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Litter decomposition of three halophytes in a Mediterranean salt marsh: Relevance of litter quality, microbial activity and microhabitat

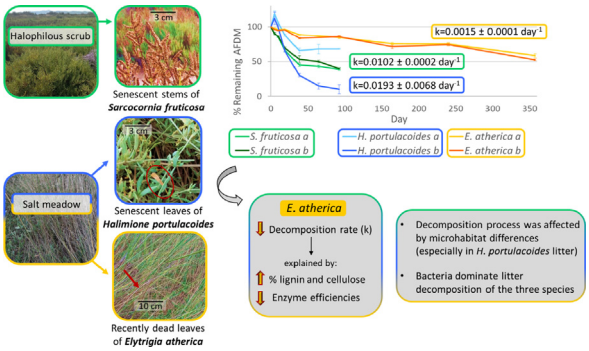
Lorena Carrasco-Barea [a,*], Laura Llorens [a], Anna M. Romaní [b], Maria Gispert [c], Dolors Verdaguer [a]

[a] Group of Soil and Vegetation in the Mediterranean (SOLIPLANT), Plant Physiology Unit, Department of Environmental Sciences, University of Girona, Campus Montilivi, 17003 Girona, Spain
[b] Group of Continental Aquatic Ecology Research (GRECO), Institute of Aquatic Ecology, University of Girona, Campus Montilivi, 17003 Girona, Spain
[c] Group of Soil and Vegetation in the Mediterranean (SOLIPLANT), Soil Science Unit, Department of Chemical Engineering, Agriculture, and Food Technology, University of Girona, Campus Montilivi, 17003 Girona, Spain

HIGHLIGHTS

- Initial lignin and cellulose better explain litter decomposition rates than C/N ratio.
- Slow litter decomposition of *Elytrigia atherica* would favour soil carbon storage.
- Enzyme efficiency, rather than enzyme activity, drive litter decomposition rates.
- Bacteria dominated litter decomposition but fungi highlight in the later stages.
- Specific microhabitat conditions determine huge differences in litter decomposition.

GRAPHICAL ABSTRACT



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ABSTRACT

Studies of litter decomposition in salt marshes have been mainly focused on the measurement of decomposition rates, being litter quality, the type of microbial decomposers and their extracellular enzyme activity, rarely considered. Moreover, most of these studies have been conducted in Poaceae and Cyperaceae species, being scarce the literature on Chenopodiaceae species, which are abundant in Mediterranean salt marshes. Here we analyse the litter decomposition process of two Chenopodiaceae (*Sarcocornia fruticosa* and *Halimione portulacoides*) and one Poaceae (*Elytrigia atherica*) species, belonging *S. fruticosa* to a halophilous scrub habitat and the other two to a salt meadow habitat of a Mediterranean salt marsh. For each species, we analysed litter decomposition rates, litter quality, fungal and bacterial biomass and potential extracellular enzymes activities. In order to embrace the spatial heterogeneity, two zones were considered within each habitat.

Litter of *E. atherica* decomposed 7- and 13-fold slower than those of *S. fruticosa* and *H. portulacoides*, respectively, suggesting that this species is the one that would favour most the carbon sequestration into the soil. The different decomposition rates would be explained by the higher initial lignin and cellulose content of *E. atherica* rather than by the initial carbon and nitrogen content and C/N ratio. Moreover, enzyme efficiency, compared to enzyme activity, better contributes to explain the different decomposition rates observed. Bacteria dominated throughout the litter decomposition process regardless the species, but fungi increased their relevance in the later stages, when the relative lignin litter content increased. Litter decomposition was affected by microhabitat spatial differences, although the responses

* Corresponding author at: Faculty of Sciences, University of Girona, Campus Montilivi, Carrer Maria Aurèlia Capmany i Farnés, 69, 17003 Girona, Spain.
E-mail address: lorena.carrasco.barea@gmail.com (L. Carrasco-Barea).

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depended on the species. Hence, flooding (in the habitat of *S. fruticosa*) or soil texture (in the habitat of *E. atherica* and *H. portulacoides*) might have modulated the decomposition process, being *H. portulacoides* the most sensitive species to the spatial differences of the salt meadow habitat.

1. Introduction

Salt marshes play an important role in climate regulation since they are considered among the ecosystems with highest carbon sequestration capacity (McLeod et al., 2011), resulting in high amounts of carbon stored in the soil (Chmura et al., 2003). Carbon (C) accumulation in the soil is mainly due to the slow transformation of plant litter into humic substances through the decomposition process (Kayranli et al., 2010). Studies up to now on litter decomposition in salt marshes have been mainly focused on litter decomposition rates, a parameter that has been broadly studied around the world, including tidal salt marshes of the Atlantic (Bouchard et al., 1998; Foote and Reynolds, 1997; Simões et al., 2011) and Pacific (Dick et al., 2002; Li et al., 2016) coasts, and non-tidal salt marshes of the Mediterranean Basin (Menéndez and Sanmartí, 2007; Scarton et al., 2002). Overall, one of the conclusions from these studies is that litter decomposition rates vary greatly, especially among species, depending also on the plant fraction analysed (leaves, herbaceous or woody stems and roots). Despite the importance of litter decomposition in soil carbon sequestration in salt marshes, other relevant parameters, such as litter quality, microbial decomposers biomass and community composition, or microbial extracellular enzyme capabilities, are still poorly studied. Thus, it is essential to increase our knowledge about these parameters in order to fully understand the role of plant litter decomposition in biogeochemical cycles such as those of carbon and nitrogen (N), and, consequently, in salt marshes functioning.

Litter quality is one of the main factors that influence decomposition rates (Duan et al., 2018; Valiela et al., 1985), being generally accepted that tissues with low C/N ratio decompose faster (Enríquez et al., 1993). Besides, the specific plant chemical composition, mainly regarding the content of polysaccharides (such as cellulose, hemicellulose), and phenolic compounds (such as lignin), determine litter hardness and affect litter decomposition (Duan et al., 2018). However, few studies have estimated the amount of these compounds in salt marsh plant species, being these studies mainly performed in monocotyledonous species from Poaceae and Cyperaceae families (Duan et al., 2018; Liao et al., 2008; Valiela et al., 1984). To our knowledge, there are no studies performed in succulent Chenopodiaceae halophytes, despite they are the dominant vegetation in Mediterranean climate salt marshes, which include salt marshes of the Mediterranean Basin, but also those of the Pacific coast of central and southern California and SW Australia (Ibañez et al., 2000).

Soil microbial communities, especially fungi and bacteria, play a key role in the litter decomposition process, since they produce extracellular enzymes that convert litter macromolecules into smaller, readily utilizable compounds (Moorhead and Sinsabaugh, 2006). The most common extracellular enzymes, related with the degradation of plant carbon-rich structural compounds, are cellulases, hemicellulases, pectinases, phenol oxidases and peroxidases (Sinsabaugh et al., 2002). Indeed, throughout the litter decomposition process there is a close relationship among extracellular enzyme activity (EEA), litter chemical quality and microbial community (Mora-Gómez et al., 2016), which is related with changes in the enzymatic profile. For instance, regarding some enzymes involved in plant fiber decomposition, β-glucosidase is active during most of the decomposition process, since it is related with the cellulolysis, splitting glucose from polysaccharide chains (such as cellulose), while β-xylosidase, involved in hemicellulose degradation, peaks approximately in the middle of the decomposition process. Oxidative enzymes, such as phenol oxidase, are more active at the last decomposition stages, when mainly the most recalcitrant compounds, such as lignin, remain (Mora-Gómez et al., 2016; Sinsabaugh et al., 2002). The activity of the enzymes involved in the acquisition of nutrients, such as leucine-aminopeptidase, would depend on the microbial threshold elemental C/N ratio at which microorganism metabolism changes from energy to nutrient supply (Sinsabaugh and Shah, 2011), being therefore highly variable along the litter decomposition process. Few studies have assessed the temporal dynamics of fungi or bacteria biomass throughout the litter decomposition process in salt marshes, being the results about their relative dominance controversial. For instance, Benner et al. (1984 and 1986) found that bacteria dominated the degradation of radiolabelled lignocellulosic from *Spartina alterniflora* leaves, which coincides with studies reporting that fungi tend to be more sensitive to soil salinity than bacteria (Sardinha et al., 2003; Wichern et al., 2006). However, in other studies with *S. alterniflora,* a fungal dominance in leaves decomposition was observed (Newell and Porter, 2000; Samiaji and Bärlocher, 1996).

Litter decomposition is also affected by environmental factors such as temperature, humidity, oxygen supply and pH (Buscot and Varma, 2005). In salt marshes, in particular, litter decomposition is slowed down in flooding periods when low oxygen availability or anoxia occur (Gingerich et al., 2014). Mediterranean Basin salt marshes are not subjected to daily flooding by tides, but the strong winds and storms that usually occur, cause the direct entrance of salty water into the marsh, where it can stay stagnant during several weeks or months influencing litter decomposition (Ibañez et al., 2000; Pascual and Martinoy, 2017). Spatial heterogeneity can also affect the process of litter decomposition, since environmental conditions, such soil oxygen, organic matter or water content, can highly vary at the microhabitat scale (Menéndez and Sanmartí, 2007; Rejmánková and Houdková, 2006). This would be especially relevant in the case of Mediterranean Basin salt marshes, since they have a high degree of patchiness (Ibañez et al., 2000), and, thus, it should be considered in litter decomposition studies.

The main aim of this study is to detail the litter decomposition process of two Chenopodiaceae (*Sarcocornia fruticosa* and *Halimione portulacoides*) and one Poaceae (*Elytrigia atherica*) species, which are dominant halophytes of a Mediterranean non-tidal salt marsh. Specifically, it is aimed to 1) investigate, on the three halophytes, the dynamics of litter quality, microbial biomass and microbial activity throughout the litter decomposition process and test differences among them, and 2) analyse the effect of the microhabitat on their litter decomposition process. To achieve these goals, litter quality parameters (carbon, nitrogen, cellulose and lignin content), fungal and bacterial biomass and potential activity of extracellular enzymes involved in plant decomposition (ß-D-glucosidase, ß-D-xylosidase, leucine-aminopeptidase and phenol oxidase), together with the litter decomposition rate, were measured for each species from litter placed at two zones within each habitat. It is expected that *E. atherica* will show the lowest decomposition rate since Poaceae species have commonly harder tissues than Chenopodiaceae ones. It is also expected that, because of the *E. atherica* litter hardness, this species will show a lower microbial colonization which could promote a lower activity and efficiency of the extracellular enzymes.

2. Material and methods

2.1. Study area

The study was performed at La Pletera, a coastal Mediterranean non-tidal salt marsh located in the north of the river Ter mouth in the municipality of Torroella de Montgrí (Girona, NE of the Iberian Peninsula, 42°1′51″N 3°11′33″E; Fig. S1). La Pletera salt marsh is composed by different natural habitats, which are typical of Mediterranean salt marshes. In particular, occupy the largest area three habitats of Community Interest (HCI; Council Directive 97/62/EC; EC, 1997), i.e., natural habitats with a high ecological value subjected to a special degree of protection and conservation by the European Commission. These habitats are the Mediterranean halophilous scrub (HCI code 1420), dominated by *Sarcocornia fruticosa* (syn. *Arthrocnemum fruticosum*); the Mediterranean salt meadow (HCI code 1410), dominated

by *Elytrigia atherica* (syn. *Elymus pycnanthus*) and *Halimione portulacoides* (syn. *Atriplex portulacoides*); and the glasswort sward (HCI code 1310), dominated by *Salicornia patula*. Litter decomposition experiments were carried out in the halophilous scrub and the salt meadow, while the glasswort sward was not considered since its soil carbon storage capacity was very low (Gispert et al., 2020) and its amount of litter was insignificant (Gispert et al., 2021).

2.2. Experimental design

To assess the litter decomposition process the litterbag technique was applied (Graça et al., 2005). Since leaves are reduced to small, hardly visible scales, senescent photosynthetic stems of *S. fruticosa* were collected in the halophilous scrub, while senescent leaves of *H. portulacoides* and recently dead leaves of *E. atherica* were collected in the salt meadow (Fig. S2). Collection took place in early November 2016, coinciding with plant senescence peak. To account for the spatial variability, plant material was collected from two zones within each habitat. Once at the laboratory, plant material of each species was mixed (to provide a uniform initial sample) and air-dried for 9 days at room temperature (around 20 °C). After this period, 3 samples per species were used to estimate initial litter quality parameters and ash free dry mass, and approximately 4 g were placed into nylon litterbags of 15 cm × 15 cm and 1 mm of mesh size. The mesh size was selected to allow the entry of edaphic mesofauna and to avoid plant material loss. To estimate the initial oven-dried weight of plant material from each litterbag, two extra samples of about 4 g of air-dried material for each species were oven-dried (70 °C) for 48 h and a moisture correction factor was calculated. In mid-November 2016, a group of 8 litterbags of each species was placed at three sites in each one of the two zones of the corresponding habitat (48 litterbags per each species) (Fig. S3).

Litterbags were situated on the soil surface under the canopy of each respective species, and they were tied to an iron stick with nylon string to allow the movement of the bags during the flooding periods. Six litterbags per species (one per site) were periodically collected for chemical and biological analyses. Litterbags of *S. fruticosa* and *H. portulacoides* remained in the field around three months (collected on days 5, 10, 19, 38, 64 and 92), while those of *E. atherica* remained almost one year (collected on days 5, 10, 19, 38, 92, 164, 240 and 357), since decomposed slowly. The litter remaining ash free dry mass and the potential extracellular enzyme activities (EEA) were measured every time litterbags were collected. The rest of the parameters (carbon, nitrogen, cellulose and lignin content and bacterial and fungal biomass) were measured three times throughout the litter decomposition period, specifically, on days 19, 64 and 92 for *S. fruticosa* and *H. portulacoides* and on days 19, 92 and 357 for *E. atherica*.

2.3. Litter ash free dry mass and decomposition rate

Plant material from field sampled litterbags was rinsed with distilled water to remove soil particles and mesofauna, oven-dried (70 °C) and weighed to assess mass loss from the start of the experiment. About 200 mg of oven-dried plant material from litterbags and from the initial oven-dried litter, were combusted at 450 °C for 4 h and weighed to estimate the ash free dry mass (AFDM, %).

Litter decomposition rates for each species were estimated from litter collected at each site (three per zone and habitat) using the single exponential decay model regression: $X_t = X_0 * e^{-kt}$, where X_t is the litter AFDM at time t (days), X_0 is the estimated initial AFDM and k is the decomposition rate coefficient.

2.4. Litter quality: carbon, nitrogen, cellulose and lignin content

An oven-dried fraction from the litter initially harvested and from litterbags were ground to a fine powder with a ball mill (Mixer Mill MM 400, Retsch GmbH, Germany) for chemical analyses. To determine total C and N content, 4 mg of fine powder were placed in tin capsules that were sent to the Stable Isotope Facility of the University of California (Davis, USA) for their analyses. The analyses were conducted using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). C and N content were expressed in percentage of litter AFDM. The C/N weight ratio was also calculated.

Cellulose and lignin content were measured gravimetrically from 240 mg of fine powder obtained from dry plant material according to the acid-detergent method described in Gessner (2005a). Briefly, samples were heated for 1 h in an acid detergent solution (hexadecyltrimethylammonium bromide at 20 g l^{-1} in 0.5 M H_2SO_4), filtered and the remained residues oven-dried (105 °C) and weighted (W1). Then, the residues were treated three times with 72% H_2SO_4, filtered again and the remained residues, which at this moment were cellulose free, were oven-dried (105 °C) and weighted again (W2). The cellulose content (%) was estimated as W1-W2 divided by the initial AFDM of the sample (W0) and multiplied by 100. Finally, the residues were combusted at 500 °C for 4.5 h and weighed again (W3). Lignin content (%) was estimated as W2-W3 divided by W0 and multiplied by 100. Lignin/N ratio was also calculated.

2.5. Litter extracellular enzyme activity and enzyme efficiency

Potential extracellular enzyme activities (EEA) were measured according to the methods described in Romaní et al. (2006). In particular, we estimated EEA of β-D-glucosidase (EC 3.2.1.21) and β-D-xylosidase (EC 3.2.1.37), which are involved in cellulose and hemicellulose hydrolytic degradation, respectively, leucine-aminopeptidase (EC 3.4.11.1) related with peptide hydrolytic degradation and phenol oxidase (EC 1.10.3.2 and 1.14.18.1) involved in lignin oxidation. The three hydrolytic enzymes were measured by fluorimetric assays using fluorescent methylumbelliferone (MUF) linked to the artificial substrates for ß-D-glucosidase and ß-D-xylosidase (MUF-ß-D-glucopyranoside and MUF-ß-D-xylopiranoside, respectively) and fluorescent 7-amino-4-methylcoumarin (AMC) linked to the artificial substrate L-leucine-4-methyl-7-coumarinylamide hydrochloride for leucine-aminopeptidase. The activity of phenol oxidase was measured by spectrophotometry using L-3,4-dihydroxyphenylalanine (L-DOPA) as oxidizable substrate. One of the products of L-DOPA oxidation is the red coloured compound 2,3-dihydroindole-5,6-quinone-2-carboxylate (DIQC), which can be quantified by measuring its absorbance at 460 nm and can be directly related to the activity of phenol oxidase. All the artificial substrates were from Sigma-Aldrich (Missouri, USA). Enzyme assays were conducted under substrate saturated conditions previously determined from saturation curves. Specifically, the final substrate saturating concentrations were 0.5 mM for β-D-glucosidase and β-D-xylosidase, 0.3 mM for leucine-aminopeptidase and 5 mM for phenol oxidase.

To analyse potential EEAs, plant material from litterbags (corresponding to about 0.04 g of oven-dry weight) was immediately incubated with 0.2 μm filtered water from a lagoon near to the sampling site, and with the artificial substrate necessary to reach the specific substrate saturation concentration, and then processed as described in Mora-Gómez et al. (2016). MUF and AMC standards (from 0 to 100 μM) were also prepared using the same 0.2 μm filtered water. After the enzyme activity analyses, samples were oven-dried (70 °C) for 48 h and weighed. Results are expressed as μmol of MUF/AMC/DIQC per g AFDM of litter and hour.

Enzyme efficiencies were calculated as turnover activities (TA), which are the inverse of the regression slope between ln % remaining AFDM regressed linearly to the accumulated enzyme activity (AEA, mmol g $AFDM^{-1}$) (Simon et al., 2009). AEA was calculated using the formula: $AEA = \Sigma_{i=0}^{n} EiTi$; where n is the number of samplings, Ei is the mean enzyme activity of two successive measurements, and Ti is the time between the two measurements. TA is expressed as mmol of enzyme produced to decompose one gram of litter, with higher values meaning lower enzyme efficiencies.

2.6. Bacterial biomass

Bacterial biomass was estimated from bacteria cell counts obtained with the flow cytometry technique. Litter directly collected from bags, corresponding to about 0.05 g of oven-dry weight, was preserved in 2% formaldehyde solution and stored until analyses. To detach bacteria from plant material, samples were sonicated (40 W, 40KHz, Selecta, Spain) for two

1 min cycles and cells were dislodged by vortexing (IKA® VORTEX, Genius 3, Sigma-Aldrich, Missouri, USA). After that, solid particles were left to sediment from the solution (1–2 min), and, to favour cell separation, 200 μl of the solution were subjected to dissolution using 0.05 M sodium pyrophosphate decahydrate until reaching the final concentration of 1:1000 sample: solution. Then, 400 μl of the sample-pyrophosphate solution were stained with 4 μl of the fluorescent Syto13 solution (5 μM, Fisher, Pennsylvania, USA) and incubated in dark conditions for 15–30 min. After that, 10 μl of bead solution (10^6 beads ml^{-1}, Fisher, 0.1 μm) was added to the samples to normalize fluorescent data from Syto 13. Bacteria cells were counted using a flow cytometer (FACSCalibur, Becton and Dickinson, New Jersey, USA), and bacterial biomass in terms of carbon was estimated as 2.2×10^{-13} gC μm^{-3} (Bratbak and Dundas, 1984), considering bacteria cell biovolume as 0.1 μm^3 (Theil-Nielsen and Sondergaard, 1998).

2.7. Fungal biomass

Fungal biomass was estimated from ergosterol concentration in the litter according to Gessner (2005b). Plant material from litterbags (corresponding to about 0.1 g of oven-dry weight) was stored in the freezer and lyophilized just before the analyses. The lyophilized samples were weighed and lipids were extracted with KOH-methanol solution heated at 80 °C for 30 min. The extracts were filtered using solid-phase extraction cartridges (Waters Sep-Pak®, Vac RC, tC18, 500 mg sorbent), and the ergosterol retained in the cartridges was eluted with isopropanol. Ergosterol was quantified by using high-performance liquid chromatography (HPLC analyzer Waters corporation, USA), equipped with a Waters Nova-Pak®C18 4 μm chromatographic column (3.9 × 300 mm). The peaks of the samples in the chromatograph were then compared with the absorption peaks of external ergosterol standards at 282 nm. Ergosterol concentration was converted to fungal biomass considering that there are 5.5 μg of ergosterol in one gram of fungal biomass (Gessner and Chauvet, 1993) and converted to carbon content considering that fungal dry mass has a 43% of carbon (Baldy and Gessner, 1997).

2.8. Data analyses and statistics

2.8.1. Differences among species

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

Differences among species in litter quality, EEA and the microbial biomass, considering all the studied decomposition period for each species, were analysed by means of generalized linear models (GLMs), using species as fixed factors, time as a covariate and including the interactions among species and time, and species and zone. GLMs were applied because normal distribution and homoscedasticity assumptions were not reached for the variables when considering all the studied decomposition period, even after being transformed. Moreover, litterbags of the different species were not sampled at the same dates and the period they were in the field also varied among the species.

Exponential regressions between the % of remaining AFDM and time and linear regressions between ln % remaining AFDM and AEA were significant using data from the 6 replicates per species. Therefore, differences among species in litter decomposition rates (k) and enzyme efficiencies (as TA) were tested by means of two-way ANOVAs, using species as fixed factor and including the interaction between species and zone.

The isolated effect of the zone factor was not included in the GLMs and the two-way ANOVAs because the zones (*a* and *b*) differed between habitats (is to say, they were not the same for the three species). Hence, only the interaction of zone with species was included in the models.

2.8.2. Within species analyses: time and zone effects

Repeated measures ANOVAs were applied for each species separately to analyse differences between zones within each habitat in litter quality parameters, EEA and microbial biomass throughout the decomposition process (time). When the interaction between time and zone was significant, one-way-ANOVAs were performed to analyse differences between zones within each sampling day. When the time factor was significant, differences among sampling dates were analysed by means of pairwise comparison for each species, considering the zones separately if the interaction among time and zone was significant. Differences in k and TA between zones of the corresponding habitat were tested using one-way ANOVAs for each species, with zone as fixed factor.

The Shapiro-Wilk test was used to test normality, while the homogeneity of variances was analysed with the Levene's test. The significance level considered for all the statistical tests was p-value <0.05. All the GLMs were chosen following the Akaike Information Criteria (AIC). R software version 4.1.1. (R Core Team, 2021) was used to perform the GLMs while repeated measures ANOVAs and one-way ANOVAs were performed with SPSS software (IBM SPSS statistics, Corporation, Chicago, USA).

3. Results

3.1. Litter quality and decomposition rates

3.1.1. Differences among species

S. fruticosa litter had the initial lowest C content and highest N content, and thus, it showed the lowest C/N ratio (half of the C/N ratio of the other two species), while no significant differences were observed in these parameters between *H. portulacoides* and *E. atherica* (Tables 1 and S2). *S. fruticosa* litter also had the lowest initial cellulose content and lignin/N ratio, while *E. atherica* litter showed the highest values of these two parameters as well as of lignin content (Table 1). Considering the whole studied period, no significant differences among the three species were observed in the litter C content, while *S. fruticosa* litter maintained the highest N

Table 1
Litter quality parameters (mean ± SE) at the beginning of the decomposition process (n = 3) and over the entire decomposition period (n = 24). Different letters indicate significant differences among species (p < 0.05) in the initial litter quality (lowercase letters) and considering the entire decomposition period (capital letters).

Litter quality	Species	C (%)	N (%)	C/N	Cellulose (%)	Lignin (%)	Lignin/N
Initial	*S. fruticosa*	45.0 ± 0.2 b	1.79 ± 0.02 a	25.1 ± 0.2 b	18.0 ± 0.4 c	8.5 ± 0.1 b	4.73 ± 0.02 c
	H. portulacoides	46.5 ± 0.3 a	0.84 ± 0.01 b	55.3 ± 0.5 a	20.6 ± 0.4 b	7.4 ± 0.2 b	8.81 ± 0.46 b
	E. atherica	46.5 ± 0.1 a	0.87 ± 0.03 b	53.3 ± 1.8 a	45.2 ± 0.6 a	10.5 ± 0.2 a	12.08 ± 0.08 a
Entire period	*S. fruticosa*	47.6 ± 0.4 A	2.00 ± 0.04 A	24.0 ± 0.4 C	26.9 ± 1.3 B	13.4 ± 1.0 B	6.6 ± 0.4 C
	H. portulacoides	47.0 ± 0.4 A	0.93 ± 0.04 C	52.1 ± 1.6 A	24.3 ± 0.7 B	11.8 ± 0.8 B	12.7 ± 0.6 B
	E. atherica	48.1 ± 0.4 A	1.08 ± 0.05 B	46.0 ± 1.4 B	44.4 ± 0.4 A	15.0 ± 0.9 A	13.8 ± 0.5 A

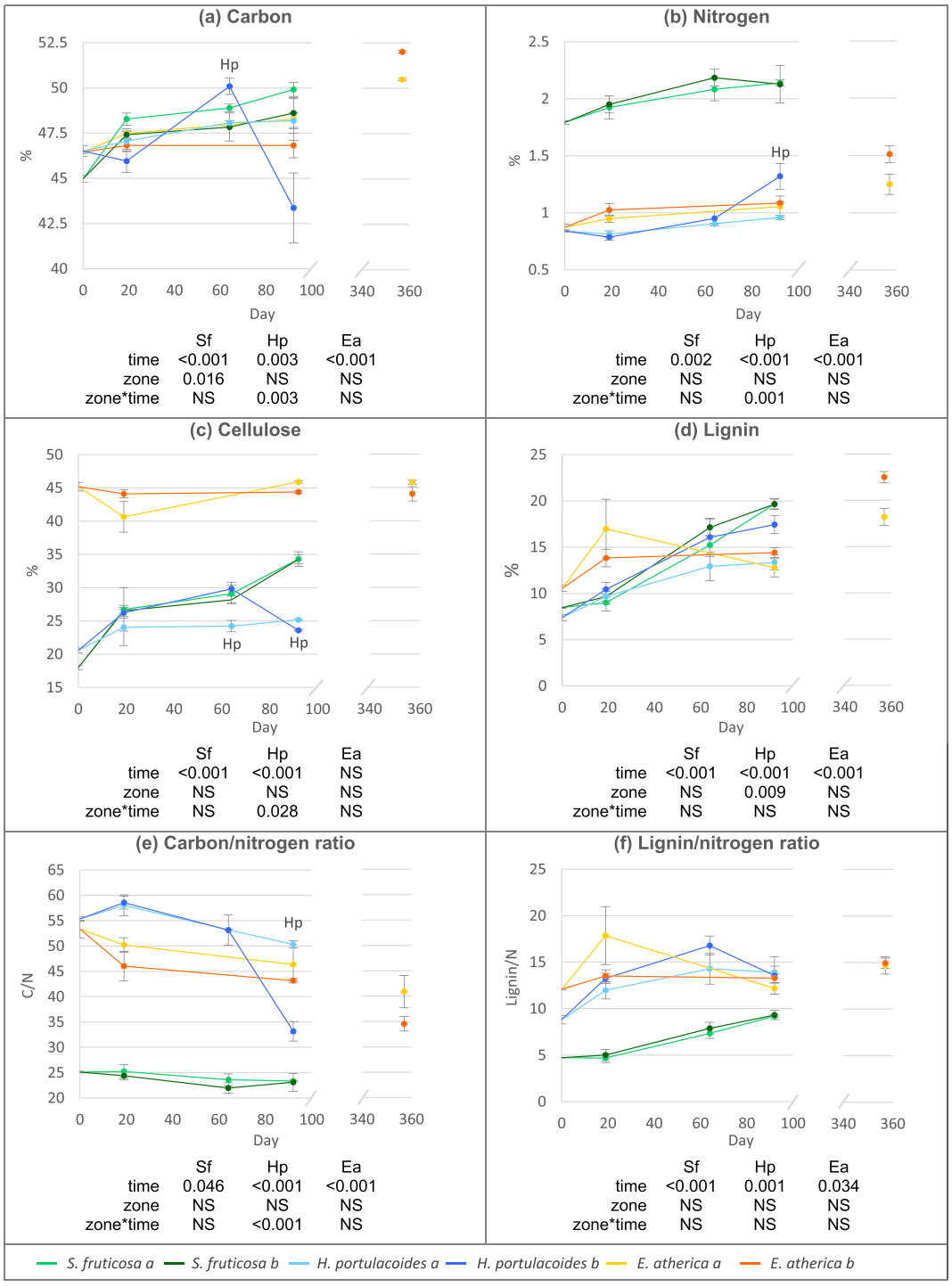

Fig. 1. Changes in litter content of carbon (a), nitrogen (b), cellulose (c) and lignin (d), carbon/nitrogen ratio (e) and lignin/nitrogen ratio (f) throughout the decomposition period of the three studied species in two zones. Bars represent standard errors ($n = 3$). For each species, significant p-values for time and zone factors and for their interaction according to the repeated measures ANOVAs are also given. The initials of the species names (Sf: *S. fruticosa*; Hp: *H. portulacoides*; Ea: *E. atherica*) are shown in the graphs when there were significant differences between zones (*a* and *b*) for the corresponding species and sampling day ($p < 0.05$), being depicted only when the interaction between zone and time was significant. NS: Not significant.

content and the lowest C/N and lignin/N ratios (Table 1, Fig. 1). *E. atherica* litter showed the highest cellulose and lignin content throughout the study (Table 1, Fig. 1).

Regarding litter decomposition rate (k), there were significant differences among the three species ($p < 0.001$), with *E. atherica* presenting the lowest values, followed by *S. fruticosa* and *H. portulacoides* (0.0015 ± 0.0001 day^{-1} 0.0102 ± 0.0002 day^{-1} and 0.0193 ± 0.0068 day^{-1}, respectively). Remaining AFDM dynamics also differed among species. *S. fruticosa* and *H. portulacoides* litter showed a fast decline of remaining AFDM (until day 38) and then, the mass loss curve stabilized (Fig. 2). In *E. atherica*, three different phases were observed: a slow initial loss of biomass (0 to 164 days), followed by a phase of stabilization (164 to 240), and then, a phase of gradual decline, with 55% of the AFDM still remaining on day 357.

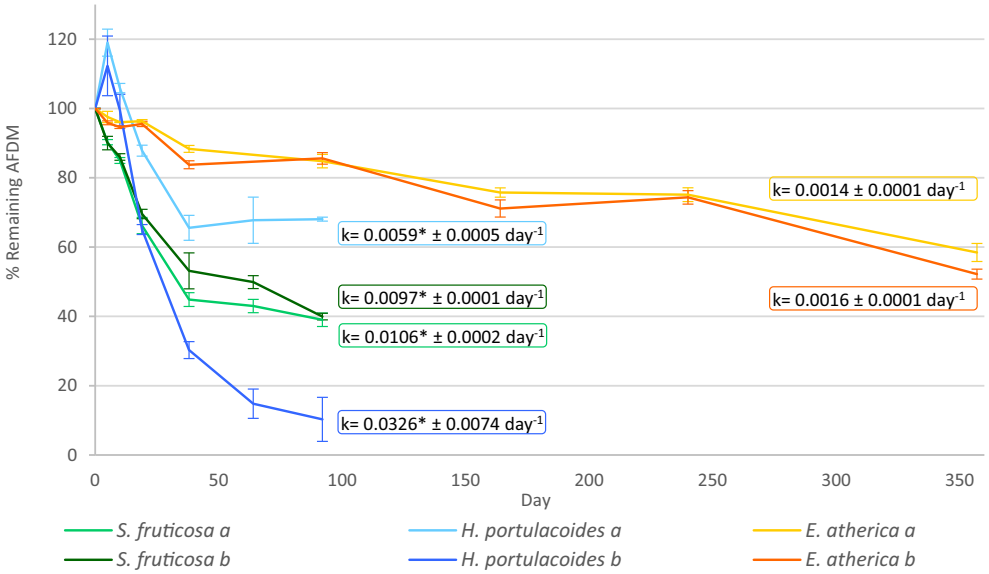

Fig. 2. Litter decomposition process for the three species expressed as remaining ash free dry mass (AFDM, % of the initial) for each sampling date. Mean ± SE of decomposition rates (k) are also shown, considering separately the two zones within each habitat (n = 3). Asterisks indicate significant differences in k between the two zones of the corresponding habitat for each species ($p < 0.05$), according to the one-way ANOVAs. Bars represent standard errors ($n = 3$).

3.1.2. Within species analyses: time and zone effects

C content of *S. fruticosa* litter increased over time, but especially at the beginning of the decomposition process, with the highest differences being observed from initial values to those of day 19. Litter C content of this species was significantly higher in zone *a* compared to zone *b* throughout the study period (Fig. 1a). The rest of the litter quality parameters (N, cellulose, lignin, C/N and lignin/N) were only affected by time, increasing throughout the decomposition process, except in the case of the C/N ratio, which decreased over time (Fig. 1b-f).

C, N, and cellulose content for *H. portulacoides* litter, as well as C/N ratio, differed depending on the zone and the date. Specifically, differences between zones appeared at the end of the decomposition process, with values being significantly higher in zone *b* on day 64 for C and cellulose, and on day 92 for N (Fig. 1a, b. c). Consequently, the lowest C/N ratio was recorded in zone *b* on day 92 (Fig. 1e). Moreover, the lignin content of *H. portulacoides* litter significantly increased over time in both zones, with overall values being also higher at zone *b* (Fig. 1d). However, its lignin/N ratio increased throughout the decomposition process independently of the zone (Fig. 1f).

E. atherica litter quality did not differ between zones, but it changed significantly over time. Indeed, increases in litter C, N, lignin and lignin/N ratio, as well as decreases in the C/N ratio, were observed throughout the decomposition period (Fig. 1a, b, d, e, f). The cellulose content was the only parameter that did not change over time (Fig. 1c).

The effect of the zone on litter decomposition rate (k) depended on the species (interaction of species and zone, $p < 0.001$). Litter decomposition rate (k) of *S. fructicosa* was significantly higher in zone *a* compared to zone *b*, although the % of remaining AFDM was similar in both zones on the last sampling day (Fig. 2). In the case of *H. portulacoides,* litter decomposition rate also differed between zones, resulting in a % of remaining AFDM of about 68% and 10% in zone *a* and *b*, respectively, on the last sampling day (Fig. 2). Conversely, in the case of *E. atherica* litter, the decomposition rate did not differ between zones, and a similar % of remaining AFDM was observed on the last sampling day Fig. 2).

3.2. Extracellular enzyme activity and enzyme efficiency

3.2.1. Differences among species

Considering the whole decomposition period studied, the highest extracellular β-glucosidase activity was found for *E. atherica* litter, although values did not differ significantly from those of *S. fruticosa* (Table 2). β-xylosidase and leucine-aminopeptidase activities did not differ among species (Table S3). Regarding phenol oxidase activity, *H. portulacoides* had the highest values while no significant differences were observed between the other two species (Table 2).

E. atherica litter showed the highest turnover activity (TA), i.e., the lowest enzyme efficiency, for the four enzymes analysed, with values being

Table 2
Potential extracellular activity (EEA) considering the whole decomposition period studied (mean ± SE; $n = 36$ for *S. fruticosa* and *H. portulacoides*; $n = 48$ for *E. atherica*) and enzyme efficiencies, expressed as turnover activity (TA; mean ± SE; $n = 6$)), for each enzymes and species studied. Different letters indicate significant differences among species ($p < 0.05$) in the EEA (lowercase letters) and in the TA (capital letters).

		β-glucosidase	β-xylosidase	Leucine-aminopeptidase	Phenol oxidase
EEA (μmol MUF, AMC or DIQC g AFDM^{-1} h^{-1})	*S. fruticosa*	2.28 ± 0.31 ab	1.45 ± 0.44 a	12.76 ± 1.59 a	0.93 ± 0.14 b
	H. portulacoides	1.80 ± 0.25 b	0.69 ± 0.12 a	17.25 ± 2.39 a	1.72 ± 0.58 a
	E. atherica	3.11 ± 0.44 a	1.95 ± 0.30 a	14.31 ± 3.03 a	0.64 ± 0.12 b
TA (mmol g AFDM^{-1})	*S. fruticosa*	7.67 ± 1.26 B	7.72 ± 1.16 B	47.04 ± 2.80 B	2.08 ± 0.38 B
	H. portulacoides	5.93 ± 1.82 B	2.20 ± 0.77 B	52.59 ± 14.19 B	2.91 ± 0.42 B
	E. atherica	73.25 ± 9.37 A	56.32 ± 8.63 A	422.16 ± 32.44 A	17.01 ± 3.44 A

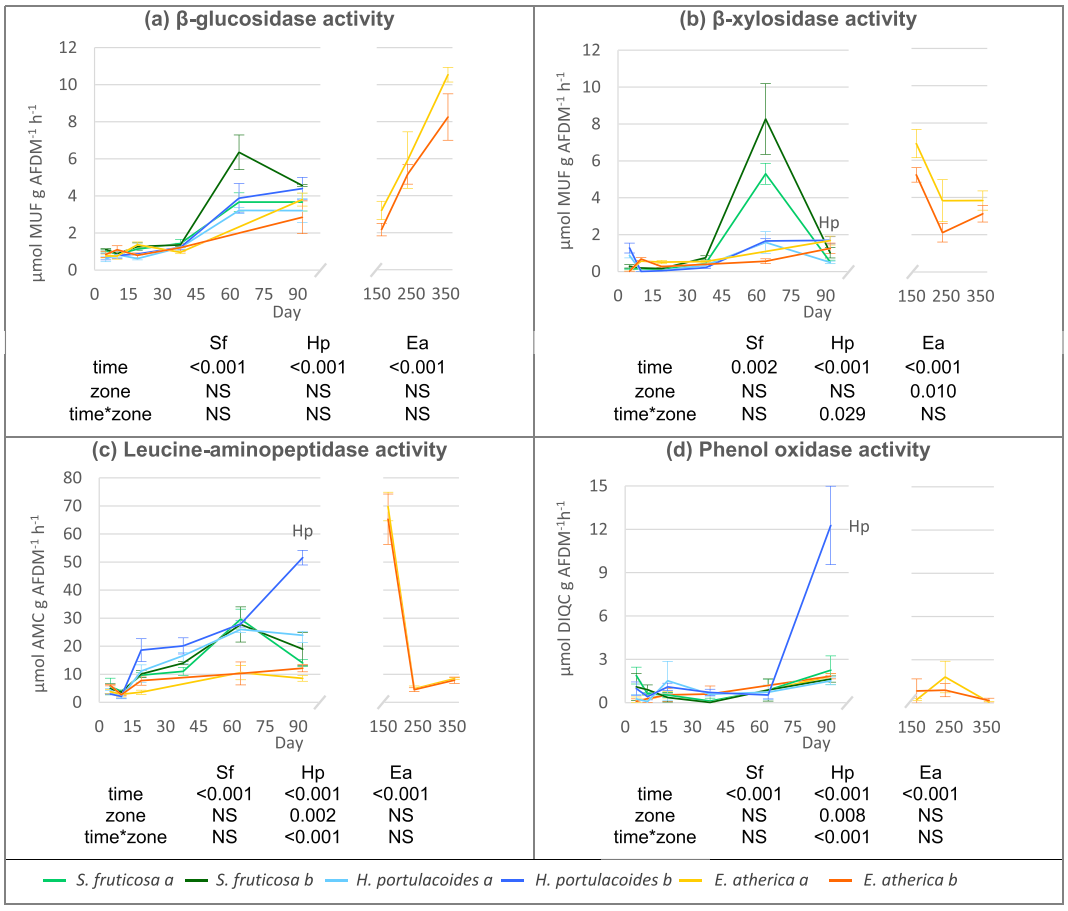

Fig. 3. Potential extracellular enzyme activities (EEA) of the four enzymes studied. Bars represent standard errors (n = 3). Significant *p*-values for time and zone factors and for their interaction according to the repeated measures ANOVAs are given. The initials of the species names (Sf: *S. fruticosa*; Hp: *H. portulacoides*; Ea: *E. atherica*) are shown in the graphs when there were significant differences between zones (*a* and *b*) for the corresponding species and sampling day ($p < 0.05$), being depicted only when the interaction between zone and time was significant.

more than one order of magnitude higher compared to those of the other two species. In contrast, TA did not differ between *S. fruticosa* and *H. portulacoides* litter (Table 2).

3.2.2. Within species analyses: time and zone effects

The temporal dynamics of EEA of the four enzymes studied differed among species, as indicated by the significant interactions between the factors species and time (Table S3).

For *S. fruticosa* litter, EEA of the four enzymes changed over time but not between zones (Fig. 3). Indeed, the activity of β-glucosidase was higher in the two last sampling dates (days 64 and 92) (Fig. 3a), while the activity of β-xylosidase and leucine-aminopeptidase peaked on day 64 and then declined (Fig. 3b, c). Phenol oxidase activity in *S. fruticosa* litter was low and quite constant throughout the decomposition period, being slightly higher on the last sampling date (Fig. 3d). TA for *S. fruticosa* litter did not differ between zones for any of the four enzymes (Fig. 4).

In *H. portulacoides* litter, changes over time in the EEA of β-xylosidase, leucine-aminopeptidase and phenol oxidase depended on the zone, with EEA values being significantly higher in zone *b* on the last sampling date (day 92), (Fig. 3b, c, d). The β-glucosidase activity increased over time in both zones (Fig. 3a).

EEA of the studied enzymes in *E. atherica* litter was affected by time but not by zone, except for of β-xylosidase, since the activity of this enzyme was higher in zone *a* throughout the whole study period (Fig. 3). Regarding changes over time, there was a steep increase in β-glucosidase activity over the entire study period (Fig. 3a), while the activities of β-xylosidase and leucine-aminopeptidase peaked on day 164 (Fig. 3b, c). Phenol oxidase activity of *E. atherica* litter was, in general, low, although it slightly increased with time (Fig. 3d).

For *H. portulacoides* litter, TA of β-glucosidase and leucine-aminopeptidase was significantly higher in zone *a*, while, for *E. atherica* litter, there were no significant differences between zones in TA for any of the four studied enzymes (Fig. 4).

3.3. Microbial biomass

3.3.1. Differences among species

Considering the entire study period, *E. atherica* litter had the lowest values of bacterial biomass, although differences with *H. portulacoides* were not significant (Table 3). No significant differences were observed among species in fungal biomass and fungal/bacterial biomass ratio (Tables 3 and S4). However, the significant interaction between species and time highlights a different microbial biomass dynamic throughout the decomposition period among the three species (Fig. 5, Table S4). On the other hand, for the entire period and for the three species, bacterial biomass was higher than fungal biomass (Fig. 5).

3.3.2. Within species analyses: time and zone effects

Bacterial and fungal biomass, and fungal/bacterial biomass ratio tended to increase throughout the decomposition period, except in the case of fungal biomass of *E. atherica* litter, which remained constant and with remarkably lower values during the entire study period, but changes through time were not significant for any of the three species studied (Fig. 5).

Regarding the differences between zones, in zone *b* of the salt meadow, litter of *H. portulacoides* had significantly higher bacterial biomass, while

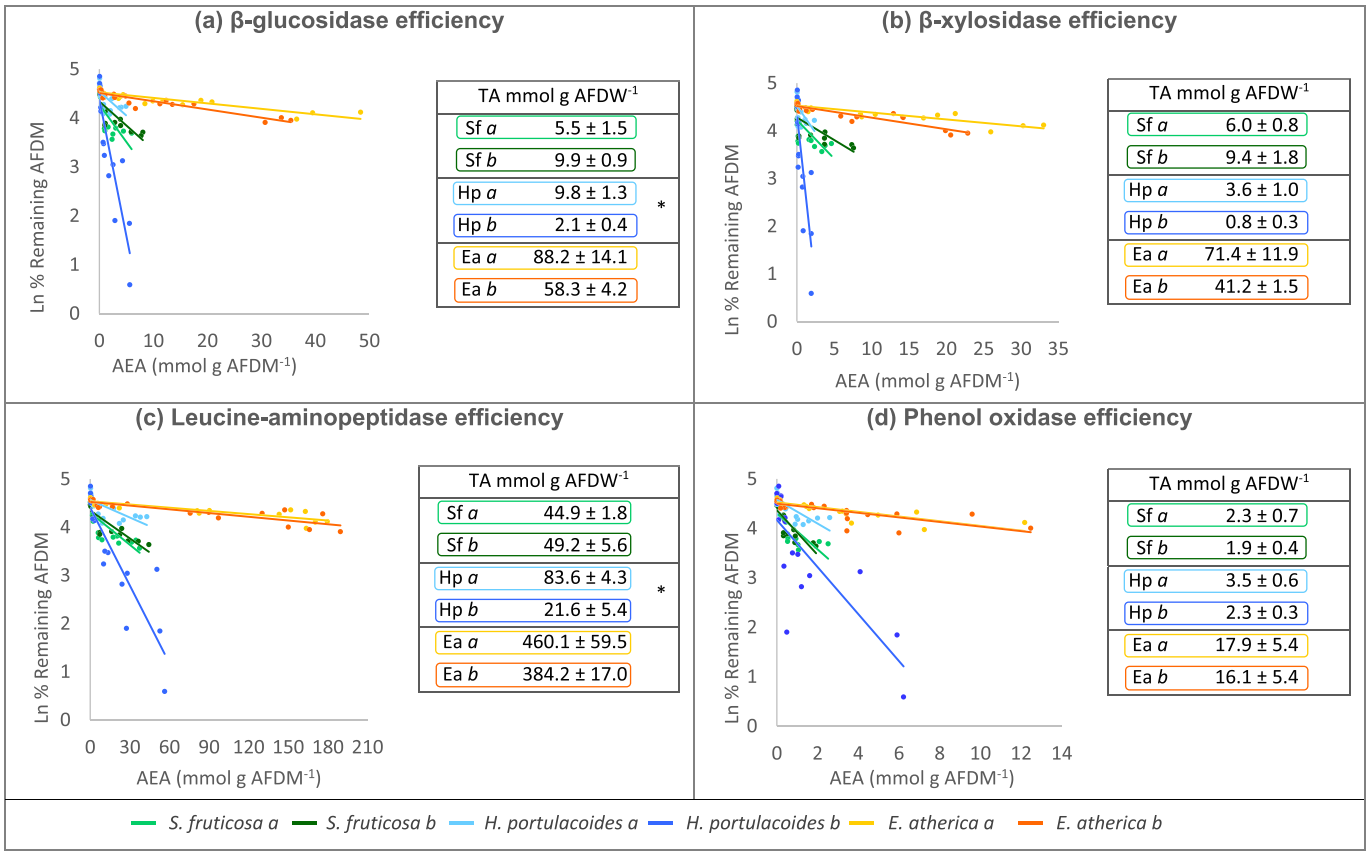

Fig. 4. Enzyme efficiencies of the four enzymes studied represented by means of linear regressions of ln of remaining ash free dry mass (Ln % remaining AFDM) against accumulated enzyme activity (AEA). Mean ± SE of turnover activity (TA) for each species (Sf: *S. fruticosa*; Hp: *H. portulacoides*; Ea: *E. atherica*) and zone within each habitat are also given. Steeper slopes and lower TA values represent higher enzyme efficiencies. Asterisks indicate significant differences in TA between the litter placed in the two zones of the corresponding habitat for each species ($p < 0.05$), according to the one-way ANOVAs.

litter of *E. atherica* showed a lower fungal/bacterial biomass ratio in comparison to values in zone *a* (Fig. 5).

4. Discussion

4.1. Differences among species in the litter decomposition dynamics

Litter decomposition rates for *Elytrigia atherica* were 7- and 13-fold lower than those of *Sarcocornia fruticosa* and *Halimione portulacoides*, respectively, which agree with lower decomposition rates reported for other salt marsh Poaceae species compared with Chenopodiaceae ones (Bouchard and Lefeuvre, 2000; Simões et al., 2011). Litter quality, such as C and N content, has been commonly defined as the main driver determining decomposition rate; for instance, it is widely accepted that a high initial N content favours decomposition, while a high C/N disfavours it (Duan et al., 2018; Enríquez et al., 1993). In this study, C, N, and C/N do not explain the lowest decomposition rates found in *E. atherica* since both *H. portulacoides* and *E. atherica* had similar initial values of C, N and C/N, although decomposition rate was much faster in *H. portulacoides*. Instead, other structural characteristics may be responsible for the low decomposition rate of *E. atherica* litter compared to *S. fruticosa* and *H. portulacoides,* such as its higher initial cellulose and lignin content, as well as its higher lignin/N ratio, since harder tissues are more resistant to be degraded and decompose slower (Curcó et al., 2002). The dynamics of extracellular enzyme activities and AFDM remaining through the decomposition process is in accordance with the hardness of *E. atherica* litter. The presence of a hard tissue structure made of cellulose, hemicellulose and lignin in *E. atherica* might be responsible for the lowest efficiency obtained for all C-degrading (β-glucosidase and β-xylosidase) and lignin degrading (phenol oxidase) enzymes, since harder tissues would need higher activity of the enzymes to breakdown these polymers (Sinsabaugh et al., 2002). The lower efficiency (high TA) of extracellular enzymes in *E. atherica* litter compared to the other two species indicates that a higher quantity of enzyme is required to decompose a certain amount of plant material. The relevance of *E. atherica* tissue composition determining its decomposition process is also highlighted by its AFDM dynamics, which showed a constant and gentle slope in contrast to the initial pronounced slope found in the other two species.

A rapid initial loose by lixiviation or by microbial decomposition of soluble and easily degradable compounds may occur in *S. fruticosa* and *H. portulacoides*, as also observed in the study of Simões et al. (2011), after which the most recalcitrant elements remain. This is shown by the progressive increment in the litter content of C, cellulose and lignin in these two species, while in *E. atherica* lignin content only increases after one year of decomposition. The tissue composition of *E. atherica* may prevent a rapid initial material loss which might be reciprocally related to a limited

Table 3
Bacterial biomass, fungal biomass and fungal/bacterial biomass ratio (mean ± SE) considering the whole decomposition period studied for each species ($n = 18$). Different letters indicate significant differences among species ($p < 0.05$).

	Bacterial biomass (mg C g AFDM^{-1})	Fungal biomass (mg C g AFDM^{-1})	Fungal/bacterial biomass ratio
S. fruticosa	50.4 ± 6.4 a	5.3 ± 1.8 a	0.11 ± 0.03 a
H. portulacoides	39.0 ± 11.0 ab	7.8 ± 2.9 a	0.27 ± 0.07 a
E. atherica	29.6 ± 9.8 b	1.0 ± 0.1 a	0.08 ± 0.02 a

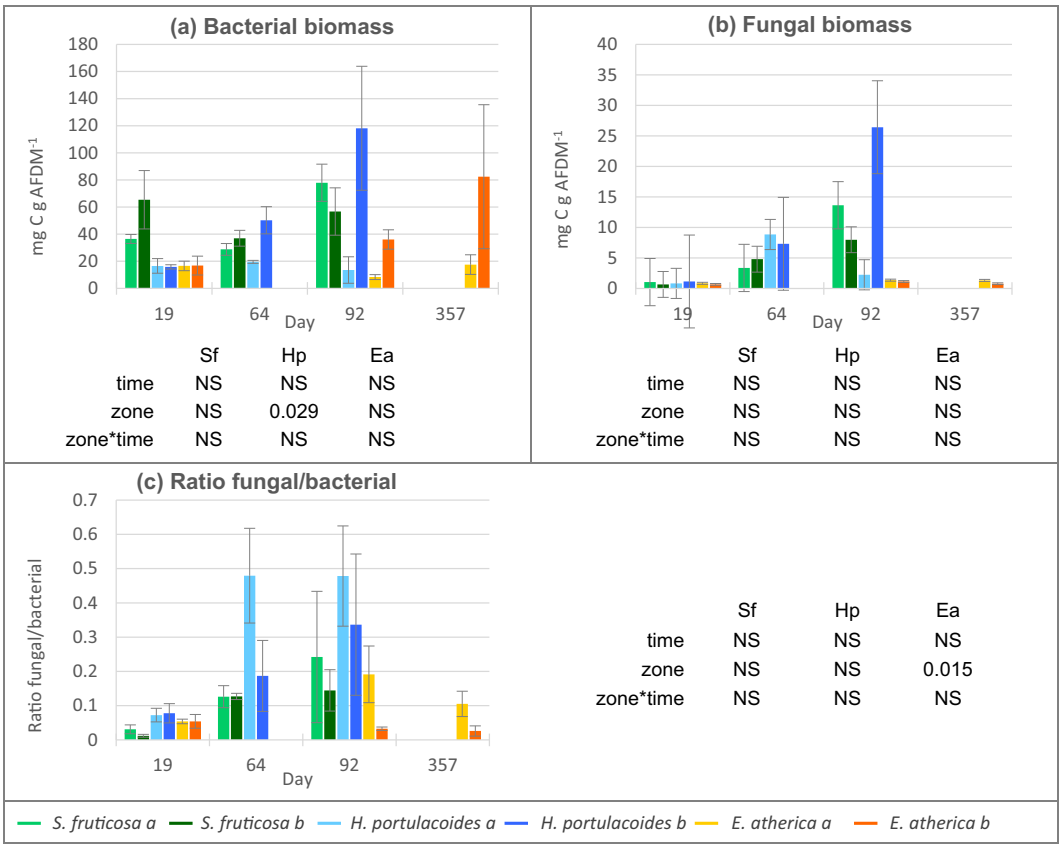

Fig. 5. Bacterial (a) and fungal (b) biomass and fungal/bacterial biomass ratio (c) for the litter of each species, sampling date and zone. Significant *p*-values for the factors time and zone and for their interaction according to the repeated measures ANOVAs are given. Sf: *S. fruticosa*; Hp: *H. portulacoides*; Ea: *E. atherica*. NS: not significant. Bars represent mean ± SE (n = 3).

microbial colonization, especially by fungi, which are considered the main microorganisms responsible of complex polysaccharides and lignin degradation (Breen and Singleton, 1999; Bugg et al., 2011; Romaní et al., 2006). In fact, estimated fungal biomass in *E. atherica* litter (1 mgC g AFDM^{-1} in average) was lower than those reported for other monocotyledonous species of the Poaceae family in a tidal salt marsh in Portugal (Castro and Freitas, 2000) or of the Cyperaceae, Juncaceae and Poaceae families in the Ebro delta (Menéndez, 2008; Menéndez and Sanmartí, 2007; Sanmartí and Menéndez, 2007), that ranged between 8 and 16 mg C g DW^{-1} for a similar period (one year). One possible explanation for this difference could be the lower initial C/N molar ratio of *E. atherica* litter (around 62) in comparison to those reported for the monocotyledonous species studied in these examples (from 160 to 200), since higher litter C/N ratios would favour fungal colonization in comparison with bacteria (Grosso et al., 2016; Rousk and Bååth, 2007). In line with this, in this study it was found a remarkably higher bacterial biomass than fungal biomass throughout the decomposition process, which would be in accordance with the previously suggested important role of soil bacteria in salt marsh litter decomposition (Benner et al., 1984, 1986; Newell et al., 1996).

Litter decomposition process differed between *S. fruticosa* and *H. portulacoides* which may be mainly related to the higher N content and lower C/N found in *S. fruticosa*. This may favour bacterial colonization of *S. fruticosa* litter (Grosso et al., 2016; Rousk and Bååth, 2007) and enhance the activity of β-glucosidase. Nevertheless, these differences did not determine a higher decomposition rate in *S. fruticosa*, since this was highly dependents on the microhabitat spatial environment (see section below).

4.2. Inter habitat-zone effect in the litter decomposition process

The litter decomposition process of the three dominant species of La Pletera salt marsh differed between the two studied zones within each habitat, indicating a microhabitat effect on litter decomposition. This microhabitat effect was huge for *H. portulacoides* litter and much less relevant, although still significant, for the other two species.

H. portulacoides and *E. atherica* developed in the salt meadow where the microhabitat conditions of zone *b* seem to favour litter microbial colonization, activity and decomposition rate. Soil in the zone *b* of the salt meadow has a sandy clay loam texture (in contrast to the clay loam texture of zone *a*), and a lower soil organic carbon and higher total nitrogen content (and thus lower C/N ratio) than in zone *a* (Table S1). The sandiest soil of zone *b* would be more drained and aerated (in accordance with lower values of soil volumetric water content; Table S1) which, together with the greater N availability, might favour microbial activity and thus litter decomposition (Kathilankal et al., 2008; Moffett et al., 2010). This was clearly observed in *H. portulacoides* litter decomposition that, at the end of the experiment, showed 10% AFDM remaining in zone *b* in contrast to around 70% AFDM remaining in zone *a*. Accordingly, microbial colonization, enzyme activities and efficiency were enhanced in *H. portulacoides* litter in zone *b*, which occurred in parallel with a distinct dynamics of structural parameters. Specifically, *H. portulacoides* showed higher bacterial biomass, and higher β-xylosidase, leucin-aminopeptidase and phenol oxidase activities and higher enzyme efficiency of β-glucosidase and leucine-aminopeptidase, together with a higher increment of lignin and N content in zone *b* compared to zone *a*, especially at the end of the decomposition experiment. The final increase of N could be explained by a higher microbial colonization (Abelho, 2001). In contrast, the more recalcitrant and slowly decomposing *E. atherica* litter was much less affected by the microhabitat, with no significant differences in the decomposition rates found between zones. Indeed, *E. atherica* litter in zone *b* only showed higher bacterial colonization resulting in a significant lower fungal/bacterial biomass ratio. Consequently, the microhabitat effect on litter decomposition would depend on the species litter composition, and, likely, differences between

zones for *E. atherica* might be observed in more advanced litter decomposition phases.

In the halophilous scrub, *S. fruticosa* also showed differences in the decomposition process between the two studied microhabitats, which resulted in higher decomposition rate at zone *a*. One of the main environmental differences between these two zones was the flooding regime. Zone *b* is close to the sea, and the flood, which mainly occurs by direct entrance of sea water during storms, can last several months. On the contrary, zone *a* is flooded for only a few days or weeks due to rainfall. During the study period (from mid-November to mid-February), seven episodes of flooding caused by sea storms, approximately two each month, occurred, resulting in a prolonged inundation of zone *b* (Pascual and Martinoy, 2017). This was probably responsible for the higher salinity in zone *b* than in zone *a* (Table S1). Furthermore, other soil characteristics differ between these two zones, such as the higher soil organic carbon and nitrogen content in zone *a*. It has been reported that inundation disfavours C mineralization in coastal wetlands (Lewis et al., 2014), and salinity disfavours litter decomposition (Mendelssohn and Slocum, 2004; Ouyang et al., 2017), which agrees with the lower decomposition rates found for *S. fruticosa* litter in zone *b* compared to zone *a*.

5. Conclusions

In the Mediterranean non-tidal salt marsh of La Pletera, the succulent Chenopodiaceae species, *Sarcocornia fruticosa* and *Halimione portulacoides*, decomposed faster than the Poaceae species, *Elytrigia atherica*. These differences were related with the initial lignin and cellulose content of the litter rather than with the initial carbon and nitrogen content and C/N ratio, highlighting the importance of tissue chemical composition in determining litter decomposability.

Besides, enzyme efficiency, rather than enzyme activity, would explain the differences observed among species in litter decomposition rates. The slower decomposition of *E. atherica* dead leaves in comparison to senescent stems of *S. fruticosa* and leaves of *H. portulacoides* would favour carbon sequestration into the soil of the non-tidal Mediterranean salt marsh studied. Regarding microbial, bacteria were dominant throughout the decomposition process of litter, although fungi increased their importance in the later stages, when the relative lignin content of litter increased.

Finally, the observed differences in litter decomposition between zones within each habitat, which were especially relevant for *H. portulacoides*, highlight the importance of considering the microhabitat heterogeneity in future experiments designed to better understand factors driving the litter decomposition process. In the salt meadow, differences in the soil texture determined huge differences in the decomposition rate of *H. portulacoides* litter, which can result in drastic differences in the C cycling and C storage between microhabitats. The high plasticity observed in the litter decomposition process of *H. portulacoides*, rapidly responding to favourable environmental conditions, may also be related to a fast change in the colonizing microbial community, which is not occurring in the more recalcitrant *E. atherica* species from the same habitat. This idea leads us to suggest that a lower content of structural compounds in the plant tissues could allow the establishment of a more plastic microbial community, which would respond differently to the diverse environmental conditions at a microhabitat level, consequently affecting the litter decomposition process.

CRediT authorship contribution statement

Lorena Carrasco-Barea: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Laura Llorens:** Conceptualization, Writing – review & editing, Supervision. **Anna M. Romaní:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision. **Maria Gispert:** Formal analysis, Writing – review & editing. **Dolors Verdaguer:** Conceptualization, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have not competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.155743.

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