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# Effects of waterlogging and increased salinity on microbial communities and extracellular enzyme activity in native and exotic marsh vegetation soils

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**Abstract**

Coastal ecosystems are vulnerable to plant invasion and expected sea level rise in China. This study explored the responses of microbial communities and extracellular enzyme activity in the marsh soils of native *Phragmites australis* and exotic *Spartina alterniflora* to waterlogging and increasing salinity (to mimic prolonged inundation and saltwater intrusion) based on the determination of phospholipid fatty acids and analysis of enzyme kinetics. The results showed that waterlogging and increased salinity treatments decreased the soil microbial biomass in both *P. australis* and *S. alterniflora* soils, with waterlogging exacerbating the negative effects of salinity. Fungi/bacteria ratios decreased under both waterlogging and salinity treatments, whereas actinomycetes/bacteria ratios increased with increasing salinity. The degree of the adverse effects of salinity on plant growth of *S. alterniflora* and soil microbial biomass was lower than that on *P. australis*. Generally, waterlogging treatment increased the activity of sucrase, cellulase, urease, and dehydrogenase in *S. alterniflora* soil. Increased salinity decreased all the assayed extracellular enzyme activity in both *P. australis* and *S. alterniflora* soils. The synergistic effects of waterlogging and increased salinity treatments on the enzyme activities in *P. australis* soil were significant, whereas only the effect on the cellulase activity was significant in *S. alterniflora* soil. This study indicated a greater ability of the microbial community and extracellular enzyme activity of *S. alterniflora* soil to adapt to waterlogging and increased salinity compared with those of *P. australis* soil due to the lower sensitivity of *S. alterniflora* growth and soil nutrients to stress.

## 1 | INTRODUCTION

Coastal salt marshes are among the most productive ecosystems and play an important role in the global C and N cycles (Chmura, Anisfeld, Cahoon, & Lynch, 2003; Duarte, Losada, Hendriks, Mazarrasa, & Marbà, 2013). Soil microbes regulate the biogeochemical processes in coastal ecosystems, including the nutrient cycling and C flux and the primary

**Abbreviations:** AN, available nitrogen; AP, available phosphorus; EC, electrical conductivity; MBC, microbial biomass carbon; ORP, oxidation-reduction potential; PLFA, phospholipid fatty acid; SLR, sea level rise; SOC, soil organic carbon; TN, total nitrogen.

production and decomposition of organic matter (Böer et al., 2009; Dini-Andreote et al., 2014; Hu et al., 2014; Ikenaga, Guevara, Dean, Pisani, & Boyer, 2010). However, salt marshes are vulnerable to the anthropogenic disturbances (e.g., plant invasion), which are widely believed to significantly alter the soil biochemical characteristics of coastal ecosystems, leading to changes in the microbial community composition and structure (Kourtev, Ehrenfeld, & Häggblom, 2003; Liao et al., 2008; Yang et al., 2015, 2016).

Furthermore, the expected sea level rise (SLR) due to global warming will change the hydrological characteristics of coastal regions, including with respect to inundation prolongation and saltwater intrusion, which is predicted to significantly affect the plant growth and biogeochemical processes in ecosystems (Chambers, Osborne, & Reddy, 2013, 2016; Morrissey, Berrier, Neubauer, & Franklin, 2014a; Neubauer, 2013; Neubauer, Franklin, & Berrier, 2013; Unger, Kennedy, & Muzika, 2009; Weston, Dixon, & Joye, 2006, 2010, 2011). The oxygen and nutrient availability profile of soil generally exhibits a decreasing trend after flooding, with some studies showing that the abundances of fungi and aerobic bacteria are reduced under inundation (Mentzer, Goodman, & Balser, 2006; Unger et al., 2009). The results of a study by Mentzer et al. (2006) indicated that prolonged inundation strongly affected microbial function (such as extracellular enzyme activity), whereas Chambers, Guevara, Boyer, Troxler, and Davis (2016) reported that both microbial biomass and community diversity increased with inundation as a result of community shifts.

Previous reports have shown that increases in salinity can decrease microbial biomass and affect microbial composition and heterotrophic metabolic capabilities, primarily due to the reductions in leaf osmotic potential, N mineralization, and plant growth (Jackson & Vallaire, 2009; Morrissey, Gillespie, Morina, & Franklin, 2014b; Neubauer, 2013; Tripathi et al., 2006; Vasquez-Cardenas et al., 2015). However, some studies have described weak (or absent) effects of salinity on the soil microbial biomass and enzyme activity (Chambers et al., 2016; Rath & Rousk, 2015; Setia et al., 2011; Wong, Dalal, & Greene, 2008). These conflicting findings could be attributed to variations in the soil texture, the quantity of soluble salts, and nutrient availability for microbial communities as well as to the adaptation to saline stress (Morrissey et al., 2014b; Neubauer, 2013; Rath & Rousk, 2015; Setia et al., 2011; Vasquez-Cardenas et al., 2015).

At the end of the 1970s, *Spartina alterniflora* from North America were intentionally introduced and planted along China's coastline with the intention of providing shore protection, resulting in a nationwide invasion (Ge, Wang, Wang, & Wang, 2008, 2015). In eastern China, the exotic *S. alterniflora* had a great competitive advantage over native vegetation, such as *Phragmites australis* and *Scirpus* species. Cheng et al. (2008) showed that invasion by *S. alterniflora* altered soil nutrient conditions because of changes in the quantity and quality of litter in soil, leading to shifts in soil microbial community structure. Therefore, determining how the community structure and function of soil microorganisms associated with native and exotic species are affected by prolonged waterlogging and increased salinity can contribute to a better understanding of the impact of SLR on biogeochemical processes in salt marshes.

**Core Ideas**

- Microbe and enzyme activity in different marsh soil under SLR projection were explored.
- Spartina growth and soil microbe was less sensitive to treatments than that for Phragmites.
- Synergistic effect of treatments on enzyme activities in Phragmites soil were notable.
- Nutrient in Spartina soil kept relatively stable under waterlogging and high salinity.

In this study, we conducted a mesocosm experiment with the field-collected marsh soil colonized by the native *P. australis* and exotic *S. alterniflora* from a coastal wetland of eastern China (Yangtze Estuary). The adaptive responses of microbial community and extracellular enzyme activity were explored in the plant–soil mesocosms treated with prolonged waterlogging, increased salinity, and their combination (to mimic the SLR conditions) over an entire growing season. We hypothesized that the prolonged inundation and increased salinity would decrease plant growth and soil microbial biomass because the vegetated marshes in the Yangtze Estuary are of the brackish type (mean water salinity <3 ppt) and because the macrophytes are generally grown in high flats with light inundation stress (mean inundation time of ~1.5–4.0 h $d^{-1}$). Previous studies have shown that the exotic *S. alterniflora* was more tolerant to prolonged tidal waterlogging and increased salinity than the native *P. australis* in terms of photosynthesis efficiency, growth rate, and reproductive perspectives (Bradley & Morris, 1991; Ge, Zhang, Yuan, & Zhang, 2014; Li et al., 2018). As found in the coastal marshes of eastern China, *S. alterniflora* stalks with lower lignin content and higher decomposition potential were better sources of microbial nutrients and C, leading to higher microbial growth rate than that of the native marshes (Chen et al., 2012; Yang & Guo, 2018; Yang et al., 2015). Accordingly, we further hypothesized that the microbial community structure and function in soil associated with the salt-tolerant exotic species could represent a better adaptation to the changing hydrological conditions compared with the native species.

## 2 | MATERIALS AND METHODS

### 2.1 | Field-collected soil and plant mesocosms

In December of 2015, when the aboveground biomass senesced, intact soil blocks with plant rhizomes were sampled from the largest salt marsh within the Chongming Dongtan (121°50′–122°05′E, 31°25′–31°28′N, also referred to as the Chongming Dongtan Nature Reserve) in the Yangtze Estuary. In the high tidal flat of the northern region of the Chongming Dongtan, 48 unbroken field-collected soil blocks associated with native *P. australis* and exotic *S. alterniflora* were removed (roughly 32 cm × 24 cm × 40 cm, length × width × height) using long-wing shovels. The sampling sites for the two species are at the same tidal line and have similar ages (~10–15 yr). The sampling sites were adjacent to each other and had similar soil properties (Supplemental Table S1). Each block used for an experimental mesocosm had a sufficient volume for root growth and was cultivated in a polyethylene container (32 cm × 24 cm × 40 cm, length × width × height). Soil material from the same sampling site was collected to fill small gaps in the containers for each mesocosm. Over January and February of 2016, all the mesocosms were watered daily using fresh water (without salt) to homogenize the soil salinity. A hose with a valve to control drainage was installed on the bottom of each polyethylene container.

### 2.2 | Experimental design for waterlogging and salinity

At the beginning of March (when buds appeared) of 2016, a total of 48 plant–soil mesocosms (24 for *P. australis* and 24 for *S. alterniflora*) were fertilized once with revised Hoagland's nutrient solution (Hoagland & Arnon, 1950) and were grown in a ventilated greenhouse (with a transparent roof film to maintain a rain-free environment) on the campus of East China Normal University. In the ventilated greenhouse, the mean daily air temperature was 16.6°C during spring (March–May), 24.9°C during summer (June–August), and 17.8°C during autumn (September–October). Following the study design described by Li et al. (2018), single-variable experiments included two waterlogging treatments, a nonwaterlogged group (control group; the water level in the container was maintained at half full) and a waterlogged group (the water level was maintained at ~100 mm above the soil surface), and four salinity treatments, including freshwater (control) and 5, 15, and 30 ppt saline water–treated groups (using sodium chloride solution). Simultaneously, the treatments for all 48 mesocosms included three replicates for two species, and eight double-variable treatments (two waterlogging × four salinity conditions) were established. The hydrological treatments for all mesocosms were renewed through drainage and irrigation every 2 wk. Freshwater was used to maintain the water level during the nonirrigation period to avoid excess salt accumulation resulting from water evaporation.

### 2.3 | Measurements of vegetation biomass and soil properties

All of the hydrological treatments continued for 8 mo (March–October 2016) from spring to autumn, with a mean air temperature of ~20°C and day length of ~16.00 h. In the latter growing season (October), the shoot organs and roots of plants were harvested to determine the biomass. A steel corer (inner diameter, 5 cm) was used to randomly extract three soil cores (40 cm depth) from each container. The soil core samples were first soaked and then successively flushed through 0.28- and 0.15-mm mesh sieves to collect all roots. All of the shoot and root materials were oven dried at 60°C until a constant weight was recorded.

Before plant harvesting, soil electrical conductivity (EC) and oxidation-reduction potential (ORP) were measured at a 10-cm depth directly using a Delta-T WET sensor (Delta-T Devices Co. Ltd.) and a portable ORP sensor (SX712, Sanxin Co. Ltd.), respectively. During the EC and ORP measurements, the soil temperature was 20.5 ± 0.2°C. The root-free soil was mixed and air-dried and then sieved to pass a 1-mm sieve to determine the conventional physical and chemical indicators. Soil pH and salinity were measured with a 1:5 soil/water suspension using an acidometer (PHS-25, INESA Co. Ltd.) and a salinometer (SYA2-2, Hwatsing Co. Ltd.), respectively. Soil organic carbon (SOC) and total nitrogen (TN) were determined using an elemental analyzer (Elementar Vario EL III CHNOS, Elementar Analysensysteme GmbH) after the samples were acidified with HCl (corresponding to the experimental standard described by Bao [2000)]. Available nitrogen (AN) was determined using the alkaline hydrolysis diffusion method, available phosphorus (AP) was determined using the molybdenum blue colorimetric method after extraction with 0.5 M sodium bicarbonate, and available potassium was determined using the flame photometric method after extraction with 1.0 M ammonium acetate (Bao, 2000).

### 2.4 | Measurements of microbial biomass and community structure

A steel corer (inner diameter, 2 cm) was used to randomly extract 10 soil cores (30 cm depth) from each container to evaluate the microbial biomass C (MBC), phospholipid

fatty acids (PLFAs), and extracellular enzyme activity (see below). Because the root density was high in both *P. australis* and *S. alterniflora* soil, the soil located 1 cm around the roots was collected and mixed. The soil samples were immediately freeze-dried, after which they were sieved to 0.6 mm and stored at 4°C, with the excess samples stored at −20°C. Microbial biomass C was measured using the chloroform fumigation and direct extraction technique (Vance, Brookes, & Jenkinson, 1987). The C was extracted with 0.5 M $K_2SO_4$ from both fumigated and nonfumigated samples and measured by an elemental analyzer (Elementar Vario EL III CHNOS, Elementar Analysensysteme GmbH), and MBC was estimated by the following equation: MBC = (C from fumigated − C from nonfumigated)/0.38 (corresponding to the experimental standard described by Lu [2000]).

The microbial community structure was assessed by analyzing the ester-linked PLFA composition of the soil using the modified method described by Bligh and Dyer (Bligh & Dyer, 1959; Frostegård, Tunlid, & Bååth, 1993). Two grams of soil were extracted with a single-phase mixture of chloroform/methanol/citrate buffer (1:2:0.8, v/v/v). The phospholipids were then separated from the neutral lipids and glycolipids via chloroform and acetone solutions, respectively. The methylation method was used to derivatize phospholipids to their respective fatty acid methyl esters. Methyl nonadecanoate fatty acid (19:0) was added as an internal standard to quantify the concentrations of phospholipids before the methylation step. The fatty acid methyl esters were identified and quantified using a gas chromatograph equipped with a flame ionization detector (Agilent Technologies) and fitted with a Sherlock Microbial Identification System (MIDI Inc.). To reduce the noise, only PLFA concentrations >0.5 mol% (relative lipid abundance) were selected for the composition analyses of microbial community (Moche, Gutknecht, Schulz, Langer, & Rinklebe, 2015). Terminal-branched saturated PLFAs (14:0 iso, 15:0 iso, 15:0 anteiso, 16:0 iso, 17:0 iso, 17:0 anteiso, 17:1 iso w9c, and 18:0 iso) were used as indicators for gram-positive (G+) bacteria; monounsaturated and cyclopropyl fatty acids (16:1 w5c, 16:1 w7c, 16:1 w9c, 17:0 cyclo w7c, 17:1 w8c, 18:1 w7c, and 19:0 cyclo w7c) mainly characterize gram-negative (G−) bacteria (Frostegård et al., 1993). Total bacteria were reported as the total of the PLFAs assayed for the G+ and G− bacteria groups and the 15:0 dimethyl acetals (White, Stair, & Ringelberg, 1996). Typical markers for fungal PLFA (18:1 w9c, 18:2 w6c, and 20:1 w9c) were associated with fungal biomass (Frostegård & Bååth, 1996), 10Me-branched PLFAs were taken as actinomycetes biomarkers (Zelles, 1999), 20:4 w6c was used as an indicator for protozoans (Ringelberg et al., 1997), and saturated straight chain fatty acids are considered universal PLFAs (Zelles, 1999).

## 2.5 | Extracellular enzyme activity assays

The activity of four extracellular enzymes was assayed in this study as key indicators of C and N cycling (sucrose [EC 3.2.1.26], cellulose [EC 3.2.1.4], urease [EC 1.11.1.6], and dehydrogenase [EC 1.3.5.1]). Sucrase activity was analyzed using 3,5-finitrosalicylic acid colorimetry and was expressed as milligrams of glucose hydrolyzed from sucrase per gram of dry soil in 24 h at 37°C (Frankeberger & Johanson, 1983). Cellulase activity was analyzed using 3,5-dinitrosalicylic acid colorimetry and was expressed as milligrams of glucose equivalents hydrolyzed from sodium carboxymethylcellulase per gram of dry soil in 72 h at 37°C (Hayano, 1986). Urease activity was analyzed using phenol-sodium hypochlorite sodium colorimetry and was expressed as milligrams of $NH_3$–N hydrolyzed from urea substrate per gram of dry soil in 24 h at 37°C (Tabatabai & Bremner, 1972). Dehydrogenase activity was analyzed using 2,3,5-triphenyltetrazolium chloride colorimetry and was expressed as micrograms of 2,3,5-tryphenyltetrazolium chloride oxidized into triphenyl formazan per milliliter of solution mixed with 2 g of dry soil in 24 h at 37°C (Casida et al., 1964). Each extracellular enzyme activity assay for each soil sample was performed in triplicate. All the assays included appropriate blanks in each group. Standard curves were prepared for extracellular enzyme activity based on colorimetric determinations.

## 2.6 | Data analysis

For each species, the differences between all of the variables under nonwaterlogging and waterlogging conditions (within the same salinity level) were tested using paired *t* tests, and the differences among all of the variables under salinity treatments (within the same water level) were tested using one-way ANOVA. The normality of data was checked with the Kolmogorov−Smirnov test, and all the data groups met the assumptions of normality. The main effects of waterlogging and salinity treatments and their interactive effects on the plant biomass, microbial biomass, and extracellular enzyme activity were tested via two-way ANOVA with Tukey's HSD test of multiple comparisons. The linear regression model with Pearson's correlation coefficient or the quadratic curve model (based on model validity at significance level) was used to describe the relationships between the plant and soil microbial variables against the levels of salinity under both water levels. We also used Pearson's correlation analysis to test the relationships between plant growth, soil microbial biomass, enzyme activity, and edaphic variables. Statistical analyses were performed using SPSS (version 23.0, IBM Inc.). Significance was determined at $P < .05$.

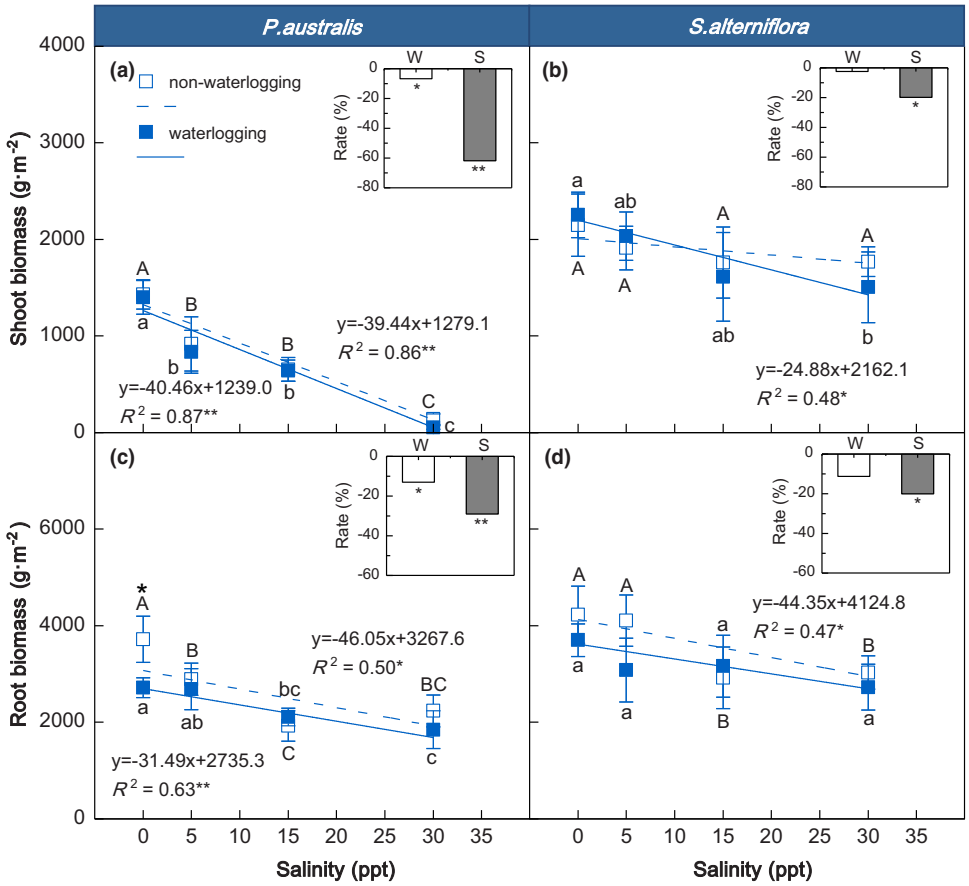


**FIGURE 1** Changes in shoot and root biomass of *Phragmites australis* (*a, c*) and *Spartina alterniflora* (*b, d*) grown under waterlogging and salinity treatments. Different uppercase and lowercase letters indicate the significant differences ($P < .05$) of the variables among salinity levels under nonwaterlogging and waterlogging conditions, respectively. Asterisks indicate the significant differences (*$P < .05$; **$P < .01$) of variables between nonwaterlogging and waterlogging conditions (within the same salinity level). The valid fitting functions ($P < .05$) are presented. Relative change rates of the variables under waterlogging (W) and salinity (S) treatments.

## 3 | RESULTS

### 3.1 | Changes in plant biomass and soil variables in response to waterlogging and salinity

The waterlogging treatments only significantly ($P < .05$) affected the shoot and root biomass of *P. australis* (Supplemental Table S2). Regardless of the salinity treatments, waterlogging decreased the shoot and root biomass of *P. australis* by 7 and 13% on average, respectively, with decreases of 2.4 and 10% for *S. alterniflora*, relative to nonwaterlogging conditions (Figure 1). The salinity treatments had significant ($P < .05$) effects on the shoot and root biomass of *P. australis* and *S. alterniflora* (Supplemental Table S2). Regardless of the waterlogging treatment, the increased salinity reduced the shoot and root biomass by 62 and 29% on average, respectively, for *P. australis* compared with nonsalinity conditions, whereas the decreases in the plant biomass of *S. alterniflora* were relatively lower (20% for both shoot and root biomass) (Figure 1). For *P. australis*, the negative linear regressions between plant biomass and salinity levels were significant ($P < .05$) (Figure 1). For *S. alterniflora*, there was only a notable negative linear relationship between salinity levels and shoot biomass under waterlogging conditions and for root biomass under nonwaterlogging conditions. The lowest shoot and root biomasses of these two plants were found under the combined treatments of waterlogging and 30 ppt salinity.

Regardless of the salinity treatments, waterlogging decreased SOC, TN, AN, AP, and available potassium of the *P. australis* soil by 9–21% on average, whereas the SOC, TN, and AN of the *S. alterniflora* soil increased by 2–12% on average relative to nonwaterlogging conditions (Table 1). Despite waterlogging treatments, increased salinity decreased SOC, TN, C/N ratio, AN, and AP of the *P. australis* soil by 10–20% on average, whereas the above variables in the

**TABLE 1** Changes in soil variables in the *Phragmites australis* and *Spartina alterniflora* soils under waterlogging and salinity treatments

| | Nonwaterlogging | | | | Waterlogging | | | |
|---|---|---|---|---|---|---|---|---|
| **Parameter** [a] | **0 ppt** | **5 ppt** | **15 ppt** | **30 ppt** | **0 ppt** | **5 ppt** | **15 ppt** | **30 ppt** |
| *P. australis* | | | | | | | | |
| SOC, % | 1.8 ± 0.1a [b] | 1.6 ± 0.2b | 1.5 ± 0.0b | 1.5 ± 0.0b | 1.7 ± 0.1a | 1.6 ± 0.2ab | 1.5 ± 0.1b | 1.0 ± 0.1c |
| TN, % | 0.2 ± 0.0a | 0.2 ± 0.01a | 0.2 ± 0.0a | 0.2 ± 0.0a | 0.2 ± 0.01a | 0.2 ± 0.02b | 0.2 ± 0.0ab | 0.13 ± 0.0c |
| C/N ratio | 9.9 ± 0.5a | 8.8 ± 0.2b | 9.0 ± 0.0b | 8.4 ± 0.3b | 9.4 ± 0.1a | 9.5 ± 0.2a | 8.9 ± 0.3b | 7.5 ± 0.3c |
| AN, mg $kg^{-1}$ | 35.0 ± 1.4a | 21.5 ± 4.7a | 27.5 ± 5.2a | 27.1 ± 4.4a | 26.7 ± 4.7a | 28.9 ± 0.5a | 25.4 ± 0.7a | 21.0 ± 3.8b |
| AP, mg $kg^{-1}$ | 194.5 ± 5.7a | 175.7 ± 25.6a | 192.1 ± 5.7a | 191.8 ± 11.3a | 170.1 ± 17.8a | 159.5 ± 17.1a | 153.6 ± 2.8a | 141.2 ± 21.8a |
| AK, mg $kg^{-1}$ | 26.8 ± 1.7a | 28.9 ± 4.4a | 29.9 ± 2.9a | 28.8 ± 0.7a | 22.7 ± 3.9a | 23.5 ± 1.9a | 24.2 ± 1.7a | 20.1 ± 1.3a |
| ORP, mV | 364.2 ± 40.1a | 356.9 ± 23.9a | 317.3 ± 43.2a | 308.3 ± 36.1a | 219.9 ± 45.2a | 152.1 ± 14.4b | 138.6 ± 16.3b | 136.4 ± 31.2b |
| EC, μS $cm^{-1}$ | 669.0 ± 82.2c | 4557.0 ± 848.9c | 7031.3 ± 604.2b | 9195.7 ± 578.8a | 613.7 ± 70.0c | 4272.0 ± 357.8b | 7471.3 ± 43.6a | 9263.7 ± 240.9a |
| *S. alterniflora* | | | | | | | | |
| SOC, % | 1.59 ± 0.3ab | 1.64 ± 0.1a | 1.4 ± 0.1b | 1.5 ± 0.1ab | 1.6 ± 0.2ab | 1.6 ± 0.1a | 1.7 ± 0.1a | 1.3 ± 0.2b |
| TN, % | 0.2 ± 0.0a | 0.2 ± 0.0a | 0.2 ± 0.0a | 0.2 ± 0.0a | 0.2 ± 0.0ab | 0.2 ± 0.0ab | 0.2 ± 0.0a | 0.2 ± 0.0b |
| C/N | 9.6 ± 0.4a | 9.7 ± 0.4a | 9.2 ± 0.2a | 9.4 ± 0.3a | 9.4 ± 0.2a | 9.7 ± 0.2a | 9.5 ± 0.2a | 9.0 ± 0.2b |
| AN, mg $kg^{-1}$ | 23.2 ± 5.2a | 25.4 ± 3.6a | 21.9 ± 0.6a | 27.1 ± 1.0a | 28.0 ± 4.4a | 24.9 ± 1.7a | 26.5 ± 2.9a | 24.5 ± 4.4a |
| AP, mg $kg^{-1}$ | 199.6 ± 16.1a | 185.1 ± 28.4ab | 163.4 ± 12.4b | 176.2 ± 10.0ab | 161.4 ± 9.7a | 163.1 ± 2.9a | 134.5 ± 8.7b | 135.0 ± 9.2b |
| AK, mg $kg^{-1}$ | 32.0 ± 1.2b | 33.7 ± 1.2ab | 33.5 ± 0.5ab | 35.4 ± 1.3a | 26.3 ± 3.5c | 28.9 ± 0.2bc | 30.5 ± 0.7ab | 32.8 ± 1.6a |
| ORP, mV | 383.9 ± 45.5a | 335.1 ± 28.8ab | 269.6 ± 36.5bc | 262.5 ± 36.7c | 113.3 ± 23.2a | 92.1 ± 10.9ab | 60.4 ± 7.9bc | 56.3 ± 23.5c |
| EC, μS $cm^{-1}$ | 668.7 ± 53.2c | 3273.0 ± 697.7b | 5936.0 ± 502.3a | 8235.0 ± 608.2a | 694.3 ± 49.2c | 3639.0 ± 168.0b | 6641.7 ± 6 74.2a | 8783.0 ± 309.0a |

*Note.* Values are means ± SE.

[a] AK, available K; AN, available N; AP, available P; EC, electrical conductivity; SOC, soil organic C; ORP, oxidation-reduction potential; TN, total N.

[b] Different letters indicate the significant differences ($P < .05$) of the variables among salinity levels under non-waterlogging and waterlogging conditions, respectively.

*S. alterniflora* soils showed smaller decreases of 4–12% on average (Table 1). For *P. australis* and *S. alterniflora* soils, both waterlogging and salinity treatments notably decreased the ORP.

## 3.2 | Changes in microbial biomass response to waterlogging and salinity

Across waterlogging and salinity treatments, the total concentration of PLFAs was strongly correlated with the MBC in the *P. australis* and *S. alterniflora* soils (Supplemental Figure S1), demonstrating the reliability of using PLFAs as indicators of microbial biomass.

Waterlogging treatments significantly ($P < .05$) affected the total PLFAs in both the *P. australis* and *S. alterniflora* soils (Supplemental Table S2). Nevertheless, the degree of decrease (average, 17%) in the total PLFAs in the *S. alterniflora* soil under waterlogging treatments was lower than that in the *P. australis* soil (average, 31%) relative to nonwaterlogging conditions (Figure 2).

Salinity treatment also had a significant ($P < .05$) effect on the total PLFAs in both the *P. australis* and *S. alterniflora* soils (Supplemental Table S2). Regardless of the waterlogging treatments, the 5-, 15-, and 30-ppt salinity treatments reduced the total PLFAs in the *P. australis* soil by an average of 10, 32, and 43%, respectively, relative to freshwater treatment. In contrast, the degree of decrease in total PLFAs in the *S. alterniflora* soil was relatively lower (by an average of 2, 17, and 20% for the 5-, 15-, and 30-ppt treatments, respectively) (Figure 2). With increases in salinity, the total PLFAs showed a strong correlation with the root biomass of plants (Figure 3), except for the *S. alterniflora* soil under waterlogging conditions.

Although the negative relation between salinity levels and total PLFAs was significant for both species, the sensitivity of the PLFAs in the *S. alterniflora* soil (regarding the slope of linear functions as 0.56–0.77) to increasing salinity was lower than that (1.11–1.23) in the *P. australis* soil (Figure 2). The lowest total PLFAs in both soils were found under the combined treatments of waterlogging and high salinity, whereas the interactive effect of waterlogging × salinity on the total PLFAs was not significant (Supplemental Table S2).

## 3.3 | Changes in microbial community composition response to waterlogging and salinity

Across all treatments, bacteria dominated the microbial community, accounting for an average of 52 and 49% of the total microbial biomass in the *P. australis* and *S. alterniflora* soils, respectively (Figure 4). Fungi and actinomycetes each accounted for ∼10% of the total microbial biomass, and protozoa accounted for <1% in both soils. Regardless of the salinity treatment, waterlogging significantly ($P < .05$) decreased the PLFAs of fungi, actinomycetes, and protozoa by 43–83% on average in the *P. australis* soil and by 0.3–33% in the *S. alterniflora* soil; these decreases were much larger than the reductions in PLFAs of G− and G+ bacteria relative to nonwaterlogging conditions (Figure 4). The ratios of fungi/bacteria and actinomycetes/bacteria under waterlogging conditions were an average of 20 and 9% lower in the *P. australis* soil and 24 and 21% lower in the *S. alterniflora* soil, respectively, than those observed under nonwaterlogging conditions (Figure 2).

Regardless of the water levels, the increased salinity significantly ($P < .05$) decreased the PLFAs of G− and G+ bacteria and fungi by 29–49% on average in the *P. australis* soil and by 16–39% in the *S. alterniflora* soil; these decreases were much larger than the reductions in PLFAs of actinomycetes and protozoa relative to the freshwater group (Figure 4). The fungi/bacteria ratio under salinity treatments decreased by 6–35% on average in the *P. australis* soil and by 18–27% in the *S. alterniflora* soil, whereas the actinomycetes/bacteria ratio increased by 5–34% on average in the *P. australis* soil and by 14–24% in the *S. alterniflora* soil relative to the freshwater group (Figure 2). The negative linear regression between salinity levels and fungi/bacteria ratio was significant ($P < .05$) for the *P. australis* soil, whereas significance was only achieved for the *S. alterniflora* soil under nonwaterlogging conditions (Figure 2). There was a significant ($P < .05$) positive linear relationship between salinity levels and the actinomycetes/bacteria ratio for the *P. australis* soil but only for the *S. alterniflora* soil under nonwaterlogging conditions.

## 3.4 | Changes in extracellular enzyme activity in response to waterlogging and salinity

There was a significant ($P < .05$) effect of waterlogging on sucrase, cellulase, and urease activity in the *P. australis* soil as well as on cellulase, urease, and dehydrogenase activity in the *S. alterniflora* soil (Supplemental Table S2). Regardless of the salinity treatments, waterlogging decreased the sucrase, urease, and dehydrogenase activity in the *P. australis* soil by an average of 36, 16, and 6% relative to nonwaterlogging conditions, respectively, whereas cellulose activity increased by an average of 63% (Figure 5). In the *S. alterniflora* soil, the cellulase, urease, and dehydrogenase activity under waterlogging increased by an average of 106, 9, and 27%, respectively, relative to nonwaterlogging conditions, whereas sucrase activity decreased slightly under waterlogging treatments.

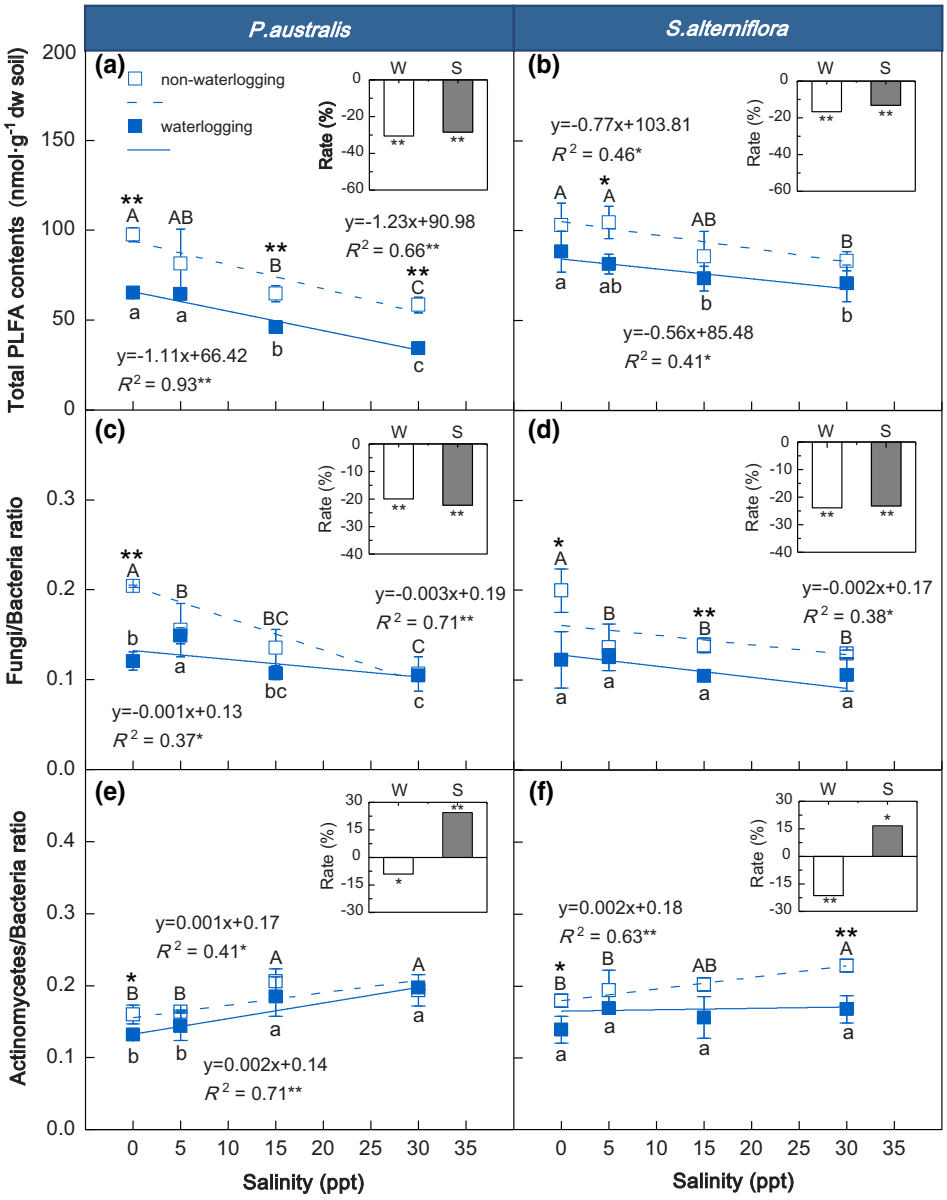


**FIGURE 2** Changes in total phospholipid fatty acid (PLFA) contents, and fungi/bacteria and actinomycetes/bacteria ratios in the *Phragmites australis* (*a, c, e*) and *Spartina alterniflora* soils (*b, d, f*) under waterlogging and salinity treatments. Asterisks indicate the significant differences ($P < .05$) of variables between non-waterlogging and waterlogging conditions (within the same salinity level). Different capital and lowercase letters indicate the significant differences ($P < .05$) of the variables among salinity levels under nonwaterlogging and waterlogging conditions, respectively. Only the valid fitting functions ($P < .05$) are presented. Insets: relative change rates of the variables under waterlogging (W) and salinity (S) treatments. *Significant at $P < .05$; **significant at $P < .01$.

The effects of salinity on the activity of all four assayed enzymes in the *P. australis* and *S. alterniflora* soils were notable ($P < .05$) (Supplemental Table S2). Regardless of the waterlogging treatments, the activity of the four assayed enzymes in the *P. australis* soil was lower under high salinity (15 and 30 ppt) than under low salinity (0 and 5 ppt) (Figure 5). There was a significant ($P < .05$) negative linear relationship between salinity levels and all four assayed enzymes in the *P. australis* soil, except for cellulase activity under nonwaterlogging conditions and urease activity under waterlogging conditions (Figure 5). With increasing salinity, cellulase, urease, and dehydrogenase activity in the *S. alterniflora* soil decreased, whereas 5 ppt salinity increased sucrase activity and then decreased under 15- and 30-ppt salinity treatments, showing a notable ($P < .05$) quadratic response trend (Figure 5). The negative linear relationship between salinity levels and cellulase and urease activity under waterlogging conditions and dehydrogenase activity under nonwaterlogging conditions was significant ($P < .05$), as observed for the *S. alterniflora* soil.

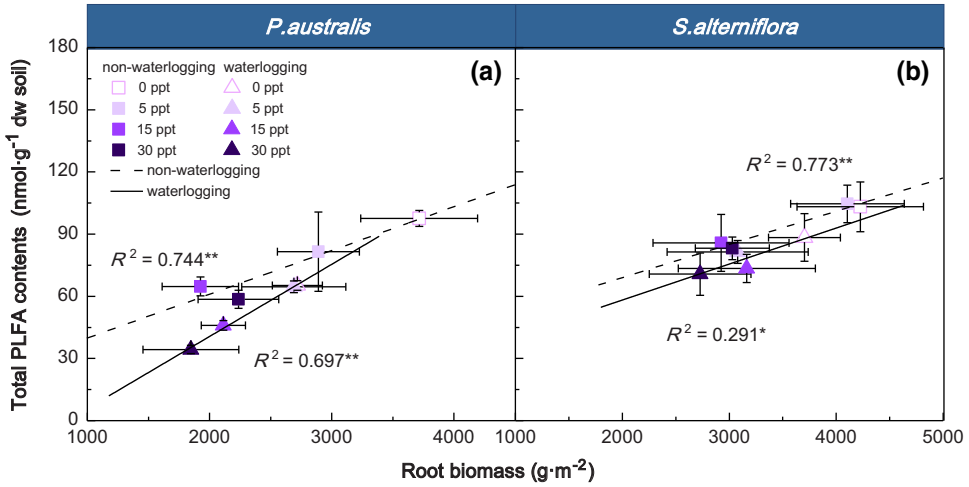


**FIGURE 3** Relationships between the total phospholipid fatty acid (PLFA) contents and root biomass in the *Phragmites australis* (*a*) and *Spartina alterniflora* soils (*b*) under waterlogging and salinity treatments. Only the valid fitting functions ($P < .05$) are presented.

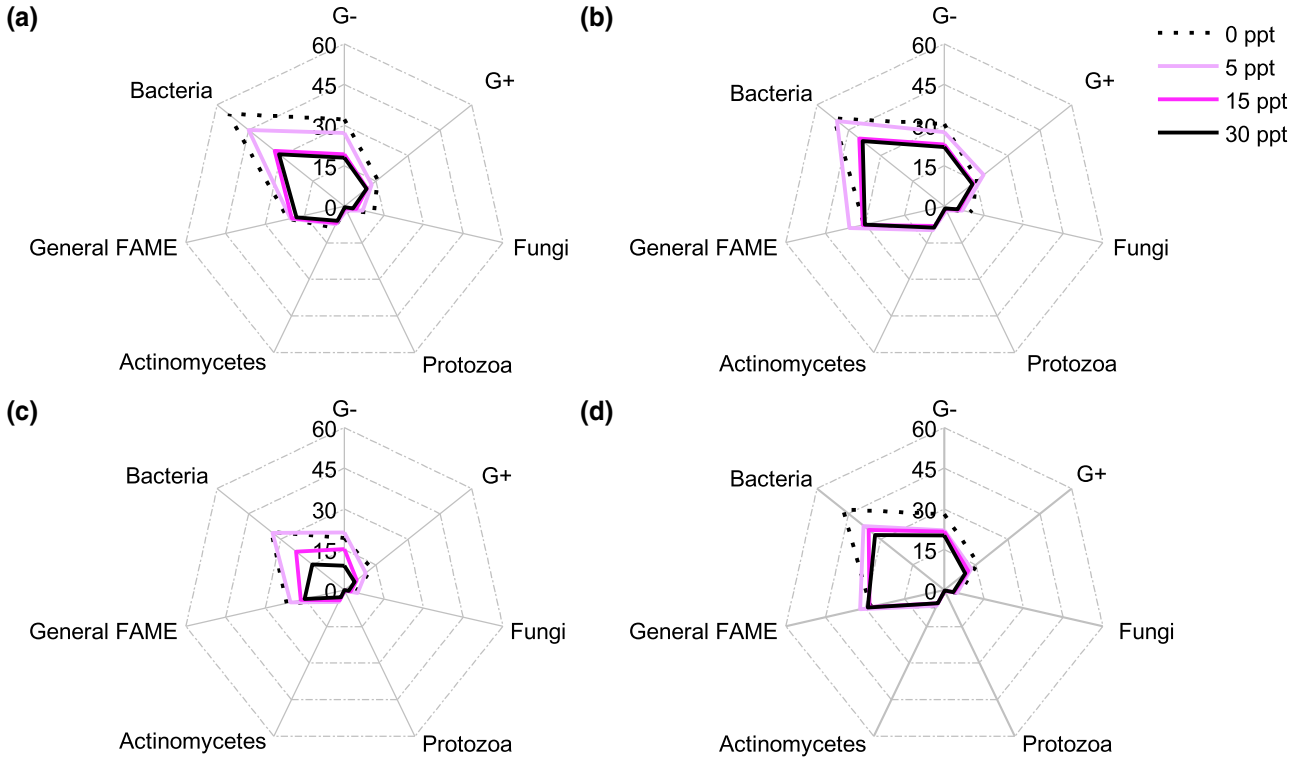


**FIGURE 4** Changes in microbial community structure (species abundance) in the *Phragmites australis* (*a, c*) and *Spartina alterniflora* soils (*b, d*) under waterlogging (upper panel: nonwaterlogging; bottom panel: waterlogging) and salinity treatments. FAME, fatty acid methyl ester; G+, gram positive; G−, gram negative.

The interactive effects of the waterlogging × salinity on the activity of all four assayed enzymes in the *P. australis* soil were significant ($P < .05$), but the interactive effects were notable ($P < .05$) for only the cellulase activity in the *S. alterniflora* soil (Supplemental Table S2). The lowest sucrase, cellulase, and urease activity rates were observed in both the *P. australis* and *S. alterniflora* soils under the combined treatments of waterlogging and the highest salinity (Figure 5), except for urease activity in the *P. australis* soil. Conversely, dehydrogenase activity in the *S. alterniflora* soil was higher under the combined treatments than under the highest salinity treatment alone.

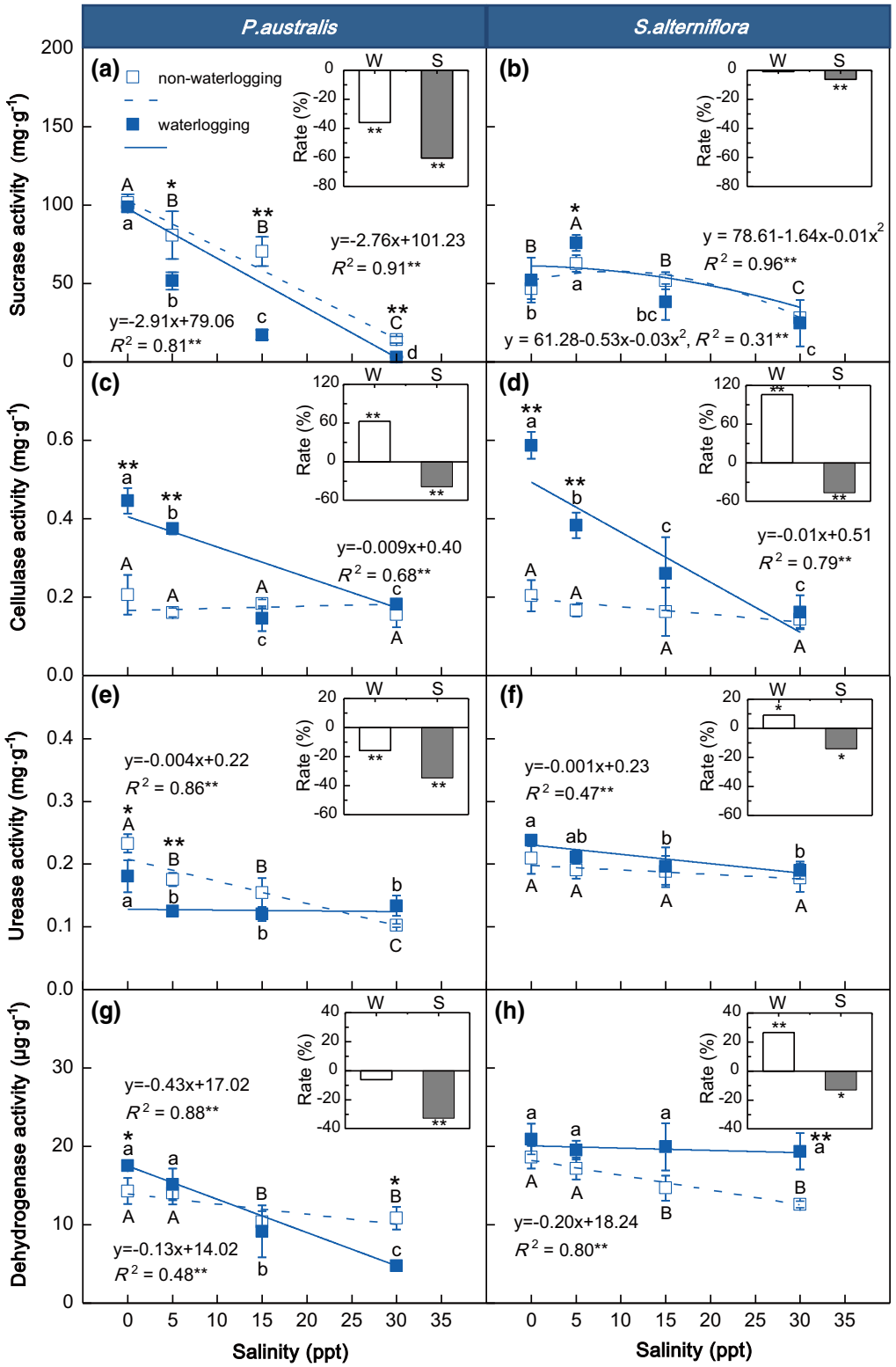


**FIGURE 5** Changes in extracellular enzyme activities in the *Phragmites australis* (*a, c, e, g*) and *Spartina alterniflora* soils (*b, d, f, h*) under waterlogging and salinity treatments. Asterisks indicate the significant differences ($P < .05$) of the variables between nonwaterlogging and waterlogging conditions (within the same salinity level). Different capital and lowercase letters indicate the significant differences ($P < .05$) of the variables among salinity levels under nonwaterlogging and waterlogging conditions, respectively. Only the valid fitting functions ($P < .05$) are presented. Insets: relative change rates of the variables under waterlogging (W) and salinity (S) treatments. *Significant at $P < .05$; **significant at $P < .01$.

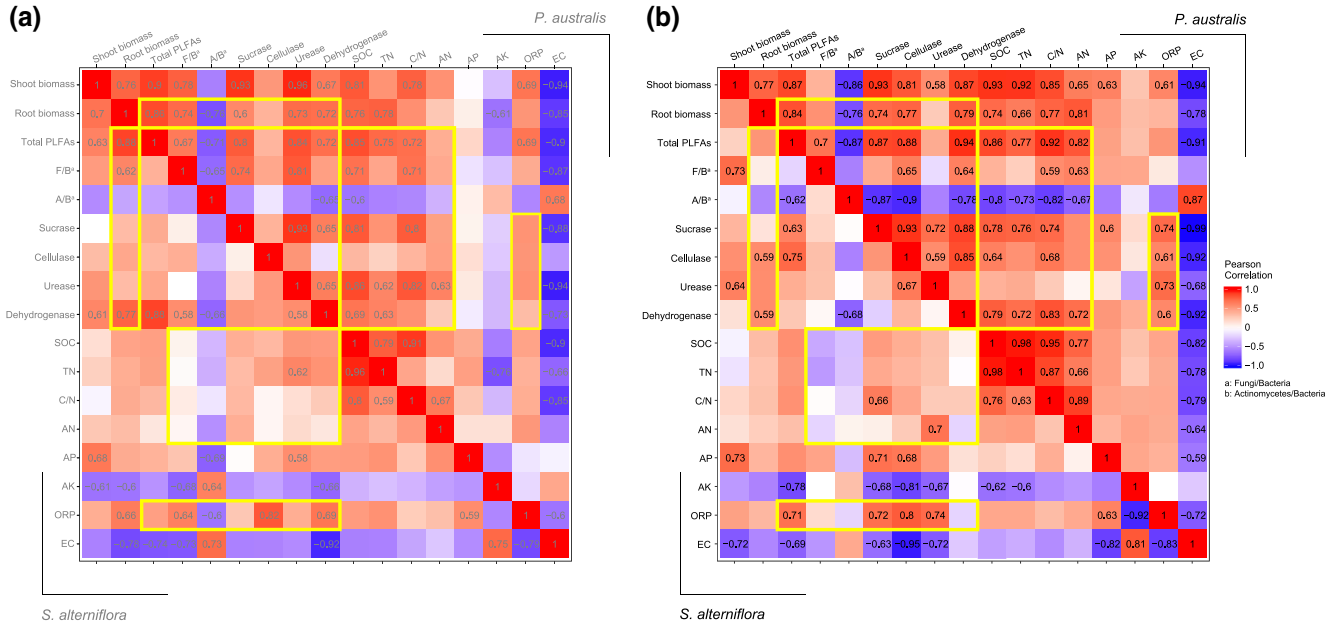


**FIGURE 6** Heat maps of Pearson's correlation coefficients between plant biomass, soil microbial biomass, extracellular enzyme activities and edaphic variables of the plant-soil mesocosms of *Phragmites australis* and *Spartina alterniflora*, within salinity gradient under nonwaterlogging (*a*) and waterlogging conditions (*b*). Only the coefficients with significant correlations ($P < .05$) are listed. The axisymmetric yellow blocks indicate the discrepancies of correlation degrees between the *P. australis* and *S. alterniflora* plant-soil mesocosms. AK, available K; AN, available N; AP, available P; EC, electrical conductivity; MBC, microbial biomass carbon; ORP, oxidation-reduction potential; PLFA, phospholipid fatty acid; SLR, sea level rise; SOC, soil organic C; TN, total N.

### 3.5 | Relationship between plant growth, soil microbe and enzyme activity, and edaphic variables

The total PLFAs, fungi/bacteria ratio, and enzyme activity were significantly ($P < .05$) positively correlated with the plant biomass and soil nutrient variables (specific SOC, TN, C/N, or AN) in the *P. australis* soil, within the salinity gradient (Figure 6). Under waterlogging treatments, there was a stronger relevance (in terms of correlation coefficients) between soil microbial biomass with enzyme activity (except for urease) and soil nutrients in the *P. australis* soil relative to nonwaterlogging treatments. For the *S. alterniflora* soil, there were only significant ($P < .05$) correlations for the total PLFAs, fungi/bacteria ratio, dehydrogenase activity *vs.* root biomass, and urease activity *vs.* TN under nonwaterlogging treatments and for the activity of cellulase and dehydrogenase *vs.* root biomass, sucrase activity *vs.* C/N, and urease activity *vs.* AN under waterlogging treatments, within the salinity gradient.

Generally, the soil EC was negatively correlated with plant biomass, soil microbial indices (except for actinomycetes/bacteria ratio), and all assayed enzyme activity rates in the *P. australis* soil, regardless of the waterlogging treatments (Figure 6). In contrast, in the *S. alterniflora* soil, only the root biomass, total PLFAs, fungi/bacteria ratio, and dehydrogenase activity under nonwaterlogging treatments. Shoot biomass; total PLFAs; and sucrase, cellulase, and urease activity under waterlogging treatment were negatively correlated with the EC.

## 4 | DISCUSSION

### 4.1 | Effects of waterlogging

We found that waterlogging treatment decreased the total PLFAs in both the *P. australis* and *S. alterniflora* soils relative to those observed under nonwaterlogging treatment. With constant flooding, the lower gaseous diffusion rates limit the supply of the dominant electron acceptors, such as $O_2$ (Banerjee et al., 2016; Blagodatsky & Smith, 2012; Weston, Vile, Neubauer, & Velinsky, 2011). In addition, the decrease in root biomass of plants under flooded conditions reduces the amount of organic C and root exudates added to the soil (Bais, Park, Weir, Callaway, & Vivanco, 2004; Boyrahmadi & Raiesi, 2018; Garcia, Roldan, & Hernandez, 2005; Westover, Kennedy, & Kelley, 1997). Our results also showed that the total PLFAs in the *S. alterniflora* soil decreased to a lesser extent than that observed in the *P. australis* soil, which can likely be attributed to *S. alterniflora* having a greater root biomass than *P. australis* (Figure 1). As

an adaptation to flooding, plants can form aerenchyma in the cortex of roots (Armstrong, 1979), which release endogenous oxygen from plants into the soil (Bais, Weir, Perry, Gilroy, & Vivanco, 2006; Bertin, Yang, & Weston, 2003; Uren, 2000).

The biomass of fungi, actinomycetes, and protozoa decreased to a greater extent than bacteria in both the *P. australis* and *S. alterniflora* soils, and there were greater decreases in the fungi/bacteria and actinomycetes/bacteria ratios under waterlogging conditions. The composition of soil bacterial and fungal communities is affected by the abundance of N and soil organic matter, and fungi, actinomycetes, and protozoa are less prevalent in inundated soils (Chambers et al., 2016; de Vries et al., 2012; Drenovsky, Vo, Graham, & Scow, 2004; Fenchel & Finlay, 1995; Moche et al., 2015; Schwarz & Frenzel, 2003; Unger et al., 2009). We observed that waterlogging did not affect the levels of SOC over 8 mo but increased that of AN in the *S. alterniflora* soil (Table 1), which could explain the lower degree to which the bacterial biomass decreased compared with fungal biomass. The remarkable decrease in the fungal biomass in the *P. australis* soil was likely due to a decrease in SOC.

Flooding affects the activity of different types of enzymes differently due to changes in the microbial communities and organic matter content in the soil (Chendrayan, Adhya, & Sethunathan, 1980; Pulford & Tabatabai, 1988; Włodarczyk, Stępniewski, & Brzezińska, 2002). The decreased sucrase activity in the *P. australis* soil under waterlogging can be attributed to the decreased plant biomass and SOC. Chang and Bandurski (1964) showed that plant roots can secrete and release sucrase. The sucrase activity in the *S. alterniflora* soil was not significantly affected, which could be explained by the high level of root biomass. We found that waterlooging treatment highly increased the cellulase activity in *P. australis* and *S. alterniflora* soils compared with that observed under nonwaterlogging conditions. Cellulase plays an important role in the formation of root aerenchyma of hygrophyte or halophyte, especially in the anaerobic environment where the roots would synthesize cellulase (Shiono, Takahashi, Colmer, & Nakazono, 2008). In addition, the accumulation of root litter in soil under flooding conditions has been shown to contribute to a high cellulose content (Allison, Gartner, Holland, Weintraub, & Sinsabaugh, 2007; Shackle, Freeman, & Reynolds, 2000). Urease activity in the *P. australis* soil was lower, whereas urease activity was higher in the *S. alterniflora* soil under waterlogging conditions than that under nonwaterlogging conditions. Urease is involved in the N circulation in soil (Saiya-Cork, Sinsabaugh, & Zak, 2002). Decreases in TN and AN in the *P. australis* soil under waterlogging conditions may have contributed to the observed decrease in urease activity. In contrast, waterlogging increased the TN and AN contents in the *S. alterniflora* soil relative to nonwaterlogging conditions, probably due to the higher N generated by N-fixing bacteria under waterlogging conditions (Currin & Pearl, 1998; Tyler, Mastronicola, & McGlathery, 2003; Welsh, 2000). In general, dehydrogenase activity is positively related to organic matter content but negatively related to ORP (Liang, Gao, Xi, Zhang, & Zhang, 2014; Nayak, Babu, & Adhya, 2007; Włodarczyk et al., 2002). Relative to the *P. australis* soil, dehydrogenase activity in the *S. alterniflora* soil was higher under waterlogging conditions than under nonwaterlogging conditions, which may be associated with stable SOC, increased TN and AN, and decreased ORP.

## 4.2 | Effects of salinity

Our study was in line with previous reports, which declared that soil salinization can inhibit the activity and growth of soil microorganisms (Bais et al., 2004; Fierer, Strickland, Liptzin, & Cleveland, 2009; Garcia et al., 2005; Mentzer et al., 2006; Neubauer et al., 2013). However, we found that the total PLFAs in the *S. alterniflora* soil was affected slightly by increasing salinity compared with that observed in the *P. australis* soil, as was the biomass of dominant microbial communities. We observed a strong correlation between root biomass and the total PLFAs across all treatments. High salinity can inhibit plant biomass production and the consequent organic matter availability for soil microorganisms (Fierer et al., 2009). Growth of *P. australis* was more sensitive to increased salinity, whereas *S. alterniflora* showed a better tolerance to salinity (except for 30 ppt) than that of the native species (Ge et al., 2014). Therefore, shoot and root biomass of *S. alterniflora* decreased to a lesser extent than that of *P. australis* under salinity treatments. The root exudates and secretions can promote the availability of substrates for microbes in soil (Motavalli, Kremer, Fang, & Means, 2004). On the other hand, the degree of variation of soil properties in the *P. australis* soil was greater than that in the *S. alterniflora* soil under salinity treatments (Table 1), which further resulted in higher sensitivity of the plant biomass of *P. australis* and soil microbial organisms to changed edaphic variables (e.g., SOC, TN, C/N ratio, AN, ORP and EC) (Figure 6).

The fungi/bacteria ratio in the *P. australis* and *S. alterniflora* soils decreased with increasing salinity. In saline soil, bacteria are more resistant to osmotic stress than fungi, primarily due to the ability of bacterial communities to alter their physiological and structural characteristics to adapt to saline stress (Pankhurst, Yu, Hawke, & Harch, 2001; Zahran, 1997). However, the actinomycetes/bacteria ratio in the *P. australis* and *S. alterniflora* soils increased with increasing salinity, demonstrating that actinomycetes were able to better adapt to the salinity treatment than the bacteria and fungi. Basilio et al. (2003) reported that actinomycetes species possess a significant capacity to produce antibacterial or antifungal compounds, which can improve their competitiveness against bacteria and fungi in a saline environment.

Many studies have described a negative effect of saline stress on soil enzyme activities (Chambers et al., 2016; Frankenberger & Bingham, 1982; Garcia & Hernandez, 1996; Neubauer et al., 2013; Saviozzi, Cardelli, & Di Puccio, 2011). In our study, the activities of all four assayed enzymes in the *P. australis* and *S. alterniflora* soils decreased under increased salinity conditions, with the exception of sucrase in the *S. alterniflora* soil. Under osmotic stress due to increased salinity, the reduction of root activity and biomass would lower soil microbial biomass and enzyme activity (Boyrahmadi & Raiesi, 2018). In addition, microbial cell lysis would increase the release of intracellular enzymes under high osmotic potential, and the presence of excessive amounts of salt ions may cause nutritional imbalances and affect the growth rates of soil microflora and their subsequent contribution to soil enzyme activity (Frankenberger & Bingham, 1982). The observed response of sucrase activity in the *S. alterniflora* soil may be attributable to the increases in SOC and the C/N ratio under moderate saline conditions (Table 1), which was in line with Yang and Guo (2018). Changes in extracellular enzyme activity can also be associated with shifts in microbial community structure. Under high salinity conditions, the fungi/bacteria ratio decreased. The reduced proportion of fungi in the microbial community may contribute to decreased enzyme synthesis. Compared with the *S. alterniflora* soil, sucrase, urease, and dehydrogenase activity in the *P. australis* soil was much lower under high saline stress (15 and 30 ppt). The lower microbial and root biomasses in the *P. australis* soil may lead to significant reductions in the synthesis and secretion of enzymes by plant root cells (e.g., Costa, Paixão, Caçador, & Carolino, 2007; Gallo, Amonette, Lauber, Sinsabaugh, & Zak, 2004). However, it is still difficult to attribute specific community-related enzymes to specific microbial communities and plant root apexes through this study.

### 4.3 | Effects of the combination of waterlogging and increased salinity

In the SLR conditions, prolonged flooding and saltwater intrusion often interact in salt marsh ecosystems, so the simultaneous effect of waterlogging and salinity on the plant–soil systems has received increasing attention (Baustian & Mendelssohn, 2018; Chambers et al., 2016). Our results indicated that waterlogging and salinity can interactively influence plant growth, soil microorganisms, and extracellular enzyme activities. The microbial biomass in both the *P. australis* and *S. alterniflora* soils was lowest under the combined waterlogging and high salinity levels. Waterlogging can promote the loss of root membrane integrity and impair the function of root organs, affecting membrane selectivity (Drew, 1983; Morard & Silvestre, 1996). The increased accumulation of salt ions in plant roots can accelerate the cell senescence and death of plants at high salt concentrations, limiting the delivery of nutrients to the soil (Barrett-Lennard, 2003). In addition to hypoxia and salt ion toxicity stress caused by waterlogging and salinity, the decrease in soil nutrients that occurs under these conditions may be one of primary reasons for the observed changes in microbial biomass and species abundance. Generally, the SOC, TN, C/N ratio, AN, and AP in both the *P. australis* and *S. alterniflora* soils were lowest under the combined treatments of waterlogging and high salinity (15 and 30 ppt) (Table 1). The lowest fungi/bacteria ratios in the *P. australis* and *S. alterniflora* soils were observed under the combined treatments of waterlogging and high salinity conditions. This result is likely attributable to the resistance of fungi to single and combined stress treatments being lower than that of bacteria (Drenovsky et al., 2004; Pankhurst et al., 2001). The actinomycetes/bacteria ratio in both the *P. australis* and *S. alterniflora* soils increased with increasing salinity, whereas it was still lower under the combined waterlogging and salinity treatments than under single salinity treatments. These results suggested that the cumulative effect of waterlogging and salinity stresses on the actinomycetes/bacteria ratio was antagonistic.

We also observed that waterlogging treatment generally increased the sensitivity (in terms of the slope of linear functions) of extracellular enzyme activities to variations in the total PLFAs under salinity treatments (Supplemental Figure S2). The lowest extracellular enzyme activity that was observed under the combined waterlogging and salinity conditions may also be associated with the lowest plant root and soil microbial biomass recorded, which was in line with previous studies (Costa et al., 2007; Włodarczyk et al., 2002). The lowest SOC, TN, C/N ratio, AN, and AP observed under the combined stresses also inhibited extracellular enzyme activities. However, relative to *P. australis*, the microbial biomass and extracellular enzyme activities in the *S. alterniflora* soil decreased to a lesser degree under the combined treatments. Compared with *P. australis* ($C_3$ plant), the $C_4$-type of photosynthesis and specialized salt-secretion glands of *S. alterniflora* can maintain the ionic balance in plants and contribute to some extent to tolerance to salt and inundation stresses (Bradley & Morris, 1991; Li et al., 2018). The stronger resilience of *S. alterniflora* (e.g., physiological tolerance and maintenance of an extensive root system) and the lower sensitivity of soil nutrient levels to flooding and salinity (Figure 6), relative to *P. australis*, play key roles in the better adaptation of soil microorganisms and extracellular enzyme activity.

## 5 | CONCLUSIONS

This study showed that the degree to which the total microbial biomass decreased in the soil of exotic *S. alterniflora* under

waterlogging conditions was less than that observed in the soil of native *P. australis* and that extracellular enzyme activity in the *S. alterniflora* soil generally increased under waterlogging treatments. High salinity decreased the total microbial biomass (as well as the dominant communities) in both the *P. australis* and *S. alterniflora* soils, with these factors being slightly less affected in the *S. alterniflora* soil. The activity of the assayed enzymes in both the *P. australis* and *S. alterniflora* soils decreased with increasing salinity (except for sucrase). The interactive effects of waterlogging and salinity on the extracellular enzyme activities in the *P. australis* soil were significant, whereas the interactive effect was only notable for cellulase activity in the *S. alterniflora* soil. This result was primarily due to the lower sensitivity of *S. alterniflora* biomass growth and soil nutrient levels to changes in the hydrological environment. Thus, the invasion of *S. alterniflora* in coastal marshes may stabilize the associated soil microbial characteristics, providing further insight into the response of soil C and N processes under conditions of rising sea level and simultaneous saltwater intrusion.

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## CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

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