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BRIEF REPORT

ENVIRONMENTAL MICROBIOLOGY

Differential response of bacteria and fungi to drought on the decomposition of *Sarcocornia fruticosa* woody stems in a saline stream

Anna Doménech-Pascual[1] | **Lorena Carrasco-Barea**[2] | **Frederic Gich**[3] | **Judit Boadella**[1] | **Zeus Freixinos Campillo**[4] | **Rosa Gómez Cerezo**[4] | **Andrea Butturini**[5] | **Anna M. Romaní**[1]

[1]GRECO, Institute of Aquatic Ecology, University of Girona, Girona, Spain

[2]Plant Physiology Unit, Department of Environmental Sciences, University of Girona, Girona, Spain

[3]Molecular Microbial Ecology Group (gEMM-IEA), Institute of Aquatic Ecology, University of Girona, Girona, Spain

[4]Department of Ecology and Hydrology, University of Murcia, Murcia, Spain

[5]Department of Evolutionary Biology, Ecology and Environmental Sciences, University of Barcelona, Barcelona, Spain

Correspondence
Anna Doménech-Pascual, GRECO, Institute of Aquatic Ecology, University of Girona, C/ Maria Aurèlia Capmany i Farnés, 69, 17003 Girona, Spain.
Email: a.domenech@udg.edu

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Abstract

Inland saline ecosystems suffer multiple stresses (e.g., high radiation, salinity, water scarcity) that may compromise essential ecosystem functions such as organic matter decomposition. Here, we investigated the effects of drought on microbial colonization and decomposition of *Sarcocornia fruticosa* woody stems across different habitats in a saline watershed: on the dry floodplain, submerged in the stream channel and at the shoreline (first submerged, then emerged). Unexpectedly, weight loss was not enhanced in the submerged stems, while decomposition process differed between habitats. On the floodplain, it was dominated by fungi and high cellulolytic activity; in submerged conditions, a diverse community of bacteria and high ligninolytic activity dominated; and, on the shoreline, enzyme activities were like submerged conditions, but with a fungal community similar to the dry conditions. Results indicate distinct degradation paths being driven by different stress factors: strong water scarcity and photodegradation in dry conditions, and high salinity and reduced oxygen in wet conditions. This suggests that fungi are more resistant to drought, and bacteria to salinity. Overall, in saline watersheds, variations in multiple stress factors exert distinct environmental filters on bacteria and fungi and their role in the decomposition of plant material, affecting carbon cycling and microbial interactions.

INTRODUCTION

Inland natural saline aquatic ecosystems are geographically widespread and specifically located in arid and semiarid zones. These ecosystems share distinct climatic and lithologic characteristics, although they usually develop in sedimentary watersheds with high accumulations of salts (such as gypsum) subjected to seasonal droughts (Farifteh et al., 2008; Gutiérrez-Cánovas et al., 2012; Luque et al., 2012). In these watersheds, the reduced precipitation and high temperatures, especially during the drying periods, lead to a progressive decrease in surface water runoff and soil humidity, so streams usually have an intermittent flow regime (Datry et al., 2014; Suárez et al., 2017). In these saline streams, seasonal drought and high

salinity conditions co-occur, and this multiple stresses may affect the community of organisms and ecosystem functioning. One of the key ecosystem functions that can be compromised is carbon cycling and specifically the process of plant litter organic matter degradation and mineralization. In this respect, it is well known that drought and salinity may lead to changes in the structure, composition, activity and growth of the microbial community (Yan et al., 2015), being this community primarily responsible for organic matter decomposition.

The plant litter decomposition process in inland saline streams may be defined by the quality of the plant material itself and by the microbial activity, both affected by the environmental conditions of high salinity and intense drought periods. These conditions result in water scarcity and osmotic stress, as well as co-occurring factors such as high sun radiation, temperatures and variability in oxygen availability (Mora-Gómez et al., 2020). The vegetation in these ecosystems is scarce and dominated by halophytic woody perennial shrubs (Gómez et al., 2016; Gutiérrez-Cánovas et al., 2012). Thus, the most significant fraction of the carbon inputs to these streams is woody material (Gispert et al., 2021; Gutiérrez-Cánovas et al., 2009), which is mainly composed by three structural polymers: cellulose, hemicellulose and lignin (Huang et al., 2016; Lionetto et al., 2012), being the latter, formed by aromatic polymers, the most recalcitrant one (Li et al., 2016; Rencoret et al., 2011). Due to its chemical structure, woody material has a low decay rate in comparison to leaf litter (Hendel & Marxsen, 2000; Rawlik et al., 2021). This, together with the expected stress effects of salinity and drought on the microbial community, may imply a slow carbon turnover in these ecosystems (Berger et al., 2019; Rothstein et al., 2004).

Fungi and bacteria are the main taxa responsible for plant litter decomposition thanks to their extracellular enzyme capabilities. Extracellular enzymes break down plant fibres and degrade polymeric compounds (such as cellulose, hemicellulose and lignin), converting them into labile small molecules that can be assimilated for fungal and bacterial growth (Mille-Lindblom & Tranvik, 2003; Romaní et al., 2006). Studying extracellular enzyme activities (EEA) provides valuable information on the cycling of nutrients in ecosystems since they represent the rate limiting step of decomposition (Rejmánková & Sirová, 2007) and can have broad implications on ecosystem biogeochemical cycling (German et al., 2011). The decomposition process of plant litter is the result of the colonization ability of fungi and bacteria, and their enzyme capabilities. Bacteria are usually the initial colonizers and are more efficient decomposers of simple polysaccharides than fungi (Romaní et al., 2006). In contrast, fungi colonize later but, thanks to their filamentous physiology and wider range of extracellular enzymes (including lignin-degrading ones), they usually achieve greater biomass than bacteria (Mille-Lindblom & Tranvik, 2003; Mora-Gómez et al., 2016). This process might be highly affected by drought, as shown for intermittent freshwater streams, where drought slows down weight loss and modifies the microbial community composition and EEA (Abril et al., 2016; Mora-Gómez et al., 2018). Effects of drought on the microbial community composition have also been found in soils (Alster et al., 2013; Sanaullah et al., 2011). In this environment, drought reduces microbial biomass and can benefit those microorganisms that are adapted to live under conditions of reduced water availability, such as Actinobacteria and Firmicutes for bacteria, or Ascomycota and Basidiomycota for fungi (Andreo-Jimenez et al., 2019; Kolb et al., 2022).

Drought conditions can also have abiotic effects on plant and woody litter decomposition. Stream intermittency and reduction of flow may reduce physical abrasion, slowing down litter weight loss (del Campo et al., 2021). Also, drought conditions may enhance the photodegradation of lignin by solar radiation, facilitating the access of decomposers to cellulose molecules (Henry et al., 2008; Mora-Gómez et al., 2020). On the other hand, salinity may inhibit the decomposition process. Rejmánková and Sirová (2007) reported that hydrolase activities were negatively correlated with salinity and showed that high salinity determined a decrease in microbial biomass and activity. Salinity may lead to microorganisms allocating energy to osmotic stress resistance strategies and this may reduce their functional capabilities (Gionchetta et al., 2020; Schimel et al., 2007). Also, Gómez et al. (2016) observed a decrease in woody stems' breakdown rate and in fungal biomass with increasing salinity, as well as changes in woody stems' chemical composition. Salinity can also influence the microbial community, as fungi are less resistant to it than bacteria, especially at a compositional level (Mohamed & Martiny, 2011; Rath et al., 2019). On the other hand, aquatic bacteria have shown to be easily adaptable to high or fluctuating salinity (Menéndez-Serra et al., 2021).

Despite the numerous studies on litter decomposition in freshwater streams and on intermittent streams in the last decade (Barthélémy et al., 2022; Bruder et al., 2011; del Campo et al., 2021), there is a lack of knowledge on the effects of drought on plant material decomposition in natural saline streams of arid and semi-arid regions. In these ecosystems, the multiple stresses imposed by high salt content and low water availability (together with high radiation and extreme temperatures) may compromise microbial decomposition and therefore carbon fluxes. At the same time, however, they may determine unique adaptations. This is especially relevant given the expected worldwide increase in areas covered by drylands (Huang et al., 2017) and anthropogenic salinization (Cañedo-Argüelles et al., 2013; Williams, 2001).

The aim of this study is to study how the microbial decomposition of woody material in an inland saline ecosystem occurs, while assessing how the different effects of multiple stresses driven by drought (such as changes in salinity, oxygen, light radiation and water availability) affect differently the functions, diversity and composition of the microbial communities. To achieve these objectives, we have conducted a 9-month decomposition experiment with woody stems from *Sarcocornia fruticosa* (~1 to 3 mm in diameter) in a saline stream from a semi-arid Mediterranean region. We placed the woody stems either permanently on the dry floodplain, submerged in the stream, or first submerged and then emerged and placed on the floodplain, simulating a seasonal drought.

We hypothesize that the decomposition of *S. fruticosa* woody stems will be slower under drought and intermittent conditions (i.e., both when placed on the floodplain or first submerged then emerged) than in wet conditions (i.e., submerged in the stream channel). Furthermore, despite several studies demonstrating that fungi are more tolerant to drought than bacteria (Abril et al., 2016; Allison et al., 2013; de Vries et al., 2018), we expect that high salinity will predominate as an environmental conditioning filter, causing a prevalence of bacterial biomass over fungal biomass.

MATERIALS AND METHODS

Study site

This study was conducted in the Rambla Salada saline stream, located in the sedimentary Fortuna Basin (Murcia, Spain), in the Protected Landscape of the Ajauque-Rambla Salada wetland. The climate in this area presents a mean annual precipitation below 300 mm (mainly concentrated in spring and autumn) and a mean annual temperature of 18°C, with long, warm, dry summers and mild winters (Gutiérrez-Cánovas et al., 2012). These climatic conditions and the high salinity of this steppe area have led to the development of scarce vegetation dominated by halophilic plants, such as *S. fruticosa* and *Arthrocnemum macrostachyum* (Gutiérrez-Cánovas et al., 2012). The soil from this watershed is composed of gypsiferous sedimentary marls (Luque et al., 2012; Oueriaghli et al., 2014) and, while the stream channel has a permanent flow, the recurrent annual droughts cause large variations in its flow and channel width, with minimum values in summer. Mean water conductivity is ca. 70 mS/cm.

Experimental design

Air-dried stems were collected from *S. fruticosa* plants from the Rambla Salada floodplain. When on the plant, these stems are initially succulent green stems that gradually undergo a transformation, becoming woody over time and dying in the process. Subsequently, they detach from the shrub, and disperse onto the floodplain or into the stream. The woody stems used for the experiment correspond to peripheral thin woody stems, with a diameter ranging from approximately 1 to 3 mm, and length varying between 4 and 8 cm. These dry stems were used to fill 40 mesh polyethylene bags (20 × 20 cm with 5 mm mesh), each containing 5.5 ± 0.4 g. The mesh bags were placed in the Rambla Salada in different habitats and under different conditions (hereafter called treatments) that integrate the natural hydrological variability of the stream. Thus, mesh bags were placed: (1) submerged in the stream channel (Submerged treatment, S); (2) on the floodplain, 10 m from the edge of the stream channel, (Exposed treatment, E); and (3) initially immersed in the stream channel water and later emerged to the floodplain-shoreline (placed 2 m from the active channel), simulating the effect of seasonal droughts (Intermittent treatment, IT). On 13 February 2020 (day 0), 36 mesh bags were placed at the study site: 24 bags submerged in the stream channel (Submerged and Intermittent treatments), and 12 bags placed on the floodplain (Exposed treatment). The four remaining mesh bags were used for the initial measurements and characterization of *S. fruticosa* stems. Bags were randomly distributed and fixed with metal sticks and fishing line at four spots with running flow along a 100 m reach in the stream, or in a parallel transect along the shoreline or floodplain, respectively, for Submerged, Intermittent and Exposed treatments. Mesh bags were then collected on three dates in 2020 (4 replicates per treatment): 19 May (day 96), 14 July (day 152) and 12 November (day 273). May 19 was also the date when bags from the Intermittent treatment were moved from the channel to the shoreline. Once in the laboratory, the collected mesh bags that were submerged were gently rinsed with distilled water while soil particles from outside the bag were gently cleaned for the mesh bags of other treatments. The subsamples of *S. fruticosa* stems were preserved as follows: samples for enzyme activity assays were freshly stored (4°C) and analysed 24 to 48 h after collection; bacterial biomass samples were fixed with formalin (2%) and stored at 4°C; samples for fungal biomass, bacterial and fungal community composition, and lignin and cellulose content were kept frozen (−20°C) until their analyses. A replica of each mesh bag was created to be used in totality for the weight loss measurement. Therefore, 36 mesh bags were created, in addition to those used in the experiment. On each collection date, physicochemical parameters from the stream water, floodplain and floodplain-shoreline soil were analysed (see below).

Physicochemical parameters

For the stream water, temperature, pH, conductivity and dissolved oxygen were measured in situ by specific probes (Intellical HQD, Hach Lange, Loveland, CO, USA). Flow velocity was measured at the location of the mesh bags with a current meter (MiniAir2; Schiltknecht Co., Zurich, Switzerland) and estimated stream discharge was measured at three sites along the stream reach based on cross-section measurements of width, water depth and water velocity. Water samples were collected and filtered (GF/F, Whatman, Maidstone, UK) and analysed within 24 h after collection for ammonium ($N\text{-}NH_4^+$), nitrate ($N\text{-}NO_3^-$), soluble reactive phosphorus (SRP) and dissolved organic carbon (DOC). In water samples, DOC was measured with a multi N/C 3100 analyser (Analytik Jena, Thüringen, Germany; detection limit 0.1 mg/L). All samples were diluted 1/5–1/20 to minimize the impact of the salts on instrument performance. In both floodplain and floodplain-shoreline soils, pH and conductivity (μS/cm) were analysed in soil diluted in distilled water (soil/water = 1:5). Organic matter (%OM, expressed as Ash-Free Dry Weight (AFDW), after drying and burning), gravimetric water content and water activity (NOVASINA, Lachen, Switzerland) of these soils were also measured. Soils were further characterized for their water extractable total nitrogen and carbon in a multi N/C analyser (Analytik Jena, Thüringen, Germany). Values for daily mean air temperature and radiation were obtained from a meteorological station located in the Fortuna Basin from the SIAM dataset ("Sistema de Información Agrario de Murcia" dataset).

Weight loss

The descomposition of *S. fruticosa* stems was estimated by measuring their weight loss over time. Subsamples from the initial pool of stems recollected for the experiment were air-dried and weighted, dried (24 h, 60°C) and combusted (4 h, 500°C) conditions, in order to calculate the relationships between air-dried weight versus dry weight (oven-dried) and organic matter content (combusted weight) needed to obtain the accurate initial organic matter content of all the stems placed in the field. At each collection, the organic matter content of the collected woody stems from all three treatments was calculated and related to the specific initial organic matter content of the bag to obtain the % OM remaining, using the formula $(OM_t/OM_0)*100$. Based on the values of %OM remaining obtained at the different time collections, the classic negative exponential decay model (Bärlocher, 2005) was applied to each treatment separately in order to obtain a decomposition rate (k) for each treatment. Apart from that function, different model decays have been tested using the R-package "litterfitter" (Cornwell & Weedon, 2014). (See Statistical analyses section for more details).

Lignin and cellulose content

The cellulose and lignin content was quantified by determining the residual weight of samples after successive removal of various tissue constituents, using an acid detergent solution to extract soluble components (Gessner, 2005b). All samples were oven-dried and ground to a fine powder with a ball mill (Mixer Mill MM 400, Retsh GmbH, Haan, Germany) to an approximate final weight of 0.2 ± 0.02 g of powdered dry stems. An acid detergent solution (20 g/L of hexadecyltrimethylammonium bromide in 0.5 M sulfuric acid) was added to the samples which were then heated for 1 h, filtered, oven-dried (105°C) and weighed. Fibre content (%) was calculated as the weight loss due to the application of the acid detergent solution divided by the initial oven-dry weight of the samples and multiplied by 100. The remaining sample residues were treated repeatedly with 72% sulfuric acid, dissolving and filtrating cellulose. The crucibles without the cellulose were again oven-dried overnight (105°C) and weighed, so the cellulose content (%) was estimated as the weight loss due to 72% H_2SO_4 treatment divided by the initial oven-dry weight of the samples and multiplied by 100. Finally, the residues that remained were combusted (500°C, 4.5 h) and weighed, obtaining the lignin content (%), estimated as the weight loss after combustion divided by the initial oven-dry weight of the sample and multiplied by 100.

Fungal and bacterial biomass

Fungal biomass was analysed by measuring the ergosterol concentration by high-performance liquid chromatography (HPLC, Waters Corporation, Milford, USA) through a lipid extraction in alkaline methanol and purification of the extract solid-phase extraction (Waters Sep-Pak, V ac RC, tC 18, 500 mg) (Gessner, 2005a). Results for fungal biomass were expressed as fungal carbon (mg) per AFDW (g) through the stoichiometric relationships described by Gessner and Chauvet (1992). Bacterial biomass was measured by flow cytometry (FACSCalibur Becton Dickinson, Franklin Lakes, USA). Bacterial cells were disaggregated with a 2% formalin solution (0.2 μm filtered water from Rambla Salada and formol) and incubated (1 h, dark conditions) (Amalfitano et al., 2009). A coagulant agent (Nycodenz Optiprep density gradient, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) was added and, after centrifugation (90 min, 14,000 rpm, 4°C), samples were stained using a nucleic acid stain, SYTO13 (5 μM; FISHER, ThermoFisher Scientific, Waltham, USA). A solution of

beads of known concentration was added to the samples as a calibration pattern. After the analyses, samples were oven-dried (48 h, 70°C) and weighed. With the corresponding conversion factors, the results were finally expressed as bacterial carbon (mg) per AFDW (g) (Bratbak & Dundas, 1984; Theil-Nielsen & Søndergaard, 1998).

Extracellular enzyme activities

Four EEA related to the decomposition of plant litter were analysed: β-D-glucosidase (GLU); cellobiohydrolase (CBH); β-D-xylosidase (XYL); and phenol oxidase (PHE). Different artificial substrates were used as proxies of the enzyme substrates for each activity (Table S1). All the hydrolytic enzyme (GLU, CBH and XYL) analyses were performed at the saturation concentration, previously determined through saturation curves (Table S1). For the PHE activity, the final concentration of the substrate was 2.5 mM, as had been commonly used in previous studies (Sinsabaugh et al., 1994). For all the incubations, subsamples were covered (4 ml) by previously filter-sterilized (0.2 μm Nylon filters) river water from Rambla Salada. Stem fragments (0.25 ± 0.1 g of DW) were incubated with the respective artificial substrates and concentrations. For the hydrolytic activities, apart from the sample incubations (1 h, agitation and dark conditions), one blank for each enzyme (with filtered water and artificial substrate) and standard concentrations of 0, 0.1, 0.25, 0.5, 2.5, 5, 10, 50 and 100 μM of MUF were also prepared. A quenching factor was calculated to correct the possible influence of particulate material and/or dissolved salts on the intensity of the MUF fluorescence by comparing the fluorescence of samples and MUF individually or when mixed. Glycine buffer (pH 10.4) was added to stop the enzymatic reactions and maximize MUF fluorescence. Fluorescence measurements were read at 365/455 excitation/emission wavelengths (Infinite M200 Pro, Tecan, Zurich, Switzerland). Results were finally expressed as μmol of MUF released per gram of AFDW and hour. For PHE analysis, apart from the sample incubations (2 h, agitation and dark conditions), a control (L-DOPA and filtered water) and blanks for the samples (stems with filtered water) were prepared. Additionally, the absorbance from the filtered water was measured separately. At the end of the incubation time, absorbance measurements were realized at 460 nm (Infinite M200 Pro, Tecan, Zurich, Switzerland). The results were expressed as μmol of DIQC (3-Dihydroindole-5,6-quinone-2-carboxylate; the product of the 3,4-Dihydroxy-L-phenylalanin reaction) released per gram of AFDW and hour.

Fungal and prokaryotic community composition

Stems from initial conditions (subsamples from the initial material) and from the last collection (day 273) from all treatments were weighed and mechanically disrupted with a high-speed homogenizer (FastPrep-24, MP Biomedicals, Santa Ana, USA). DNA was extracted from 0.66 g (on average) of disrupted stems with the FastDNA® SPIN Kit for Soil (MP Biomedicals, Santa Ana, USA) in accordance with the manufacturer's instructions including a treatment with the FastPrep-24 cell disruptor (3 cycles at a 5.5 power during 30 s) to lyse the cells. DNA was quantified by Qubit dsDNA HS Assay Kit (ThermoFisher Scientific, Waltham, USA). The sequencing of the 16S rRNA and 18S rRNA genes was performed at the MSU Genomics Core (Michigan, USA) with a 2 × 250 bp paired-end Illumina MiSeq platform using a v2 500-cycle reagent cartridge (Mardis, 2008). The prokaryotic 16S rRNA gene V4 region and the eukaryotic 18S rRNA gene V9 region were amplified using dual-indexed, Illumina-compatible primers 515F/806R (Caporaso et al., 2011; Walters et al., 2016) and Euk1391F/EukBR (Stoeck et al., 2010), respectively. Raw sequence data from this study was deposited via the BioSample Submission Portal (National Center for Biotechnology Information) under accession number PRJNA985427. The quality of the raw reads was initially checked using the FastQC application (Andrews, 2010). The mothur software package version 1.46.1 (Kozich et al., 2013) was used to conduct all the downstream sequencing analyses, namely, chimera detection, sequence classification with a threshold of 80% using the SILVA database 132 (https://mothur.org/wiki/silva_reference_files/) and clustering into operational taxonomic units (OTUs) (97% cut-off), following the MiSeq SOP manual (www.mothur.org/wiki/miseq_sop/). Gini–Simpson (1-D) and Chao as diversity indices and S_{obs} (observed sequences, number of OTUs) as a richness indicator as well as coverage were calculated in mothur after normalizing the number of sequences in each sample by randomly selecting a subset corresponding to the lowest number of sequences found in a sample. All 18S rRNA gene sequences not belonging to fungi were eliminated. The number of initial 18S rRNA gene sequences varied between 13,412 and 101,954 sequences depending on the treatment, with a total number of 553,346 sequences. After eliminating all the non-fungi eukaryote, alpha-diversity results of the 18S rRNA gene for the Submerged treatment were obtained separately from the other treatments due to lower number of sequences recovered (S = 313 ± 19.52) compared to the other treatments (Initial (IN) = 59,120 ± 36,389, E = 23,306 ± 13,990, IT = 37,779 ± 20,492).

Statistical analyses

Differences between treatments in weight loss were tested by ANCOVA (with the natural logarithm of % remaining weight against treatment and collection time as covariable) and further tested by Tukey's HSD test. Different decay models (discrete parallel, discrete series and negative exponential) were tested using the R-package "litterfitter" (Cornwell & Weedon, 2014), and compared through their AIC and BIC values. The models were performed using the proportion of remaining organic matter of the samples through time (days), for each one of the three treatments (Exposed, Intermittent and Submerged), and were produced with a number of random starts for the fitting of 900. Linear mixed models were applied to test the effect of the treatment, the sampling time and the interaction between these two factors on microbial biomasses, EEA and the content of fibre, cellulose and lignin. ANOVAs were performed to analyse potential differences between treatments for the alpha-diversity indices of the microbial communities, with post hoc tests (Tukey's HSD test) to analyse differences between treatments. Differences among microbial community composition were analysed using a permutational multivariate analysis of variance (PERMANOVA) complemented with a pairwise test with PRIMER (ver. 7) and analysed by the neighbour-joining method using the UPGMA algorithm based on the distance between communities as calculated by Jaccard with MEGA-X (ver. 10.0.5). Network analyses were performed with PAST (ver. 4.16c) using the OTU relative abundance from bacteria and fungi together. Prior to analyses, filtration of taxa on the whole dataset was performed by removing taxa with relative abundance <1% (Abdelhak et al., 2023). Network graphs, one for each treatment, were plotted at order level with PAST using Bray-Curtis as a similarity index and with an edge cutoff of 75%. To test the relationships between microbial biomass and the microbial community composition with the EEA, two Principal Components Analyses (PCAs) were applied. Data from the biomasses and the EEA were shown on the first PCA while variables such as the structural compounds (cellulose and lignin content) were not shown due to non-significant correlations with the analysis. For the second PCAs (one for 16S one for 18S), samples were distributed according to the total of OTUs observed for each treatment, and an environmental fitting was performed afterward with the EEA data. All the analyses were conducted with RStudio (version 4.1.2).

RESULTS

Physicochemical characteristics of the study site

During the study period, Rambla Salada water showed an average conductivity of 77.89 mS/cm and a mean pH and oxygen concentration of 7.75 and 9.46 mg/L, respectively (Table S2). From February to July, temperature and DOC increased while dissolved oxygen, NH_4^+ and PO_4^{3-} decreased together with the flow reduction. The highest conductivity (119.20 mS/cm) and total dissolved nitrogen (9.05 mg/L) were measured in July (Table S2). Floodplain soil was characterized by mean values of conductivity, organic matter (OM), and water content (estimated as gravimetric humidity) of 0.82 mS/cm, 1.25% and 2.61%, respectively. The floodplain-shoreline soil showed higher values for these variables than those in the floodplain soil (16.70 mS/cm, 4.06% and 10.74% in average, respectively, Table S2), and a similar pH (7.7), on average. Meteorological station data showed the highest values for maximum and mean solar radiation, 1447 and 322.59 W/m^2, respectively, in May–June (Figure S1).

Weight loss, lignin and cellulose content

The three treatments experienced little weight loss after 273 days: the Exposed treatment lost around 20% of organic matter and the Submerged and Intermittent treatments lost around 23% (Figure 1). The dynamics of organic matter loss for the samples subjected to the Exposed treatment presented a constant decrease, while the samples under the Submerged and Intermittent treatments showed the lowest %OM remaining on day 96, only to increase slightly on day 152. However, no significant %OM weight loss differences among the treatments were detected for each factor and the interaction between them (ANCOVA, p >0.05 for time, treatment and time × treatment factors). As the dataset for the Intermittent and Submerged treatments clearly violated the assumptions to use a negative exponential decay model, with the increase in the organic matter content at day 152 (Figure 1), different model fits widely used for decay models have been tested (Figure S2). One major limitation of the negative exponential decay model is that it considers the decaying material (woody stems in our study) as a homogeneous mass throughout all the decomposition process. Alternative models to the negative exponential decay, as the discrete series or the discrete parallel models (Manzoni et al., 2012), consider different decomposing pools along the decomposition process that can describe non-linear behaviours, providing different decomposition rates for each pool (Table S3). Results from the model comparison (Figure S2) confirmed that the datasets for the Intermittent and Submerged treatments better fit to the discrete parallel and discrete series models than to the exponential decay, indicating a two-pool weight loss dynamics (Table S3). In contrast, the Exposed treatment fit to the exponential decay model and thus to a homogenous single-pool decomposition dynamics. However, despite these limitations, the negative exponential decay model was still applied to all

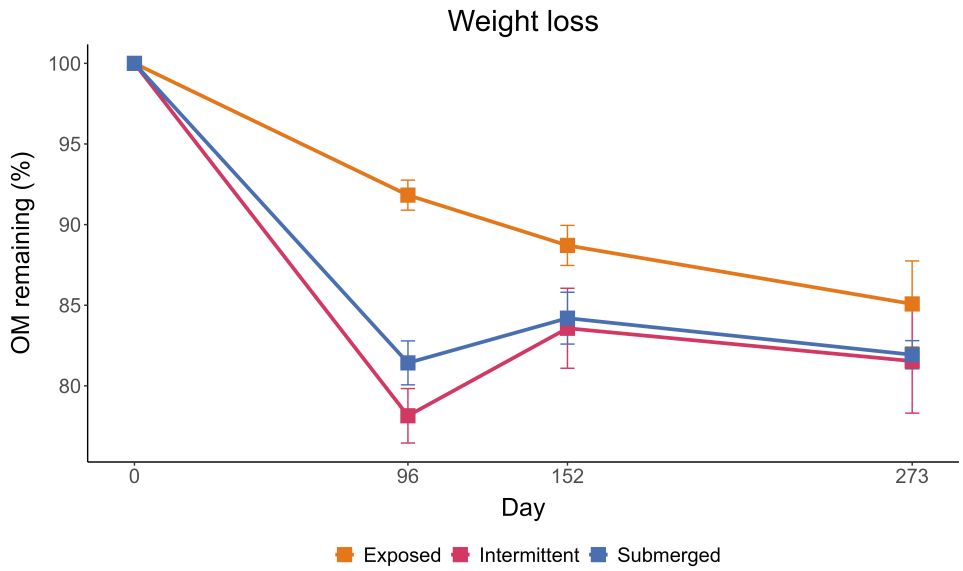

FIGURE 1 Organic matter remaining (%) for each treatment through the decomposition experiment: day 0 (13 February 2020), day 96 (19 May 2020), day 152 (14 July 2020) and day 273 (12 November 2020). Values are means ± SE ($n = 4$).

TABLE 1 Results of the Linear Mixed Models (LMM), indicating the significance (p value) for each factor and the interaction between them for the microbial biomass, extracellular enzyme activities and structural compounds content of the *S. fruticosa* stems.

	Treatment	Day	Treatment × day
Structural compounds content (%)			
Fibre	n.s.	0.04	n.s.
Cellulose	n.s.	n.s.	n.s.
Lignin	n.s.	n.s.	n.s.
Microbial biomass (mg C·g AFDW^{-1})			
Bacterial biomass	0.005	0.05	n.s.
Fungal biomass	0.006	0.01	0.01
Extracellular enzyme activities (μmol·g AFDW^{-1}·h^{-1})			
β-Glucosidase	<0.001	<0.001	<0.001
β-Xylosidase	<0.001	<0.001	<0.001
Cellobiohydrolase	<0.001	<0.001	<0.001
Phenol oxidase	0.002	0.03	n.s.

Note: "n.s." stands for non-significant differences (p value >0.05).

treatments to obtain the exponential decay rates, which can be—cautiously—compared with other bibliography. Therefore, the decomposition rates from the negative exponential decay model for each treatment were of 0.19 ± 0.05, 0.09 ± 0.10 and 0.13 ± 0.08 year^{-1} for the Exposed, Intermittent and Submerged treatments, respectively.

The fibre, cellulose and lignin content remained stable during all the experiments at approximate values of 70%, 50% and 20%, respectively, for all treatments (Figure S3A–C), and only a significant increase in fibre over time was detected (Table 1): from 69.36% (initial content of fibre) to 72.17%, 75.71% and 71.27% for the Exposed, Intermittent and Submerged treatments, respectively.

Bacterial and fungal biomass

Bacterial biomass increased over time in the Submerged and Intermittent treatments, reaching significantly higher values than those in the Exposed treatment on days 96 and 152, and decreased on the last sampling day (Figure 2A and Table 1, Tukey-test p <0.05). In contrast, in the Exposed treatment, similar values were maintained throughout the experiment with no significant differences between sampling days (Figure 2A and Table 1). Fungal biomass did not increase until day 152 when significantly higher values were measured in Exposed and Submerged treatments and the lowest ones were measured in the Intermittent treatment (Figure 2B and Table 1, Tukey-test p <0.05).

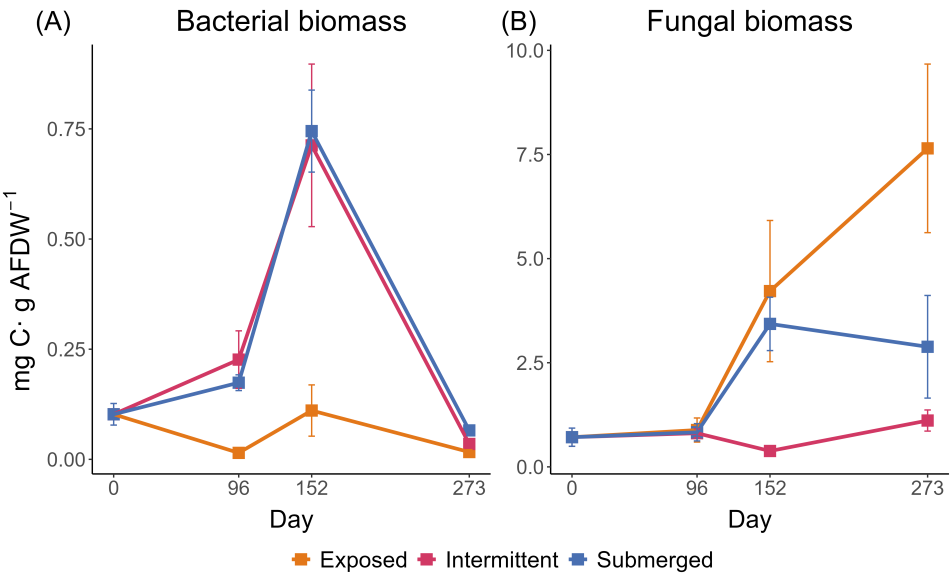

FIGURE 2 Bacterial (A) and fungal (B) biomass for each treatment through the decomposition experiment: day 0 (13 February 2020), day 96 (19 May 2020), day 152 (14 July 2020) and day 273 (12 November 2020). Values are means ± SE ($n = 4$).

On the last sampling day, the highest fungal biomass was measured in the Exposed treatment, followed by the Submerged and Intermittent treatments. However, statistical differences between treatments were only observed between the Exposed and Intermittent treatments.

Extracellular enzyme activities

Higher hydrolytic EEA were measured in the Exposed treatment than in the Submerged and Intermittent treatments and significant differences already appeared on day 96 for CBH and XYL and on day 152 for GLU (Figure 3A–C, Table 1, Tukey-test p <0.05). Time dynamics were also different between treatments: in the Exposed treatment, XYL progressively increased and GLU and CBH were maintained at initial values, while all three hydrolytic EEA decreased during the experiment in the Submerged and Intermittent treatments (Figure 3A–C). Conversely, PHE activity was significantly higher in the Submerged and Intermittent treatments than in the Exposed treatment, showing a peak on day 96 (Figure 3D and Table 1, Tukey-test p <0.05). In the Exposed treatment, PHE activity remained low during the entire experiment (Figure 3D).

Bacterial and fungal community composition

The final sequence data set consisted of 360,000 high-quality reads, which passed quality filtering for both 16S rRNA and 18S rRNA genes, and, on average, were distributed between 10,000 and 58,000 reads per sample. The sampling effort was enough to recover a reasonable coverage of the bacterial and fungal diversity, as shown in the rarefaction plots (Figure S4). Although using primers 515F/806R, which are suitable to analyse both bacterial and archaeal members of the microbial community (Caporaso et al., 2011), and have been used for detecting them in other studies from saline environments (Ghori et al., 2021; Menéndez-Serra et al., 2020; Sáenz de Miera et al., 2021), no archaea have been detected on the samples. Microbial alpha-diversity parameters were calculated after being subsampled in 495,694 and 361,537 sequences for 16S and 18S rRNA genes, respectively, at a distance cut-off level of 0.03 (Table 2). The observed number of operational taxonomic units (OTUs, S_{obs}) showed higher richness in the bacterial than in the fungal community. The richness (S_{obs}), diversity (Gini–Simpson index), and Chao index of the bacterial community were significantly highest in the Submerged treatment and lowest in the Intermittent treatment, with values in the Exposed treatment falling in between (Table 2). Conversely, in the fungal community the lowest richness and Chao diversity were measured in Submerged treatment, which showed significantly lower values than those in the Initial samples (Table 2). However, the fungal community in the Submerged treatment showed the highest value for the Gini–Simpson index (significantly higher than in the Initial and the Exposed treatments), indicating higher evenness (although lower richness) in the Submerged fungal community.

The bacterial community composition varied among treatments (Figures 4A and S5A). In the Exposed treatment, the order Sphingomonadales accounted for up to

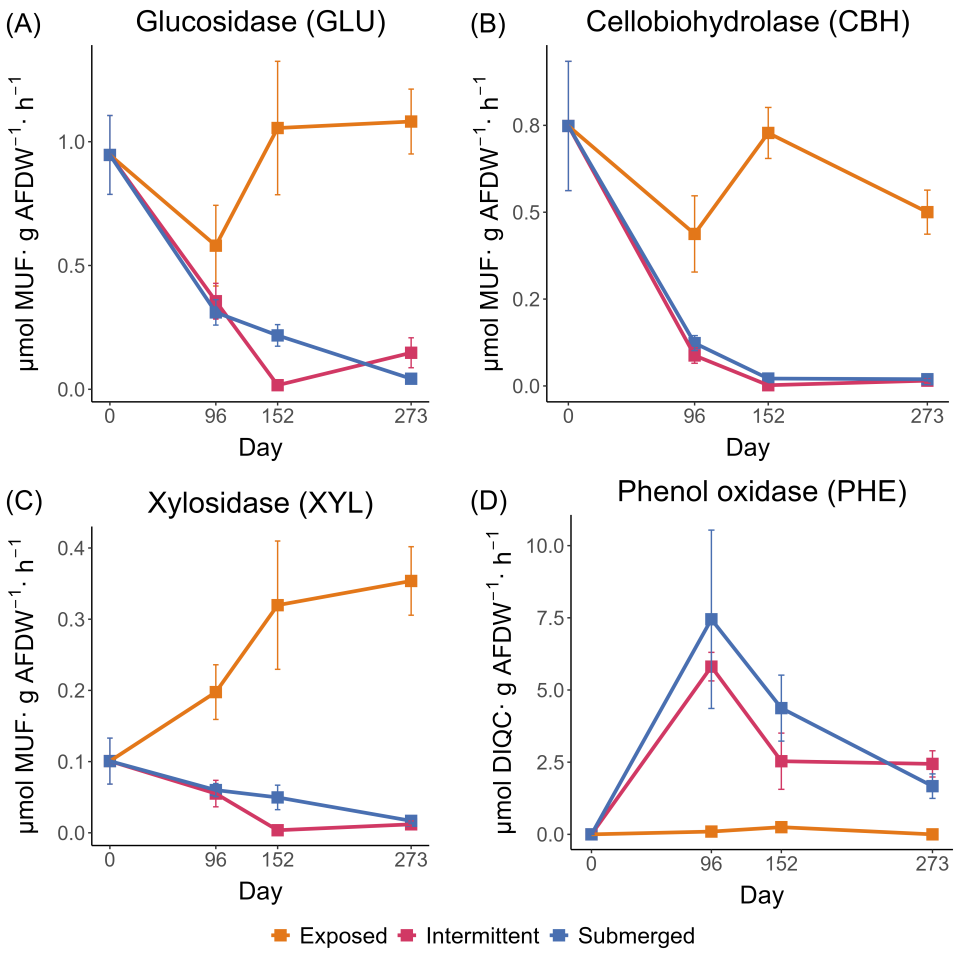

FIGURE 3 Extracellular enzyme activities (A: GLU, B: CBH, C: XYL, D: PHE) for each treatment through the decomposition experiment: day 0 (13 February 2020), day 96 (19 May 2020), day 152 (14 July 2020) and day 273 (12 November 2020). Values are means ± SE ($n = 4$).

TABLE 2 Microbial alpha-diversity results (average ± SD) with estimated coverage of DNA for all treatments plus the initial samples, and for both 16S rRNA gene ($n = 14$) and 18S rRNA gene ($n = 12$).

Treatment	Total number of sequences	Coverage	S_{obs}	1-D	Chao
16S rRNA					
Initial	35,137 ± 19,740	0.99 ± 0.01	385 ± 41.90 ^{a}	0.94 ± 0.01 ^{ab}	533.32 ± 138.98 ^{ab}
Exposed	31,506 ± 17,558	0.99 ± 0.00	282 ± 67.48 ^{ab}	0.86 ± 0.20 ^{ab}	378.60 ± 152.33 ^{bc}
Intermittent	40,148 ± 14,856	0.99 ± 0.01	132 ± 92.64 ^{b}	0.49 ± 0.14 ^{a}	201.03 ± 161.14 ^{c}
Submerged	34,556 ± 13,480	0.98 ± 0.00	657 ± 81.91 ^{c}	0.97 ± 0.01 ^{b}	871.64 ± 74.97 ^{a}
18S rRNA (fungi)					
Initial	59,120 ± 36,390	1.00 ± 0.00	92 ± 31.43^{a}	0.31 ± 0.03^{a}	136.79 ± 65.50^{a}
Exposed	23,306 ± 13,990	1.00 ± 0.00	73 ± 7.94^{ab}	0.42 ± 0.14^{a}	85.87 ± 19.28^{ab}
Intermittent	37,773 ± 20,493	1.00 ± 0.00	74 ± 26.84^{ab}	0.53 ± 0.23^{ab}	85.42 ± 27.36^{ab}
Submerged	313 ± 20	0.99 ± 0.00	22 ± 3.06^{b}	0.83 ± 0.06^{b}	24.14 ± 2.29^{b}

Note: S_{obs} and 1 – *D* stand for the number of observed OTUs and the Gini–Simpson index, respectively. Superscript letters indicate statistical differences between values (Tukey Post Hoc, *p* value <0.05). Results for the 18S rRNA gene for Submerged were calculated independently of the other treatments due to a lower value of final sequences obtained regarding the other treatments.

32.5% of total sequences followed by Rhizobiales (14.3%), while in the Intermittent treatment, the community was clearly dominated by the order Bacillales (79.1%). The Submerged treatment presented a higher number of taxonomic groups than the other treatments, which was consistent with the higher number of OTUs

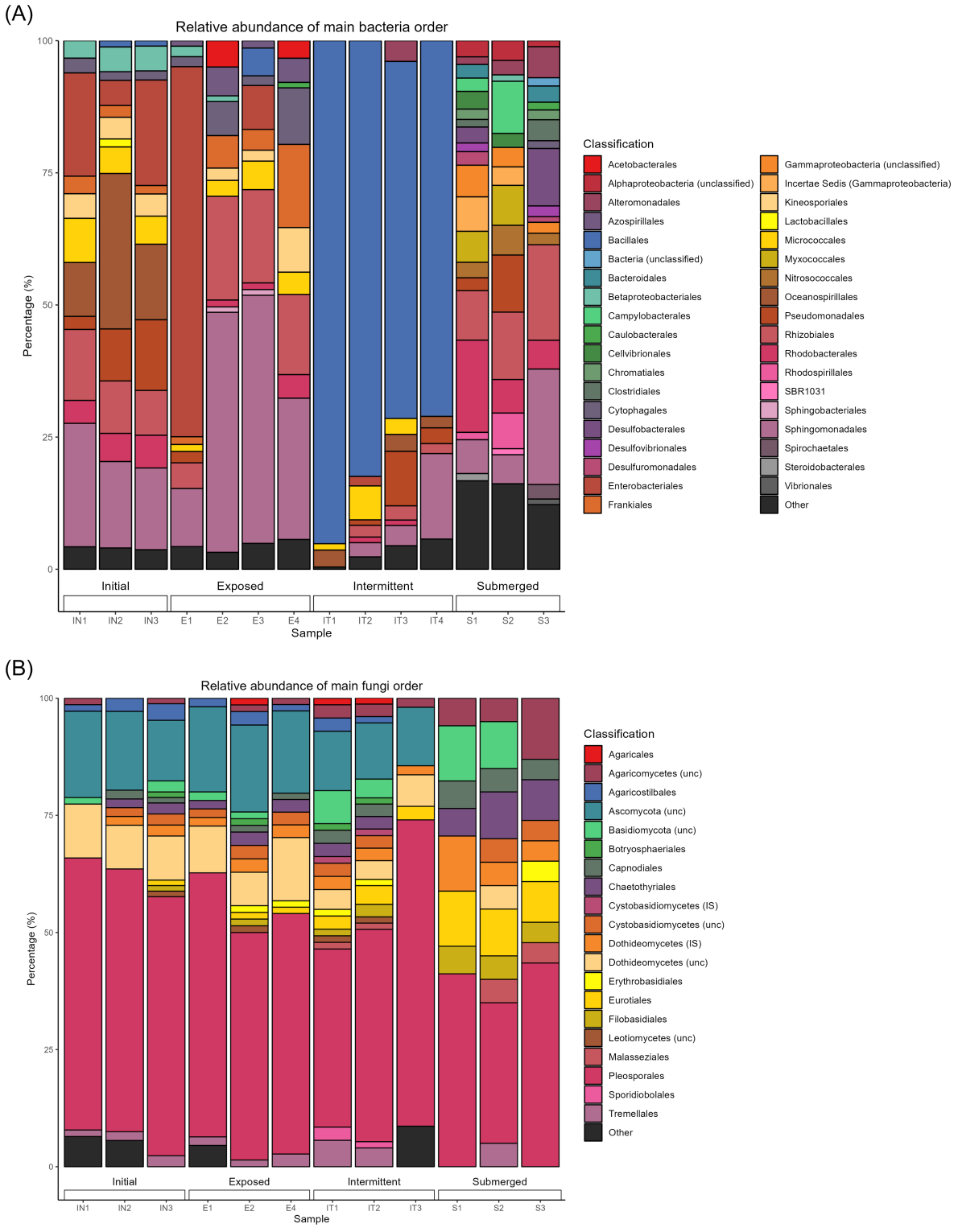

FIGURE 4 Relative abundance of main order (found >1% of sequences) from bacteria (A) and fungi (B) in each replicate from the initial samples (Initial) and the last collection samples (Exposed, Intermittent and Submerged). Some replicates for fungi (E3 and IT4) were eliminated due to a low abundance of sequences detected (<800 final sequences). "Other" refers to orders that represented <1% of total sequences in all samples.

reported (Table 2), and with Rhizobiales, Sphingomonadales and Rhodobacterales being the most abundant (13.4%, 11.2% and 9.7%, respectively) (Figure 4A). Initial, Exposed and Intermittent treatments harboured bacterial orders of gram-positive bacteria (Lactobacillales, Bacillales, Micrococcales, Kineosporiales) which

were not detected in the Submerged treatment. In contrast, only the Submerged treatment presented sulfate-reducing bacteria (orders Desulfobacterales and Desulfovibrionales) and Clostridiales as the only order of gram-positive bacteria. Dominant OTUs (OTUs with a >30% of relative abundance per sample) were only detected in samples from the Intermittent and Exposed treatments. Specifically, in the Intermittent treatment an unclassified *Planococcaceae* appeared as a dominant member of the community (69.4%) while in the Exposed treatment only one replicate showed an unclassified *Enterobacteriaceae* as a dominant member (66.5%).

In all treatments, the fungi phyla detected were Ascomycota and Basidiomycota (Figure S6B), with the former dominated by the order Pleosporales (between 30% and 65.4% of relative abundance depending on the treatment). The Submerged treatment had low relative abundance of Pleosporales, but had other orders that, although also present in the other treatments, were represented at higher relative abundances. These orders, such as Dothideomycetes incertae sedis, Eurotiales and unclassified Agaricomycetes (the latter within the phylum Basidiomycota) accounted for up to 13%. Interestingly, unclassified Ascomycota were absent in the Submerged treatment but were relevant in the Exposed and Intermittent treatments (18.1% and 12.4%, respectively). In addition, unclassified Dothideomycetes also showed higher abundances in the Exposed and Intermittent treatments (10.2% and 5%, respectively) compared to the Submerged treatment (Figure 4B). Surprisingly, although a similar number of total 18S rRNA gene sequences were recovered for the Submerged treatment in comparison to the other treatments (111,116 sequences obtained red for the Submerged), fungal 18S rRNA gene sequences accounted for <25% of the total 18S rRNA gene sequences in the Submerged treatment (Figure S7).

Beta-diversity for the bacterial community showed significant differences among the three treatments with the Exposed community more similar to the Initial than to the other treatments (PERMANOVA pairwise test, p value <0.05, Figure S5A), probably due to harbouring a higher proportion of the Actinobacteria phylum (Figure S6A). The fungal community composition of the Submerged treatment was significantly different from the Exposed and Intermittent treatments (PERMANOVA pairwise test, p value <0.05, Figure S5B). Fungal communities of the Initial samples were not significantly different from the Exposed treatment but differed from the Intermittent treatment (Figure S5B).

Network analyzes (Figure S8) showed that the Exposed treatment had a greater number of total co-occurrences, followed by the Intermittent and the Submerged treatments (41, 35 and 32, respectively). However, when assessing exclusively the between bacteria-fungi co-occurrences for each of the treatments separately, the Submerged treatment had the most edges between them (10 co-occurrences out of a total of 32), followed by the Exposed (9 out of 41) and the Intermittent (2 out of 35). Results also showed that co-occurrences between fungi from the Intermittent treatment were more similar to the ones from the Exposed than to the Submerged one.

Relationships between microbial biomass, enzyme activities and microbial community composition

Principal Component Analysis performed with EEA, %OM remaining and microbial biomasses for the entire study period showed how the Exposed treatment separated from the Submerged and Intermittent treatments while these latter two remained overlapped. The PC1 that separated the Exposed treatment to the left side was characterized by higher hydrolytic EEA and fungal biomass, while the Submerged and Intermittent treatments to the right were characterized by higher PHE activity and bacterial biomass (Figure 5). PC2, correlated with %OM remaining and with PHE activity, seems to be related to the experimental time period, with samples from the last collection located at lower parts of the plot in comparison with the other samples, and characterized by a low amount of %OM remaining (Figure 5).

The relationships between the community composition and EEA from the last collection day indicate that, for the 16S rRNA gene, the community composition

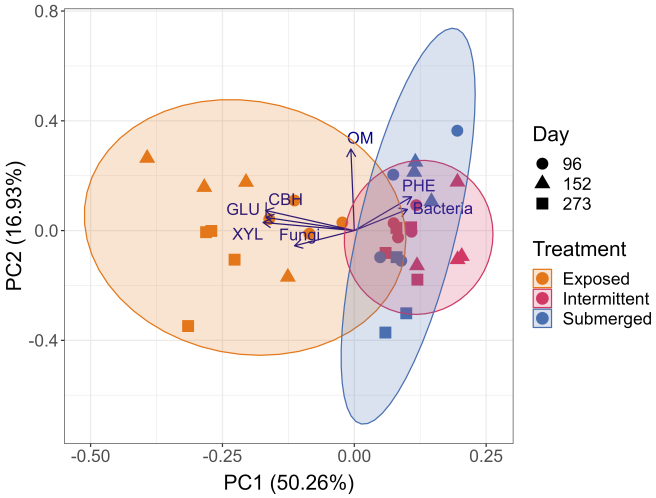

FIGURE 5 Principal Component Analysis (PCA) of the functional and biomasses variables (B, bacterial biomass; CBH, cellobiohydrolase activity; F, fungal biomass; GLU, β-glucosidase activity; OM, organic matter content (%); PHE, phenol oxidase activity; XYL, β-xylosidase activity) with data of the three collections. For each treatment, a concentration ellipse covering 95% of the data was created.

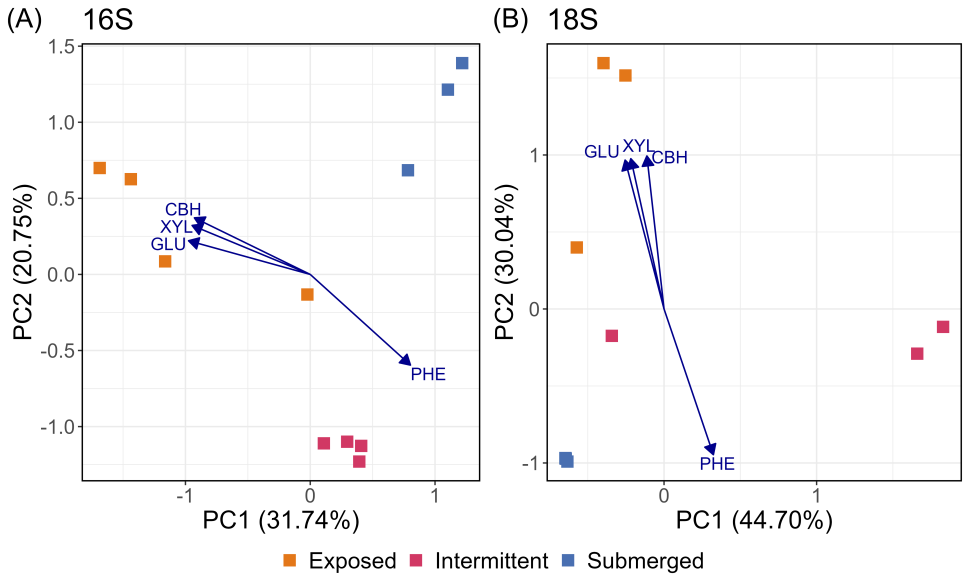

FIGURE 6 PCA distribution samples according to the total of OTUs observed for each treatment from the last collection, represented as squares. Vectors represent the two-dimensional correlation structure among the EEA results from the last collection (day 273) for both 16S rRNA gene (A) and 18S rRNA gene (B).

was correlated with all the EEA in the first axis and with PHE in the second axis (Figure 6A and Table S4). Among the enzymes, CBH and PHE were the most significant for the PCA, and higher values of hydrolytic EEA were related to the Exposed bacterial community while higher PHE was related to Submerged and Intermittent communities. For the 18S rRNA gene, the enzymes most significantly correlated were GLU and XYL, while PHE was not (Table S4). Fungal communities in the Exposed treatment were related to higher GLU and XYL activities (Figure 6B).

DISCUSSION

Suppressed decomposition of woody material in a saline and arid environment

After 273 days, the loss of organic matter of *S. fruticosa* woody stems did not surpass 20%, with decomposition rates between 0.09 and 0.19 year^{-1} (considering the negative exponential decay model). As expected, decomposition rates were low, as when considering other studies of woody tissue decomposition in freshwater streams (including intermittent ecosystems), decomposition rates vary between 0.26 and 1.2 year^{-1} (Abril et al., 2015; Golladay & Sinsabaugh, 1991; Melillo et al., 1983). Woody tissue decomposition studies in terrestrial semi-arid environments are not as common, but Erdenebileg et al. (2020) detected degradation rates between 0.08 and 0.12 year^{-1} for stems of diameters (between 0 and 4 mm) similar to the *S. fruticosa* ones studied here (1–3 mm). This suggests that the saline environment has a further inhibition effect on woody tissue decomposition. On one hand, salinity can act as a preserver of the basic structural components of the woody stems (Fojutowski et al., 2014; Wróblewska & Owczarzak, 2008), which is supported by the non-significant changes in lignin, cellulose and only a 3.7% increase in fibre content after 273 days. On the other hand, osmotic stress caused by the natural salinity and enhanced by water scarcity can cause changes in the abundance and/or composition of the microbial community, which must relocate energy to resistance/adaptation strategies, reducing organic matter degrading capabilities, such as the EEA (Allison et al., 2013; Alster et al., 2013; Wichern et al., 2006). In fact, the values obtained for the EEA of *S. fruticosa* stems in this study were far lower than those found in another study performed in a salt marsh (between approximately 1.5 to 6 μmol*g AFDW^{-1}*h^{-1} for GLU and 0.5 to 8 μmol*g AFDW^{-1}*h^{-1} for XYL) (Carrasco-Barea et al., 2022). Apart from that, other stress factors such as high temperature and UV radiation can play a role in the ecosystem functioning of these saline environments, affecting the decomposition process directly (Gostinčar et al., 2011).

Within this context of slow decomposition, and despite the similar amount of weight loss after 273 days in the three habitats, the weight loss dynamics of the woody stems was different between habitats, suggesting a qualitative difference in the organic matter decomposition process among them. The woody stems in the Intermittent and Submerged treatments fit to a two-pool dynamic, with a faster initial decomposition rate (until day 96), probably due to the wet conditions enhancing

lixiviation and microbial utilization of the more labile compounds (such as non-structural carbohydrates). This first pool in the decomposition process may determine the second pool, consisting of a more recalcitrant fraction, characterized by a higher percentage of fibre material (as shown by the slight increase in fibre content) and showing almost no decrease in weight, or even an increase. This unexpected increase in organic matter content in this second pool (day 152) was probably related to stress factors occurring at the Submerged and especially at the Intermittent conditions. In part, this gain in organic matter content can be explained by the microbial colonization that occurred on the same collection day, especially for bacteria. However, this increase in organic matter content on day 152 (July), which was higher for the Intermittent samples, could also be explained by the possible secretion of extracellular polymeric substances (EPS), which play a crucial role in mediating bacterial cell interactions with their environment (Di Martino, 2018), particularly when microorganisms are stressed (i.e., due to the environmental perturbations as water availability interruption and UV radiation). When stressed, microorganisms invest more resources in producing EPS compounds, using them to counteract for example desiccation (Guo et al., 2018) and UV radiation (Flemming and Wingender, 2010). In contrast, the decomposition of the Exposed woody stems followed a single-pool model (i.e., exponential decay), indicating that throughout the 273 days of experiment, the decomposing material was relatively homogenous in terms of composition and decomposability. Therefore, in this context of a general suppression of organic matter from microbial decomposing activity, our results show how specific habitat conditions, mainly derived from changes in water availability, determine a different decomposition path and distinct colonization of fungi and bacteria, as discussed below.

Different decomposition paths and microbial colonization under drought and wet conditions

A priori we expected decreased microbial biomass, EEA and weight loss on the dry floodplain, as several studies have observed a negative relationship between drought and EEA (Adetunji et al., 2017; Alster et al., 2013; Mora-Gómez et al., 2018; Sardans & Peñuelas, 2005); and as it is commonly accepted that decomposition rates are lower in terrestrial ecosystems than in aquatic ones (Yue et al., 2018), and in intermittent streams (Mora-Gómez et al., 2018). However, in this study, although weight loss was slightly lower on the dry floodplain, it did not significantly differ from the wet conditions. At the same time, microbial biomass and EEA were not reduced, but the respective biomass of fungi and bacteria, and the specific enzymes expressed in the dry floodplain decomposing stems, clearly differed from those stems immersed in the stream. This suggests that, in this arid saline environment, other factors in addition to water availability drive the decomposition process of woody material in both dry and wet habitats, determining distinct decomposition processes. On the dry floodplain, *S. fruticosa* stems were mainly colonized by fungi and showed high hydrolytic enzyme activity involved in cellulose and hemicellulose degradation while showing very low lignin-degrading capacity (low PHE activity). The main EEA expressed were the opposite of what was expected, given the functional roles classically assigned to bacteria and fungi. That is, bacteria mainly decompose simple polysaccharides (therefore mainly contributing to GLU and XYL), while fungi more actively degrade complex molecules and recalcitrant compounds (such as lignin, linked to PHE activity) (Romaní et al., 2006). This unexpected result can be linked to the colonization of stems on the floodplain by the specific microbial community composition showing these specific enzyme capabilities (as discussed in the next section), but it can also be related to the effect of environmental factors on the decomposition process. The high incident sunlight radiation on the dry floodplain could have been responsible for the reduced PHE activity. UV radiation can promote photodegradation of some components, especially accelerating the breakdown of lignin (Mora-Gómez et al., 2020), so microorganisms do not need to produce as much PHE (Gallo et al., 2009). Furthermore, with this lignin degradation, the celluloses and hemicelluloses that were protected structurally by the lignin compounds could have been available (Kirk, 1984), stimulating hydrolytic enzymes such as CBH and XYL. Although this could be a possible explanation, the photodegradation effect was probably low since no significant changes in lignin and cellulose content were observed. A further reason for the high hydrolytic activities could be that the decomposition process during these 273 days was still in an initial phase and little lixiviation (loss of water-soluble compounds) could have occurred on the dry floodplain. Being in an initial phase of the decomposition process implies that there is still labile material (i.e., simple polysaccharides) of easy utilization available (Bani et al., 2018), which is prioritized by microorganisms, so they are not being forced to create PHE to degrade lignin in order to survive.

In contrast, *S. fruticosa* stems submerged in water (permanently or temporarily) were mainly colonized by bacteria and showed much higher lignin degradation than simple polysaccharide degradation activities. The lignin degradation activity being greater than the hydrolytic enzyme activity could be due to the already lost simple dissolved polysaccharides after 96 days of immersion, suggesting immersed stems being in a slightly more advanced state of decomposition than the

ones from the dry floodplain (as shown by their greater weight loss on day 96). This is linked to the loss of a first pool of material of the immersed stems, as suggested by the weight loss dynamics, being the remaining organic matter pool more structurally complex and thus requiring the action of enzymes such as the lignin-degrading PHE activity. However, the high bacterial biomass is surprising, due to the expected limited capability of bacteria to degrade lignin, and suggests that the specific high saline conditions of the stream water determine that mainly bacteria, and specifically those adapted to this environment, colonize the immersed steams first. Accordingly, previous studies have shown that salinity conditions imply an increment of the bacteria-to-fungi biomass ratio since fungi tend to be more sensitive to salt stress than bacteria (Buchan et al., 2003; Sardinha et al., 2003; Wichern et al., 2006; Yan et al., 2015). In the Rambla Salada water, extremely high salinity was measured, reaching 120 mS/cm in conductivity and 37 g/L of salinity in summer. Also, low oxygen conditions in the stream water could negatively affect fungal colonization. Low oxygen acts as an inhibiting fungal growth and decomposition rate (Pascoal & Cássio, 2004), and some microorganism decomposers of woody tissues are only active with an ample supply of oxygen (Björdal & Nilsson, 2008; Huisman et al., 2008; Lee, 1992). However, fungal biomass in the submerged stems increased on day 152 and, at the end of the experiment, bacteria drastically decreased while fungal biomass was maintained. This replacement of bacteria by fungi along with the degradation process is commonly observed in stream leaf litter decomposition (Frey-Klett et al., 2011; Suberkropp & Weyers, 1996). Both high salinity and decreased oxygen may be responsible for this reduced decomposition underwater (Freixa et al., 2016; Yan et al., 2015). Surprisingly, the intermittency simulated by placing the immersed *S. fruticosa* stems on the dry floodplain (Intermittent treatment) did not produced an expected delay in the decomposition process, as observed in a freshwater intermittent stream (Mora-Gómez et al., 2020). In contrast, weight loss, enzyme activities, and bacterial colonization were similar to the wet conditions. Results suggest that the decomposition process has some inertia in the function of the extracellular enzymes, of which the ones previously synthesized by the microbiota remained active, despite the effect of the changing conditions, so the decomposition process did not cease temporarily. Some microbial descriptors (i.e., bacterial biomass and phenol oxidase activity) have shown to be resistant after changing conditions of water availability (Mora-Gómez et al., 2018). However, the water content of the sediment from the floodplain-shoreline treatment was not as low as that on the floodplain, and the humidity was possibly enough to maintain decomposition and microbial activity. Furthermore, previous studies in hypersaline systems suggest that salt crystallization on the woody stems could be especially enhanced in this treatment since the habitat change may favour salt crystal formation and thus physical abrasion effects. Dissolved salts can penetrate the porous woody stems, and when moisture evaporates due to the exposure to air, salt crystals are formed inside the woody tissues, causing a mechanical fragmentation of the fibre compounds of the samples, and thus accelerating the decomposition process (Blanchette et al., 2002; Gómez et al., 2016). However, water intermittency did not imply an increase in fungal biomass once woody stems were placed at the floodplain-shoreline. The change in the environmental conditions (from submerged to dryness) could have greatly perturbed the fungal biomass, which did not start increasing until the last collection. It is possible that, after the perturbation, a transition between different types of fungal communities is happening in the samples from the intermittent treatment, and that this new fungal community adapted to dryness had not yet been established, so biomass values could not thrive at the same level as those found in drought conditions. The possible disturbance effect of crystallization, enhancing the salt content of the stems, could also inhibit fungal colonization of intermittent stems once placed at the floodplain-shoreline.

Bacterial and fungal community composition: Differential stress of drought and salinity

The bacterial community colonizing *S. fruticosa* stems might be determined by the saline conditions, as salt-tolerant bacterial groups (such as *Pseudomonas*, *Bacillus*, *Enterobacter* and others) are found in the three habitats (Kim et al., 2021; Mei et al., 2009; Rima et al., 2018; Yuan et al., 2016; Zhao et al., 2016). However, the permanent aquatic conditions were best for the development of a rich and diverse bacterial community, as also shown by the greater abundance of low representative OTUs (“Other”, <1% of total sequences) than in the other treatment conditions, therefore providing an environment with reduced environmental stress. Also, in the immersed stems, bacteria from the order Corynebacteriales and Vibrionales (only present in samples from the wet treatment), as well as from some Alphaproteobacteria (especially from the Sphingomonadales order), are reported to have PHE activities (Gaur et al., 1992; Sinsabaugh, 2010; Woo et al., 2014), which could contribute to this activity not commonly expressed by bacteria. Although no differences in bacterial biomass and EEA were found for the permanently and temporarily submerged treatments, the bacterial community composition was significantly different between the two, and was also clearly distinct from the Exposed treatment. Bacterial richness and

diversity significantly decreased in the intermittent treatment, suggesting that this intermittence creates stress for bacteria due to the transition from water to the floodplain, as occurs during seasonal drought events. This also suggests bacteria being sensitive to water availability, and therefore, to the disturbance of intermittency. However, functions were not lost. The intermittent condition provides a habitat for the development of a distinct bacterial community with the lowest richness and diversity, characterized by the high abundance of Bacillales. This intermittent condition could have enhanced the sporulation of members of Bacillales, that harbour some genera (i.e., *Bacillus*), which are well known to be spore-forming bacteria, and thus adapted to the intermittent conditions (Gong et al., 2017). These bacteria are also known to provide high EEA (Ettoumi et al., 2013). Thus, although this bacterial community of the stems that were first submerged then emerged appears to be compromised, it maintains similar enzymatic and degrading capabilities to the one developing in the wet stems. In contrast, a totally distinct bacterial community was found in the woody stems from the dry floodplain. This was characterized by a high proportion of Actinobacteria, perhaps due to their physiological adaptation to drought conditions. These organisms possess the capability to enhance the transcription of specific genes and produce spores that exhibit high resistance to dehydration (Gionchetta et al., 2020; Kolb et al., 2022). Apart from Actinobacteria, orders from other phyla present in samples from the initial and Exposed treatments, such as Enterobacterales and Azospirillales have also been reported to increase when effects of heat and drought occur altogether (Kolb et al., 2022).

Intriguingly, no archaeal taxa represented a significant fraction of the prokaryotic community in *S. fruticosa* woody stems across any of the treatments. This result was unexpected, as it is known that archaea thrive under saline conditions due to their high adaptability to extreme conditions (Andrei et al., 2012; Zhao et al., 2022). The same primer pair has been previously used in many microbial diversity surveys which recovered archaeal sequences from oligotrophic high-altitude lakes (Ortiz-Ãlvarez et al., 2020; Ortiz-Alvarez & Casamayor, 2016), inland saline lakes (Ghori et al., 2021; Huang et al., 2020; Menéndez-Serra et al., 2020), and saline sediments under environmental disturbances (Sáenz de Miera et al., 2021). However, all the above studies focused on sediment or water samples, which comparatively harbour higher numbers of prokaryotic cells than the woody stems. Our results suggest that archaea were underrepresented as colonizers of *S. fruticosa* woody stems, being fungi and bacteria the main players in their microbial colonization and degradation dynamics. Also, our results could suggest that the universal primer combination 515/806R might not be suitable for the recovery of archaeal sequences when underrepresented, and this might probably need the use of archaeal specific primers to be further tested.

No other fungal phyla were found in the fungal community apart from Ascomycota and Basidiomycota, which have been reported to thrive in abundance under drought and salinity conditions, unlike other main fungal phyla (Andreo-Jimenez et al., 2019; Chen et al., 2022). Most fungi are moderate or extreme xerophiles (tolerating or needing low-water activity) so they have several strategies to survive under these dry conditions, such as osmotic regulation (accumulating solutes like glycerol) or increased membrane fluidity (Vinnere Pettersson & Leong, 2011). Among several of these terrestrial fungi there is an indirect relationship between their growth and salinity conditions, that is, their growth is significantly lower in waters with high salinity than in distilled waters (Jones et al., 2022). Accordingly, the fungi observed in the submerged stems may be highly stressed, as shown by the limited diversity of the community. In contrast to bacteria, the fungal community in the Submerged treatment was different from those in the Exposed and Intermittent treatments. This pattern was also captured by the network co-occurrence analyzes, which showed a similar co-occurrence network for these later two treatments, differing to the Submerged one. The observed sequences suggest a progressive reduction of richness from the Initial to the Submerged treatment, confirming that fungi are better adapted to dry conditions than to wet ones. Few fungi survive being submerged in high saline water conditions, as evidenced by the low number of OTUs identified (no low representative OTUs have been found), which also suggests a greater sensitivity of fungi to salinity compared to bacteria. In fact, 18S rRNA gene sequencing revealed that fungi were a low percentage of all the diversity found in the wet samples. It is interesting to see how water intermittency caused a mismatch between the type of community and the quantity of fungal biomass. The samples from the Intermittent treatment had a similar community diversity and composition to those from the Exposed treatment, as expected since both had been on the floodplain before the analyses. However, at a biomass level, temporarily immersed stems showed a low fungal biomass, similar to the permanently immersed ones. This suggests that saline water acts as a stressor for fungal growth and that this effect is enhanced in the Intermittent treatment probably due to the disturbance of drying (including possible salt crystallization). It could be expected that if more collections had been conducted, the community present in the Intermittent treatment would have finally recovered from the stressor and thrived, so the fungal biomass of these samples could have ended up being similar to the ones permanently placed on the floodplain as well. Finally, when regarding specially the Intermittent treatment, it is also noticeable that water

intermittency has also negatively affected the co-occurrences between bacterial and fungal communities. These outcomes have been observed in other studies, where, independently of the type of sample studied, drought in general disrupted microbial networks, specially between bacteria-fungi taxa, but also between bacteria-bacteria (Gao et al., 2022). Overall, these co-occurrences analyzes determined another evidence of bacteria being more sensitive to drought when compared to fungi, as well as they could help to interpret the bacteria-fungi relationships when considering enzymatic activities. For example, bacteria have the potential to eliminate the by-products resulting from the decomposition of fungal enzyme activities (Johnston et al., 2016). Therefore, if less co-occurrence between bacteria and fungi, due to drought or water availability intermittency, fungi would be subjected to less substrate competition and consequently, more enzyme production from the fungi community (in this study, the hydrolytic enzymes GLU, CBH and XYL) could be expected.

CONCLUSION

Woody stems from halophyte vegetation are the major carbon input in saline streams, but their decomposition is severely compromised by the conjunction of salinity and drought extremes, resulting in a slow carbon acquisition process from soils/sediments and water. The expected higher decomposition of woody material in aquatic than in terrestrial environments did not occur in the studied decomposition of *S. fruticosa* woody stems. Although this statement is constrained to the length of the study (273 days, ca. 20% organic matter lost), results suggest that contrasting factors such as inhibition of microbial degradation by high salinity and low oxygen under wet conditions as well as enhanced photodegradation under drought conditions may eclipse the effect of water availability. These differential factors acting under wet and dry conditions may be responsible for two distinct degradation paths of woody stems: on the dry floodplain, dominated by cellulose and hemicellulose degradation; and at both the stream channel and shoreline, dominated by lignin degradation. At the same time, the different conditions exert a strong environmental filter on the microbial biomass and communities: a drought adapted fungal community mainly colonizing the stems on the dry floodplain suggests their resistance to drought (and sensitivity to salinity), while a diverse bacterial community mainly colonizing stems submerged in the saline water, together with a low diverse fungal community, suggests bacteria being more resistant to salinity (and more sensitive to drought). At the shoreline, when woody stems were first submerged then emerged, the enzymatic decomposition was similar to that of wet conditions, despite the community composition at the end of the experiment being more similar to the one in dry stems, with the lowest bacterial diversity and lowest fungal biomass. This indicates that while function had some inertia, the surviving community was already adapted by shifting after the environmental change (from wet to dry), thereby suggesting functional redundancy. Overall, in saline streams from arid regions, drought and intermittency conditions, in contrast to wet ones, do not reduce plant carbon cycling, while water availability (and co-occurring stress factors) exerts a distinct environmental filter on bacteria and fungi and their role in the decomposition of plant material. This may affect global carbon cycling and microbial interactions in arid inland saline ecosystems.

AUTHOR CONTRIBUTIONS

Anna Doménech-Pascual: Conceptualization; methodology; data curation; writing – original draft; writing – review and editing. **Lorena Carrasco-Barea:** Methodology; data curation; writing – review and editing. **Frederic Gich:** Methodology; data curation; writing – original draft; writing – review and editing. **Judit Boadella:** Conceptualization; methodology; writing – review and editing. **Zeus Freixinos Campillo:** Conceptualization; methodology. **Rosa Gómez Cerezo:** Conceptualization; methodology; data curation; funding acquisition; writing – review and editing. **Andrea Butturini:** Conceptualization; methodology; funding acquisition; writing – review and editing. **Anna M. Romaní:** Conceptualization; methodology; data curation; funding acquisition; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

Corresponding author declares that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

Raw sequence data from this study are openly available in the NCBI repository under the accession number PRJNA985427: "https://www.ncbi.nlm.nih.gov/bioproject/PRJNA985427".

ORCID

Anna Doménech-Pascual https://orcid.org/0000-0002-1459-6736
Lorena Carrasco-Barea https://orcid.org/0000-0001-5091-9785
Frederic Gich https://orcid.org/0000-0002-0941-634X
Judit Boadella https://orcid.org/0000-0002-6029-2493
Zeus Freixinos Campillo https://orcid.org/0000-0002-5275-1215
Rosa Gómez Cerezo https://orcid.org/0000-0001-5501-7692
Andrea Butturini https://orcid.org/0000-0002-7712-6328
Anna M. Romaní https://orcid.org/0000-0001-5183-8231

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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