al., 2016; Li et al., 2019) or other terrestrial ecosystems (Merbold et al., 2011; Shi et al., 2014). In this sense, at La Pletera salt marsh, the halophilous scrub and the salt meadow had higher content of SOC and TN (0-20 cm) than the glasswort sward (% SOC: 1.16 ± 0.14, 0.87 ± 0.14, 0.40 ± 0.06 and % TN: 0.045 ± 0.005, 0.046 ± 0.005, 0.023 ± 0.003 for the halophilous scrub, salt meadow and glasswort sward, respectively, Study 1), with SR being positively correlated with SOC (R^2: 0.997; p=0.045) and with TN (R^2: 0.999; p=0.026). Previous studies have shown that most of the CO_2 produced during decomposition is derived from organic material recently incorporated to the soil, being only a small fraction (approx. 10%) of soil respiration derived from decomposition of older, more recalcitrant carbon compounds (Trumbore, 2000; Giardina et al., 2004). Considering this, the lower values of soil CO_2 emission found in the glasswort sward compared to the other two habitats would be in accordance to the less active nature of the soil organic matter (Gispert, unpublished data) and the scarce amount of litter of this habitat (571 ± 98, 419 ± 68, 44 ± 11 g m^{-2} in the halophilous scrub, the salt meadow and the glasswort sward, respectively; Study 1). Soil respiration was measured in plots in which vegetation was previously (6 or 18 months before) harvested. However, the regrown of plants in the surrounding of the cover-boxes (observed especially in the salt meadow) and the fact that *S. fruticosa*, *A. portulacoides* and *E. pycnanthus* have a superficial root system with a great horizontal development (personal observations), support that in the halophilous scrub and the salt meadow habitats, the respiration of surrounding root systems could also contribute to explain the high soil respiration values found. In the glasswort sward, the scarcity of vegetation (which is only alive during few months) and the poorly developed root system of *S. patula* (personal observations) would make negligible the contribution of roots to the soil respiration.

Daily soil respiration presented a similar seasonal pattern in the three habitats, with the highest values being recorded in summer (July) in all the habitats, which agrees with the positive correlation found between SR and soil temperature (Ts) ($p < 0.01$). These findings are consistent with numerous previous studies in which the highest soil CO_2 emissions are produced in the warmest season, since high temperatures enhance metabolic activity of soil microbes (Wang et al., 2016; Hu et al., 2017; Chen et al., 2018). Contrary to the results found for the daily SR, instantaneous soil CO_2 respiration (iSR) did not seem to be related with soil temperature. This lack of relation could be attributed to the sampling size and the short measures duration (one minute) not being enough large to collect a clear relationship between the relatively stable soil temperature and a more heterogeneous soil metabolic activity.

Previous studies have shown that electrical conductivity affects negatively soil respiration (Neubauer, 2013; Wilson et al., 2015; Hu et al., 2017; Chen et al., 2018). Accordingly, we found that iSR was negatively correlated with electrical conductivity ($p = 0.007$). On the contrary, daily SR and EC were not significantly correlated ($p = 0.613$), which suggests that rather than salinity, other environmental factors, as the temperature, would have a more important role in determining daily SR

In general, our estimations of daily soil CO_2 emissions were in the upper part of the range previously published for salt marshes (Table 5.1), excepting for the study of (Hu et al., 2017). One relevant difference between La Pletera salt marsh and all the salt marshes considered in Table 5.1, except the one studied by Hirota et al. (2007), is the daily tidal flood. In the tidal salt marshes, flooding occurs one or two times every day, while in La Pletera flooding is infrequent and the soil becomes more aerated, being the microbial respiration favoured because of the high oxygen availability. In fact, studies of the tidal effect on CO_2 fluxes under field (Kathilankal et al., 2008; Moffett et al., 2010) or laboratory (Jones et al., 2018; Wang et al., 2019) conditions support a negative effect of flooding on soil CO_2 emissions. At La Pletera, the reduction of soil CO_2 emissions by flooding becomes widely evident when comparing the SR values of flooded and non-flooded soils (Figure 4.8), although a possible underestimation of the CO_2 emissions in flooded soils due the use of the gas chromatography method, compared with the non-flooded soils in which soda-lime method was used, could also be contributing to these differences (Lou & Zhou, 2006). Regarding the study of Hirota et al. (2007), also in a non-tidal salt marsh, daily soil CO_2 emissions are similar to those found at La Pletera, which also agree with higher soil CO_2 emissions observed in the unflooded upper parts of some tidal salt marshes compared to the lower part (Chmura et al., 2011; Khan, 2016; Wang, 2018).

Methane fluxes in salt marshes are the result of CH_4 production, consumption and diffusion from soil anaerobic zones to the atmosphere (Bodelier & Laanbroek, 2004; Sun et al., 2013). A strict condition for the growth of methanogen microorganisms is the complete absence of oxygen, which is common in ecosystems where soil is periodically flooded, as occurs in wetlands and salt marshes (Kayranli et al., 2010). Methane generated in the anoxic layers of soil diffuses to more superficial and aerated soil layers or to the overlying water column were it can be oxidized, thereby reducing the amount of methane that eventually reaches the atmosphere (Dean et al., 2018). In La Pletera salt marsh, some negative values of soil methane flux (SMF) (indicating net CH_4 consumption) were found in all the habitats, but especially in the halophilous scrub (Figure 4.10). CH_4 absorption have also reported in other salt marshes (Bartlett & Harriss, 1993; Hirota et al., 2007; Sun et al., 2013; Chen et al., 2018). On the contrary, at La Pletera, peaks of SMF emission were also found for all habitats (271 ± 90, 161 ± 58 and 110 ± 59 mg CH_4 m^{-2} d^{-1}, for the halophilous scrub, salt meadow and glasswort sward, respectively). In the glasswort sward, peaks were observed either when soil was not flooded (110 ± 59 mg CH_4 m^{-2} d^{-1}) as when soil was flooded (131 ± 45 mg CH_4 m^{-2} d^{-1}) highlighting that methane oxidation in the overlying water column would not be happening.

The highest soil methane fluxes were detected in the warmest months, being recorded in June in the halophilous scrub and in the glasswort sward, and in July in the salt meadow. The same seasonal trend has been broadly found in other studies in salt marshes, and would respond to the fact that higher temperatures would favour microbial activity (Bartlett et al., 1987; Yuan et al., 2015; Wang et al., 2016; Hu et al., 2017; Chen et al., 2018). Despite no soil anaerobic conditions would be expected during summer, because the low soil VWC, the high ground water level at La Pletera salt marsh (which ranges from around 30-40 cm depth; Soler and Menció, unpublished data), would cause anaerobic conditions at the water table level, which could promote CH_4 production, since, at these depths, soil organic matter to be decomposed is still present (Amorós, 2018). The CH_4 produced in the anaerobic zone can easily diffuse through air-filled macropores, especially during summer when high temperatures in the soil surface promote soil water evaporation to the atmosphere (Denier Van Der Gon et al., 1996).

CH_4 emissions are, in general, negatively affected by salinity, being usually higher in freshwater wetlands than in salt marshes (Bartlett & Harriss, 1993; Poffenbarger et al., 2011; Hu et al., 2017). Indeed, after reviewing data of CH_4 emissions in marshes with different grade of salinity, Poffenbarger et al. (2011) found significantly lower values (3 ± 5 mg CH_4 m^{-2} d^{-1}) in polyhaline salt marshes (salinity >18 ‰) compared to the rest of the marshes studied, which were mesohaline (salinity 5-18 ‰), oligohaline (salinity 0.5-5 ‰) and freshwater (salinity 0-0.5 ‰) (44 ± 30, 411 ± 578 and 115 ± 208 mg CH_4 m^{-2} d^{-1}, respectively). Hence, they concluded that CH_4 emissions are only reduced when soil salinity is higher than 18 ‰. In the present study, we detected relatively high soil CH_4 emissions, similar to those reported for freshwater marshes by Poffenbarger et al. (2011), although there were also some studies which presented higher values than those of La Pletera (Table 5.1). Likely, in these studies, the low salinity of the water table, which was 2‰ for Hirota et al. (2007); 0-12‰ for Bartlett et al. (1987); 4‰ for Hu et al. (2017) and 13-21‰ for Wang et al. (2016), could explain the high CH_4 emission found (Table 5.1), as it could also be the case of La Pletera (Menció et al., 2017). On the other hand, as summer progresses (from July onwards), the absence of important rainfall episodes at La Pletera salt marsh together with the sea water intrusion moves the saltwater wedge inland, increasing consequently the groundwater salinity until levels similar to those of the sea (Menció et al., 2017), i.e., approximately 38 ‰. Therefore, the patterns in soil CH_4 emissions observed in La Pletera might be related with this movement of the saltwater wedge inland, since the sharp decrease in CH_4 emissions recorded in the halophilous scrub and in the glasswort sward (the closest habitats to the sea) in July might be the consequence of more saline conditions in the water table.

Conversely, this was not the case of the salt meadow, the most distant habitat from the sea, where maximum CH_4 emissions were recorded in July.

Table 5.1. Published data on daily soil CO_2 and CH_4 emissions from salt marshes at different locations. Values without parentheses indicate maximum values of CO_2 and CH_4 emissions while values in parentheses indicate annual averages. A rank of mean maximum values is given when emissions have been measured in more than one area or habitat within the same salt marsh. When at the same tidal salt marsh C emissions were measured in zones subjected to different tidal influence, non-tidal regime is indicated when flooding occurs only in some moments of the year (instead of being daily flooded by tides). ND: No data.

Location	Tidal regime	Sampling frecuency	Climate zone	CO_2 emissions (g CO_2 m^{-2} d^{-1})	CH_4 emissions (mg CH_4 m^{-2} d^{-1})	References
La Pletera salt marsh, Spain	**Non-tidal**	**Seasonal**	**Temperate (Mediterranean)**	**13.2–19.4 (8.4–12.3)**	**109.6–270.5 (22.0–36.5)**	**This study**
Carpinteria salt marsh, California (USA)	Non-tidal	Seasonal	Temperate (Mediterranean)	5.3 (3.7)	(-0.08)	Wang, (2018)
Carpinteria salt marsh, California (USA)	Tidal	Seasonal	Temperate (Mediterranean)	2.8–3.8 (2.0–2.7)	(1.9–2.2)	Wang, (2018)
Lake Nakaumi salt marsh, Japan	Non-tidal	August	Temperate	17.4	845	Hirota et al. (2007)
Gulf of St. Lawrence, New Brunswick (Canada)	Non-tidal	August	Temperate	11.6	0.5	Chmura et al., (2011)
Bay of Fundy, New Brunswick (Canada)	Tidal	August	Temperate	9.5	0.8	Chmura et al., (2011)
Bay of Fundy, New Brunswick (Canada)	Tidal	July-September	Temperate	2.3–2.8	0.5–3.7	Magenheimer et al. (1996)
York River delta, Virginia (USA)	Tidal	Seasonal	Temperate	ND	46-259 (5.6-22)	Bartlett et al. (1987)
Soenke-Nissen-Koog, Germany	Non-tidal	Seasonal	Temperate	4.7 (2.4)	ND	Khan (2016)
Soenke-Nissen-Koog, Germany	Tidal	Seasonal	Temperate	0.2 (-0.03)	ND	Khan (2016)
Min River estuary, China	Tidal	Seasonal	Subtropical	84 (34)	382	Hu et al. (2017)
Jiulong River estuary, China	Tidal	Seasonal	Subtropical	4.3 (1.1)	480 (153)	Wang et al. (2016)
Yellow River delta, China	Tidal	Seasonal	Subtropical	0.7–0.7 (0.1-0.4)	7.2–9.6 (1.7-1.8)	Chen et al. (2018)
Yellow River delta, China	Tidal	Seasonal	Subtropical	ND	-9.4–12	Sun et al. (2013)
Yellow River delta, China	Tidal	Seasonal	Subtropical	(0.13–0.41)	(17–18)	Chen et al. (2013)
Mississippi River delta, Louisiana (USA)	Tidal	Seasonal	Subtropical	4.4–17.6	ND	DeLaune & Pezeshki (2003)

Soils of the halophilous scrub and the salt meadow showed higher carbon losses as CO_2 and CH_4 emissions than the glasswort sward. However, when comparing mineralization quotients among habitats, the glasswort sward had the highest values (Figure 4.11). Hence, despite the higher soil carbon emissions of the halophilous scrub and the salt meadow, soils of these habitats would present a higher carbon sequestration potential, since the organic carbon stored in these soils is subjected to a lower mineralization.

5.3. Study 3: Litter decomposition of the dominant species of the halophilous scrub and the salt meadow

5.3.1. Differences among species in the litter decomposition process

Results reported in this study agree with previous ones in which litter of Poaceae salt marsh species decompose slower in comparison with the litter of succulent Chenopodiaceae species (Bouchard & Lefeuvre, 2000; Simões et al., 2011). Indeed, litter decomposition rates for *Elymus pycnanthus* were 7- and 13-fold lower than those of *Sarcocornia fruticosa* and *Atriplex portulacoides*, respectively (Table 4.20), requiring about 400 days more than these succulent species to decompose half of the initial material. This would explain why, after almost one year, more than 50% of the *E. pycnanthus* litter was still present (Figure 4.15). In line with this, the results of the Principal Components Analysis showed that *E. pycnanthus* data were located in the most negative part of the first principal component (PC1), implying a high % remaining AFDM. The initial content of C and N, and thus the C/N ratio, have been considered important features of litter, since they determine its decomposability, with a high initial N content affecting positively and thus a high C/N affecting negatively (Enríquez et al., 1993; Rejmánková & Houdková, 2006; Simões et al., 2011; Duan et al., 2018). In this study, however, the initial content in C and N, and the C/N ratio, did not contribute to explain the differences of decomposition rates among species, since the litter of *A. portulacoides* and *E. pycnanthus* had initially similar values of C, N and C/N, but their decomposition rates highly differed (Table 4.18). Other studies have not found a clear relationship between the C/N ratio and the decomposition rate of the litter (Sowerby et al., 2000; Van Ginkel et al., 2000; Ross et al., 2002). These results suggest that other structural parameters may be determining the differences observed in the litter decomposition process of the three studied species. For instance, low decomposition rates of *E. pycnanthus* litter could likely be related with its high initial cellulose and lignin content (and lignin/N ratio) in comparison to that of *S. fruticosa* and *A. portulacoides* (Table 4.18). Harder tissues are more resistant to be degraded and decompose slower (Curcó et al., 2002; Hoorens et al., 2003), in accordance with the negative correlation reported between the lignin/N ratio and the decomposition rates of the litter (Duan et al., 2018). Nevertheless, comparing the initial amount of cellulose and lignin, and the lignin/N ratio, of the litter of the three species with their respective decomposition rates, it seems that the initial lignin content would be the parameter that would better explain the differences in the decomposition rates among the three species (Table 4.20). Therefore, our results suggest that the initial litter lignin content, instead of the initial content of C, N or the C/N ratio, would be the most suitable parameter to be used as an initial litter quality indicator of the observed differences in the decomposition rates of the three studied species.

Contrary to the results obtained by Rejmánková & Sirová (2007), the lower decomposition rate of *E. pycnanthus* litter did not coincide with lower extracellular enzyme activities. In fact, *E. pycnanthus* presented the highest activity of the C-degrading enzymes β-glucosidase and β-xylosidase (Table 4.22). One possible explanation for the discrepancy with the results obtained by Rejmánková & Sirová (2007) might be that these authors worked with only one species (*Eleocharis cellulosa*), and thus the initial content of structural polymers, such as cellulose or lignin, was always similar (Rejmánková & Houdková, 2006). In the present study, the higher relative content of cellulose in *E. pycnanthus* litter compared with the other two species would justify the higher β-glucosidase and β-xylosidase activities, since harder tissues would need higher activity of the enzymes to breakdown these polymers (Sinsabaugh et al., 2002). Nevertheless, the lowest decomposition rates of *E. pycnanthus* litter might be explained by the lowest efficiency (high TA) of all the measured enzymes (Table 4.23), which would indicate that a higher quantity of enzyme is required to decompose a certain amount of plant material. Hence, our results suggest that, rather than the activity of extracellular enzymes, the enzyme efficiencies would better explain the differences in litter decomposition rates among the three species studied and probably among other species showing different structural polysaccharide content.

On the other hand, according to the results obtained by Rejmánková & Sirová (2007), *E. pycnanthus* showed the lowest values of microbial biomass, especially for fungi (Table 4.25). Fungal biomass was previously estimated in some studies with monocotyledonous species of the Poaceae family in a tidal salt marsh in Portugal (Castro & Freitas, 2000) and of the Cyperaceae, Juncaceae and Poaceae family in the Ebro delta (Menéndez & Sanmartí, 2007; Sanmartí & Menéndez, 2007; Menéndez, 2008). In these studies, maximum values of litter fungal biomass (8 to 16 mg C g DW^{-1}) were higher than those obtained in *E. pycnanthus* litter in this study (1.3 mg C g DW^{-1}) for a similar period (one year). One possible explanation for this difference could be the lower C/N ratio of *E. pycnanthus* litter in comparison to the other monocotyledonous species studied in these examples, since higher litter C/N ratios would favour fungal colonization (Rousk & Bååth, 2007; Grosso et al., 2016). Comparing the studied species at La Pletera salt marsh, fungal biomass values for *S. fruticosa* and *A. portulacoides* were five and seven times higher, respectively, than that of *E. pycnanthus* (Table 4.25). Fungi have been traditionally considered the main responsible microorganisms of lignin degradation (Breen & Singleton, 1999; Bugg et al., 2011), being more relevant at the later stages of litter decomposition due to the increase in the relative content of the most recalcitrant compounds such as lignin. Hence, higher fungal biomass in the Chenopodiaceae species in comparison to *E. pycnanthus* could be attributed to faster decomposition rate in the former. Indeed, whereas, initially, *S. fruticosa* and *A. portulacoides* had lower lignin content than *E. pyncnanthus*, no differences were observed when the entire period was considered, since the relative content of lignin of *S. fruticosa* and *A. portulacoides* increased over the decomposition period. This decomposition dynamics is corroborated by the PCA results, where the fungal biomass had the highest contribution to PC1 (positive) followed by the % remaining AFDM (negative) (Figure 4.18).

Regarding the bacterial biomass, the high values found in *S. fruticosa* litter could be related with its higher N content, and thus its lower C/N ratio, during the entire period studied, since, contrary to fungal colonization, low C/N ratios would favour bacterial colonization (Rousk & Bååth, 2007; Grosso et al., 2016). Accordingly, *E. pycnanthus* litter, with a remarkably lower N content and higher C/N than *S. fruticosa,* showed the lowest bacterial biomass overall the study period (Table 4.25). *A. portulacoides* litter, however, showed intermediate bacterial biomass values despite having a similar N content and C/N ratio than *E. pycnanthus.* This was probably due to the high variability found for *A. portulacoides* bacterial biomass between zones (Figure 4.17). Literature reporting bacterial biomass in the litter of salt marsh species is really scarce. Samiaji & Bärlocher (1996), studying the leaf litter of *Spartina alterniflora* from a tidal salt marsh in Canada, found bacterial biomass values (0.05 mg C g AFDM^{-1}) about three orders of magnitude lower than the ones we obtained for La Pletera salt marsh species. These differences could be due to the low temperature (from 1 to 14 °C) in which the litter of *S. alterniflora* decomposed in the study by Samiaji & Bärlocher (1996), since this might have disfavoured microbial development. Overall, the values obtained for the bacterial biomass and for the fungal/bacterial biomass ratio in the present study support a dominant role of bacteria in the litter decomposition of the three species studied at La Pletera salt marsh. These results would be in accordance with the previously suggested important role that bacteria from salt marsh soils would play in litter decomposition (Benner et al., 1984, 1986; Newell et al., 1996).

5.3.2. The effect of the spatial heterogeneity on the litter decomposition process

The litter decomposition process of the three dominant species of La Pletera salt marsh differed between the two studied zones, indicating a microhabitat effect on litter decomposition, which was most evident in the case of *A. portulacoides*.

Decomposition rates found for *S. fruticosa* (0.0102 ± 0.0002 day^{-1}) were slightly higher than the range obtained for this species (0.0038 to 0.0067 day^{-1}) in other Mediterranean salt marshes, as the Ebro Delta (Curcó et al., 2002), Po Delta (Scarton et al., 2002), and Rhône Delta (Ibañez et al., 1999), but similar to the values reported for the close species *Arthrocnemum macrostachyum* (0.0090 day^{-1}) from a tidal salt marsh in Portugal (Simões et al., 2011). The decomposition rates of *S. fruticosa* at La Pletera salt marsh varied between the two studied zones of the halophilous scrub habitat, being about 9% higher in zone *a* than in zone *b* (Table 4.21). Considering that the initial litter placed at both zones was the same, and that the overall salt marsh macroclimate was equal for both areas, results support an effect of the microenvironment of each zone on *S. fruticosa* litter decomposition, as has been reported for other species in diverse studies (Rejmánková & Houdková, 2006; Joly et al., 2017). The main difference between these two zones of the halophilous scrub habitat is the flooding regime. Zone *b* is close to the sea and is mainly flooded by direct entrance of the sea during storms; in this zone, flooding can last several months. On the contrary, zone *a* is mostly flooded for few days or weeks due to rainfall. During the studied period (from mid-November to mid-February), seven flooding episodes caused by sea storms, approximately two each month, occurred, resulting in a prolonged inundation of zone *b* (Pascual & Martinoy, 2017). Inundation disfavours C mineralization in coastal wetlands (Lewis et al., 2014), which agrees with the lower decomposition rates and the lower C content throughout the decomposition period found for *S. fruticosa* litter in zone *b* compared to zone *a*. Conversely, in the litter from zone *a*, there is a greater loss or consumption of the most labile and soluble compounds as the decomposition process progresses, remaining the most recalcitrant compounds with a higher C content. Prolonged flooding periods can also create anaerobic conditions that slowdown microbial efficiency and, consequently, disfavour litter decomposition (Atkinson & Cairns, 2001; Gingerich et al., 2014), which would be in accordance with the lower β-glucosidase efficiency found in *S. fruticosa* litter from zone *b*. Besides, the higher soil salinity (or water salinity when it was flooded) of zone *b* of the halophilous scrub as a result of the flood (sea water salinity about 38‰), could also affect negatively the decomposition of *S. fruticosa* litter. In previous studies, a negative relationship between salinity (30‰ and over) and decomposition rates was reported (Mendelssohn & Slocum, 2004; Ouyang et al., 2017).

Regarding the decomposition rates of *A. portulacoides* litter from the salt meadow habitat, to our knowledge, this is the first study providing data from this species in a non-tidal salt marsh subjected to Mediterranean climate. Hence, when overall litter decomposition rate for *A. portulacoides* at La Pletera (0.0193 ± 0.0068 day^{-1}) was compared with data reported for the same species but from two temperate Atlantic tidal salt marshes (k values being from 0.0104 to 0.0504 day^{-1}), one in Portugal (Simões et al., 2011) and another in France (Bouchard et al., 1998, 2003; Bouchard & Lefeuvre, 2000), it is clear that the decomposition rate of *A. portulacoides* litter from the non-tidal salt marsh La Pletera is in the lower part of the range reported for the Atlantic tidal salt marshes. These results could be related with the daily tidal movements. Indeed, Bouchard et al. (2003) studied the litter decomposition of *A. portulacoides* along an elevation gradient subjected differentially to tides and they found that decomposition rates were up to 4-fold higher in the areas exposed to a higher tidal influence compared to the upper part of the salt marshes, which were almost not affected by daily tides. On the other hand, higher litter decomposition rates in the tidal salt marshes could also be a consequence of an overestimation, since the tidal movement would contribute to the loss of material from litterbags (White & Trapani, 1982; Bouchard et al., 2003). Bouchard et al. (2003) estimated that 60-80% of the decomposition rates would be due to microbial and macroinvertebrate activity, while the rest would be losses of fine litter material.

When the decomposition rates of *A. portulacoides* litter were compared between the two considered zones of the salt meadow habitat, a remarkably difference between zones was found, since the litter decomposition rate of this species in zone *b* was 5.5-fold higher than in zone *a* (Table 4.21). The initial litter was the same in both zones and the two zones were subjected to the same overall climatic conditions, including flooding, presenting also a similar plant cover (Study 1 of this thesis). Thus, differences between zones in the litter decomposition rates of *A. portulacoides* could result from local edaphic features, which, in turn, would affect the composition of microbial communities and their activity. Zone *b* of salt meadow has a soil with a sandy clay loam texture, whereas the soil of zone *a* has a clay loam texture (Table 3.4). The sandiest soil of zone *b* would be more drained and aerated (in accordance with lower values of soil volumetric water content; Table 3.4) which would favour microbial activity (Kathilankal et al., 2008; Moffett et al., 2010). Indeed, a remarkably higher bacterial biomass was found in *A. portulacoides* litter from zone *b*, and fungal biomass also tended to be higher. In accordance, the extracellular enzyme activity (EEA) of β-xylosidase, leucine-aminopeptidase and phenol oxidase at the last sampling date, as well as the efficiency of the four studied enzymes, were higher in the *A. portulacoides* litter of zone *b*. This would explain the lower values of cellulose and C/N ratio, and the higher N content, of the litter found in zone *b* at the last sampling date, as well as the higher increase of lignin over time in the litter of this zone compared to that of zone *a*. These results agree with the fact that only around 10% of AFDM of *A. portulacoides* litter remained in zone *b* at the last sampling date. Indeed, as the litter decomposition progresses to more advanced stages, the availability of labile compounds is reduced, while the most recalcitrant compounds remain, being necessary a higher activity of extracellular enzymes to degrade polymers as lignin (Sinsabaugh et al., 2002) and to obtain N from more unavailable compounds (Valiela et al., 1984). A higher microbial heterotrophic activity promoting a higher organic matter mineralization instead of humification, would also be in accordance with the lower soil organic carbon content (SOC) found in zone *b* (Table 3.4). However, other edaphic parameters that were not measured could have affected the microbial activity and consequently the decomposition rates; for example, the availability of nutrients such as phosphorous (Gijsman et al., 1997), calcium or magnesium (García-Palacios et al., 2016). Hence, more research would be needed to better understand the high spatial variability found in the *A. portulacoides* litter decomposition process at La Pletera salt marsh.

The decomposition rate of *E. pycnanthus* litter at La Pletera salt marsh (0.0015 ± 0.0001 day^{-1}) was within the range (0.0007 to 0.0030 day^{-1}) reported for other Poaceae salt marsh species in tidal salt marshes (Valiela et al., 1985; Foote & Reynolds, 1997; Bouchard & Lefeuvre, 2000; Simões et al., 2011) and in the Ebro Delta (Menéndez & Sanmartí, 2007). Conversely to *A. portulacoides,* the decomposition rate of *E. pycnanthus* litter did not differ between the two zones. Only the fungal/bacterial biomass ratio was lower in *E. pycnanthus* litter from zone *b* compared to that of zone *a*. Since the fungal biomass remained constant throughout the studied decomposition period, differences in this ratio between zones would be attributed to the observed tendency of bacterial biomass to increase over time in zone *b*, as in the case of *A. portulacoides*. Besides, β-xylosidase activity in the litter of *E. pycnanthus* was higher in zone *a*, and, coinciding with the results obtained for *A. portulacoides* litter (Figure 4.16), enzyme efficiencies of β-glucosidase and β-xylosidase were higher in zone *b* than in zone *a*. These results suggest that the spatial heterogeneity of the salt meadow affects the microbial colonization, and the production and efficiency of some extracellular enzymes, of *E. pycnanthus* litter. Taking into account that the decomposition process of the litter of this species is slow, differences in decomposition rates, and likely in litter quality, between zones might probably be observed in more advanced decomposition phases. Indeed, in *A. portulacoides* litter, differences between zones in most of the studied parameters (litter quality and enzyme activities) were only observed at the end of the studied decomposition period. The differences in *E. pycnanthus* litter decomposition process between zones are shown in the PCA ordination plot (Figure 4.18), where

E. pycnanthus litter from zone *b* is located more to the right side of PC1 (mainly related with litter weight loss and microbial colonization).

5.3.3. Changes in the litter decomposition process over time

The temporal decomposition pattern of *S. fruticosa* and *A. portulacoides* litter differed from that of *E. pycnanthus* litter. In *S. fruticosa* and *A. portulacoides,* the temporal pattern of litter decomposition would fit into a two-phase decomposition process, as it was previously described for both species in other Mediterranean salt marshes (Curcó et al., 2002; Scarton et al., 2002; Simões et al., 2011). Indeed, during the early stages of litter decomposition, the percentage of remaining ash free dry mass (AFDM %) declined quickly (until day 38), and, then, the mass loss curve stabilized. These changes over time might be explained by an initial fast leaching of water-soluble compounds, remaining the most recalcitrant elements and thus slowing down the decomposition process (Simões et al., 2011). This is in accordance with the progressive increment in the content of C, cellulose and lignin that *S. fruticosa* and *A. portulacoides* litter showed throughout the decomposition period (Figure 4.14).

The temporal dynamics of litter decomposition of *E. pycnanthus* was similar to that reported for other Poaceae salt marsh species (Valiela et al., 1985; Foote & Reynolds, 1997; Simões et al., 2011). Contrary to the other two species studied, litter of *E. pycnanthus* showed a slow weight loss at the initial phase of the decomposition (with 73% remaining AFDM at day 164). From day 164 to 240 no weight changes were observed and, after that, a decrease of weight reaching the 55% of remaining AFDM on day 357 was registered, which might correspond with the more refractory phase of the decomposition, where only the most recalcitrant compounds remain, being in agreement with the high content of lignin found on day 357 (Figure 4.14).

In general, all hydrolytic enzymes tend to increase through time, which could be explained by a decrease over time in the availability of soluble compounds that can be taken by microorganisms with no enzymatic breakdown (Rinkes et al., 2011; Glanville et al., 2012). In the litter of the three species studied, the peak of β-xylosidase activity (Figure 4.16c) (on day 64 in *S. fruticosa* and in *A. portulacoides* from zone *a*, and on day 164 in *E. pycnanthus*) could be related with the presence of the most recalcitrant compounds, such as hemicellulose. In fact, concomitantly to the β-xylosidase activity peaks, the % remaining AFDM remained fairly stable (Figure 4.15), suggesting the dominance of more recalcitrant compounds difficult to decompose. It is known that heterotrophic microorganisms increase enzyme secretion when a certain polymer becomes abundant and, then, enzyme secretion decrease when the products of the enzyme activity that can be consumed directly by microorganisms increase (Hanif et al., 2004; Kobakhidze et al., 2016). In *S. fruticosa* and *E. pycnanthus* litter, coinciding with the β-xylosidase peak, a peak in the activity of the enzyme leucine-aminopeptidase was also observed (Figure 4.16e). Likely, this could be related with the threshold elemental C/N or C/P ratio at which microorganism metabolism changes from energy supply (C) to nutrient supply (N, P) (Doi et al., 2010; Sinsabaugh and Shah, 2011). It would be expected that an increment of C-degrading enzymes (β-glucosidase and β-xylosidase) would be accompanied by an increment of N-acquiring enzymes in order to maintain these threshold elemental ratio and to avoid that C-degrading enzyme production could be constrained by N supply (Allison, 2005).

The temporal patterns of the studied enzyme activities were, in general, similar for *S. fruticosa* and *E. pycnanthus* litter, regardless of the zone of the habitat in which litterbags were placed, and also for the litter of *A. portulacoides* from zone *a*, but not for that of zone *b*. Differences between zones in the temporal patterns found for the activities of the studied enzymes, especially β-xylosidase, leucine-aminopeptidase and phenol oxidase, in the case of *A. portulacoides* litter were in agreement with the results obtained regarding its decomposition rates and quality parameters. As commented before, *A. portulacoides* litter from zone *b* decomposed very fast, which gave a decomposition curve with a sharp decay (Figure 4.15). In zone *b*, the observed continuous increment of β-glucosidase and leucine-aminopeptidase activities until the last sampling date, and of β-xylosidase activity until day 64 (being similar afterwards), could be due to a high microbial heterotrophic activity provoking that final products (monomers able to be directly consumed) would not be enough to promote enzyme suppression (Hanif et al., 2004; Kobakhidze et al., 2016). Besides, a marked increment of the phenol oxidase activity (from day 64 to day 92) was recorded only in *A. portulacoides* litter of zone *b*, being phenol oxidase the enzyme responsible to degrade lignin. This agrees with the low AFDM of *A. portulacoides* litter remaining at the end of the study period in zone b, and with the final decrease and increase in the relative content of cellulose and lignin, respectively.

Although bacterial and fungal biomass, as well as the fungal/bacterial ratio, did not change significantly over time for any of the three studied species, there was a tendency to increase the fungal/bacterial biomass ratio during the decomposition process, especially in *S. fruticosa* and *A. portulacoides* (Figure 4.17c), which might support an important role of fungi in the decomposition of the more recalcitrant organic compounds, like lignin. Fungi are able to produce and secrete a great group of extracellular oxidative enzymes involved in lignin degradation (Breen & Singleton, 1999; Bugg et al., 2011). In this sense, the high increment of phenol oxidase activity observed on the last sampling date for *A. portulacoides* litter located in zone *b* of the salt meadow could indicate a fungi direct secretion of the lignin-degrading enzyme. Accordingly, in an experiment in which interactions between fungi and bacteria, as well as their extracellular enzyme activities on litter decomposition, were studied, it was found that when bacteria were incubated alone, phenol oxidase activity was almost inexistent, but the opposite happened when fungi were present (Romaní et al., 2006). The changes along the decomposition process are summarized in the results obtained from PCA, mainly those related with PC2 axis, indicating, from negative to more positive values of this axis, the increase in the capacity to degrade cellulose and hemicellulose (β-glucosidase and β-xylosidase), as well as in the relative lignin and C content throughout the decomposition process. It was not until the end of the decomposition process (as seen for *A. portulacoides* litter of zone *b*) that the highest weight loss and the maximum values of fungi and phenol oxidase and leucine aminopeptidase activities occurred (positive values in PC1).

5.4. General discussion

Salt marshes are important ecosystems for global climate regulation because of their high primary production (Mitsch & Gosselink, 2015) and low rates of organic matter decomposition, promoting the carbon accumulation into the soil (Chmura et al., 2003; Kayranli et al., 2010). However, these ecosystems have suffered large area losses (McLeod et al., 2011), being subjected to restoration efforts in the last decades because of their high ecological value (Craft et al., 1999; Warren et al., 2002). Literature concerning the carbon sequestration capacity of salt marshes have been mainly centred on the estimations of carbon stocks from vegetation and soil and their changes over time. However, few studies on carbon dynamics in salt marshes include measurements of plant community structure and composition, although it could be a close relationship among them (Elsey-Quirk et al., 2011; Ford et al., 2019). Moreover, the seasonal pattern of carbon fluxes from soil and, especially, from vegetation are poorly known. Litter decomposition rates in these ecosystems have been broadly documented, but other key parameters, such as the microbial activity, remain understudied. Most of the studies on the carbon sequestration capacity and litter decomposition have been mainly performed in tidal salt marshes, especially from North America, being studies from non-tidal salt marshes, such as those of the Mediterranean Basin, really scarce. Taken into account all this, the goal of this thesis was to study the structure and composition of plant communities, the soil and vegetation carbon dynamics (stocks and seasonal fluxes) and the litter decomposition process of the main habitats of a Mediterranean non-tidal salt marsh (La Pletera).

The one-year study of the structure and composition of the plant communities of the three well-preserved habitats of La Pletera salt marsh, i.e. the halophilous scrub, the salt meadow and the glasswort sward, revealed that the first two habitats presented the most mature and stable plant communities. In contrast, the plant community of the glasswort sward was the most dynamic, and it will probably evolve towards a plant community dominated by late-successional species similar to that of the halophilous scrub. In accordance with the results obtained in other salt marshes (Elsey-Quirk et al., 2011; Ford et al., 2019), the halophilous scrub and the salt meadow had the higher amount of carbon stored in vegetation and soil compared with the pioneer community of the glasswort sward. Concretely, the halophilous scrub stored more C in the aboveground biomass, especially in their woody and standing dead fraction, and both the halophilous scrub and the salt meadow had the highest amount of C stored in belowground biomass, litter and soil.

Regarding carbon fluxes from the dominant plant species of each habitat, *Elymus pycnanthus* was the perennial species with the highest photosynthetic carbon sequestration capacity, although, in the growing period, values were also high for the annual *Salicornia patula*. However, since *Sarcocornia fruticosa* had the highest photosynthetic biomass (green plus thin woody stems), the halophilous scrub became the habitat with the highest CO_2 sequestration from vegetation. Nevertheless, the halophilous scrub and the salt meadow had higher soil CO_2 emissions than the glasswort sward, although when considering the carbon emitted to the atmosphere (mainly as CO_2) in relation to the amount of organic carbon stored in the soil (i. e. the soil carbon mineralization quotient) the former habitats showed the lowest values. This indicates the high carbon sequestration potential of the halophilous scrub and the salt meadow, since the organic carbon stored in their soils is subjected to a lower mineralization.

The litter decomposition at La Pletera was influenced by the microenvironment, which affected the soil carbon sequestration. The importance of the site spatial heterogeneity in litter decomposition was previously reported (Rejmánková & Houdková, 2006), and in environments with high patchiness, as occurs in the Mediterranean Basin salt marshes (Ibañez et al., 2000), it could become especially important. In the present study, results suggest that flood duration (in the halophilous scrub) or soil texture (in the salt meadow) might be the main factors explaining the spatial heterogeneity in litter decomposition. However, it cannot be disregarded that other parameters, such as the soil content in phosphorous (Gijsman et al., 1997), calcium or magnesium (García-Palacios et al., 2016), could also affect the organic matter decomposition. In addition, in the salt meadow, the high plant species richness and diversity might have determined different microbial decomposers communities and this might have also contributed to explain the litter decomposition differences among zones (Sinsabaugh et al., 2002). Finally, results indicate that the decomposition of dead leaves of *E. pycnanthus* would contribute to a greater extend to the carbon sequestration into the soil than the decomposition of senescent stems of *S. fruticosa* and leaves of *A. portulacoides*, since *E. pycnanthus* leaves decomposed remarkably slower.

The carbon dynamics found in the non-tidal Mediterranean salt marsh La Pletera, when compared to other salt marshes (mainly tidal salt marshes), suggest a lower carbon sequestration capacity of the former. La Pletera salt marsh, as other Mediterranean non-tidal salt marshes, has lower values of aboveground standing biomass than tidal salt marshes (Ibañez et al., 2000; Curcó et al., 2002), mainly because of the high soil hypersalinity and thus the enhanced salt and water stresses that plants experience (Cameron, 1972; Berger et al., 1978). Moreover, soil CO_2 emissions in La Pletera were higher than those reported for temperate and subtropical tidal salt marshes (Table 5.1). This is probably due to the more aerated and thus oxygenated conditions at La Pletera as a consequence of the absence of tides, which agree with previous studies on the tidal effect in soil respiration (Kathilankal et al., 2008; Moffett et al., 2010).

An improvement in the carbon sequestration capacity of La Pletera salt marsh can be expected in the next decades/years due to the restoration of some perturbed zones that formerly were part of the salt marsh. In particular, the restoration works carried out until now focused on the removal of the remaining urban infrastructures and piles of debris, the reestablishment of the natural hydrology, and the creation of new lagoon systems. The increase in water movement and connectivity will improve the dispersion of the seeds from halophytic vegetation, favouring the recolonization of the restored zones and the transformation of the glasswort sward into an halophilous scrub. In fact, the present study detected the appearance of *S. fruticosa*, the dominant species of the halophilous scrub, in the glasswort sward and in the restored zone (two years after the restoration). Hence, it is expected that, in the near future, the evolution of the glasswort sward and the restored zone towards a more complex halophilous scrub would result in an increase of organic matter, raising the carbon stored in vegetation and soil. Besides, results suggest that longer flooding periods (as it occurs in the restored zone) would slow down litter decomposition, as it has been reported in previous studies (Atkinson & Cairns, 2001; Gingerich et al., 2014), thus favouring the integration of organic matter into the soil.

To sum up, results reported in this thesis contribute to better understand the carbon dynamics and the litter decomposition process in non-tidal salt marshes of the Mediterranean Basin. Salt marshes are important ecosystems for global climate regulation through the capture of high amounts of carbon, being finally stored in vegetation and soil. The conservation of these ecosystem and the recovery of their degraded areas, jointly with the reestablishment of the natural hydrologic fluxes, will contribute to enhance the amount of carbon stored, avoiding its release to the atmosphere, which will guarantee the continuation of their valuable ecosystem services, including climate regulation.

6. CONCLUSIONS

- The halophilous scrub and the salt meadow habitats of La Pletera salt marsh presented a mature plant community with no changes in its structure and composition between the two studied years, while the plant community of the glasswort sward would be evolving from pioneer vegetation to a community similar to that of the halophilous scrub.
- In the disturbed zone the ruderal plant community has been replaced after the restoration by annual pioneer vegetation of saline soils after one year, but with the presence of the dominant species of the halophilous scrub (*Sarcocornia fruticosa*) after two years.
- The halophilous scrub is the salt marsh habitat that stores more C in vegetation while both the halophilous scrub and the salt meadow show the highest amount of C in soil.
- *Elymus pycnanthus* was the dominant species of La Pletera salt marsh with the higher atmospheric CO_2 removal capacity through photosynthesis during the entire year, although, the high photosynthesis rates of *Salicornia patula* in summer was also remarkable. However, since *Sarcocornia fruticosa* have the highest photosynthetic biomass, the vegetation of the halophilous scrub is the one that most contribute to CO_2 sequestration.
- The four species studied showed daily net CO_2 uptake from the green fraction during most of the year, except in December when *Sarcocornia fruticosa* and *Atriplex portulacoides* showed net CO_2 emission.
- The thin woody stems of *S. fruticosa* and *A. portulacoides* showed net CO_2 uptake in winter and spring and in winter, spring and summer, respectively, highlighting the importance of this fraction in the characterization of the daily and seasonal CO_2 fluxes from these ecosystems.
- The halophilous scrub and the salt meadow showed higher soil CO_2 emissions than the glasswort sward, and, in general, these values were higher than those reported for temperate and subtropical tidal salt marshes.
- At La Pletera, CH_4 absorption and emission were detected, being CH_4 emissions remarkably high, and, in general, higher than those of other salt marshes with high water table salinity and similar to those of salt marshes with low salinity.

- The soils of the halophilous scrub and the salt meadow presented lower mineralization quotients than the glasswort sward soil suggesting that they have higher carbon sequestration potential.
- The litter decomposition process was different for the three dominant species of La Pletera salt marsh. The two succulent Chenopodiaceae species, *Sarcocornia fruticosa* and *Atriplex portulacoides*, likely due to the low lignin content of their litter, decomposed faster than the Poaceae species, *Elymus pycnanthus*.
- *E. pycnanthus* presented the highest activity of the C-degrading enzymes β-glucosidase and β-xylosidase and the lowest enzyme efficiency for all the enzymes studied. The activity of phenol oxidase showed a high increase only when the litter was in the most advanced stages of the decomposition.
- Throughout the process of litter decomposition, bacteria dominated, although fungi became especially important in the later stages, when the relative lignin content of the litter increased.
- Despite the importance of litter quality and overall climatic conditions in litter decomposition, differences between the two zones studied per habitat suggest that flooding (in the case of *S. fruticosa*) or soil texture (in the case of *A. portulacoides* and *E. pycnanthus*) might have significantly influenced the process of decomposition.
- Decomposition of *E. pycnanthus* dead leaves would favour soil carbon sequestration more than the decomposition of senescent stems or leaves of *S. fruticosa* and *A. portulacoides*, respectively.

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Supplementary material

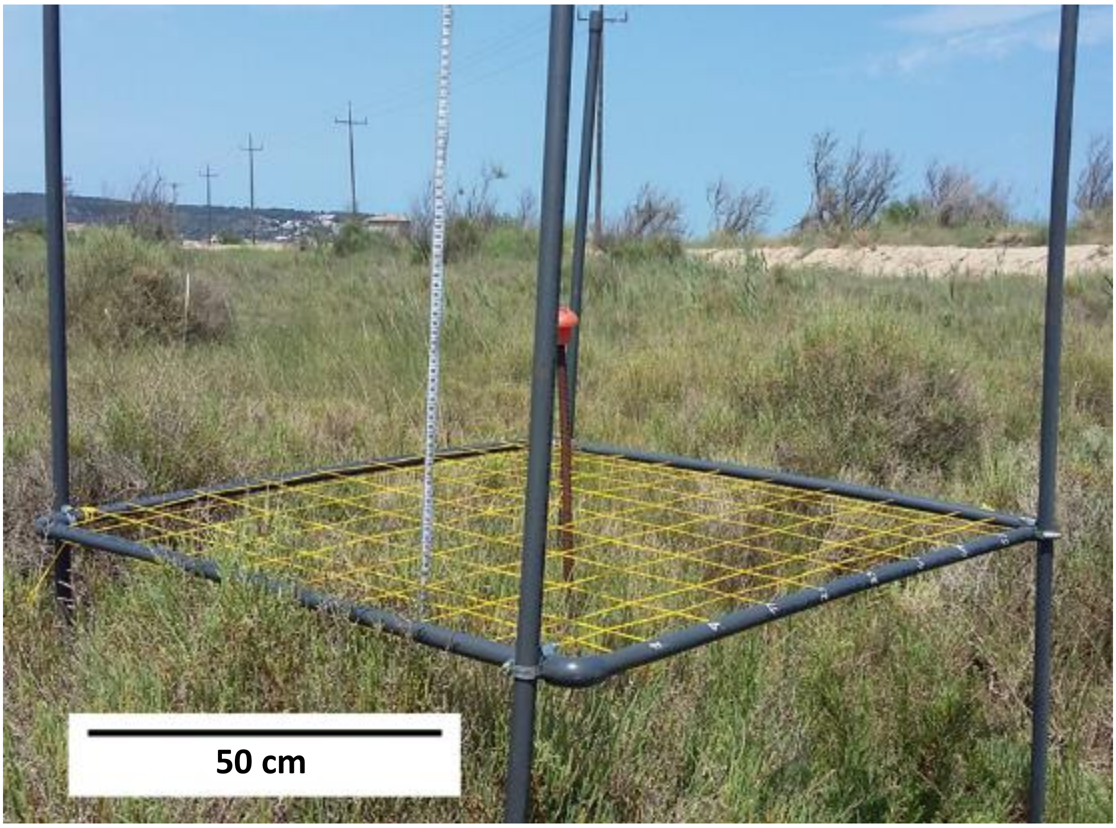
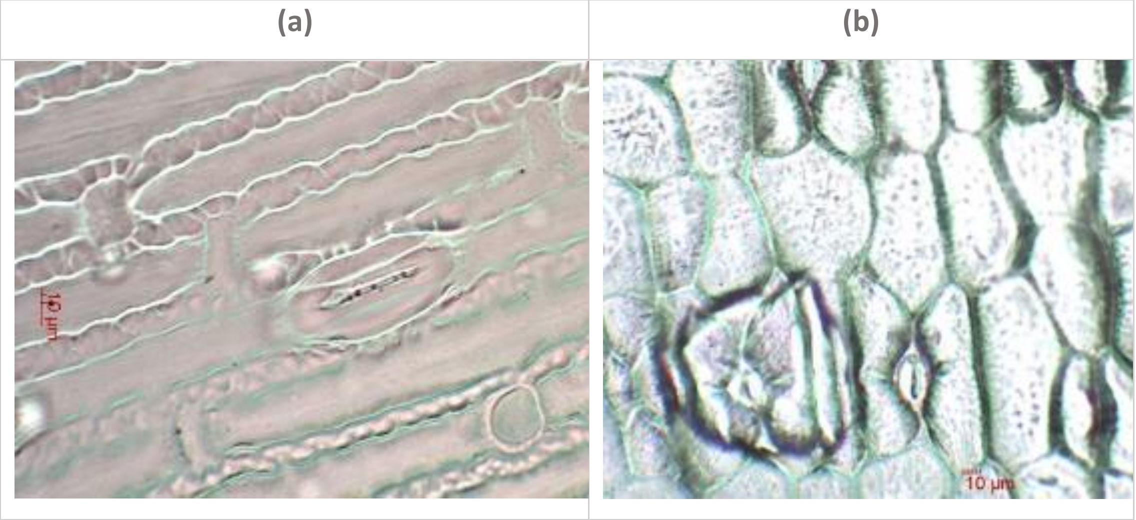


Figure SM 1. Grid frame and metal pin (Ø =7 mm) used to study the structure and composition of plant communities by means of the point quadrat method.


(a)

(b)

Figure SM 2. Images of dumbbell-shaped stomata observed on the *E. pycnanthus* leave (abaxial face) (a) and kidney-shaped stomata observed on the green stem of *S. fruticosa* (b).

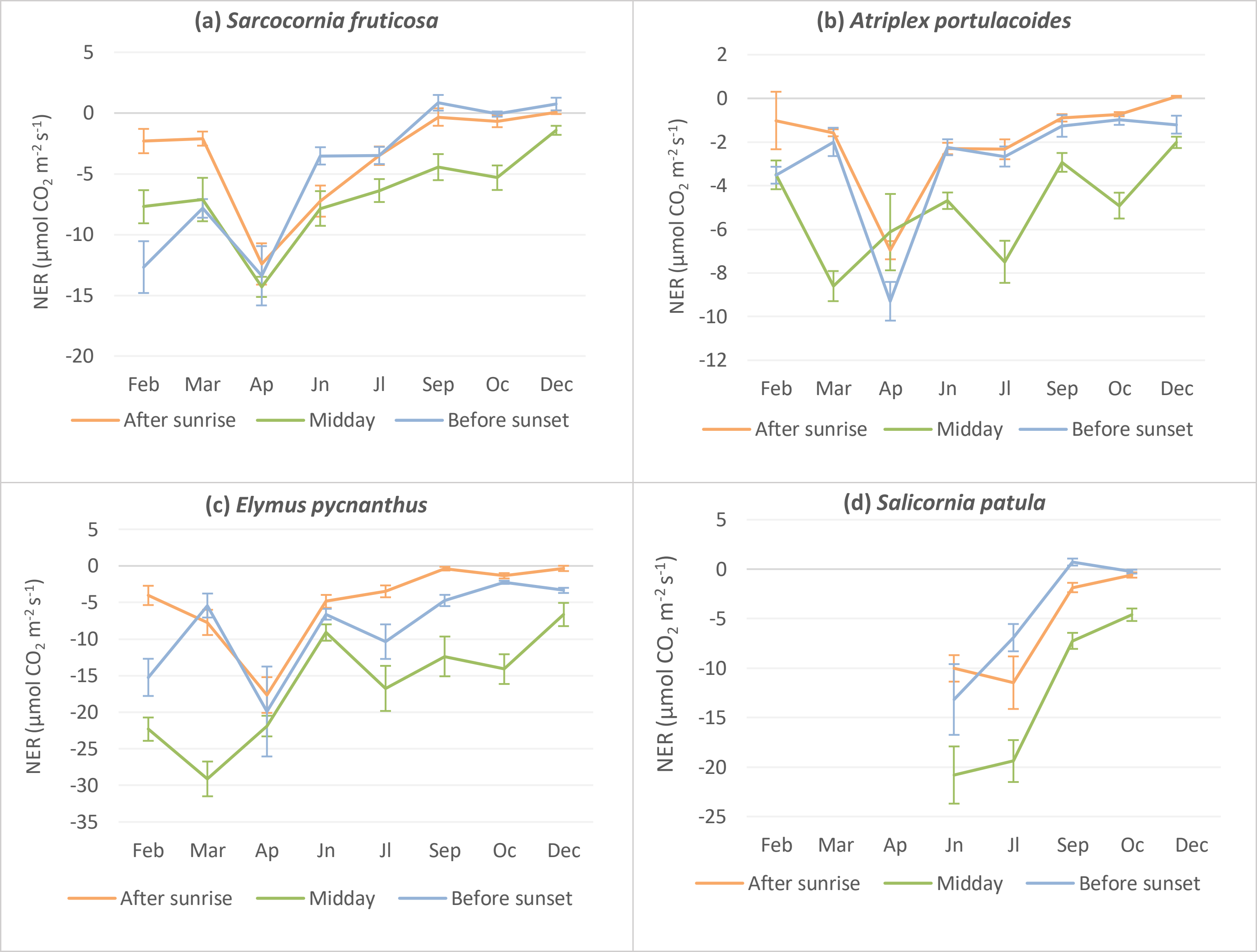


Figure SM 3. Instantaneous net CO$_2$ exchange rate (NER) of the three daily moments (after sunrise, at midday and before sunset) measured in green tissues of *S. fruticosa* (a), *A. portulacoides* (b), *Elymus pycnanthus* (c) and *Salicornia patula* (d). Negative values indicate net photosynthetic activity, while positive values indicate net respiration. Bars represent standard errors (n = 4 for the period after sunrise, before sunset and night, and n = 6 for midday).

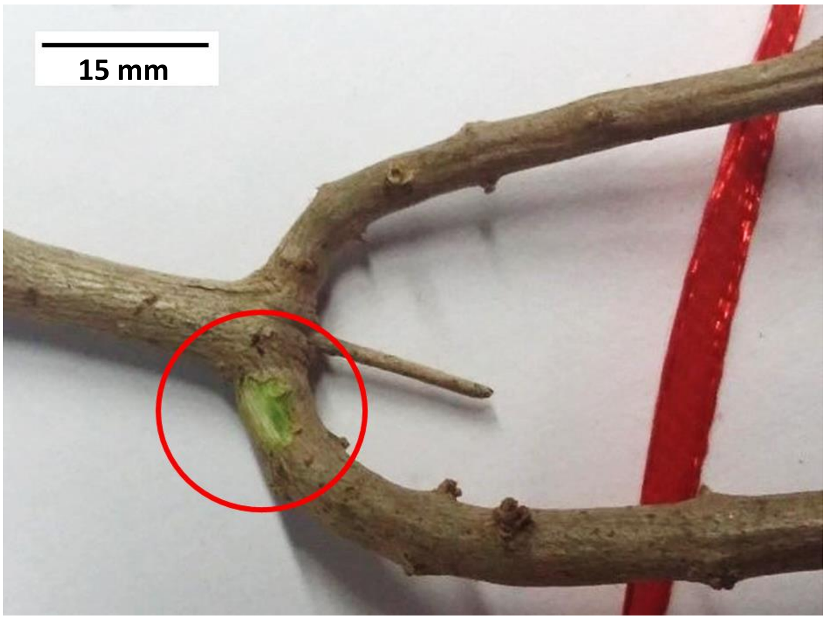


Figure SM 4. Thin woody stems of S. fruticosa where the green tissue under the bark can be observed.

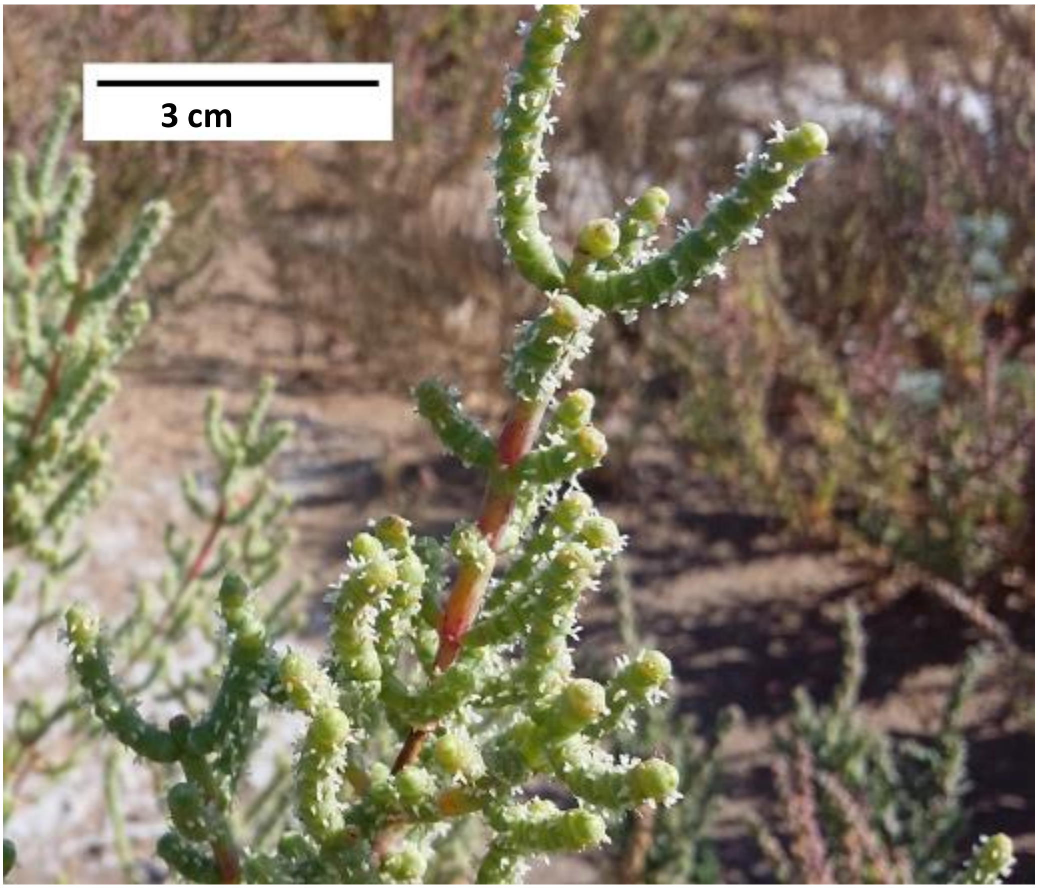


Figure SM 5. Flowered herbaceous stem of *S. fruticosa.*

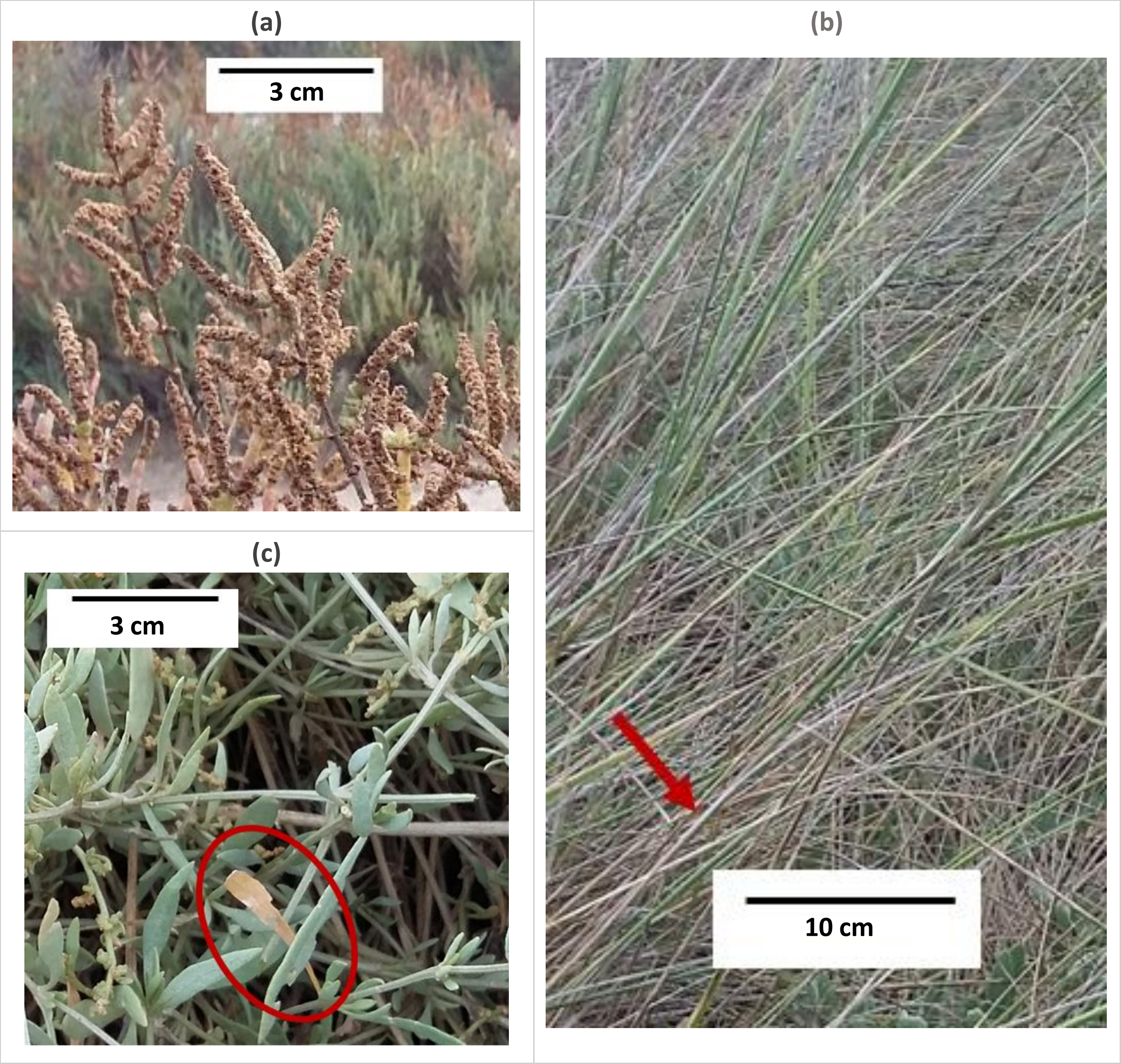


Figure SM 6. Vegetal material used in the litter decomposition study (Study 3). Senescent stems of *S. fruticosa* (a), Recently dead leaves of *E. pycnanthus* (b) and senescent leaves of *A. portulacoides* (c).

Figure SM 7. Measurement of instantaneous net CO_2 exchange rate (NER) in vegetation (woody stem of *S. fruticosa*).