Deurbanization and restoration of La Pletera salt marsh

Action D4 (Carbon balance)

FINAL REPORT

LIFE 13 NAT/ES/001001





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July 2018

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SUMMARY ACTION D4: CARBON BALANCE

The aim of action D4 was to assess the role of La Pletera salt marsh in the emission and/or capture of greenhouse gases (CO_2 and CH_4). To achieve this goal, two methods have been used (according to the Toolkit for Ecosystem Service Site-based Assessment or TESSA tool; Peh et al., 2013): a) the estimation of carbon stored in the different systems of the salt marsh (vegetation, soil and lagoons) and b) the analysis of dynamic fluxes (CO_2 and CH_4) from these systems throughout the year.

The first method gives information about the amount of carbon contained in every system (carbon stock), allowing the estimation of net primary production. Related with carbon storage into the soil, biomass decomposition of the dominant species has been studied in order to understand the incorporation of carbon from vegetation into the soil, since this is an important process for carbon burial at the long-term. The second method (the analysis of dynamic fluxes of CO_2 and CH_4) gives information about how the carbon sink/source capacity of the ecosystem is affected by seasonal or daily variations in the environmental factors (temperature, solar radiation, humidity, etc.).

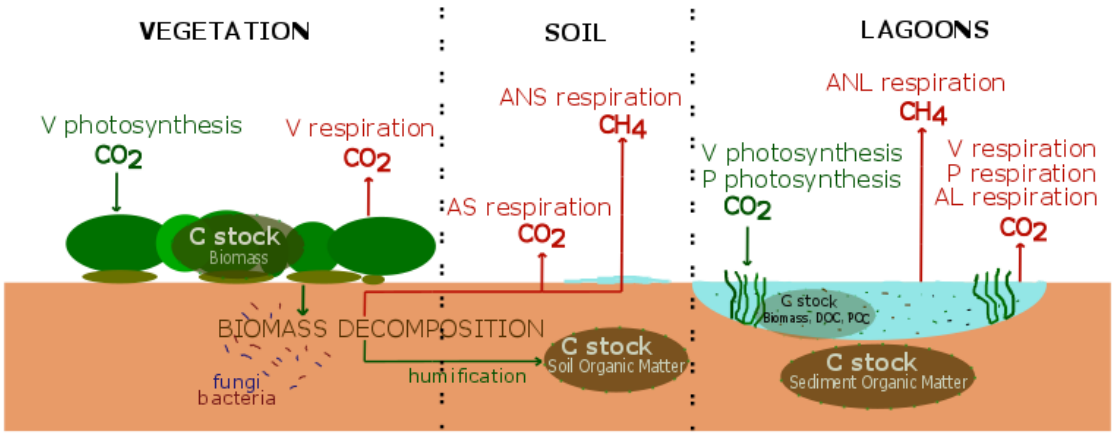


Figure 1: Scheme of the main processes related with the carbon balance in coastal salt marshes. V photosynthesis: photosynthesis of salt marsh vegetation and macrophytes of lagoons; P photosynthesis: photosynthesis of the phytoplankton; V respiration: respiration of salt marsh vegetation and macrophytes of lagoons; P respiration: respiration of phytoplankton; AS respiration: soil aerobic respiration; ANS respiration: soil anaerobic respiration; AL respiration: aerobic respiration of lagoons; ANL respiration: anaerobic respiration of lagoons.

In coastal salt marshes, carbon is stored as organic carbon in vegetation, soil and in sediments of lagoons. Besides, in lagoons, there is also a carbon fraction under the forms of dissolved organic carbon (DOC) and particulate organic carbon (POC) (Figure 1). To estimate the amount of carbon stored in the different systems of La Pletera salt marsh determinations of carbon concentrations in vegetation, soil and sediments of lagoons (as compartments in which carbon is stored for long-term) were done.

Carbon stored in the vegetation has been estimated in the same habitats of European Community interest studied in action D2, section 3.1. These habitats, called habitats 1, 2 and 3, belong to the well-conserved zone of the salt marsh, being dominated by shrubland, grassland and annual

halophytic vegetation, respectively. A fourth habitat (called habitat 4) was also studied, since this was a disturbed habitat with dominance of ruderal vegetation, which was subjected to restoration and, after, it was let to be naturally revegetated. In the well-conserved zone, aboveground and belowground biomass, as well as superficial litter, were collected in summer of two consecutive years (2015 and 2016). In habitat 4, the samplings were performed in summer 2015 (before restoration) and in summer 2017 (one year after the restoration). Aboveground biomass was separated by species and by living green (photosynthetic), living woody, and standing dead tissue. All the plant material was dried and total C was determined. The shrubland habitat (habitat 1) had the highest amount of carbon stored in vegetation, specifically in the woody and standing dead tissues of its dominant species *Sarcocornia fruticosa.* Both the shrubland and the grassland (habitat 2) showed the highest values of carbon stored in dead aboveground biomass, belowground biomass and litter. In the case of the disturbed habitat 4 (ruderal vegetation), the amount of carbon accumulated in the living and total aboveground biomass was similar to that of habitat 3. Nevertheless, carbon stored in the belowground biomass and litter in habitat 4 was considerable higher than that of habitat 3, although much lower than in habitat 1 and 2. One year after the restoration, the amount of carbon stored was very low in all the studied fractions of the habitat 4, since vegetation was still very scarce.

Litter decomposition of the dominant species of habitats 1 and 2 (*Sarcocornia fruticosa, Atriplex portulacoides* and *Elymus pycnanthus*) was also studied, since this important process mediated by microorganism contributes to enhance soil organic carbon content. Litterbags were used and litter quality, decomposition rates, extracellular enzyme activities and carbon of fungal biomass were studied throughout the decomposition process of these species. *E. pycnanthus* litter had the highest values of carbon, cellulose and lignin, being more recalcitrant than the litter of the other two species. Accordingly, the litter of this species had the lowest carbon of fungal biomass and decomposition rates. When comparing extracellular potential enzyme activities (EEA), there were few significant differences among species, which means that similar enzyme activities decompose a lower amount of litter in the case of *E. pycnanthus*. Overall, results indicate that *E. pycnanthus* would favour the incorporation of organic carbon into the soil more than the other two species due to its lower litter decomposition rates and consequently lower mineralization of the organic carbon.

The decomposition rate of *A. portulacoides* showed a remarkable variability. In fact, differences in decomposition rates, extracellular enzyme activities and fungal biomass were found between litter samples of *A. portulacoides* placed in two different zones of habitat 2. This suggests that this species is highly sensitive to edaphic heterogeneity, and thus it can contribute differently to the incorporation of organic carbon into the soil depending on the edaphic properties of the zone.

Carbon stored in the soil was estimated by means of the determination of soil oxidizable organic carbon. Besides, other parameters that also give information about the carbon stored in the soil, such as glomalin content and the composition of soil organic matter, were also analyzed. In the well-conserved zone, habitats 1 and 2 had the highest amount of glomalin and organic carbon stored in the soil. This agrees with the dominant vegetation of these habitats, since both the shrubland (habitat 1) and grassland (habitat 2) have dense vegetation with a higher amount of plant biomass and litter on the soil surface compared to habitat 3 (annual vegetation). Litter acts as an important

source of soil organic carbon, improving also parameters associated with the carbon dynamics, such as the glomalin content of the soil. Besides, the composition of soil organic matter in habitats 1 and 2 revealed a fresher nature of this organic matter compared to habitat 3, which agrees with the idea that, in these two habitats, litter is contributing to enhance soil carbon content. Similar values of SOC, SOM and glomalin were found in the soils of the three habitats of the well-conserved zone compared to the soil of the disturbed habitat (habitat 4). However, this result may be misleading because of the origin of the disturbed soil. In fact, the soil of habitat 4 was rubble coming from different places without any structure and just used to fill and rise the cote of the salt marsh area.

To estimate the carbon stored in the sediment of lagoons, nine lagoons were analysed: three old permanent (two of them being naturally-formed and the third created in 2002); three new permanent and three new temporary lagoons (all created in 2016). Organic and inorganic carbon concentrations were estimated, and the highest values were found in the old permanent naturally-formed lagoons. The permanent lagoon created in 2002 did not reach the levels of carbon stored in the sediment that the other two permanent lagoons had. Hence, the age of the lagoons is an important factor determining the storage of carbon in these systems.

To estimate the net carbon flux and its temporal fluctuations (daily and seasonal), carbon dioxide (CO_2) fluxes from the vegetation, soil and lagoons were measured during 2017, as well as methane (CH_4) emissions from soils and lagoons. Vegetation and phytoplankton, through photosynthesis, fix atmospheric CO_2 and transform it into biomass. Through vegetal respiration, a part of this fixed CO_2 is returned to the atmosphere. When biomass dies and decomposition process starts, a part of this carbon is humificated and integrated into the soil as organic matter, while another part is mineralized to inorganic forms. Under aerobic conditions, soil emits CO_2 to the atmosphere as final product of the decomposition. However, during the flooding periods, CH_4 emission occurs under anaerobic conditions, being CH_4 a greenhouse gas with a global warming potential much higher than CO_2. In lagoons, DOC and POC can also be integrated in the sediment or decomposed to inorganic forms and, depending on the partial pressures of CO_2 and CH_4 between water and air, can be diffused to the atmosphere.

Regarding vegetation, net CO_2 exchange rates (NER) from the dominant species of each habitat (*Sarcocornia fruticosa*, *Atriplex portulacoides*, *Elymus pycnanthus* and *Salicornia patula*) were monitored using a PLC3 conifer leaf chamber (80x40 mm) connected to an InfraRed Gas Analyser (IRGA). NER was measured at different times of the day (sunrise, midday, sunset and night) in green and thin woody living tissues, except for *E. pycnanthus* and *S. patula*, in which only green living tissues were present. *E. pycnanthus,* the dominant species of the grassland (habitat 2), presented the highest photosynthetic activity (negative NER values) during most of the year. However, since the shrub *S. fruticosa* had a much larger amount of biomass per area, habitat 1 had the highest CO_2 uptake rates in spring, while CO_2 uptake values were similar between habitat 1 and 2 in winter and summer. In autumn, both habitats, 1 and 2, emitted CO_2. Habitat 3 acted as a small sink of CO_2 in spring and summer, the two seasons with photosynthetically active annual plants in this habitat. To sum up, taking into account that habitat 1 is the most extensive habitat in La Pletera salt marsh, our results indicate that the vegetation of this salt marsh would be a sink of CO_2 most of the year, but this situation could be reversed in autumn, when it might be a net source.

In soil, daily CO_2 flux was measured by the soda-lime method. When the soil was flooded, CO_2 and CH_4 fluxes were measured by gas chromatography. The highest soil CO_2 emissions were found in the habitats where the soil organic carbon content was higher (i.e., habitat 1 and 2). CH_4 emissions from soils were registered, even when soils were not flooded. For all the habitats, the highest carbon emissions (CO_2 and CH_4) from soils were found in summer, when temperatures were higher. In summer, CH_4 emissions from non-flooded soils were explained by the proximity of the water table to the soil surface and by the fact that soils were dry, which allows the diffusion of CH_4 to the surface. The values of CH_4 emission in La Pletera salt marsh are higher than those expected in salt marshes. Despite the highest CO_2 and CH_4 emissions were found in habitats 1 and 2, these habitats have lower mineralization coefficients, and thus a higher potential of carbon sequestration, than habitat 3.

When vegetation and soil CO_2 fluxes were integrated, habitat 1 (shrubland with *S. fruticosa* as dominant species) and habitat 2 (grassland with *E. pycnanthus* and *A. portulacoides* as dominant species) were a net sink of CO_2 in all the seasons, except in autumn when net CO_2 emissions were registered. In habitat 3 (annual vegetation with *S. patula* as dominant species), soil CO_2 emissions could not be counteracted by CO_2 uptake from vegetation, even in spring and summer when *S. patula* was photosynthetically active, and, thus, this habitat was a net CO_2 source throughout the year, and specially in spring and summer when temperatures were higher.

In the same nine lagoons where the carbon stored in the sediment was determined, the net CO_2 exchange rate was measured, at midday, using an opaque closed chamber connected to an IRGA. Among permanent lagoons, differences in CO_2 water-air fluxes between old and new lagoons were found only in March (highest values in old lagoons) and September (highest values in new lagoons). Permanent and temporary new lagoons had similar CO_2 water-air flux throughout the year. Throughout the year, eight of the nine lagoons studied were net emitters of CO_2, and only the old permanent naturally-formed lagoon called Fra Ramon sequestered CO_2 most of the year, probably due to its high abundance of macrophytes.

EXTENT REPORT ACTION D4: Carbon Balance

1. General introduction

The increase of anthropogenic greenhouse gas emissions since the pre-industrial era is the main cause of the increase in the global warming registered in the last decades (IPCC, 2014). In particular, it has been a continuous increase of carbon dioxide concentrations in the atmosphere, exceeding nowadays 400 parts per million (ppm) (412.6 ppm in May 2018, ESRL/NOAA 2018). In a context of continuous global warming associated to the increase in 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.

Salt marshes, and wetlands in general, play an important role in global climate regulation. In fact, according with the synthesis report of the Millennium Ecosystem Assessment "Ecosystems and human well-being: wetlands and water", climate regulation through the capture and emission of high proportions of carbon fixed in the biosphere is one of the ecosystem services strongly associated to the human well-being in these ecosystems. Even if salt marshes cover a relatively small portion of the earth surface (4–6%) (Mitsch & Gosselink, 2000), an estimated 25–30% of the global soil carbon reservoir is stored in these ecosystems (Lal, 2008). This large amount of stored carbon is due to high primary production and slow rates of soil organic matter decomposition caused by anaerobic soil conditions during flooding periods, that avoid the return of carbon dioxide (CO_2) to the atmosphere promoting its accumulation into the soil (Chmura et al., 2003; Kayranli et al., 2010). However, one of the final products of the organic matter decomposition under anoxic conditions is methane (CH_4), which presents a warming potential higher than that of CO_2. It has been estimated that 20-25% of the global methane emissions are produced by wetlands (Whiting and Chanton, 2001; Conrad, 2009).

The aim of the action D4 was to assess the role of La Pletera salt marsh in the emission and/or capture of greenhouse gases (CO_2 and CH_4). To achieve this goal, two methods have been used (according to the Toolkit for Ecosystem Service Site-based Assessment or TESSA tool; Peh et al., 2013): the estimation of carbon stored in the different systems of the salt marsh (vegetation, soil and lagoons) and the analysis of dynamic fluxes of CO_2 and CH_4 from these systems throughout the year. The first method gives information about the amount of carbon contained in every system allowing the estimation of net primary production. Related with carbon storage into the soil, the biomass decomposition of the dominant species has been studied in order to understand the incorporation of carbon from vegetation into the soil, since this is an important process for carbon burial at the long-term. The second method (the analysis of dynamic fluxes of CO_2 and CH_4) gives information about how the carbon sink/source capacity of the ecosystem is affected by seasonal or daily variations in the environmental factors (temperature, solar radiation, humidity, etc).

2. Carbon stored in La Pletera salt marsh

2.1. Carbon stored in vegetation and net primary production

2.1.1 Characterization of the study area

To determine the carbon stored and carbon dynamic fluxes from vegetation and soil of the La Pletera salt marsh, two zones were differentiated: the disturbed and finally restored zone and the reference zone, which corresponds to the well-preserved salt marsh. The disturbed zone was considered as a single type of habitat, while in the reference zone three habitats of European Community interest were differentiated according to the dominant plant species (Table 1) (more detailed information in Action D2).

			Area
Reference zone	Habitat 1 (shrubland)	Perennial succulent halophilic vegetation, dominated by *Sarcocornia fruticosa* with presence of *Atriplex portulacoides*	15.0 ha
	Habitat 2 (grassland)	Mediterranean halophilic meadows, dominated by *Elymus pycnanthus* and *Atriplex portulacoides*, with the presence of *Juncus acutus* and *Phragmites australis*	10.8 ha
	Habitat 3 (pioneer vegetation)	Annual pioneer vegetation of saline soils, dominated by *Salicornia patula,* with the presence of *Suaeda maritima* and *Sarcocornia fruticosa*	4.1 ha
Disturbed/ restored zone	Habitat 4	Disturbed: Ruderal vegetation dominated by *Foeniculum vulgare* and *Inula viscosa* Restored: Annual pioneer vegetation of saline soils dominated by *Suaeda maritima*	10.0 ha

Table 1. Study zones and habitats differentiated according to the dominant vegetation

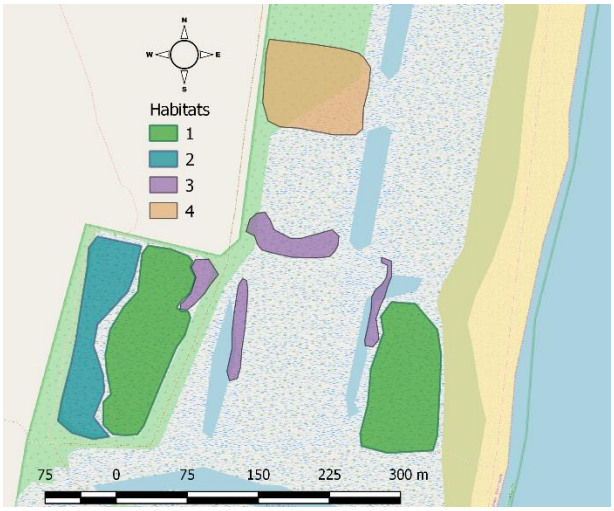


Figure 2. Distribution of the four studied habitats at La Pletera salt marsh

2.1.2. Sampling, sample treatment of vegetation and data analyses

The amount of carbon stored was estimated for the plant aboveground (life and dead) and belowground biomass, as well as for the litter, according with the methods recommended by TESSA tool (Peh et al., 2013). To estimate the carbon accumulation rate (net primary production) in the reference zone, we compared the estimated carbon stored in the vegetation in two consecutive years (2015 and 2016).

Biomass samples of the reference zone were collected in summer in 2015 and 2016, while biomass of the disturbed zone was sampled only in summer of 2015 (before the restoration) and in summer 2017 (after the restoration). For each sampling period, five plots in each one of the three well-conserved habitats and ten in the disturbed zone were randomly selected. For each plot (0.8 m x 0.8 m), plant aboveground biomass was harvested and superficial litter was collected in a quarter of the plot. Plant roots were sampled in each one of the three well-preserved habitats at 0-20 cm and 20-40 cm depth in 2015 and at 0-20 cm in 2016 by the extraction of three soil cores (8 cm diameter, 20 cm long) per plot. In 2016 sampling was only performed at 0-20 cm depth because data from 2015 demonstrated that 94%, 83% and 75% of the belowground biomass of habitats 1, 2 and 3, respectively, was located at the first 20 cm. Plant roots of the disturbed habitat was also collected in summer 2015 at 0-20 cm, but sampling was performed by removing the soil from one fourth of the zone 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 that the individuals presented, root sampling was done by collecting the entire radicular part of each individual (in all cases roots were not deeper than 20 cm).

Samples were taken to the laboratory where aboveground material was separated by species and by living green (photosynthetic), living woody, and standing dead tissue. Roots were 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 70ºC to constant weight and two subsamples of each fraction were ground to a fine powder for elemental composition analysis. Total C and N were determined using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK), by the Stable Isotope Facility of University of California (Davis).

Carbon stored in the vegetation of the different habitats was estimated multiplying the carbon amount of each fraction (living green, living woody, standing dead tissues, root and litter) by the biomass that the different fractions represented in each habitat. Net aboveground primary production (NAPP) was estimated comparing carbon stored in living and total aboveground biomass in 2015 and 2016. Net belowground primary production (NBPP) was also estimated comparing belowground biomass in 2015 and 2016.

To determine whether the differences between years, habitats and species in the estimated carbon stored in the five fractions measured (living green, living woody, standing dead, roots and litter) were significant, we performed ANOVAs and, when needed, Tukey's HDS post-hoc tests. In case that data did not accomplish normality and homoscedasticity non-parametric tests (Kruskal-Wallis and Mann-Whitney U test) were applied.

2.1.3. Results

2.1.3.1. Carbon stored in vegetation

In the well-preserved zone, the shrubland (habitat 1) had the highest amount of carbon stored in living and total aboveground biomass (Table 2). In addition, both the shrubland and the grassland (habitat 2) showed the highest values of carbon stored in the other three compartments considered (dead aboveground biomass, belowground biomass and litter). Habitat 3 (pioneer annual vegetation) had the lowest amount of carbon stored in all fractions, except for living and total aboveground biomass.

Carbon amount g C m^{-2}	Living aboveground biomass	Dead aboveground biomass	Total aboveground biomass	Belowground biomass	Litter
Habitat 1 (shrubland)	**808.09 ± 87.95 a**	**654.69 ± 120.24 a**	**1462.78 ± 178.84 a**	**103.43 ± 8.95 a**	**258.15 ± 45.47 a**
Habitat 2 (grassland)	206.83 ± 51.43 b	**297.44 ± 40.21 a**	504.27 ± 60.70 b	**103.31 ± 12.26 a**	**184.14 ± 29.80 a**
Habitat 3 (pioneer)	59.31 ± 9.85 c	36.35 ± 7.39 c	95.67 ± 14.25 c	18.16 ± 1.78 c	15.12 ± 4.16 c
Habitat 4 (ruderal)	54.51 ± 13.58 c	99.90 ± 14.79 b	154.41 ± 26.34 c	49.53 ± 17.94 b	44.18 ± 5.73 b

Table 2. Carbon stored in living, dead and total aboveground biomass, belowground biomass and litter in the 4 studied habitats (mean ± SE, n=10). Different letters and colors indicate significant differences ($p < 0.05$) between habitats within the carbon content of each fraction.

Carbon amount g C m^{-2}	Living aboveground biomass	Dead aboveground biomass	Total aboveground biomass	Belowground biomass
Habitat 4 (restored)	0.83 ± 1.97	0.24 ± 0.73	1.07 ± 2.03	0.17 ± 0.31

Table 3. Carbon stored in living, dead and total aboveground biomass and belowground biomass in habitat 4 one year after the restoration (mean ± SE, n=10). There was no litter.

In the case of the disturbed habitat 4 (ruderal vegetation), the amount of carbon accumulated in the living and total aboveground biomass was similar to that of habitat 3. Nevertheless, carbon stored in the belowground biomass and litter was considerable higher (about 250%) than that of habitat 3, although it was much lower than in habitat 1 and 2 (Table 2). One year after the restoration, the amount of carbon stored in habitat 4 was very low for all the fractions, because the vegetation was still very scarce (Table 3).

	Percentage of total biomass	
Habitat 1		
Sarcocornia fruticosa	**93.5 ± 2.9**	
Habitat 2		
Elymus pycnanthus	**68.4 ± 7.6 a**	
Atriplex portulacoides	17.2 ± 6.7 b	
Others sp	14.4 ± 4.6 b	
Habitat 3	2015	2016
Salicornia patula	**98.9 ± 0.5**	**71.8 ± 15.3 a**
Sarcocornia fruticosa		21.3 ± 15.7 b
Habitat 4	2015	2017
Foeniculum vulgare	32.9 ± 9.6 a	
Inula viscosa	19.1 ± 9.9 a	
Others sp	47.9 ± 10.2 a	
Suaeda maritima		73.6 ± 21.7 a
Cynodon dactylon		22.7 ± 22.7 a

Table 4. Percentage of total biomass accounted for the dominant species of each habitat (mean ± SE). In habitats 1 and 2 no significant differences were found between years, and, because of that, the average is given. Different letters indicate significant differences between species in the percentage of biomass within the same habitat ($p < 0.05$).

Considering the percentage of biomass that dominant species represent in each habitat, *S. fruticosa* accounted for ≈ 94% of the total biomass of habitat 1, with the highest amount of carbon being stored in both woody and standing dead tissues (Tables 4, 5). In habitat 2, *E. pycnanthus* had the highest percentage of biomass with carbon being stored mainly in dead tissues. In habitat 3, *S. patula*, an annual species, accounted for the highest percentage of biomass with carbon being stored either in living or dead tissues.

	Carbon (g C m^{-2})
Habitat 1	
Sarcocornia fruticosa	
Green	120.1 ± 17.4 a
Woody	**663.9 ± 78.1 b**
Standing dead	**652.2 ± 121.0 b**
Habitat 2	
Elymus pycnanthus	
Green	77.2 ± 7.8 a
Standing dead	**260.0 ± 45.9 b**
Habitat 3	
Salicornia patula	
Green	49.2 ± 11.4
Standing dead	33.2 ± 6.2

Table 5. Carbon content in green, woody (in case of woody species) and standing dead tissues of the dominant species of the three well-preserved habitats (mean ± SE, n=10). Different letters indicate significant differences between fractions in stored carbon within the same species ($p<0.05$).

In habitat 4, the dominant species in 2015 (i.e, before the restoration) were *Foeniculum vulgare* and *Inula viscosa*, which showed similar relative values of biomass (Table 4). One year after the restoration, the dominant species was *Suaeda maritima*, accounting for ≈ 74% of the total biomass. However, results obtained after the restoration must be interpreted with caution since only four of the ten plots sampled at that moment had vegetation. A much longer period is needed to evaluate correctly the results of the natural revegetation process.

2.1.3.2. Net aboveground primary production (NAPP)

In the well-preserved habitats 1 and 2 no significant differences were found between the two years of the study (2015 and 2016) in the living, dead or total aboveground biomass and neither in their carbon content (Figure 3a,b); thus, NAPP of these two habitats was considered zero. In habitat 3, dominated by annual pioneer vegetation, there was a significant increment in the carbon stored in total biomass between 2015 and 2016 (Figure 3c). This increment occurred in the living biomass (green and woody together), indicating that NAPP was positive in this habitat (Figure 3c). The estimated NAPP for habitat 3 was 59.36 g C m^{-2} y^{-1} for total biomass and 37.93 g C m^{-2} y^{-1} for living biomass.

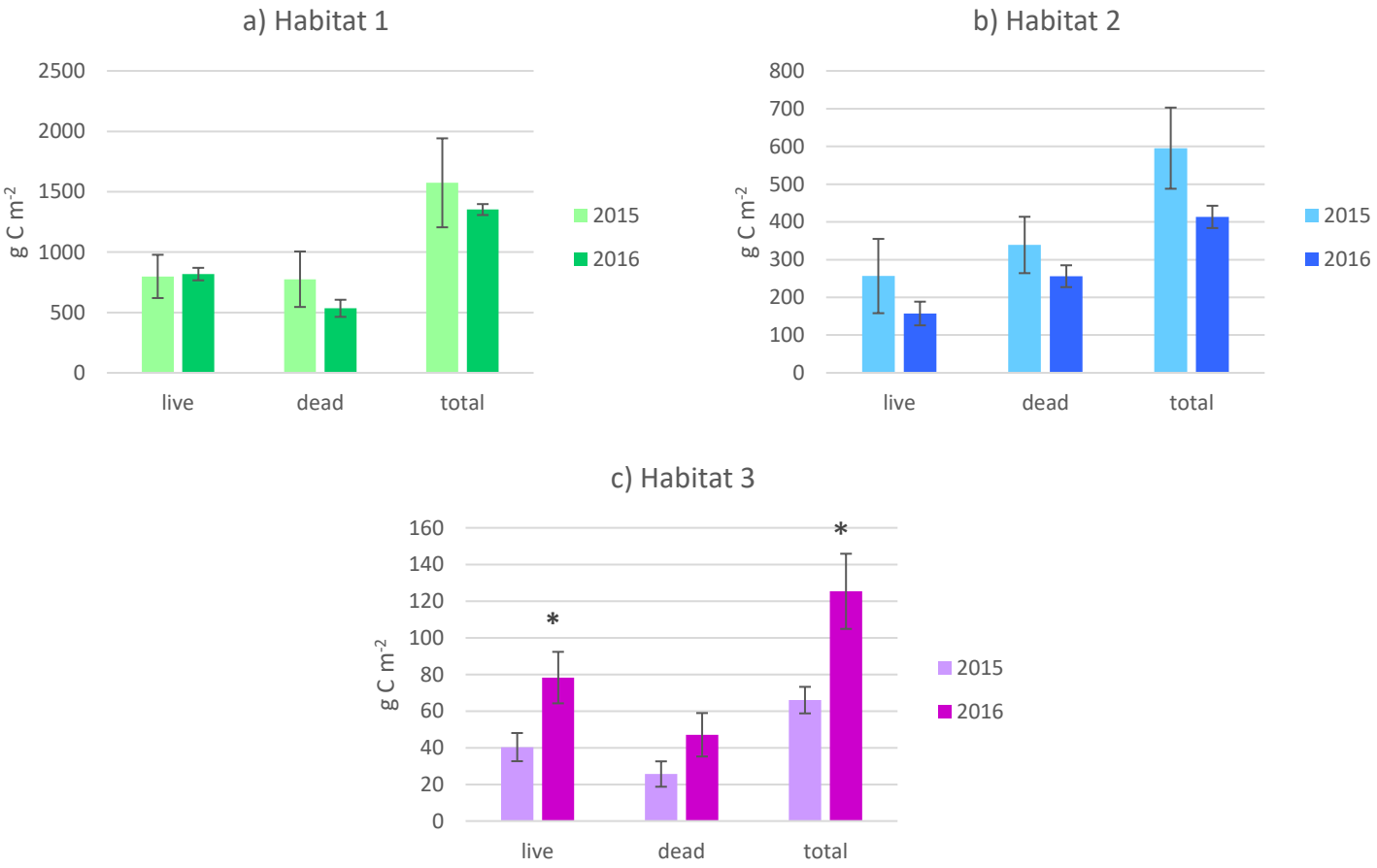


Figure 3. Averaged carbon stored in living, dead and total aboveground biomass for habitat 1 (a), habitat 2 (b), and habitat 3 (c) in 2015 and 2016. Bars represent ± standard errors. Asterisks indicate significant differences in carbon content between years ($p < 0.05$).

2.1.3.3. Net belowground primary production (NBPP)

Significant differences in the belowground biomass, as well as in its carbon content, were not detected between the two years of the study in any of the three well-preserved habitats considered. Therefore, NBPP was not significant during the study period.

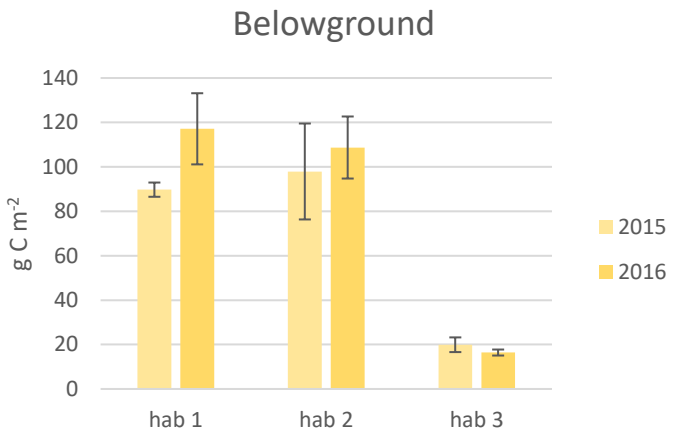


Figure 4. Carbon stored in total belowground biomass for the three well-preserved habitats in 2015 and 2016. Bars represent ± standard errors.

2.1.3.4. Annual changes in the amount of carbon stored in the litter

The amount of litter, as well as the litter carbon content, were similar between the two studied years in the three well-preserved habitats (Figure 5).

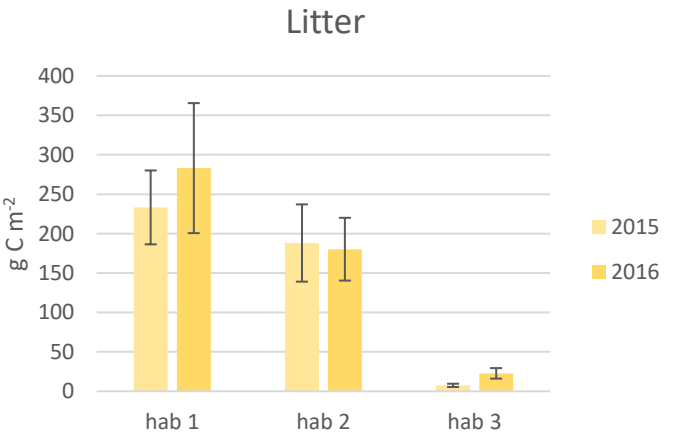


Figure 5. Carbon amount of litter for the three well-preserved habitats in 2015 and 2016. Bars represent ± standard error.

2.2. Litter decomposition

2.2.1. Sampling and litterbag method

Litter decomposition analyses were carried out only in the shrubland and grassland habitats (1 and 2, respectively), because the amount of litter was insignificant in habitat 3 (dominated by annual pioneer vegetation). To assess the litter decomposition process, senescent or recently dead plant material of the dominant species of these two habitats (Table 6) was collected at the beginning of November 2016, coinciding with the peak of autumn senescence of these plants. Specifically, plant material used was, for habitat 1, senescent stems of *S. fruticosa* and, for habitat 2, senescent leaves of *A. portulacoides* and recent dead leaves of *E. pychnantus*.

(a) (b)

Figure 6. Sites where litterbags were placed (represented by dots) in order to study the litter decomposition process in habitat 1 and habitat 2 (a); litterbags placed in the field (b).

Plant senescent/dead material collected in the field was brought to the laboratory, mixed to provide a uniform initial sample for each species, air-dried for 9 days and placed into litterbags (15 cm x 15 cm, 1 mm of mesh size). For each species, two extra samples were oven dried (70ºC) to constant weight, being used to calculate a conversion factor to estimate the oven dry weight of plant material in each litterbag at the start of the experiment. In the middle of November 2016, litterbags were placed in six sites per habitat, which were selected in order to cover the edaphic heterogeneity of each habitat (Figure 6a). Litterbags of each species (36 for *S. fruticosa* and *A. portulacoides* and 48 for *E. pycnanthus*) were attached with nylon string in groups, one per site for habitat 1 (*S. fruticosa*) and two per site for habitat 2 (*A. portulacoides* and *E. pycnanthus*). Litterbags were placed on soil surface under the canopy of each respective species (Figure 6b) and six litterbags per species and habitat were periodically collected for laboratory analyses (Table 6).

Habitat	Specie	Days of litterbag recollection
1	*Sarcocornia fruticosa*	5, 10, 19, 38, 64, 92
2	*Elymus pycnanthus*	5, 10, 19, 38, 92, 164, 240, 357
2	*Atriplex portulacoides*	5, 10, 19, 38, 64, 92

Table 6. Days of litterbag collection, counted from the start of the experiment, for each one of the species selected in each habitat.

2.2.2. Laboratory and data analyses

Plant material from each litterbag was rinsed with distilled water to remove soil particles and mesofauna, dried (70 ◦C) and weighed to assess mass loss from the beginning. The initial and the decomposed oven-dried plant material were ashed at 450 °C for 4h, and weighed again to calculate the ash free dry weight (AFDW). Litter decomposition rates (k day^{-1}) per species were calculated using a single exponential decay model regression $X_t=X_0e^{-kt}$; where X_t = litter mass at time t (days) and X_0= initial mass. Total C and N were determined from the dried, ground material at the Stable Isotope Facility of the University of California, Davis (see section 2.1.2). Cellulose and lignin content were estimated also in dried, ground material by gravimetric determination following Gessner (2005a).

The activity of four extracellular enzymes related to litter decomposition was measured in the plant material from the litterbags immediately after collection. Two hydrolytic enzymes involved in cellulose and hemicellulose degradation (ß-D-glucosidase and ß-D-xylosidase, respectively); one hydrolytic enzyme related with peptide breakdown (leucine-aminopeptidase) and one oxidative enzyme involved in lignin degradation (phenol oxidase). Hydrolytic enzymes were measured by fluorimetric assays using the methylumbelliferyl (MUF)-substrate method for β-glucosidase and ß-D-xylosidase, and the methylcoumain (AMC)-substrate method for leucine-aminopeptidase. Phenol oxidase was measured using L-DOPA (L-3,4-dihydroxyphenylalanine). Enzyme efficiencies were calculated as Turnover Activities (TA), i.e. TA = $1/k_2$, where k_2 is the slope of Ln % remaining AFDW regressed linearly to the accumulated enzyme activity (AEA). AEA was calculated using the formula $AEA=\sum_{i=0}^{n} E_i T_i$ where n is the number of sampling dates, E_i is the mean enzyme activity of two successive measurements, and T_i is the time between the two measurements (Waring, 2013). Turnover Activity is expressed as mmol of enzyme produced to decompose one gram of litter (mmol g^{-1}), and higher values mean low enzyme efficiencies. Carbon of fungal biomass was estimated from ergosterol concentration in the litter according to Gessner (2005b), since this compound has been demonstrated to be a useful indicator of fungal biomass (Lee et al., 1980). Ergosterol concentration was converted to fungal biomass considering that a) there are 5.5 µg of ergosterol in one gram of fungal biomass (Gessner and Chauvet 1993) and b) fungal dry mass has a 43% of carbon content (Baldy and Gessner 1997).

A Principal Component Analysis (PCA) was performed using soil parameters to analyse the edaphic heterogeneity between and within the two habitats. After performing the PCA two zones were differentiated within each habitat (Figure 7). In habitat 1, soil electric conductivity and silt content explained the two zones separation. In habitat 2, volumetric water content, soil organic

carbon, total glomalin, total nitrogen and potential respiration determined the differences between the two zones. Differences between species, habitats and zones within each habitat for the different parameters measured during the litter decomposition process (mass loss, AFDW, litter decomposition rate, TA, AEA, and litter content in cellulose, lignin, C and N) were evaluated by means of ANOVAs (and post-hoc tests) and, when necessary, using the non-parametric Kruskal-Wallis and Mann-Whitney U tests.

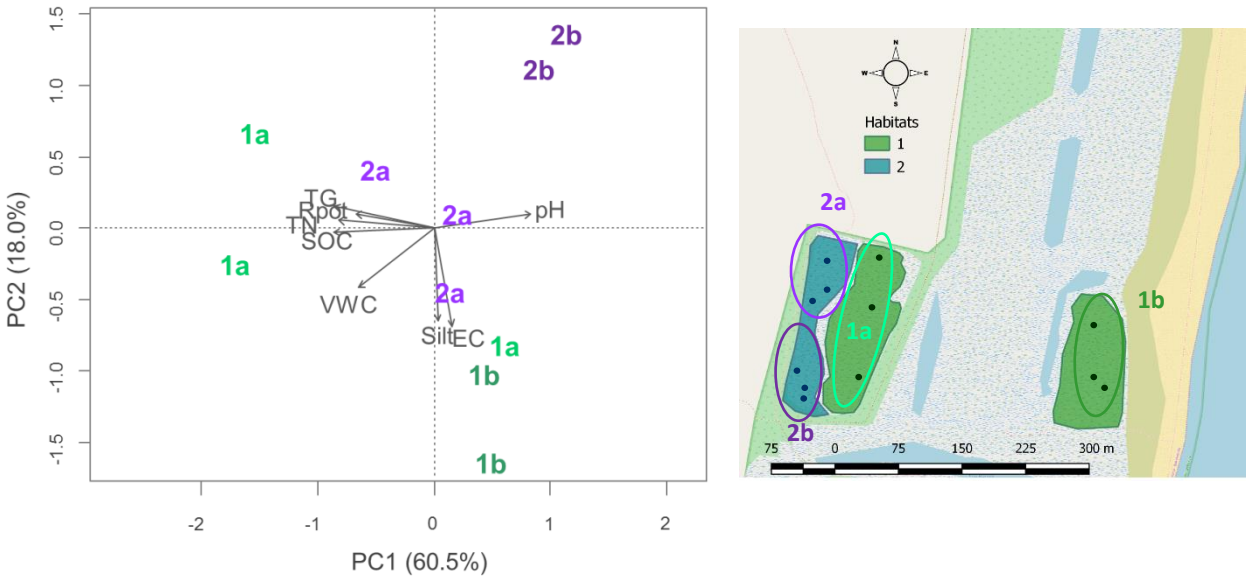


Figure 7. (Left) Principal Component Analysis (PCA) performed with the following edaphic parameters: total glomalin (TG), potential respiration (Rpot), total nitrogen (TN), soil organic carbon (SOC), volumetric water content (VWC), silt content, electric conductivity (E.C.) and pH. Habitat 1 is represented in green and Habitat 2 in blue. Letters a and b represent the different zones distinguished within each habitat. (Right) Distribution of the zones differentiated within each habitat.

2.2.3. Results

2.2.3.1. Litter quality

E. pycnanthus litter had higher initial carbon content than the other species, whereas *A. portulacoides* litter had the lowest values in all the sampling dates (Figure 8a). Carbon concentration of the litter tended to increase throughout the decomposition process in *S. fruticosa* and *A. portulacoides*. Regarding nitrogen concentration, litter of *S. fruticosa* had the highest values (Figure 8b) and consequently, the lowest values of C:N ratio throughout the decomposition process (Figure 8c). *A. portulacoides* showed the highest initial C:N ratio in the litter, which tended to decrease at the end of the decomposition period due to the increase in the N content.

Regarding cellulose and lignin content, litter of *E. pycnanthus* had always the highest values of cellulose, having also the highest initial content of lignin (Figure 8). However, at the end of the decomposition period, the highest values of lignin were found in *S. fruticosa* litter. In both *S.*

fruticosa and *A. portulacoides* litter the content of cellulose and lignin increased throughout the decomposition process.

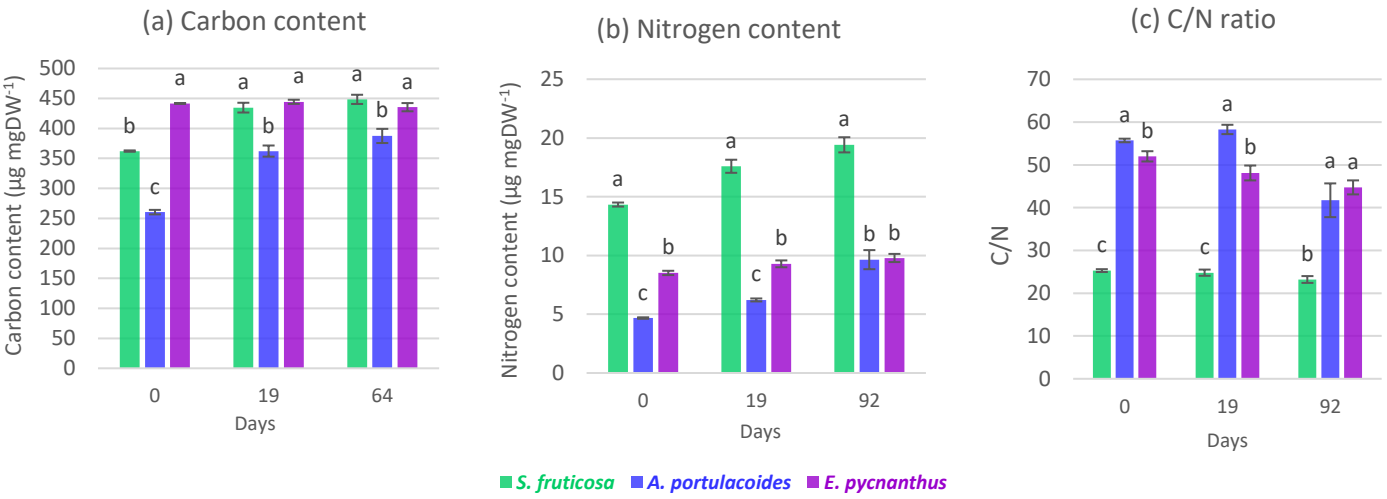


Figure 8. Concentrations of carbon (a), nitrogen (b) and C:N ratio (c) at the beginning, middle and end of the studied decomposition period. Bars represent ± standard error (n=6). Different letters indicate significant differences between species within each date (p<0.05)

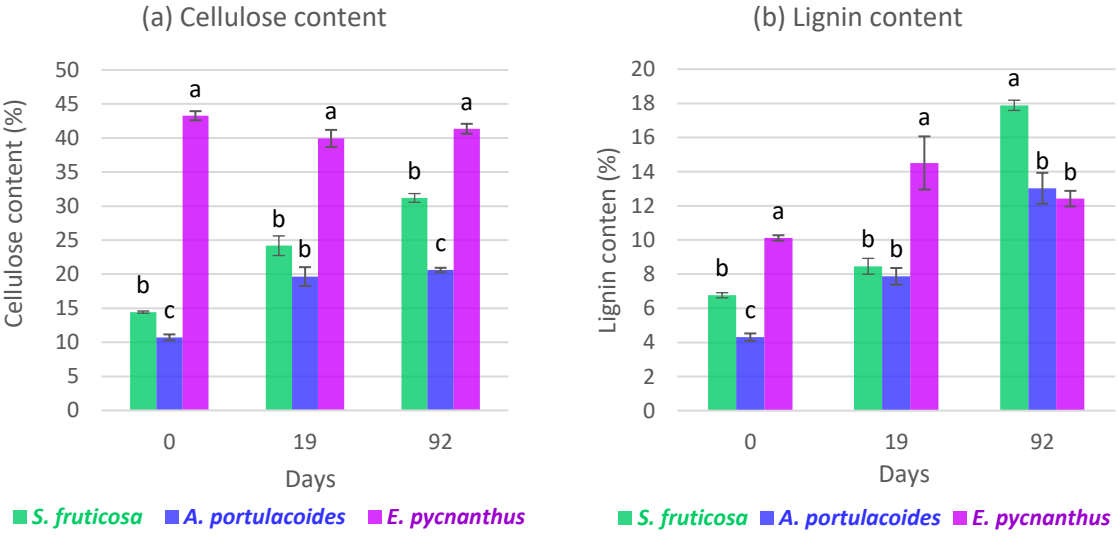
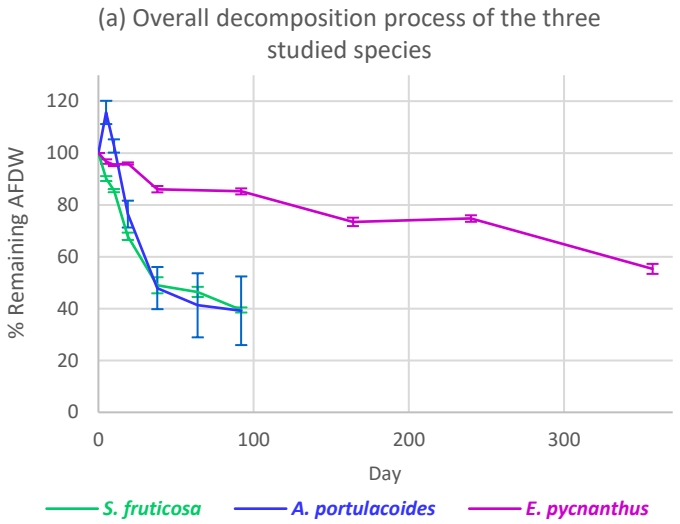


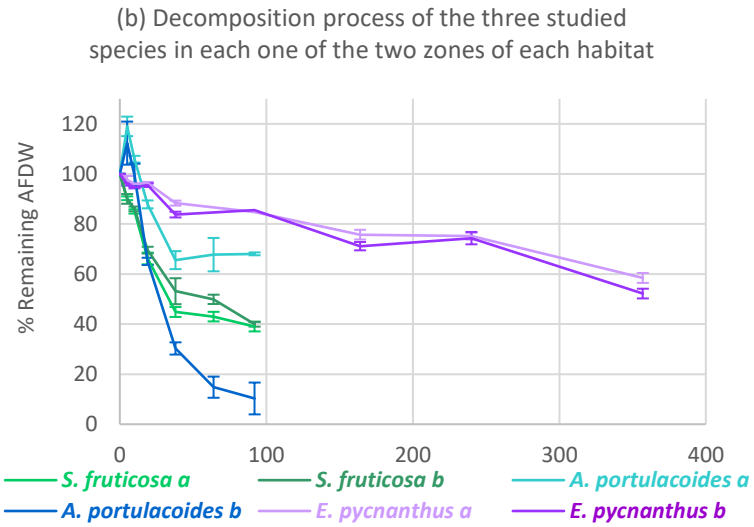
Figure 9. Cellulose (a) and lignin (b) content (%) in the litter at the beginning, middle and end of the studied decomposition period. Bars represent ± standard error (n=6). Different letters indicate significant differences between species within each date (p<0.05)

2.2.3.2. Litter decomposition rates

Among the three species analyzed, *E. pycnanthus* of habitat 2 had the lowest decomposition rates (Figure 10a). Indeed, after almost a year, more than 50% of the *E. pycnanthus* litter was still present.

On the other hand, decomposition rates of *A. portulacoides* showed a high variability (Figure 10b) due to big differences between the two zones of habitat 2 (Figure 7).





Species	k (d^{-1}) ± SD
S. fruticosa	0.0102 ± 0.0002 a
A. portulacoides	0.0193 ± 0.0068 ab
E. pycnanthus	0.0015 ± 0.0001 b

Species	Zone	k (d^{-1}) ± SD
S. fruticosa	1a	0.0106 ± 0.0002 *
	1b	0.0097 ± 0.0001 *
A. portulacoides	2a	0.0059 ± 0.0005 *
	2b	0.0326 ± 0.0074 *
E. pycnanthus	2a	0.0014 ± 0.0001
	2b	0.0016 ± 0.0001

Figure 10. Decomposition process of the three studied species expressed as remaining ash free dry weight (% of initial) in each sampling date (a) and separating the two zones within each habitat (b). Bars represent ± standard error. Under each graph, values (mean ± SE) of decomposition rates (k) are presented. Different letters indicate significant differences between species (n=6, p<0.05). Asterisks indicate significant differences in decomposition rates between zones for each species (n=3, p<0.05)

2.2.3.3. Potential extracellular enzymatic activity

In general, enzyme activity increased with time, although some differences were found among species. Specifically, it is remarkable the pronounced increase in the activity of leucine-aminopeptidase and phenol oxidase in *A. portulacoides* litter (Figure 11c, d). However, when these enzyme activities in *A. portulacoides* litter were analyzed considering the two different zones within habitat 2, it was observed that the high values were mainly due to the enzyme activities in one of the zones (zone 2b) (Figure 12).

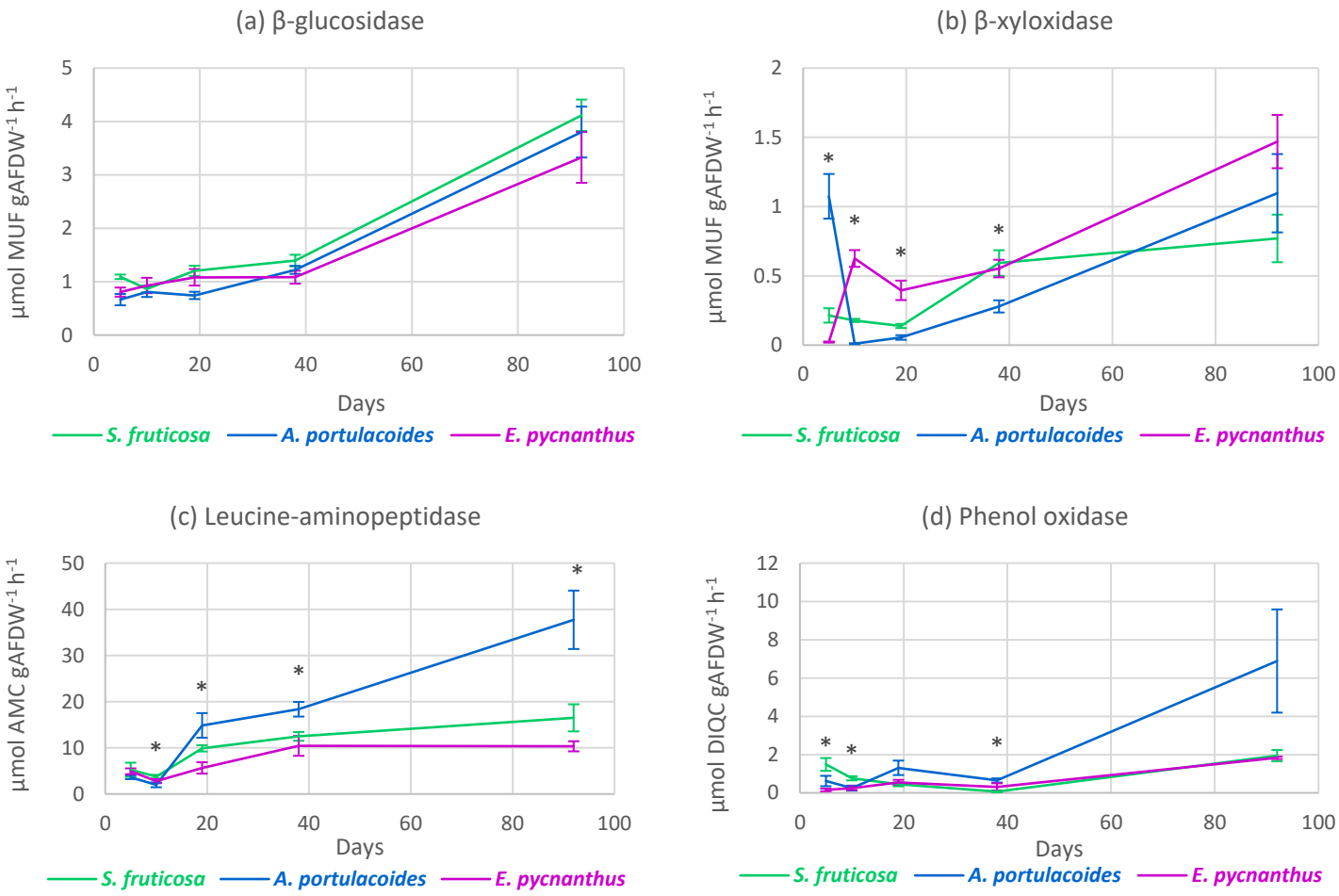


Figure 11. Data from potential extracellular enzymatic activities of β-glucosidase (a), β-xyloxidase (b), leucine-aminopeptidase (c) and phenol oxidase (d) from litter of the three species studied (n=6). Bars represent ± standard error Asterisks indicate significant differences among species within each day ($p<0.05$).

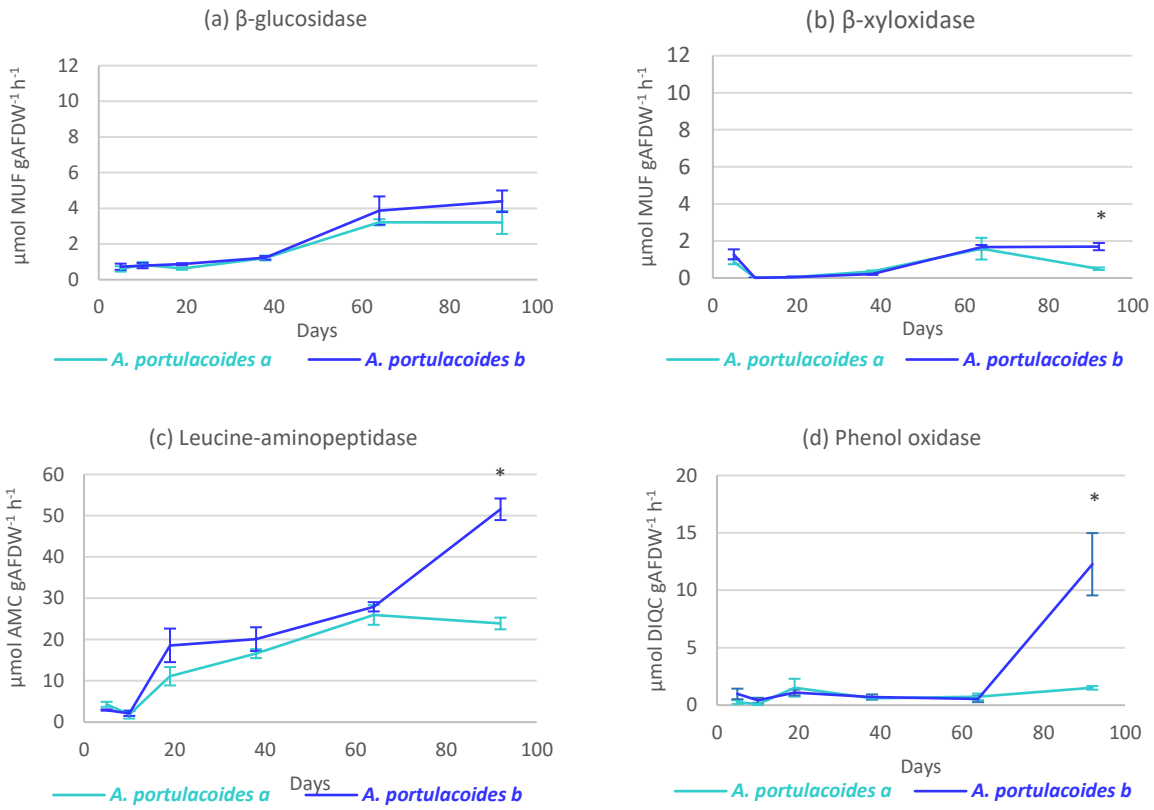


Figure 12. Potential extracellular enzymatic activities of β-glucosidase (a), β-xyloxidase (b), leucine-aminopeptidase (c) and phenol oxidase (d) in *Atriplex portulacoides* litter located at the two zones (a and b) distinguished within habitat 2 (n=3). Bars represent ± standard error. Asterisks indicate significant differences between zones in each date (p<0.05).

2.2.3.4. Carbon of fungal biomass

Carbon of fungal biomass at the beginning of the decomposition process was similar for the litter of the three studied species (Figure 13a). However, at the end of the studied decomposition period, *S. fruticosa* and *A. portulacoides* presented higher values than *E. pycnanthus.* In the case of *A. portulacoides,* as occurred in the other parameters, differences in carbon of fungal biomass were found between the two zones of habitat 2 at the end of the study, having higher values in zone 2b (Figure 13b).

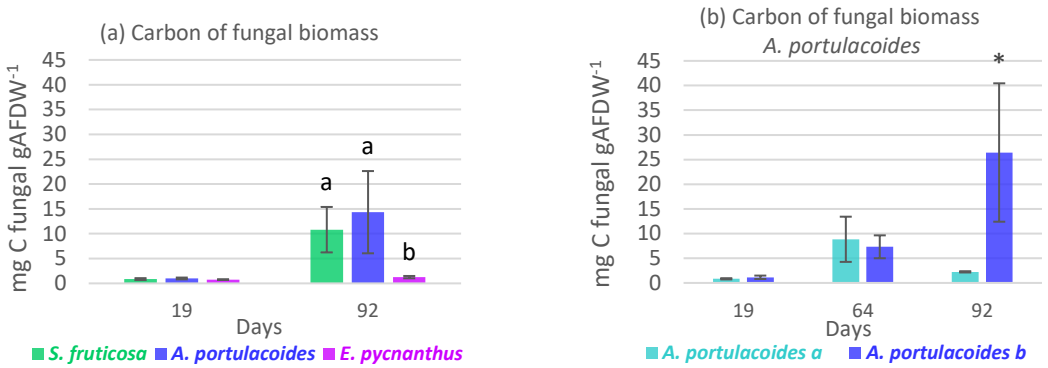


Figure 13. Carbon of fungal biomass in the litter at the beginning and end of the decomposition period for the three studied species (a) and for *Atriplex portulacoides* distinguishing the two zones considered within habitat 2 (b). Bars represent ± standard error. Different letters indicate significant differences between species within each date ($p<0.05$). Asterisks indicate significant differences in carbon of fungal biomass in *A. portulacoides* litter between the two zones of habitat 2 and per each date ($p<0.05$).

2.3. Soil parameters and carbon stored in soil

2.3.1. Sampling

Soil samples were taken by manual drilling (auger set; Eijkelkamp Inc.) during summer of 2015 and 2016 in plots coinciding with those in which biomass was harvested. At each plot, three soil samples were taken to give a composite soil sample per plot. Samples of 2015 were initially taken from 0-20 cm and 20-40 cm depth. However, the first 0-5 cm was very different than the below ones, so an additional sampling of 0-5 cm depth were done for every plot. Samples of 2016 were only taken at 0-5 cm and 5-20 cm depth, since results of 2015 showed that soil characteristics of the first 20 cm mostly determine soil carbon parameters.

In 2015 soil samples were taken in three plots of each four habitats studied and principal soil parameters were analysed (pH, electric conductivity (EC), total glomalin (GRSP) and easily extracted glomalin content (EE-GRSP), oxidizable organic carbon and total nitrogen content). In summer of 2016, it was decided to extend the soil sampling and analysis. Soil samples were taken in all of the five plots of each habitat of the well-conserved zone (habitats 1, 2 and 3) used for biomass harvesting in that year and, besides the repetition of the analyses already done for the samples of 2015, some new parameters were analysed. Within them, bulk density (BD), water stability of aggregates (WSA), calcium carbonate content, soil textural classes, exchangeable sodium percentage (ESP) and the composition of the organic matter by pyrolysis-gas chromatography (Py-GC). The increment in the number of sampling points (from three to five) and soil parameter analyses was done to improve the soil characterization of the different habitats, since results of 2015 showed that there was a high heterogeneity within habitats. Consequently, this would improve our understanding about the relations between soil parameters and soil carbon storage, but also about how these parameters could be affecting the dynamic of carbon fluxes between soil surface and atmosphere in these habitats (section 3.2).

In addition, soil profiles in each habitat of the well-conserved area were open and characterized for soil classification at the beginning of 2018. For that, some field determination was done and soil samples at each horizon were taken to analyse soil parameters.

2.3.2. Laboratory and data analyses

Samples were air-dried in the lab and belowground biomass was separated from soil. The estimation of the major part of physicochemical and biochemical parameters were done according with the standard procedures for the soil analysis and characterization (ISRIC, 2002). The three samples taken at each depth for every plot were bulked to give one composite sample per plot and depth. Air-dry composite soil samples were sieved to 5.6, 2.0 and 0.25 mm. Soil aggregates of the fractions 2 - 5.6 mm and 0.02 - 2 mm were used to estimate the WSA. The rest of the soil properties were determined on the fine earth (soil fraction sieved to 2 mm). Measurement of WSA were determined following Kemper and Rosenau (1986) using a wet sieving apparatus Model: 08.13 by Eijkelkamp Inc. This parameter gives information about the resistance of the aggregates after immersion/emersion in water and classes or resistance can be determinate (0-20% very low resistance; 20-40% low resistance; 40-60% moderate resistance; 60-80% high resistance; 80-100% very high resistance). Regarding soil textural classes, sand was determined using sieves with different mesh sizes (200 µm and 20 µm) and silt and clay using a calibrated volumetric pipette and considering the principle of sedimentation of soil particles. Soil pH was determined potentiometrically in distilled water and with soil:solution ratio 1:2.5 and electrical conductivity was measured with soil:water ratio 1:5. A known volume of every air-dried sample was weight and then used for bulk density estimation, after doing an adjustment by humidity, taken into account the weight of the samples after 105ºC to constant weight. 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). Calcic carbonate content was measured by means of the Bernard calcimeter (Eijkelkamp 08.53). ESP was calculated using the formula described by the United Stated Department of Agriculture (USDA, 1954), from values of sodium absorption ratio (SAR) obtained by the determination of Na^{+}, Ca^{2+} and Mg^{2+} concentrations of water-saturate soil paste.

Regarding biochemical parameters, soil organic carbon (SOC) was quantified by the dichromate wet oxidation method (Walkley and Black, 1934) in presence of concentrated sulfuric acid. Soil organic matter (SOM) was estimated from SOC values multiplying by the conversion factor of 1.724 (Van Bemmelen factor), considering that organic carbon represents 58% of organic matter. Total nitrogen (TN) was determined by means of the standard Kjeldahl method. Potential respiration was measured by the soda lime technique (Grogan, 1998). Easily extractable glomalin related soil protein (EE-GRSP) and total glomalin (GRSP) contents were quantified using the method described for Wright (1996). The composition of organic matter was analyzed at 0-5 cm depth by pyrolysis-gas chromatography (Py-GC). Pyrograms were interpreted by quantifying the seven characteristic peaks corresponding to the major volatile components (Table 7). The peak areas were standardized so that the area under each peak referred to the percentage of the total all peaks (relative abundances).

Volatile component	Cod.	Component of SOM from which derived
Acetonitrile	E_1	Aminoacids, proteins, microorganism cells
Furfural	N	Cellulose and others aliphatic organic compounds
Acetic acid	K	Lipids, fats, waxes, cellulose
Phenol	Y	Fresh or condensed (humic) lignocellulosic structures
Benzene	B	Condensed aromatic structures of stable (humified) organic matter
Toluene	E_3	Aromatic uncondensed rings with aliphatic chains.
Pyrrole	O	Nucleid acids, proteins, microbial cells, condensed humic structures

Table 7. Volatile components derived from the pyrolysis-gas chromatography of the SOM, codes used for identification and principal SOM components from which derived according to Bracewell and Robertson (1976).

Some ratios between relative abundances of some of the volatile components were also determined:

- **O/N**: Mineralization index of labile fractions of SOM. The larger ratio, the larger rates of SOM mineralization
- **O/Y**: Mineralization index of stable fractions of SOM. The larger ratio, the larger rates of SOM mineralization
- **B/ E_3**: Humification index. Higher ratios indicate higher condensation of organic matter
- **AL/AR**: Index of energetic reservoir. It expresses the ratio between the sum of aliphatic (acetonitrile E_1, acetic acid K and furfural N) and aromatic (benzene B, toluene E_3, pyrrole O and phenol Y) components of SOM

Data was statistically analyzed by means of ANOVAs and Tukey's HDS post-hoc tests to determine differences between habitats. In case that data did not accomplish normality and homoscedasticity non parametric tests (Kruskal-Wallis and Mann-Whitney U test) were applied.

2.3.3. Results

2.3.3.1. Soil physicochemical parameters

All the soils showed slightly alkaline pH values, with differences between habitats, but only at 0-5 cm, having the grassland habitat (3) the highest values (around 8.5) in both years (Table 8, 9). Electrical conductivity, also in both years, was very variable between habitats at all the depths, being habitat 1 and 3 the most saline because in this habitats flood comes mainly from the sea water. In the soil samples of 2016, habitat 1 and 2 had the lowest bulk density values (only at 0-5 cm) and the highest WSA for both aggregates classes and depths, with values corresponding to the class "very high resistance" at 0-5 cm and "low resistance" in the deeper samples (Table 9).

Habitat	Depth cm	pH	E.C. dSm^{-1}
1	0-5	**7.35 ± 0.24 a**	**12.23 ± 2.03 a**
2	0-5	**8.02 ± 0.34 ab**	**0.25 ± 0.10 b**
3	0-5	**8.72 ± 0.13 b**	**5.87 ± 3.43 a**
4	0-5	**8.26 ± 1.33 ab**	**0.15 ± 0.05 b**
1	0-20	8.11 ± 0.16	**4.26 ± 0.48 a**
2	0-20	8.40 ± 0.18	**0.31 ± 0.14 b**
3	0-20	8.71 ± 0.27	**3.14 ± 0.99 a**
4	0-20	8.36 ± 0.25	**0.12 ± 0.03 b**
1	20-40	8.51 ± 0.10	**2.87 ± 0.48 a**
2	20-40	8.71 ± 0.31	**0.44 ± 0.32 b**
3	20-40	8.73 ± 0.19	**2.28 ± 0.69 a**
4	20-40	8.50 ± 0.09	**0.01 ± 0.02 b**

Table 8. Soil physicochemical parameters of the sampling in 2015 (mean ± SD, n=3). Different letters indicates significant differences between habitats, separating by depth (p<0.05).

Habitat	Depth cm	pH	EC dSm^{-1}	B.D. gcm^{-3}	WSA 0.25 - 2.0 %	WSA 2.0 - 5.6 %	$CaCO_3$ %	Silt %	Clay %	Sand %	ESP* %
1	0-5	**7.84 ± 0.19 a**	**12.74 ± 5.58 a**	**0.81 ± 0.17 a**	**80.33 ± 8.23 ab**	**90.60 ± 1.29 ab**	7.56 ± 4.06	32.35 ± 10.40	38.78 ± 11.87	28.87 ± 18.60	53.59 ± 7.2
2	0-5	**7.92 ± 0.30 a**	**2.78 ± 2.29 b**	**0.89 ± 0.10 a**	**93.84 ± 1.97 a**	**92.83 ± 1.59 a**	7.36 ± 1.94	27.08 ± 16.46	30.86 ± 8.32	42.06 ± 23.93	54.49 ± 10.0
3	0-5	**8.44 ± 0.13 b**	**12.64 ± 3.17 a**	**1.20 ± 0.10 b**	**69.87 ± 16.10 b**	**54.74 ± 21.38 b**	8.41 ± 2.84	27.08 ± 11.03	33.24 ± 5.42	39.69 ± 16.09	55.6 ± 4.5
1	5-20	8.37 ± 0.24	**4.22 ± 1.87 a**	1.04 ± 0.11	44.88 ± 20.00	**29.65 ± 9.83 ab**	9.95 ± 1.24	34.11 ± 9.07	36.31 ± 8.12	29.58 ± 17.06	-
2	5-20	8.55 ± 0.25	**1.24 ± 0.99 b**	1.07 ± 0.12	64.30 ± 22.41	**36.85 ± 19.11 a**	9.35 ± 2.76	25.39 ± 12.19	27.87 ± 5.97	46.74 ± 18.05	-
3	5-20	8.60 ± 0.11	**4.50 ± 1.12 a**	1.13 ± 0.07	40.13 ± 14.57	**10.27 ± 13.48 b**	7.80 ± 2.46	28.36 ± 11.17	33.73 ± 5.75	37.91 ± 16.27	-

Table 9. Soil physicochemical parameters of the sampling in 2016 (mean ± SD, n=5). Different letters indicate significant differences between habitats within each depth (p<0.05) *ESP was determined at 0-20 depth.

There were no significant differences between habitats in $CaCO_3$ content nor in ESP. In the case of ESP, all habitats of the well-conserved area had high values (>15%). This, together with the high EC (EC >1dS m^{-1}), allowed us to classify these soils as saline-sodic soils. The textural classes presented a remarkably high variation that masked the differences between habitats and corroborated the high spatial heterogeneity of the area.

2.3.3.2. Soil biological and biochemical parameters

Similar values of SOC, SOM, TN and glomalin content were found in habitats 1 and 2 of the well-conserved area and in the disturbed habitat, being higher in this habitats than in the habitat 3

dominated by annual herbaceous species (Table 10, 11). The potential respiration was lower in habitat 3, which agrees with the results of soil respiration measured in the field (section 3.2).

Habitat	Depth cm	**SOC %**	**SOM %**	TN %	C:N	GRSP mg g^{-1}	EE-GRSP mg g^{-1}
1	0-5	**4.00 ± 2.12 a**	**6.89 ± 3.66 a**	**0.188 ± 0.082 a**	**20.52 ± 3.34 a**	**8.87 ± 2.93 ab**	**1.14 ± 0.19 a**
2	0-5	**2.36 ± ± 0.74 a**	**4.07 ± 1.28 a**	**0.074 ± 0.020 b**	**31.60 ± 2.02 a**	**5.31 ± 2.09 ab**	**1.02 ± 0.12 a**
3	0-5	**0.44 ± 0.10 b**	**0.76 ± 0.17 b**	**0.022 ± 0.006 c**	**21.30 ± 8.98 a**	**0.46 ± 0.08 a**	**0.26 ± 0.05 b**
4	0-5	**1.60 ± 0.60 a**	**2.76 ± 1.03 a**	**0.034 ± 0.002 bc**	**46.76 ± 2.87 b**	**2.66 ± 1.55 b**	**0.86 ± 0.18 a**
1	0-20	**1.11 ± 0.5 ab**	**1.92 ± 0.9 ab**	0.046 ± 0.018	**35.91 ± 12.26 ac**	**2.89 ± 0.80 a**	**0.70 ± 0.14 a**
2	0-20	**0.75 ± 0.41 ab**	**1.29 ± 0.71 ab**	0.039 ± 0.010	**15.00 ± 4.94 b**	**2.02 ± 0.58 ab**	**0.42 ± 0.13 ab**
3	0-20	**0.35 ± 0.23 b**	**0.60 ± 0.40 b**	0.017 ± 0.014	**23.09 ± 6.06 ab**	**0.82 ± 1.09 b**	**0.17 ± 0.20 b**
4	0-20	**1.53 ± 0.44 a**	**2.64 ± 0.76 a**	0.030 ± 0.008	**50.90 ± 6.35 c**	**2.32 ± 0.07 ab**	**0.78 ± 0.03 a**
1	20-40	**0.60 ± 0.16 ab**	**1.04 ± 0.28 ab**	0.024 ± 0.004	24.96 ± 5.72	**1.03 ± 0.51 a**	**0.25 ± 0.06 a**
2	20-40	**0.23 ± 0.04 ac**	**0.40 ± 0.08 ac**	0.013 ± 0.007	27.97 ± 24.60	**0.89 ± 0.37 a**	**0.15 ± 0.09 a**
3	20-40	**0.20 ± 0.14 c**	**0.34 ± 0.25 c**	0.012 ± 0.010	20.70 ± 9.47	**0.74 ± 0.44 a**	**0.30 ± 0.25 a**
4	20-40	**0.93 ± 0.35 b**	**1.61 ± 0.60 b**	0.028 ± 0.005	35.30 ± 16.88	**2.43 ± 0.54 b**	**0.83 ± 0.10 b**

Table 10. Soil biological and biochemical parameters of the sampling in 2015 (mean ± SD, n=3). Different letters indicates significant differences between habitats within each depth (p<0.05)

Habitat	Depth cm	SOC %	SOM %	TN %	C:N	Potential respiration $mgCO_2\ g^{-1}dia^{-1}$	GRSP mg g^{-1}	EE-GRSP mg g^{-1}
1	0-5	**1.86 ± 0.65 a**	**3.21 ± 1.13 a**	**0.09 ± 0.02 a**	**21.42 ± 4.40 a**	**0.507 ± 0.028 a**	**3.29 ± 0.82 a**	**0.63 ± 0.02 a**
2	0-5	**1.65 ± 0.63 a**	**2.85 ± 1.09 a**	**0.09 ± 0.03 a**	**18.10 ± 1.12 ab**	**0.493 ± 0.016 a**	**3.28 ± 1.20 a**	**0.63 ± 0.08 a**
3	0-5	**0.57 ± 0.11 b**	**0.99 ± 0.18 b**	**0.04 ± 0.01 b**	**15.38 ± 1.58 b**	**0.348 ± 0.027 b**	**0.76 ± 0.08 b**	**0.31 ± 0.03 b**
1	5-20	**0.66 ± 0.32 ab**	**1.13 ± 0.54 ab**	0.03 ± 0.01	20.23 ± 4.32	0.073 ± 0.031	**1.17 ± 0.60 ab**	**0.19 ± 0.09 ab**
2	5-20	**0.80 ± 0.33 a**	**1.38 ± 0.57 a**	0.04 ± 0.01	21.82 ± 2.20	0.213 ± 0.146	**1.39 ± 0.61 a**	**0.30 ± 0.05 a**
3	5-20	**0.37 ± 0.15 b**	**0.64 ± 0.26 b**	0.02 ± 0.01	16.66 ± 2.91	0.103 ± 0.064	**0.48 ± 0.33 b**	**0.12 ± 0.05 b**

Table 11. Soil biological and biochemical parameters of the sampling in 2016 (mean ± SD, n=5). Different letters indicates significant differences between habitats within each depth (p<0.05).

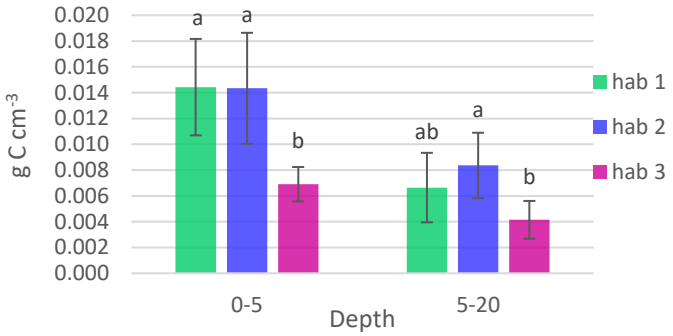


Figure 14. Soil organic carbon content, of the sampling in 2016 (mean ± SD, n=5), expressed in g C cm^{-3} at 0-5 cm and 5-20 cm depth for each habitat. Different letters indicates significant differences between habitats within each depth (p<0.05).

The composition of organic matter in habitats 1 and 2 showed lower values of benzene, toluene and higher values of acetic acid and phenol than in habitat 3 (Table 12). This indicates that the organic matter in habitat 1, and especially in habitat 2, is fresher. Comparing the ratios between relative abundances of some of the volatile components, habitat 3 had the higher values of O/Y ratio (mineralization index of stable fractions of SOM) (Table 13).

Habitat	Depth cm	**Acetonitrile E_1**	**Acetic acid K**	**Benzene B**	**Pyrrole O**	**Toluene E_3**	**Furfural N**	**Phenol Y**
1	0-5	**9.5 ± 1.2 a**	**10.7 ± 1.6 a**	**10.1 ± 3.0 ab**	16.4 ± 1.5	**19.7 ± 1.8 ab**	14.8 ± 4.0	**18.7 ± 4.4 ab**
2	0-5	**11.3 ± 0.8 ab**	**11.3 ± 2.7 a**	**8.8 ± 1.1 a**	18.0 ± 1.2	**18.6 ± 1.9 a**	15.4 ± 2.1	**16.5 ± 0.9 a**
3	0-5	**14.4 ± 2.7 b**	**4.7 ± 1.6 b**	**13.0 ± 1.4 b**	16.0 ± 1.4	**22.7 ± 2.1 b**	16.6 ± 2.1	**12.5 ± 1.2 b**

Table 12. Relative abundances (%) of volatile components derived from the pyrolysis-gas chromatography of the SOM (mean ± SD, n=5). Different letters indicates significant differences between habitats (p<0.05)

Habitat	Depth (cm)	**O/N**	**B/E_3**	**O/Y**	**AL/AR**
1	0-5	1.15 ± 0.24	0.52 ± 0.17	**0.92 ± 0.24 a**	0.54 ± 0.09
2	0-5	1.19 ± 0.19	0.48 ± 0.10	**1.10 ± 0.11 ab**	0.61 ± 0.02
3	0-5	0.97 ± 0.09	0.57 ± 0.03	**1.28 ± 0.11 b**	0.56 ± 0.05

Table 13. Index calculated from ratios between relative abundances of some of the volatile components of SOM (mean ± SD, n=5). Different letters indicates significant differences between habitats (p<0.05)

2.3.3.3. Soil classification

Following the Soil Taxonomy System, soils of habitats 1, 2 and 3 (Appendix I) would correspond to a Xeropsamment Aquic (Soil Survey Staff, 2014). In the map of the soils of Catalonia (scale 1:25000, published in 2007), the soils of La Pletera are included in the category Eo2 “Les Vernedes-sandy-loam”. The definition of this category is: “Deep soil, imperfectly drained, with moderately coarse texture and without gross elements, pending <1%. Xerofluvent Aquic, gross loam, mixed (calcaric), thermic”. However the large scale of this map and the soil heterogeneity of the area (especially regarding soil texture) make possible to find soils of other categories (Ew, Xeropsamment Aquic as Soil Taxonomy Classification) as inclusions.

2.4. Carbon stored in lagoon sediments

2.4.1 Selected lagoons

To assess the carbon stored in the sediments of lagoons, nine lagoons with different age and water regimes were selected (Figure 15). We studied three old permanent, three new permanent and

three new temporary lagoons. Within the three old lagoons, two were naturally formed (P and FR) while the third was created in 2002. Conversely, all the new lagoons were created in 2016.

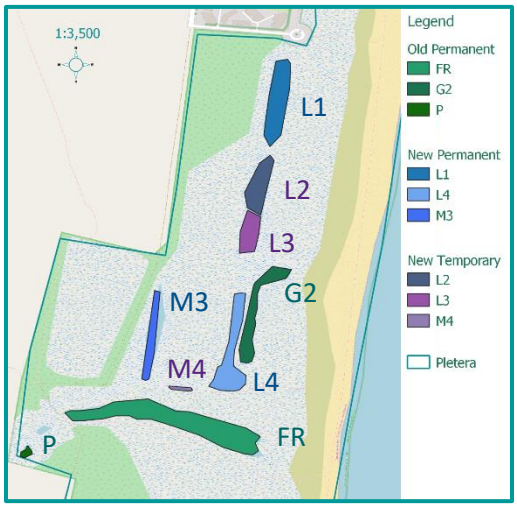


Figure 15. Distribution of the nine lagoons studied at La Pletera salt marsh

2.4.2. Sampling and carbon analyses

In summer 2017, three points were chosen in each one of the nine lagoons and, in each point, three samples of sediment were collected at 0-5 cm depth. The three samples per point were pooled together to give one composite sample. Samples were oven dried (105 ºC) and then combusted in a muffle furnace, first at 400 ºC to determine the organic matter content and then at 1200 ºC to determine the inorganic carbon content (Loss-on-ignition method). To transform organic matter to organic carbon the equation of Craft et al (1991) was applied. Differences in organic and inorganic carbon content among the different typologies of lagoons were evaluated by means of ANOVAs and posterior post-hoc tests.

2.4.3. Results

The organic and inorganic carbon content of permanent lagoons was about three-fold higher in old than in new lagoons (Figure 16a). Among new lagoons, no significant differences were found between permanent and temporary ones (Figure 16b).

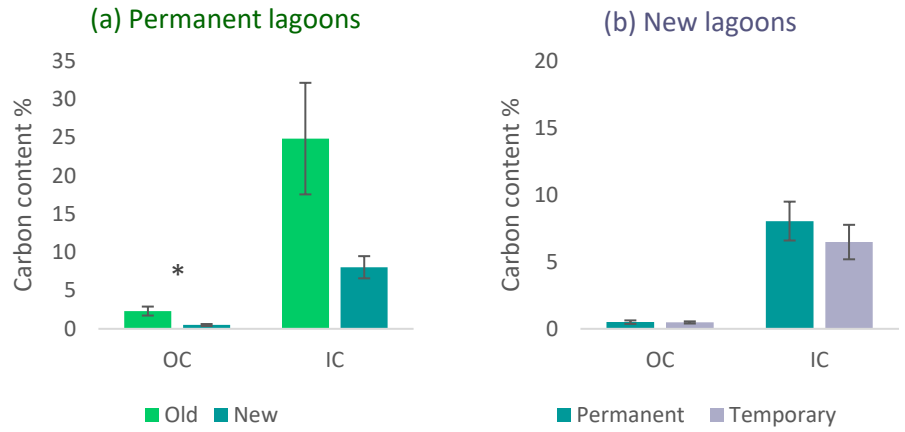


Figure 16. Mean content of organic (OC) and inorganic (IC) carbon in sediments of permanent (old and new) lagoons (a) and new (permanent and temporary) lagoons (b) (n=3). Bars represent ± standard errors. Asterisks indicates significant differences among lagoon typologies (p<0.05).

Among permanent lagoons, the two old natural lagoons (FR and P) had higher amounts of organic and inorganic carbon than the others, even than the old one created in 2002 (G2) (Figure 17). When considering all the new lagoons, values of organic carbon stored in the sediment were similar, while some differences for inorganic carbon were observed among them (Figure 18).

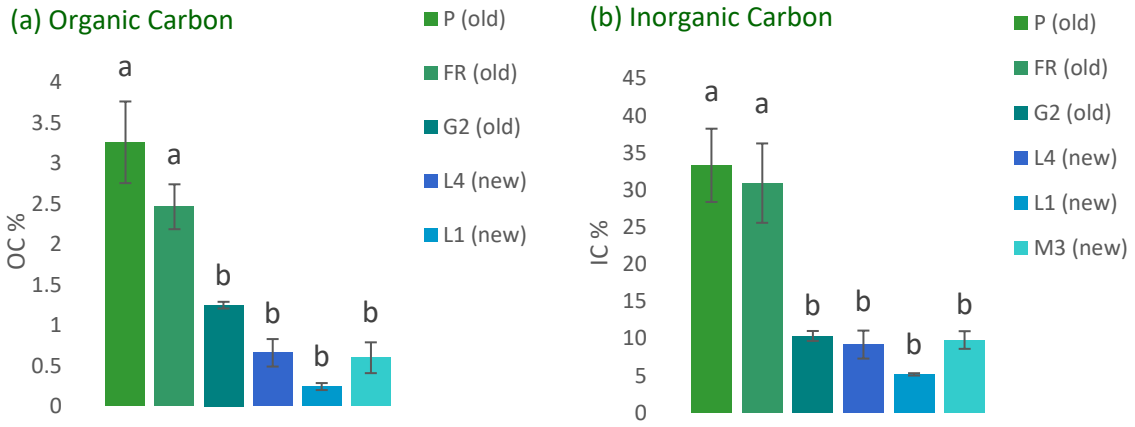


Figure 17. Mean content of organic (a) and inorganic (b) carbon in the sediment of permanent (old and new) lagoons. Bars represent ± standard errors. Different letters indicate significant differences among lagoons (p<0.05).

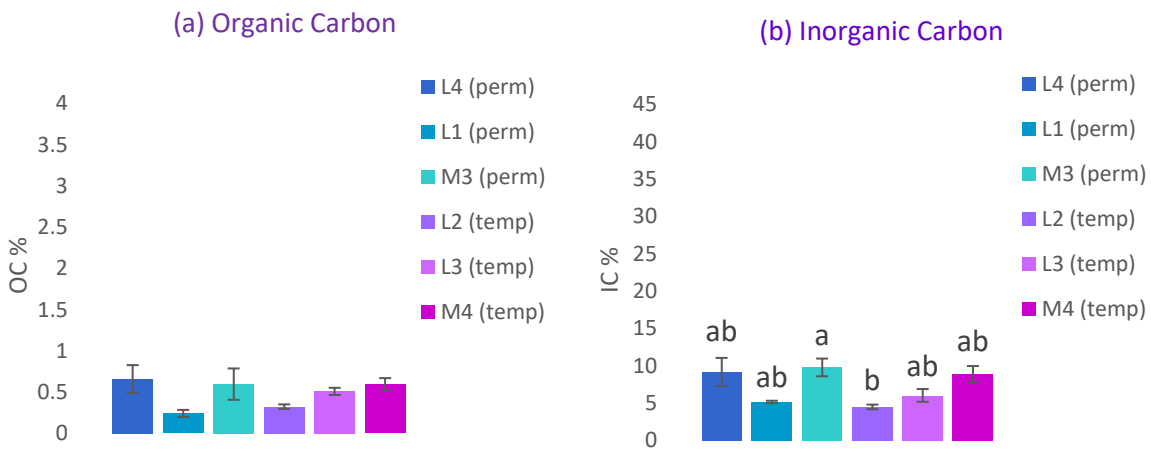


Figure 18. Mean content of organic (a) and inorganic (b) carbon in the sediment of new (permanent and temporary) lagoons. Bars represent ± standard errors. Different letters indicate significant differences among lagoons (p<0.05).

2.5. General discussion of carbon stored

The amount of carbon stored in the vegetation (aboveground, belowground and litter biomass) of the disturbed habitat (habitat 4) was only ≈ 14% and 31% of that stored in the well-preserved habitats 1 and 2, respectively. Conversely, no differences were found between habitat 4 and 3 (pioneer halophytic vegetation) regarding the carbon stored in the aboveground biomass, while habitat 4 had a higher amount of carbon in belowground biomass and litter compared to habitat 3. These results clearly indicate that both well-preserved shrublands and grasslands of this salt marsh accumulate much more carbon than ruderal vegetation appeared in the disturbed area, which support the idea that these salt marsh habitats need to be preserved since they are important carbon reservoirs. Among well-preserved habitats, the shrubland (habitat 1) had the highest amount of carbon stored in the aboveground biomass (1462.78 ± 178.84 g C m^{-2}), especially in the woody and standing dead part of *S. fruticosa.*

When comparing the two studied years, net primary production was only detected for the pioneer halophytic vegetation of habitat 3 (37.93 g m^{-2} y^{-1}) due to an increase in the aboveground biomass. Habitats 1 and 2 are mature plant communities (see Action A2) and, thus, one-year changes in biomass are negligible and extremely difficult to detect if they exist. On the contrary, habitat 3 is a pioneer community, being in a very early state of succession, and, for this reason, we could detect increases in biomass in this habitat from one year to the other, mainly due to the colonisation of the habitat by *S. fruticosa*.

Regarding litter decomposition, *E. pycnanthus*, the dominant species of habitat 2 (grassland), had the lowest decomposition rates, probably due to its high initial concentrations of cellulose and lignin, being thus more recalcitrant than the other studied species. The low decomposition rates of *E. portulacoides* litter are also in accordance with the lower carbon amount of fungal biomass detected in this species at the end of the study. Overall, results indicate that *E. pycnanthus* would favor the incorporation of organic carbon into the soil more than the other two species due to its lower litter decomposition rates and, consequently, lower mineralization of

organic carbon. The differences in decomposition rates, extracellular enzyme activities and carbon of fungal biomass found between litter samples of *A. portulacoides* placed in the two different zones of habitat 2 suggest that this species is highly sensitive to edaphic heterogeneity, and thus it would contribute differently to the incorporation of organic carbon into the soil depending on edaphic properties.

Moving to soil parameters, the high values of exchangeable sodium percentage classifies the soils of all the habitats of the well-conserved area as "saline-sodic". Thus, plants growing in these soils must face saline stress. The halophytic vegetation of La Pletera salt marsh is adapted to these conditions but another type of vegetation (such as agricultural crops) would have low production in these habitats. Soil physicochemical parameters, such as the higher resistance of water stability of aggregates (WSA) in the shrubland (1) and grassland (2) habitats agree with the values of soil organic matter and total and easily extractable glomalin in these habitats, since SOM and glomalin act as cementing agents in soil, enhancing soil structure. Related to these concepts, low values of bulk density in habitat 1 and 2 are explained by the higher values of SOM, and glomalin, which improve soil structure and increase porosity.

The composition of organic matter in the habitats of the well-preserved zone revealed that habitat 1 and 2 presented a fresher nature of SOM (lower relative abundances of benzene and toluene and higher abundances of acetic acid and phenol). This is probably due to the fact that in habitat 1 and 2 there is an important input of fresh organic matter from plant litter that do not occur in habitat 3. In this sense, these results would indicate that habitat 3 presents a more humified and therefore more stable SOM, which is corroborated by the highest values of O/Y ratio (mineralization index of stable fractions of SOM) in this habitat. However, it is necessary to consider that the composition of organic matter has been estimated in terms of relative abundances and therefore no quantitative values of humification have been obtained.

Regarding the capacity of the habitats of the well-conserved zone to store soil carbon, habitats 1 and 2 had the highest amount of carbon stored in the soil, especially, in the first 5 cm. The mean value of soil organic carbon content in these two habitats (0.014 g C cm^{-3}) was within the range found in other salt marshes of the world, from 0.009 to 0.121 g C cm^{-3} (Chmura et al., 2003). The highest values of SOC in habitats 1 and 2 compared to habitat 3 can be explained by the dominant vegetation of each habitat. Both, the shrubland (habitat 1) and grassland (habitat 2) have dense vegetation which higher biomass and higher litter amount on soil surface than in the habitat of annual herb species (3) (see section 2.1.3.1.). The high amount of litter acts as an important source for soil carbon content (Carrasco-Barea et al., 2018). Litter accumulation is especially important in Mediterranean basin salt marshes as La Pletera, where the absence of tides allows the accumulation of important amounts of litter (Ibañez et al., 2000). Similar values of SOC, SOM and glomalin were found between the habitats of the well-preserved zone and the disturbed habitat (habitat 4). However, this result may be misleading because of the origin of the disturbed soil. In fact, soil of habitat 4 was rubble coming from different places that was used to fill and rise the cote of the salt marsh area.

When comparing carbon stored in vegetation (above- and below-ground) and soil (0-20 cm depth) in the habitats of the well-preserved zone (Figure 19), the highest amount of carbon was found in the soil, according to data previously reported regarding carbon stocks in wetlands (Dalal

and Allen, 2008). However, in the shrubland (habitat 1), no significant differences were found between the amount of carbon stored in the soil and in the vegetation for any of the studied years, showing the importance that the vegetation of this habitat (dominated by *S. fruticosa*) have in the maintenance of the carbon stored in La Pletera salt marsh.

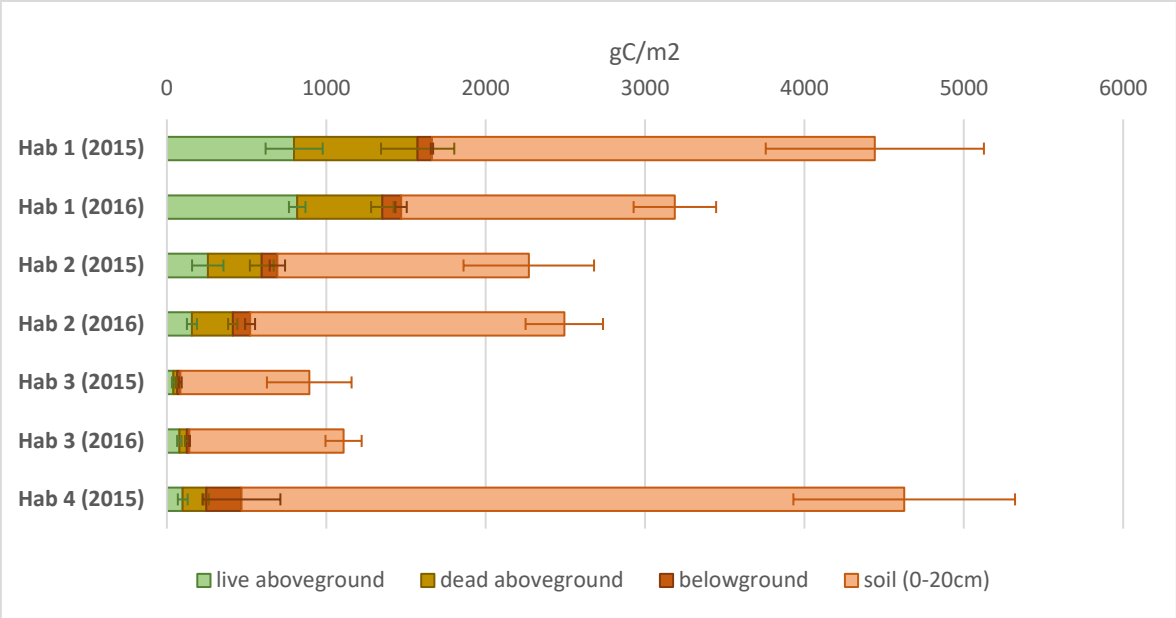


Figure 19. Carbon content in vegetation (living and dead aboveground and belowground biomass) and in soil (0-20 cm depth). Bars represent ± standard errors.

Regarding carbon stored in the sediments of lagoons, the highest values of organic and inorganic carbon were found in the old permanent naturally-formed lagoons. The permanent lagoon created in 2002 did not reach the levels of carbon stored in the sediment that the other two permanent lagoons had. Hence, the age of the lagoons is an important factor determining the storage of carbon in these systems.

Finally, comparing carbon pools in vegetation, soil (0-5 cm) and sediment of lagoons (0-5 cm), similar proportions have been found (37%, 31% and 32%, respectively) (Figure 20). However, taking into account that only the first 5 cm of soil and lagoon sediments have been considered, higher percentages of carbon stored in these compartments would be expected.

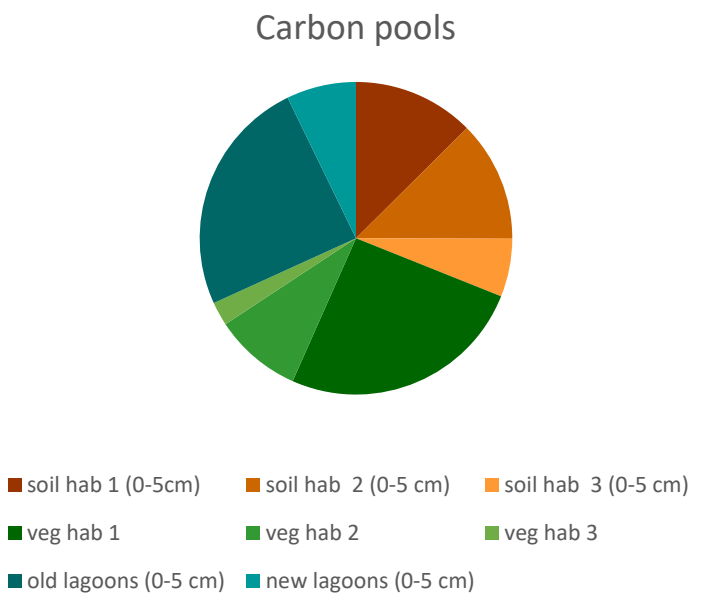


Figure 20. Percentage of overall carbon pools (g C m^{-2}) stored in the vegetation (green), soil (orange) and sediments of the lagoons (blue) of La Pletera salt marsh.

3. Carbon flux dynamics in La Pletera salt mash

3.1. Carbon fluxes from vegetation

3.1.1. Sampling and data analyses

During 2017, carbon dioxide (CO_2) fluxes were monitored for the dominant species of each habitat (*Sarcocornia fruticosa*, *Atriplex portulacoides*, *Elymus pycnanthus* and *Salicornia patula*) (see action D2, section 3). Measurements were performed in living green and woody plant tissues (except for *E. pycnanthus* and *S. patula* in which only green tissues were present) using a PLC3 conifer leaf chamber (80 x 40 mm) connected to an InfraRed Gas Analyser (IRGA; CIRAS-II, PPsystems USA) (Figure 21a,b). Measurements in woody tissues were only performed in stems with a diameter of 3 mm as maximum, which represents the 35% and the 100% of the total woody living biomass of *S. fruticosa* and *A. portulacoides* respectively. Thicker woody fractions were not measured because they did not fit in the conifer's leaf chamber. To assess daily and seasonal changes, measurements were carried out at different times of the day in clear sky days and throughout the year (Table 14). Plant fractions used to measure CO_2 fluxes were taken and stored in a fridge until being processed in the lab to determine tissue area and dry weight (oven dried at 75 ºC for 48 h).

Times of the day	Green tissues		Woody tissues	
	Frequency	**n**	**Frequency**	**n**
sunrise	Every month and a half (twice/season)	4	Every three months (once/season)	4
midday	Every month and a half (twice/season)	6	Every three months (once/season)	6
sunset	Every month and a half (twice/season)	4	Every three months (once/season)	4
night	Every three months (once/season)	4	Every three months (once/season)	4

Table 14. Summary of CO_2 flux samplings from living green and thin woody plant tissues throughout one year considering: the times of the day in which measurements were taken, the frequency of samplings per season, and the number of plants used per species (n).

Instantaneous CO_2 flux measurements from living green and thin woody tissues taken at sunrise, midday, sunset and night once or twice per season were multiplied by the number of hours per day corresponding to each time range to roughly scale these data to daily and seasonal level. To scale the CO_2 fluxes from leaf to habitat level, all the aboveground vegetation of three plots per habitat and season (0.5 m x 0.5 m per plot) was harvested (Figure 21c). In the case of habitat 3, samplings were only performed in spring and summer since the dominant species of this habitat, *S. patula*, is annual. Sampling plots were randomly selected, and the plant material harvested was separated in the laboratory by species and by living (distinguishing between green and woody tissue) and dead biomass. Biomass from each fraction was oven-dried at 75°C during 48 hours and weighed. To calculate the net CO_2 flux per habitat, daily net CO_2 exchange rates, calculated by dry

weight for the dominant species of each habitat, were multiplied by the amount of biomass of each species per square meter in each season.

(a) (b) (c)

Figure 21. InfraRed Gas Analyser (IRGA) (a) connected to a PLC3 conifer leaf chamber (b) to measure net CO_2 flux from vegetation. Example of one of the squares (0.5 m x 0.5 m) in which plant biomass was sampled to assess CO_2 flux per area in the distinct habitats (c).

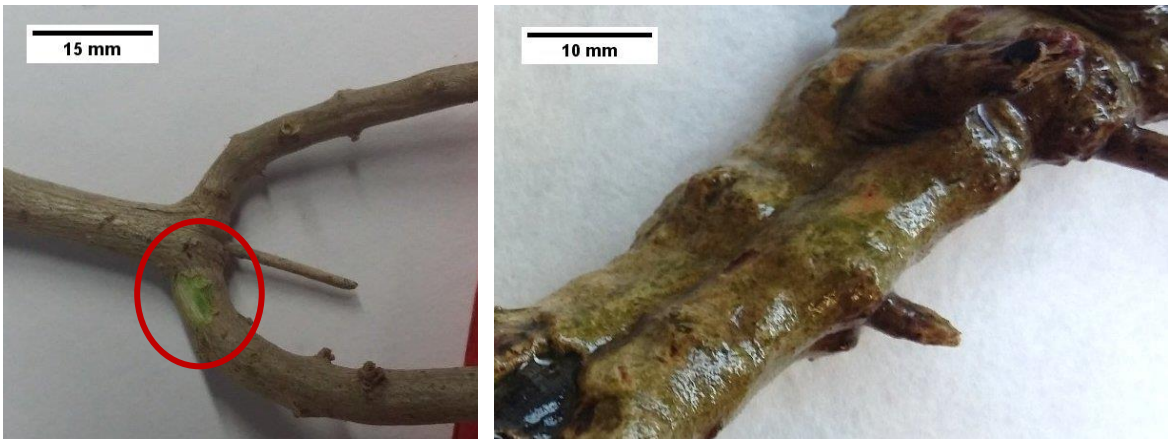
To determine differences among species, ANOVAs and Tukey's HDS post-hoc tests (when necessary) were performed. When data did not accomplish normality and homoscedasticity, non-parametric tests (Kruskal-Wallis and Mann-Whitney U test) were applied.

3.1.2. Results

Differences among species in the net CO_2 exchange rate (NER) of green tissues depended on the time of the day (Figure 23). At sunrise, differences in NER were only observed in spring and summer. Specifically, *E. pycnanthus* had the highest photosynthesis values (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 23a). At midday, *E. pycnanthus* had the highest photosynthetic rates during all the year, except in June and July when *S. patula* showed the highest values (Figure 23b). At sunset, *E. pycnanthus* also had the highest photosynthetic rate during most of the year, but from February to April values were not statistically different from those measured in *S. fruticosa* (Figure 23c). At night, the highest respiration values (positive NER) were found in August and November for *E. pycnanthus* and *S. fruticosa* (Figure 23d). Overall, sunrise was the time of daylight hours with the lower photosynthetic rates for the four species, ranging from 1.2 to -22.1 µmol CO_2 m^{-2} s^{-1}, although for *S. fruticosa* and *A. portulacoides* the differences among sunrise, midday and sunset were not as pronounced as for *E. pycnanthus* and *S. patula* (Figure 23).

The NER of the thin woody tissue of *S. fruticosa* and *A. portulacoides* did not differ throughout daytime (sunrise, midday and sunset) independently of the season (Figure 24a,b,c). However, at night, significantly higher respiration values were found for *A. portulacoides* in June, and for *S. fruticosa* in November (Figure 24d). The high photosynthetic rates found in woody tissues

of these species, especially at sunset in March and May could be explained by the presence of photosynthetic cells under the thin bark layer of the stem (Figure 22a) and/or by the presence of microalgae that would colonize the stems during flooding (Figure 22b) (Sullivan and Currin, 2000).



(a) (b)

Figure 22. Woody stems of *S. fruticosa* in which it was observed: a) green tissue under the bark, and b) microalgae biofilm covering the stem surface.

When daily CO_2 fluxes from thin woody and green tissues of the four studied species were estimated, results show that all the species had a net CO_2 uptake during most part of the year, being remarkably high in the case of *E. pycnanthus* in winter and spring, and in *S. patula* in spring and summer (Figure 25a). Autumn was the season in which CO_2 emission predominates, being positive the daily CO_2 fluxes from all the species and fractions, except for *E. pycnanthus*. Scaling up to habitat level, there was a net CO_2 uptake in habitat 1 and 2 during winter, spring and summer, and in habitat 3 during the entire period in which *S. patula* was alive (spring and summer). However, during autumn, net CO_2 emission was recorded in habitats 1 and 2.

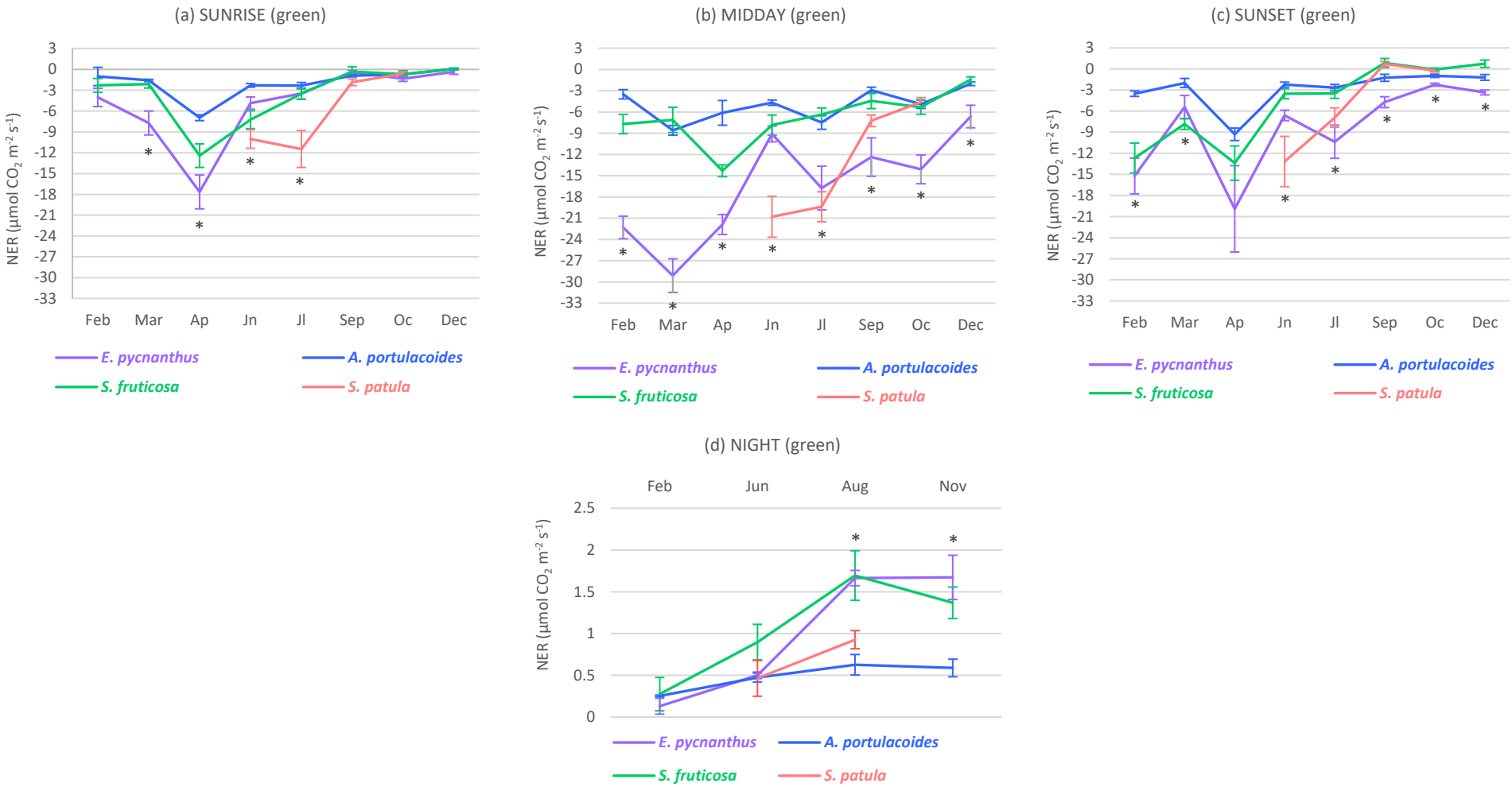


Figure 23. Instantaneous net CO_2 exchange rate (NER) from the green part of the four studied species at different times of the day: sunrise (a), midday (b), sunset (c) and night (d). Negative values indicate net photosynthetic activity, while positive values indicate net respiration. Bars represent ± standard errors. Asterisks indicate significant differences among species in each sampling date ($p < 0.05$).

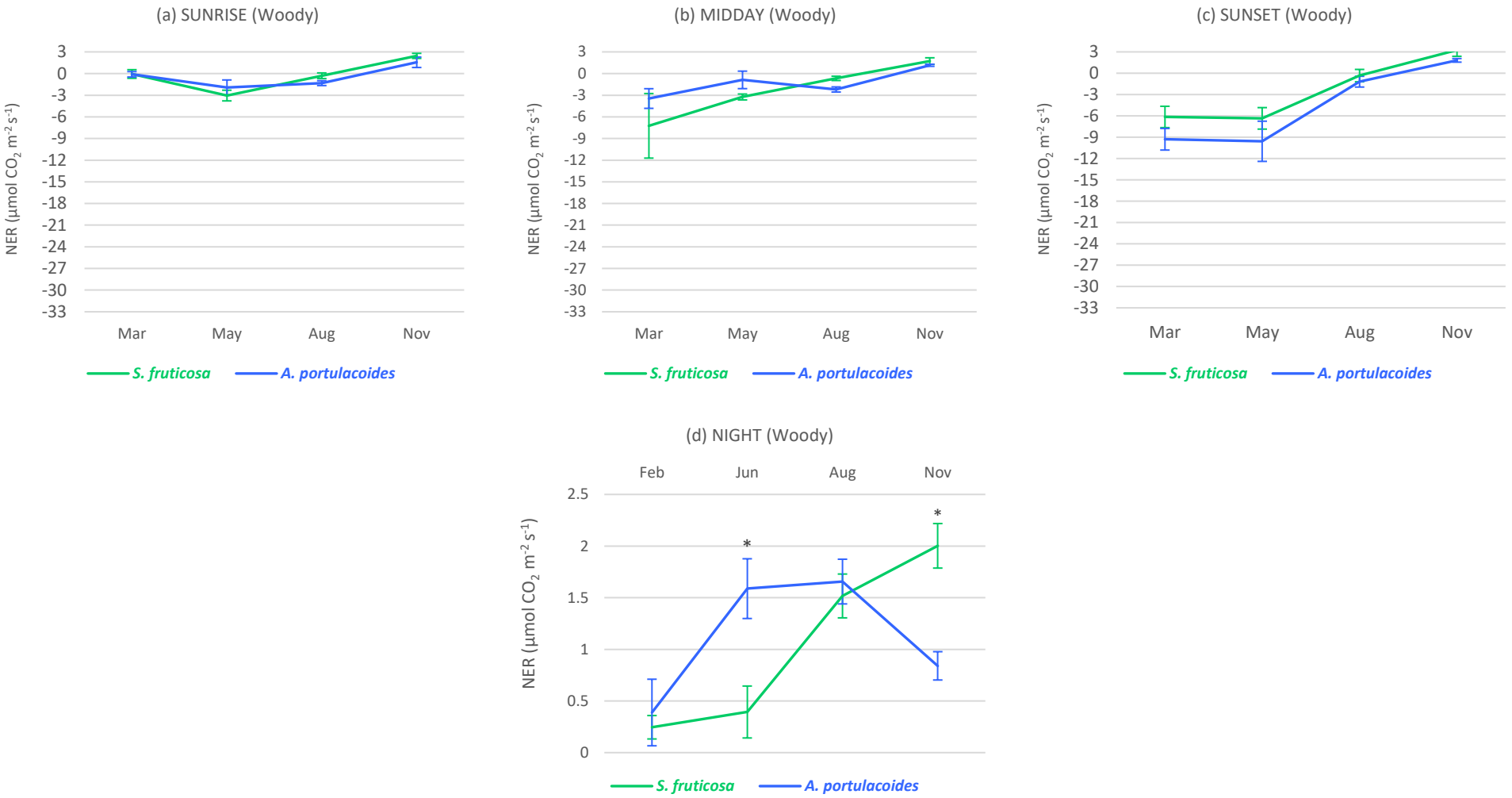
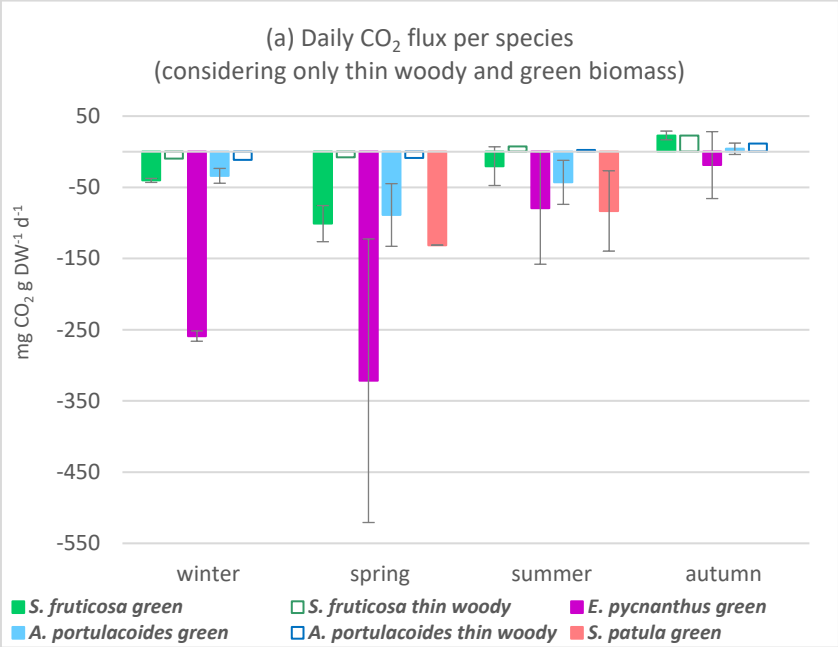


Figure 24. Instantaneous net CO_2 exchange rate (NER) from thin woody parts of *S. fruticosa* and *A. portulacoides* plants at different times of the day: sunrise (a), midday (b), sunset (c) and night (d). Negative values indicate net photosynthetic activity, while positive values indicate net respiration. Bars represent ± standard errors. Asterisks indicate significant differences between species in each sampling date ($p < 0.05$).



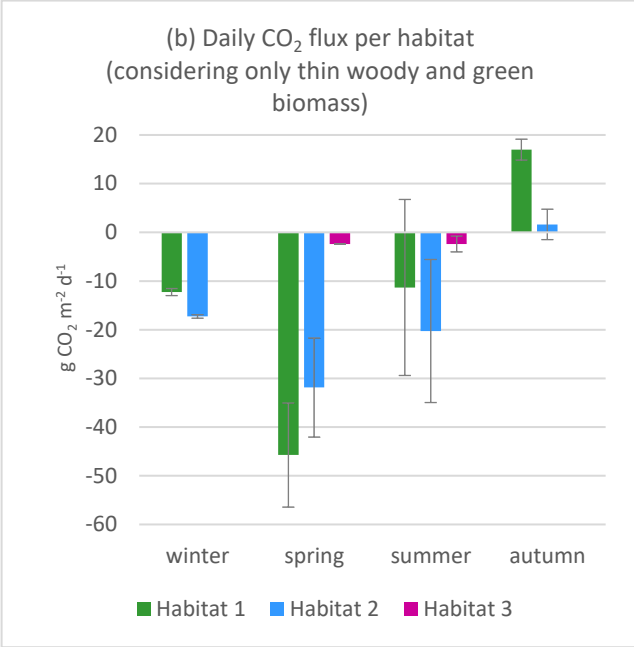
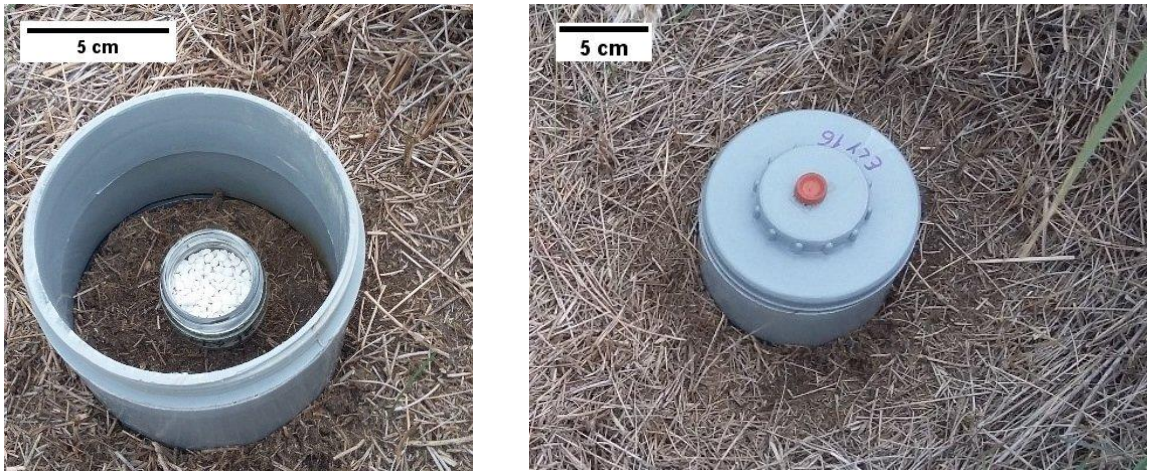


Figure 25. Estimated daily net CO_2 exchange rates from thin woody and green biomass for each species and season (a); estimated daily CO_2 fluxes from thin woody and green tissues of the vegetation of the three well-conserved habitats for each season (b). Negative values indicate CO_2 uptake, while positive values indicate CO_2 emissions. Bars represent ± standard error. Data from woody tissues and for *S. patula* in spring have no standard error because, in these cases, measurements were performed only in one sampling day per season.

3.2. Carbon fluxes from soil

3.2.1. Sampling and data analysis

Measurements of CO_2 fluxes from the soil were performed during 2017 every month and a half. In each habitat, five randomly selected points were measured. Measurements of CO_2 were carried out by the soda-lime method (Grogan, 1998). In the field, this method consists on placing a "cover box" (PVC cylinders) of 11 cm of diameter and 8 cm of height above soil surface, putting inside glass vessels containing soda-lime (which have the capacity to absorb CO_2), previously oven-dried at 105 °C and weighted, and closing the cover-box with a hermetic cover (Figure 26b,c). After 24 h, the soda-lime was collected and weighed again (after drying). Since the absorption of CO_2 increases its weight, the difference between initial and final weights allows the estimation of the quantity of CO_2 emitted by the soil during 24 h.



(a) (b)

Figure 26. Measurement of net CO_2 flux from soil. Soda-lime placed inside de cover box (a) and cover box hermetically closed (b)

When soil was flooded, the measurements with cover-box using soda-lime couldn't be made. In this cases a slightly variation of the cover-box method was performed. Cover box with height enough to allow that a part of the cylinder stays outside of the water (i.e., around 40 cm height) were placed in the same points where cover-boxes were (Figure 27). The level of water in the cylinder was measured to know the air volume. Cylinders were hermetically closed for 24 h and after this time an air sample was taken from the cylinder, passing a syringe thought the rubber septum on the top of the cylinder and passing it to a 5-ml vacuum vial. Control air samples were also taken on the day of closing the cylinders to know the initial value of CO_2. The samples were kept at low temperatures and later they were analysed by gas chromatography at the Chemical and Environmental Engineering laboratory of the Research Technical Services of the University of Girona.

(a) (b)

Figure 27. Longer cover box used for flooding periods, placed in a non-flooded soil (a) and in a flooded soil (b).

Methane flux between the soil surface (or between water surface when soil was flooded) and atmosphere was estimated using the cover-box method. Coinciding with the CO_2 sampling, cover-boxes were hermetically closed for 24 h and after this time an air sample was taken from the cylinder and passing it to a 5-ml vacuum vial. Control air samples were also taken on the day of closing the cylinders to know the initial value of CH_4. The samples were kept at low temperatures and later analysed by gas chromatography at the Chemical and Environmental Engineering laboratory of the Research Technical Services of the University of Girona. When soil was flooded the same air samples taken to estimate the CO_2 flux were used to determine the CH_4 flux.

At each sampling date and measurement location, soil temperature was also monitored with a portable thermometer (Digital Portable Thermometer AI 368, Acez), as well as soil volumetric water content (VWC) (FieldScout TDR 300 soil moisture meter, Spectrum technologies, Inc), and soil conductivity (conductivity meter 254, CRISON instruments). As the high levels of electric conductivity found in these soils could affect the measurements of VWC, a correction of these values was done by calculating calibrating lines from TDR readings and humidity content measured directly by weight loss over time (taking into account the soil bulk density) in undisturbed soil samples.

Mineralization coefficients (Q_{min}, mg C g^{-1} soil d^{-1}) were calculated for each sampling date following this equation:

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

Were C_CO2 is the carbon emitted as CO_2 form (mg C g^{-1} soil d^{-1}); C_CH4 is the carbon emitted as CH_4 form (mg C g^{-1} soil d^{-1}) and SOC is soil organic carbon (g SOC g^{-1} soil).

Data was statistically analyzed by means of ANOVAs and Tukey's HDS post-hoc tests to determine differences among habitats. In case data did not accomplish normality and homoscedasticity non-parametric tests (Kruskal-Wallis and Mann-Withney U test) were applied.

3.2.2. Results

Daily soil CO_2 emissions were higher in habitat 1 and 2 than in habitat 3 between June and October, whereas for the rest of the year no significant differences among habitats were found (Figure 28). When soils were flooded, emissions were remarkably lower.

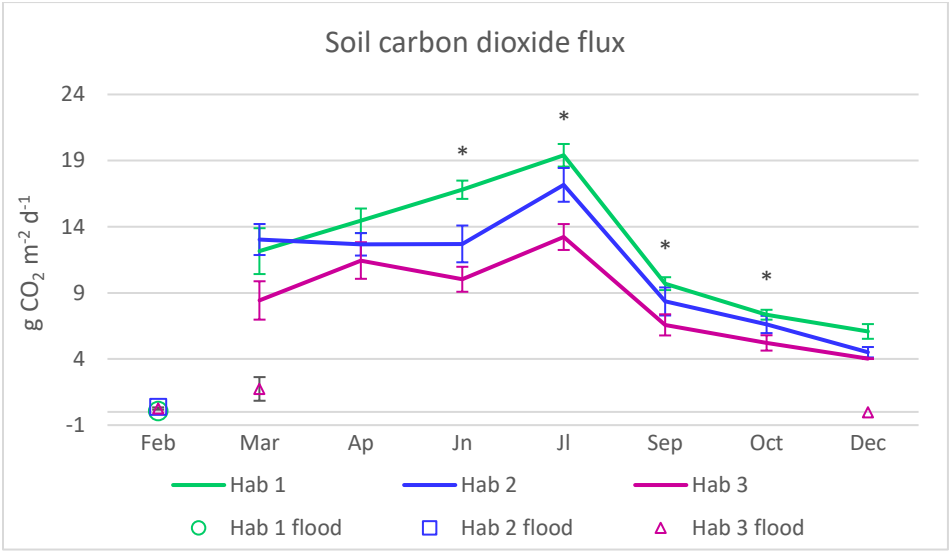


Figure 28. Soil CO_2 flux (respiration) measured with the soda-lime method (when soil was not flooded) and with gas chromatography method (when soil was flooded). Lines represent CO_2 flux measured in non-flooded soils and symbols represent CO_2 flux measured in flooded soils. In March and December, habitat 3 had flooded and non-flooded plots. Bars represent ± standard errors. Asterisks indicate significant differences in CO_2 flux among habitats in non-flooded soils ($p<0.05$).

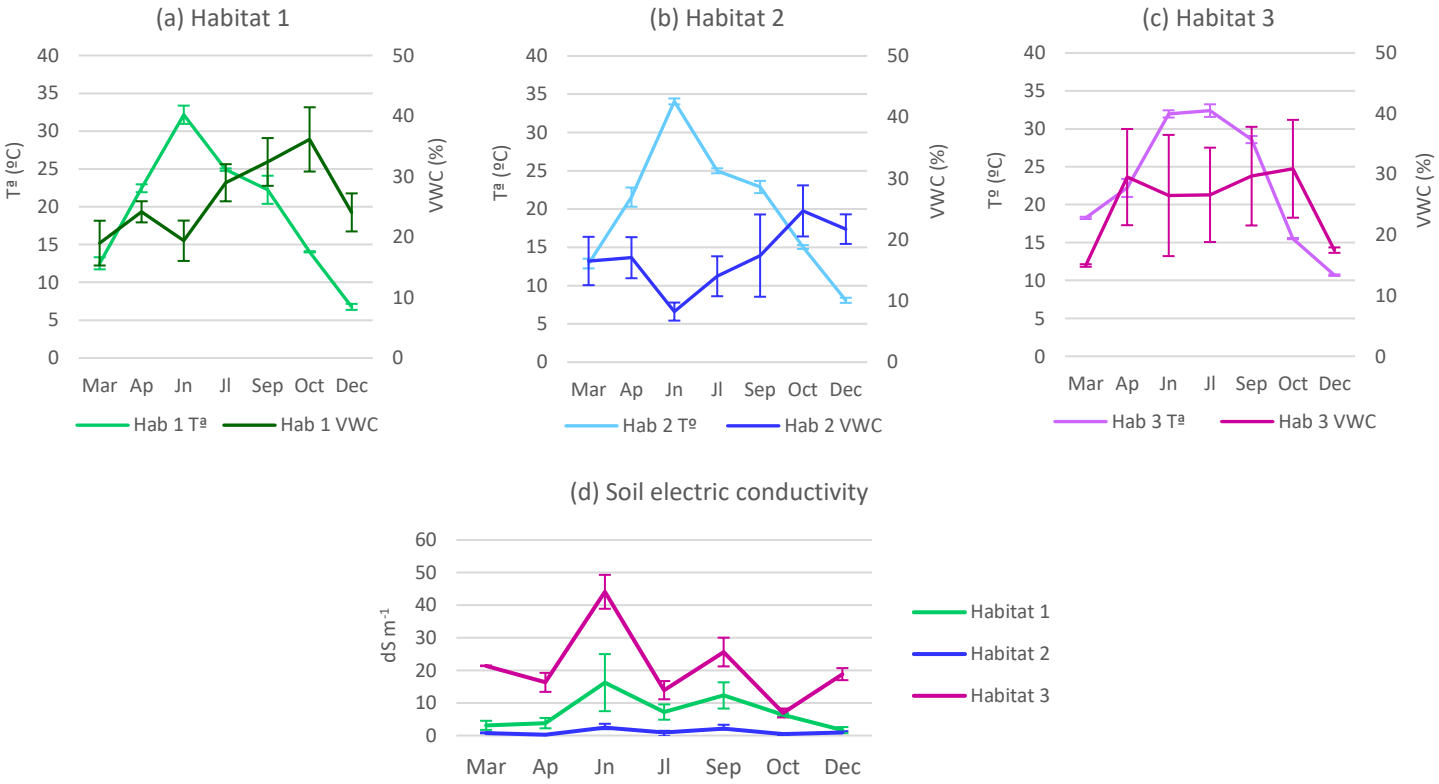


Figure 29. Soil temperature and volumetric water content (VWC) measured in habitat 1 (a), habitat 2 (b) and habitat 3 (c) and soil electric conductivity in the three habitats (d). Bars represent ± standard errors

The highest soil temperature was registered during spring and summer (Figure 29). The lowest value of volumetric water content in habitat 1 and 2 occurred in June, coinciding with the date in which maximum values of soil temperature were registered. Regarding volumetric water content, in habitat 3 there was a remarkably high variability and seasonal patterns were less evident than in the other habitats. Electric conductivity was lower in habitat 2 for all the sampling dates.

In some dates during the sampling period peaks of CH_4 emissions were detected. In habitat 3 CH_4 emissions occurred in March and June. In habitat 1 and 2 CH_4 emissions were in June and July, respectively (Figure 30). Differences among habitats were found, being each habitat responsible of the highest emission rates in different dates (habitat 3 in March, with values ranging from 0.029 to 0.215 g CH_4 m^{-2} d^{-1}, and in June, with values from 0.021 to 0.342 g CH_4 m^{-2} d^{-1}; habitat 1 in June, with values ranging from 0.068 to 0.422 g CH_4 m^{-2} d^{-1}; and finally, habitat 2 in July, with values ranging from 0.029 to 0.358 g CH_4 m^{-2} d^{-1}).

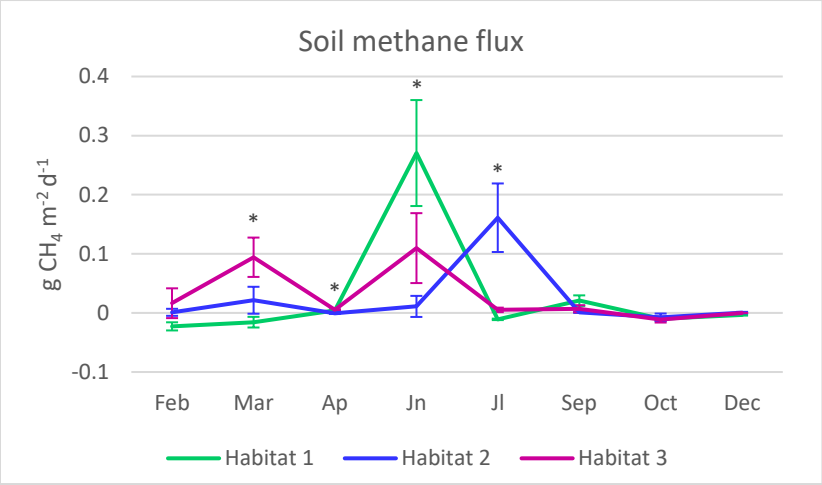


Figure 30. Soil CH_4 flux. Bars represent ± standard errors. All plots (flooded and non-flooded) have been considered together because the same method have been used for CH_4 flux measurements. Asterisks indicate significant differences in CH_4 flux among habitats ($p<0.05$).

Habitat 3 had the highest mineralization coefficient during the entire year while no differences between habitat 1 and 2 were found at any date (Figure 31).

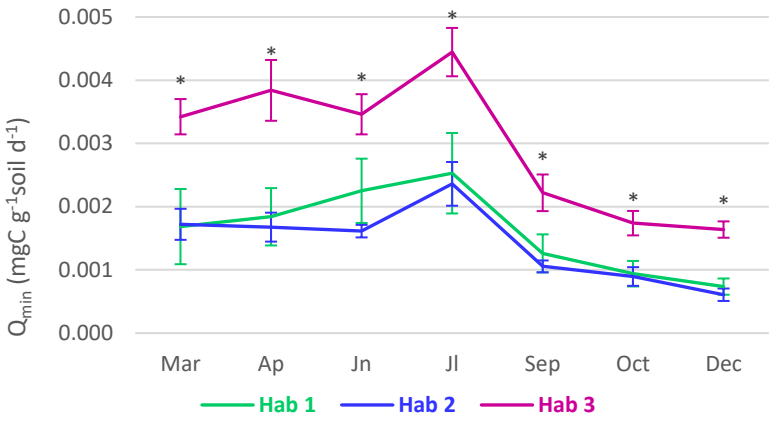


Figure 31. Mineralization coefficients for habitat 1, 2 and 3. Bars represent ± standard errors. Asterisks indicate significant differences in mineralization coefficient among habitats (p<0.05).

3.3. Carbon fluxes from lagoons

3.3.1 Sampling and data analyses

Measurements of net CO_2 exchange rates (NER) were performed at midday in the nine lagoons previously selected to assess the amount of carbon in the sediment (see Figure 15, section 2.4.1.). Three of the lagoons were old permanent, three were new permanent and three more were new temporary. CO_2 flux measures were performed during 2017, every month and a half, between the lagoon water surface and the air, or, in temporary lagoons, between the lagoon sediment surface and the air when they were dry. In each lagoon, three measurements (5 min. each one) were taken at different places using an opaque closed chamber connected to an infrared gas analyzer (IRGA, CIRAS-2, PP-Systems, Amesbury, USA). Two models of chambers were used, one to measure the CO_2 water-air flux (Figure 32a) and the other to measure the CO_2 sediment-air flux (Figure 32b). The air CO_2 concentration was also measured with the IRGA.

(a) **(b)**

Figure 32. Chambers to measure the CO_2 water-air flux (area: 0.139 m^2, volume: 19.5 l) (a) and the CO_2 sediment-air flux (area: 0.007 m^2, volume: 1.2 l) (b).

To calculate the CH_4 flux, data from dissolved CO_2, dissolved CH_4, atmospheric CO_2 and CO_2 water-air flux (NER) were necessary. Dissolved CO_2 and CH_4 were obtained using the headspace equilibrium

technique and gas chromatography (Striegl et al., 2012). Briefly, 40 ml of water and 20 ml of air were collected in a syringe and then stirred vigorously to force the equilibrium between water and air phase. After that, an air sample from the syringe was taken and transferred to a 5-ml vacuum vial. The samples were kept at low temperatures and later they were analysed by gas chromatography at the Chemical and Environmental Engineering laboratory (Research Technical Services, University of Girona, Spain). The CO_2 transfer velocity (kCO_2) should be calculated from Fick's law of gas diffusion:

$$kCO_2 = \Delta CO_2 / FCO_2$$

where FCO_2 is the CO_2 water-air flux (NER) and ΔCO_2 is the CO_2 concentration difference between water and air. From kCO_2, kCH_4 should be estimated following Wanninkof (1992), and then, CH_4 water-air flux can be calculated as:

$$FCH_4 = kCH_4 \times (\Delta CH_4)$$

where the ΔCH_4 is the difference between the CH_4 concentration in the water (measured by headspace and gas chromatography) and in the atmosphere. However, we could not estimate CH_4 fluxes since kCO_2 could not be correctly determined. This was because gas chromatography analyses gave inconsistent values for most of the samples taken in the different days.

Differences in net CO_2 exchange rates (NER) among lagoon typologies were analyzed by means of ANOVAs and Tukey's HDS post-hoc tests. When data did not meet normality and homoscedasticity non-parametric tests (Kruskal-Wallis and Mann-Withney U test) were applied.

3.3.2. Results

Differences in net CO_2 exchange rates between old and new permanent lagoons were found only in March (with higher values in old lagoons) and September (with higher values in new lagoons) (Figure 33a). Permanent and temporary new lagoons had similar CO_2 water-air flux throughout the year (Figure 33b). When analyzing lagoons individually, all lagoons were net emitters of CO_2, except the old permanent naturally formed lagoon called Fra Ramon, which sequestered CO_2 during most of the year (Figure 34). Among new permanent lagoons, the lagoon L4 had the lowest CO_2 emissions during most of the year (Figure 34b). Regarding new temporary lagoons, there was a peak of CO_2 emission in June when these lagoons were dry (especially in the case of L2 and L3; Figure 34c).

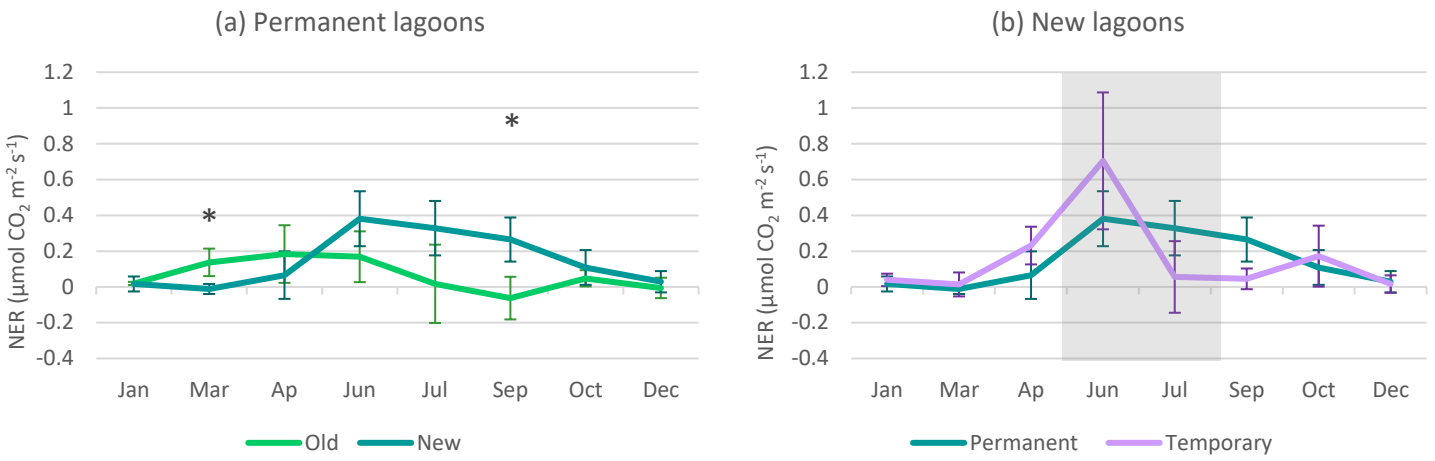


Figure 33. Instantaneous net CO_2 exchange rate (NER) at midday from old and new permanent lagoons (a) and from permanent and temporary new lagoons (b). Positive values indicate net CO_2 emissions, while negative values indicate net CO_2 uptake. The shaded strip indicates the period in which temporary lagoons were dry. Bars represent ± standard errors. Asterisks indicate significant differences between lagoon typologies in each sampling date ($p < 0.05$)

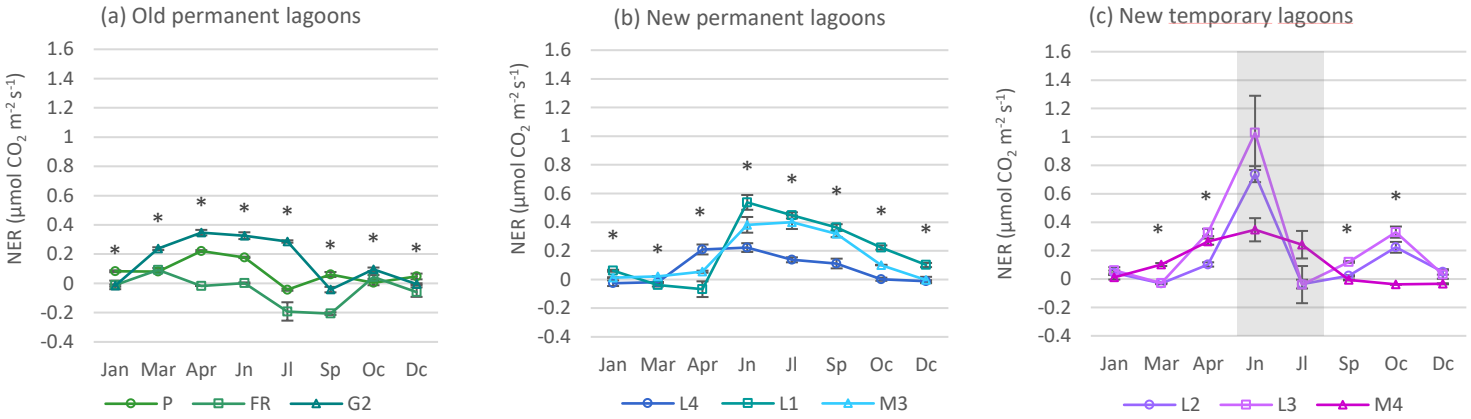


Figure 34. Instantaneous net CO_2 exchange rate (NER) at midday from old permanent (a), new permanent (b) and new temporary lagoons (c). Positive values indicate net CO_2 emissions, while negative values indicate net CO_2 uptake. The shaded strip indicates the period in which temporary lagoons were dry. Bars represent ± standard errors. Asterisks indicate significant differences among lagoons in each sampling date ($p < 0.05$).

3.4. General discussion of carbon flux dynamics

Vegetation

The comparison of the net CO_2 exchange rates (NER) from the green biomass of the four dominant species of the three studied habitats of La Pletera salt marsh revealed that *E. pycnanthus,* the dominant species of the grassland (habitat 2), presented the highest photosynthetic rates during most of the year, with values from green tissues reaching -30 µmol CO_2 m^{-2} s^{-1} in March. Conversely, *S. fruticosa*, the dominant species of the shrubland (habitat 1), showed maximum photosynthetic rates in April, being half of those found for *E. pycnanthus*. NER from the thin woody fraction was

very similar for *S. fruticosa* and *A. portulacoides* throughout the day and the year, being negative (CO_2 sequestration) throughout most of the year, except in autumn when positive NER (CO_2 emission) was recorded. The much larger *S. fruticosa* biomass per area, considering both green and thin woody tissues, explains that, although photosynthetic rates of *E. pycnanthus* were 3 to even 5 times higher than those of *S. fruticosa*, habitat 1 had the highest CO_2 uptake rates in spring (about -55 g CO_2 m^{-2} d^{-1}). In winter and summer, CO_2 uptake rates were similar between habitats 1 and 2. For these habitats (1 and 2), CO_2 emission was only found in autumn, being the highest values for habitat 1 (20 g CO_2 m^{-2} d^{-1}). Nevertheless, taking into account the great extension of habitat 1 in La Pletera salt marsh, our results suggest that the vegetation of this salt marsh would probably be a sink of CO_2 most of the year, except in autumn when the situation could be reversed. However, values provided here must be taken with caution since they are roughly estimates of CO_2 fluxes being the result of an over-simplification of the reality; among other reasons, because:

a) CO_2 fluxes were only estimated from the vegetation of the three most extensive habitats of La Pletera salt marsh, and, thus, minority habitats present in the salt marsh have not been taken into account.
b) Night CO_2 fluxes were measured at the beginning of the night, when night temperatures were higher, and, thus, the whole night respiration was probably over-estimated.
c) The effect of flooding on the CO_2 fluxes from vegetation was disregarded.
d) Due to methodological limitations, to estimate CO_2 fluxes at habitat level only the green and the thin woody fractions of biomass were considered, even though the thick stems of *S. fruticosa* represented the 65% of the total woody fraction. Hence, respiration values would be under-estimated.

Soil

Soil CO_2 emission presents a dynamic trend during the year with a peak of emissions in July for all the habitats, when the conditions of soil temperature and humidity were most favourable for the activity of soil microorganisms.

Comparing among habitats, the highest soil CO_2 emissions were found in the habitats where the soil organic carbon content was higher (habitat 1 and 2). Electrical conductivity did not affect soil respiration activity, since there was a clear difference in this parameter between habitat 1 and 2 during the whole year.

CH_4 emissions in La Pletera salt marsh have been reported, even when soil was not flooded. The highest emissions were found in summer, when temperatures where higher. This behavior could be explained because, although these soils were not flooded, the water table was close to the surface. In summer, the soil surface was dry and, thus, the diffusion of CH_4 from the water table was possible in these sandy soils.

The values of CH_4 emission in La Pletera salt marsh (0.021-0.342 g CH_4 m^{-2} d^{-1} from habitat 3; 0.068 to 0.422 g CH_4 m^{-2} d^{-1} from habitat 1; 0.029 to 0.358 g CH_4 m^{-2} d^{-1} from habitat 2) were higher than those expected in salt marshes (Bartlett and Harris, 1993).

Although habitat 1 and 2 had higher carbon losses as CH_4, but especially as CO_2 emissions, than habitat 3, they had lower mineralization coefficients. Hence, soils of habitat 1 and 2 had a higher carbon sequestration potential than habitat 3, since the total amount of organic carbon stored in the soil of these habitats was subjected to lower mineralization.

Vegetation and soil CO_2 fluxes

When comparing daily CO_2 fluxes from vegetation (thin woody and green biomass) and soil among habitats, it is notorious that the three habitats followed different trends. In habitat 1 (shrubland with *S. fruticosa* as dominant species) and habitat 2 (grassland with *E. pycnanthus* and *A. portulacoides* as dominant species), CO_2 uptake was favored most of the year, with the only exception being in autumn (Figure 35). On the contrary, habitat 3 was a net source of CO_2 throughout all the year, even in spring and summer when *S. patula* was present.

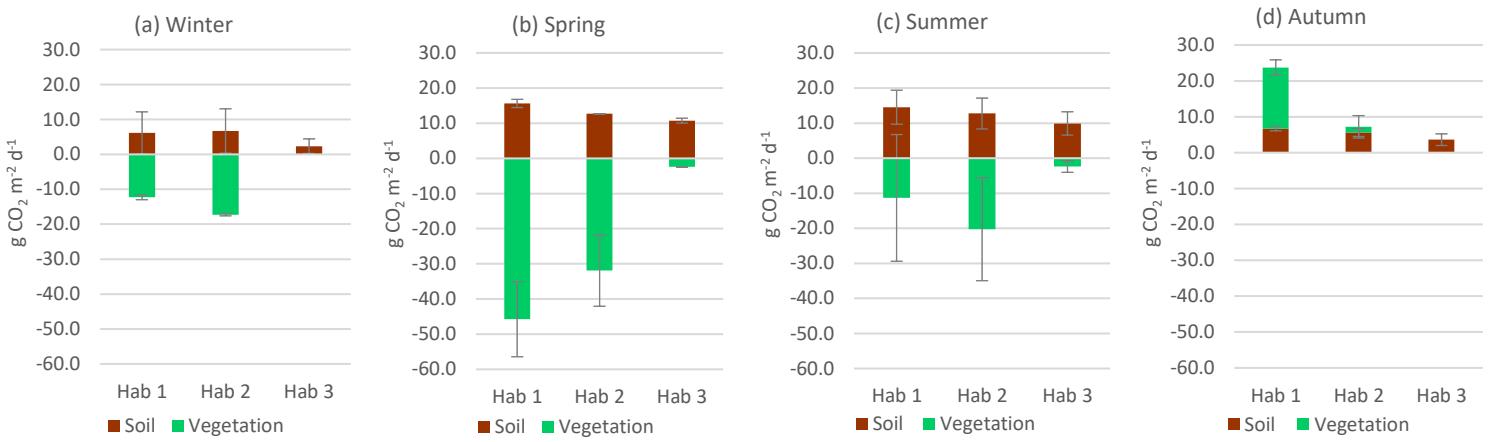


Figure 35. Daily CO_2 fluxes from vegetation (green and thin woody biomass) and soil for each habitat in winter (a), spring (b), summer (c) and autumn (d) (n=2). Negative values indicate CO_2 uptake, while positive values indicate CO_2 emission. Bar represents ± standard errors.

CO_2 fluxes from the lagoons

The nine studied lagoons were net emitters of CO_2, except the old permanent naturally-formed lagoon called Fra Ramon, which sequestered CO_2 during most of the year, probably due to the high abundance of macrophytes found in this lagoon (Figure 36). Nevertheless, it is important to highlight that CO_2 fluxes from the lagoons were only measured at midday, and thus we did not have any information about night respiration.

Figure 36. Banks of *Ruppia cirrhosa* in the old permanent lagoon Fra Ramon during the July sampling.

4. Summary of the Carbon Balance results

In this section, we present a summary of the results obtained regarding carbon balance in La Pletera salt marsh, either carbon stored in the different systems (vegetation, soil and lagoon sediment) or carbon fluxes from these systems and the atmosphere (Table 15). However, when interpreting the results it should be considered that several assumptions had to be done in order to estimate these parameters. First, carbon stored in lagoon sediments and soils corresponds only to 5 cm and 20 cm depth, respectively; therefore, the real amount of carbon stored in these two compartments should be higher than the values presented here. Second, measurements of CO_2 fluxes from vegetation were performed in sunny days and in sun-exposed green leaves/stems; hence, the amount of CO_2 fixed by vegetation was probably overestimated, since cloudy days and shaded parts of the plants were not considered.

	Area[a] (ha)	CARBON STORED[b,c] ($g\ C\ m^{-2}$)		CARBON FLUXES ($g\ CO_2\ m^{-2}d^{-1}$ or $g\ CH_4\ m^{-2}d^{-1}$) [d,e]					
						WINTER	SPRING	SUMMER	AUTUMN
Habitat 1	15.0	Vegetation	1566 ± 178	*S. fruticosa*	Green CO_2	-10.5 ± 0.7	-42.5 ± 10.7	-13.5 ± 18.1	8.0 ± 2.2
					Woody CO_2	-1.8	-3.2	2.2	9.0
		Soil (0-20 cm)	2248 ± 388	Soil	CO_2	6.1 ± 6.0	15.6 ± 1.2	14.5 ± 4.9	6.7 ± 0.6
					CH_4	-0.019 ± 0.004	0.137 ± 0.133	0.005 ± 0.016	-0.007 ± 0.003
Habitat 2	10.8	Vegetation	608 ± 64	*E. pycnanthus*	Green CO_2	-12.5 ± 0.3	-16.4 ± 10.2	-14.8 ± 14.7	-1.3 ± 3.1
				A. portulacoides	Green CO_2	-3.3 ± 1.0	-13.0 ± 6.4	-5.8 ± 4.1	1.1 ± 2.1
					Woody CO_2	-1.5	-2.4	0.3	1.8
		Soil (0-20 cm)	1774 ± 234	Soil	CO_2	6.7 ± 6.3	12.7 ± 0.01	12.8 ± 4.4	5.6 ± 1.0
					CH_4	0.011 ± 0.010	0.005 ± 0.006	0.081 ± 0.080	-0.003 ± 0.004
Habitat 3	4.1	Vegetation	114 ± 14	*S. patula*	Green CO_2	-	-2.4	-2.4 ± .6	-
		Soil (0-20 cm)	897 ± 127	Soil	CO_2	2.3 ± 2.1	10.7 ± 0.7	9.9 ± 3.3	3.6 ± 1.6
					CH_4	0.055 ± 0.039	0.057 ± 0.052	0.006 ± 0.001	-0.006 ± 0.006
	Area[a] (ha)	CARBON STORED[b] ($g\ C\ m^{-2}$)		CARBON FLUXES ($mg\ CO_2\ m^{-2}h^{-1}$) [f]					
						WINTER	SPRING	SUMMER	AUTUMN
Old permanent lagoons	1.3	Sediment (0-5 cm)	1421 ± 283	Water surface	CO_2	12.4 ± 9.3	27.9 ± 1.2	-3.6 ± 6.3	3.3 ± 4.3
New permanent lagoons	1.8	Sediment (0-5 cm)	415 ± 100	Water surface	CO_2	0.4 ± 2.2	35.4 ± 25.0	47.0 ± 5.0	10.9 ± 6.3
New temporary lagoons	1.0	Sediment (0-5 cm)	413 ± 57	Water surface	CO_2	4.2 ± 2.0	74.1 ± 37.5	8.0 ± 0.8	14.9 ± 12.4

Table 15. Summary of results about Carbon Balance in La Pletera (Mean ± SE) considering C stored (n=10 in vegetation and soil; n=3 in sediment of lagoons) and C fluxes (n=2, i.e., two sampling days per season). Negative values indicate CO_2 uptake, while positive values indicate CO_2 emissions. [a]: Estimations of the area occupied by each habitat in 2015-2016 and by the different types of lagoons in 2017. [b]: Estimated values of C stored in the vegetation and soil of each habitat for 2015-2016 (no significant differences were found between years) and in the sediment of the different types of lagoons in 2017; [c]: Vegetation estimations integrate the C stored in aboveground (green, woody and standing dead) and belowground biomass; [d]: Woody measurements have been performed in woody stems of 3 mm of diameter as maximum [e]: There is no standard error for woody measurements neither for *S. patula* in spring, because in these cases measurements were only performed one day per season. [f]: CO_2 fluxes from lagoons are not expressed per day because measurements were only performed at midday.

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APPENDIX. Soil Profiles Characterization

SOIL CHARACTERIZATION _ GENERAL INFORMATION_ PEDON: Habitat 1

Location: La Pletera
Municipality: Torroella de Montgrí (l'Estartit)
Date of description: 08/02/2018
Prospectors: Marc Amorós and Lorena Carrasco

CARTOGRAPHY:

Editor: ICGC (http://www.icgc.cat)
Map/Scala: Orthophoto 1:5000
Coordinates UTM 31N ETRS89: 515723 m E, 4653301 m N
Altitude:1,3m

CLIME AND WATER OF THE SOIL:

Soil temperature regime: Thermic
Soil humidity regime: Aquic
Water table: Accessible, 0.35 m
Drainage class: moderately well drained

GEOMORPHOLOGY:

Observation scale: Hectometric
Surface type: Flood plain
Dynamic of the form: -
Intensity of the process: -
Local morphology: rectilinear area
Situation within the form: central
General slope: 0%
Orientation: West

SUPERFICIAL PEDREGOSITY: 0%

ROCKY OUTCROP: No outcrops

EROSION: No erosion

EFFECTIVE DEPTH: >0,35 m

GEOLOGY: Cord of coastal dunes. Holocene (Qd)

ORIGINAL MATERIAL: Marshes deposits

VEGETATION: Halophytic shrubland , dominated by *Sarcocornia fruticosa* with presence of *Atriplex portulacoides.* Vegetal cover: 100%

TECNOLOGY:

LAND USE: Natural use, protected area

CLASSIFICATION*:

(SSS, 1999) **Xeropsamment Aquic**

OBSERVACTIONS: The organic horizon (O) is formed by rest of stems and roots of *Sarcocornia fruticosa.*

Figure S1. Soil profile of habitat 1

MACROMORPHOLOGIC DESCRIPTION OF THE HORIZON

NAME (ABC)	O	1	A2
DEPTH (cm)	(-3)-0	0-12	12-(>30)
TEXTURE		Clay-loam	Sandy
HUMIDITY	Humid	Wet	Humid
OXIDE-REDUCTION STATE	Oxydated with reduction signs	Oxydated with reduction signs	Oxydated with reduction signs
α-α-dipyridyl REACTION	mild	mild	mild
COLOUR (DRY)		10YR 5/4	10YR 6/4
COLOUR (WET)		10YR 6/4	10YR 5/4
STAINS	No	No	No
THICK ELEMENTS	No	No	No
STRUCTURE	-	In blocks	Apedal (without structure)
CONSISTENCY	-	Plastic	Loose
MI (Kg cm^{-2})	-	2,5	2
FAUNAL ACTIVITY	Yes	No	No
ANTHROPOGENIC ACTIVITY	No	No	No
ROOTS	Yes	Yes	Yes
REACTION HCl	No	Strong	Very strong
FORM LIMIT	Flat	Flat	-
LIMIT-SHARPNESS	Very abrupt	Very abrupt	-
BD (g cm^{-3})	-	1,39	1,69

Table S1. Description of the horizons identified in the habitat 1. MI: Mechanic impedance; BD: Bulk density.

SOIL CHARACTERIZATION _ GENERAL INFORMATION_ PEDON: Habitat 2

Location: La Pletera
Municipality: Torroella de Montgrí (l'Estartit)
Date of description: 08/02/2018
Prospectors: Marc Amorós and Lorena Carrasco

CARTOGRAPHY:

Editor: ICGC (http://www.icgc.cat)
Map/Scala: Orthophoto 1:5000
Coordinates UTM 31N ETRS89: 515681 m E, 4653370 m N
Altitude: 0,8 m

CLIME AND WATER OF THE SOIL:

Soil temperature regime: Thermic
Soil humidity regime: Aquic
Water table: No accessible
Drainage class: moderately well drained in the surface

GEOMORPHOLOGY:

Observation scale: Hectometric
Surface type: Flood plain
Dynamic of the form: -
Intensity of the process: -
Local morphology: rectilinear area
Situation within the form: central
General slope: 0%
Orientation: West

SUPERFICIAL PEDREGOSITY: 0%

ROCKY OUTCROP: No outcrops

EROSION: No erosion

EFFECTIVE DEPTH: >0,5 m

GEOLOGY: Cord of coastal dunes. Holocene (Qd)

ORIGINAL MATERIAL: Marshes deposits

VEGETATION: Halophytic grassland, dominated by *Elymus pycnanthusn* and *Atriplex portulacoides* with presence of *Juncus acutus.*
Vegetal cover: 100%

TECNOLOGY:

LAND USE: Natural use, protected area

CLASSIFICATION*:

(SSS, 1999) **Xeropsamment Aquic**

OBSERVACTIONS

Figure S2. Soil profile of habitat 2

MACROMORPHOLOGIC DESCRIPTION OF THE HORIZON

NAME (ABC)	A1		A2	
DEPTH (cm)	0-10	10-20	20-25	>35
TEXTURE	Sandy-loam	Sandy-loam	Sandy	Sandy
HUMIDITY	Humid	Humid	Humid	Wet
OXIDE-REDUCTION STATE	Oxydated with reduction signs	Oxydated with reduction signs	Oxydated with reduction signs	Oxydated with reduction signs
α-α-dipyridyl REACTION	Very mild	Very mild	Very mild	Very mild
COLOUR (DRY)	2.5Y 5/3	2.5Y 6/3	2.5Y 7/3	10YR 6/3
COLOUR (WET)	2.5Y 4/3	2.5Y 5/3	2.5Y 5/4	10YR 4/4
STAINS	No	No	No	No
THICK ELEMENTS	No	No	No	No
STRUCTURE	Crumb	Crumb	In blocks	Apedal (without structure)
CONSISTENCY	Friable	Friable	Friable	Loose
MI (Kg cm^{-2})	2,5	3	5	2
FAUNAL ACTIVITY	Yes	No	No	No
ANTHROPOGENIC ACTIVITY	No	No	No	No
ROOTS	Yes (thick)	Yes (thick)	Yes (fine)	Yes (fine)
REACTION HCl	Very strong	Very strong	Very strong	Strong
FORM LIMIT	Irregular	Irregular	Irregular	-
LIMIT-SHARPNESS	Diffuse	Very abrupt	Very abrupt	-
BD (g cm^{-3})	1.09	1,47	1.69	1,43

Table S2. Description of the horizons identified in the habitat 2. MI: Mechanic impedance; BD: Bulk density.

SOIL CHARACTERIZATION _ GENERAL INFORMATION_ PEDON: Habitat 3

Location: La Pletera
Municipality: Torroella de Montgrí (l'Estartit)
Date of description: 01/02/2018
Prospectors: Marc Amorós and Lorena Carrasco

CARTOGRAPHY:

Editor: ICGC (http://www.icgc.cat)
Map/Scala: Orthophoto 1:5000
Coordinates UTM 31N ETRS89: 515866 m E, 4653381 m N
Altitude: 1,7 m

CLIME AND WATER OF THE SOIL:

Soil temperature regime: Thermic
Soil humidity regime: Aquic
Water table: Accessible, 0,5 m
Drainage class: Impeded

GEOMORPHOLOGY:

Observation scale: Hectometric
Surface type: Flood plain
Dynamic of the form: -
Intensity of the process: -
Local morphology: rectilinear area
Situation within the form: central
General slope: 0%
Orientation: West

SUPERFICIAL PEDREGOSITY: stony, with gravel and stones (10-15% of volume)

ROCKY OUTCROP: No outcrops

EROSION: No erosion

EFFECTIVE DEPTH: 0,5 m

GEOLOGY: Cord of coastal dunes. Holocene (Qd)

ORIGINAL MATERIAL: Marshes deposits

VEGETATION: Pioneer annual vegetation of saline soils, dominated by *Salicornia patula* with presence of *Suaeda maritima.*
Vegetal cover: 80%

TECNOLOGY: partial affectation by movements of surrounding lands

LAND USE: Natural use, protected area

CLASSIFICATION*:

(SSS, 1999) **Xeropsamment Aquic**

OBSERVACTIONS: Presence of vegetal carbon from 30 cm depth

Figure S3. Soil profile of habitat 3

MACROMORPHOLOGIC DESCRIPTION OF THE HORIZON

NAME (ABC)	A	A_b	A_{2b}
DEPTH (cm)	0-30	30-40	>40
TEXTURE	Sandy	Fine	Sandy
HUMIDITY	Wet	Humid	Wet
OXIDE-REDUCTION STATE	Oxydated	Oxydated	Reduced
α-α-dipyridyl REACTION	Mild	Mild	Mild
COLOUR (DRY)	10R 5/3	10R 5/3	10R 5/2
COLOUR (WET)	10R 4/3	10R 5/3	10R 4/2
STAINS	No	Yes	No
THICK ELEMENTS	No	No	No
STRUCTURE	Apedal (without structure)	Laminar	Apedal (without structure)
CONSISTENCY	Loose	Friable	Loose
MI (Kg cm^{-2})	2	6	2
FAUNAL ACTIVITY	No	No	No
ANTHROPOGENIC ACTIVITY	No	Presence of vegetal carbon	Presence of vegetal carbon
ROOTS	Yes (10 cm)	No	No
REACTION HCl	Strong	Extremly strong	Extremly strong
FORM LIMIT	Flat	Irregular	
LIMIT-SHARPNESS	Very abrupt	Very abrupt	
BD (g cm^{-3})	1.69	1,96	1.61

Table S3. Description of the horizons identified in the habitat 3. MI: Mechanic impedance; BD: Bulk density.

CHARACTERIZATION OF THE HORIZONS OF THE SOIL PROFILES

		Physicochemical parameters				Biochemical parameters			
	Horizon	pH	EC (dS m^{-1})	BD (g cm^{-3})	$CaCO_3$ (%)	TN (%)	SOC (%)	C:N	SOM (%)
Habitat 1	0-12	8,51	0,215	1,39	8,920	1,673	0,725	4,331	1,249
Habitat 1	12-(<30)	8,61	0,195	1,69	5,891	0,847	0,116	1,369	0,200
Habitat 2	0-10	8,60	0,167	1,09	5,294	2,114	1,275	6,033	2,199
Habitat 2	10-20	8,60	0,235	1,47	7,861	1,715	0,667	3,887	1,149
Habitat 2	20-35	8,60	0,299	1,69	7,914	1,211	0,261	2,154	0,450
Habitat 2	>35	8,32	0,260	1,43	8,770	0,742	0,101	1,367	0,175
Habitat 3	0-30	8,25	1,209	1,70	10,000	1,001	0,188	1,882	0,325
Habitat 3	30-40	8,29	1,508	1,96	9,037	0,924	0,217	2,353	0,375
Habitat 3	> 40	8,18	0,836	1,61	4,278	1,260	0,188	1,495	0,325

Table S4. Physicochemical and biochemical parameters of the soil profiles studied in habitat 1, 2 and 3. EC: electric conductivity, BD: bulk density, TN: total nitrogen, SOC: soil organic carbon, C:N: ratio SOC and TN, SOM: soil organic matter.

	Horizon	CS	FS	S	C	Textural class
Habitat 1	0-12	35,85	19,18	36,89	8,09	Sandy loam
Habitat 1	12-(<30)	64,35	17,38	7,35	10,93	Loamy sand
Habitat 2	0-10	30,25	21,93	23,33	24,50	Sandy clay loam
Habitat 2	10-20	29,90	30,58	21,79	17,74	Sandy loam
Habitat 2	20-35	48,05	18,50	16,62	16,83	Sandy loam
Habitat 2	>35	84,10	1,30	3,01	11,59	Loamy sand
Habitat 3	0-30	82,60	1,03	4,34	12,03	Loamy sand
Habitat 3	30-40	56,15	13,33	13,38	17,15	Sandy loam
Habitat 3	> 40	78,40	4,30	4,12	13,18	Sandy loam

Table S5. Granulometric fractions and textural classes of each horizon in the soil profiles of habitat 1, 2 and 3. CS: coarse sand, FS: fine sand, S: silt, C: clay.