Spartina Alterniflora Raised Soil Sulfide Content by Regulating Sulfur Cycle-Associated Bacteria in the Jiuduansha Wetland of China

Chiquan He

Shanghai University

Liyu Cheng

Shanghai University

Daoyuan Wang

Shanghai University

Zhenzhen Zhao (✉ zhaozhen0828@126.com)

Shanghai University <https://orcid.org/0000-0001-8268-2698>

Zhengyu Wang

Shanghai University

Feifei Wang

Shanghai University

Xiaoxi Wang

Shanghai University

Pu Zhang

Shanghai University

Xueping Chen

Shanghai University

Xiaoyan Liu

Shanghai University

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Abstract

Purpose The *Spartina alterniflora* invasion across the southeast coast in China significantly reduced vegetation diversity and generated associated ecological problems. Sulfur (S) is a vital nutrient, while sulfide is phytotoxic and the impact of *S. alterniflora* invasion on soil S cycle remains unclear. Therefore, this study aims to investigate the impacts of *S. alterniflora* invasion on the S cycle and associated soil microbial communities.

Methods Both field investigation and lab-scale experiments were conducted, analyzing soil sulfide and sulfate contents, soil properties over four seasons in the Jiuduansha Wetland of Shanghai, China, the high-throughput sequencing of soil microbial communities, S cycle-related functional genes and seed germination experiments.

Results The contents of sulfide, soil organic carbon (SOC), and total nitrogen (TN) in the bulk soil of *S. alterniflora* invaded area were higher than those in the native species *S. maritima* habitat and bare mudflat soils. *Spartina alterniflora* invasion increased the abundance of the *Nitrospiraceae* and *Desulfarculaceae* families and reduced that of *Hydrogenophilaceae*. The relative abundance of the S reduction functional genes (*dsrA* + *dsrB*) in the soil was increased after *S. alterniflora* invasion, while that of the S oxidation functional genes (*yedZ* + *soxY*) in the soil was reduced. Seed germination experiments with different sodium sulfide concentrations (Na_2S) revealed that the phytotoxicity of sulfide caused more lethal damage to *S. maritima* than to *S. alterniflora*.

Conclusion The *S. alterniflora* invasion significantly increased SOC and TN contents and reduced the abundance of sulfur-oxidizing functional genes, which led to the accumulation of soil sulfide.

1. Introduction

Plant invasion can dramatically reduce the biodiversity of the native ecosystem by dramatically reducing the abundance and survival rate of native species (Mack et al. 2000). It also generate ecosystem degradation by altering both soil nutrient cycling processes and hydrological parameters (Sanderson et al. 2001; Wang et al. 2020; Li et al. 2021). The *Spartina alterniflora* is an invasive plant in coastal wetlands of China across climate zones, which was introduced to China to reduce soil erosion in the 1990s (Chung et al. 2004; Zhou et al. 2009). As a C4 plant, the *S. alterniflora* has higher photosynthetic efficiency and water use efficiency than the native species *Scirpus maritima* (Cheng et al. 2006; Liao et al. 2007; Ge et al. 2015). Previous researches revealed that plant invasion could have distinct impacts on soil elemental distribution and transformation (Yu et al. 2015; Feng et al. 2018).

Sulfur (S) plays a vital role in plant physiological processes, while high soil S levels can be phytotoxic (Lamers et al. 2013). The *S. alterniflora* can raise soil S contents and generate environmental stress on native species during its invasion (Xia et al. 2015). Soil organic matter and total S content increase can promote the reduction of sulfate, leading to phytotoxic sulfide accumulation in soil (Li et al. 2019). Soil sulfide content can alter the composition and distribution of vegetation depending on its sulfide tolerance

level (Li et al. 2009). High soil sulfide levels can inhibit plant growth by reducing oxidase activity in cell mitochondria (Bagarinao 1992). Previous research indicated that increased organic matter input could result in higher soil sulfide levels by increasing S mineralization and offering substrate for sulfur-reducing bacteria (Scherer 2009). *S. alterniflora* is a high sulfur-tolerant wetland plant than lots of native plants since it can absorb sulfides and perform sulfide oxidation at the root zone (Carlson and Forrest 1982; Holmer et al. 2002; Martin et al. 2019). The plant S storage in *S. alterniflora* tissues was found significantly higher than that in native species (Chambers et al. 1998; Zhou et al. 2009).

The *S. alterniflora* invasion impacts soil S cycle-associated microbial activities. Sulfate can be reduced to sulfide by sulfur-reducing bacteria (SRB) in anoxic environments (Niu et al. 2021). The sulfur-oxidizing bacteria (SOB) changes S^{2-} to a higher valence state (Xing et al. 2007). Both SRB and SOB play significant roles in the S cycle and the distribution of soil S forms. Our previous research found that *S. alterniflora* invasion into coastal salt marsh promoted SRB activities and soil S accumulation, which promotes the invasion of the sulfur tolerant plant (Zheng et al. 2017). Zeleke et al. (2013) found that the *dsrB* (SRB) gene copy number in the soil of *S. alterniflora* invaded area was 2.9 times higher than that in the soil of *P. australis*. Cui et al. (2017) found that *S. alterniflora* invasion significantly increased the abundance and diversity of soil SRB community in China's eastern coast. There is still a lack of systematic research on how *S. alterniflora* initiates its invasion by affecting microorganisms related to the S cycle.

We aim to investigate the impacts of *S. alterniflora* invasion on the S cycle and associated soil microbial communities. We hypothesized that (1) the invasion of *S. alterniflora* could increase SOC and TN; (2) *S. alterniflora* invasion altered soil S cycling associated microorganisms and resulted in an increase in sulfate reduction and a change in sulfide in the Jiuduansha Wetland; (3) increased soil sulfide level can environmentally stress on native species *S. maritima*, whereas *S. alterniflora* can survive under high sulfide levels. To test these hypotheses, a field investigation was carried out in the Jiuduansha Wetland, and seed germination experiments were conducted in the laboratory. This study will provide a new perspective for explaining the rapid invasion of *S. alterniflora* and provide a detailed reference for subsequent ecological protection.

2. Materials And Methods

2.1. Site description

The study was conducted in the Zhongxiasha area (30°10'N, 122°01'E) of the Jiuduansha Wetland National Nature Reserve in China (Fig. 1). The Jiuduansha Wetland is approximately 423 km² and has a subtropical monsoon climate with an average annual temperature of 15–17°C and annual precipitation of about 1200 mm. Before the introduction of *S. alterniflora*, *S. maritima* was the local pioneer species in this region. After introducing *S. alterniflora*, it quickly underwent an ecological invasion and was widely distributed in the Jiuduansha Wetland. It is now a widespread intertidal species (Huang and Zhang 2007). *S. alterniflora* is mainly distributed in medium-low tidal flats of the Zhongxiasha area, and *S.*

mariqueter is the pioneer plant in the Jiuduansha Wetland. It is distributed in low tidal flats (Yang and Guo 2018).

2.2. Sample collection and analyses

As shown in Fig. 1, we chose a mudflat without vegetation, a community containing the native species *S. mariqueter*, and a community containing the invasive plant *S. alterniflora*. The mudflat, *S. mariqueter*, and *S. alterniflora* sites were distributed successively in the same region from sea to land. The elevation of the offshore site was low, while that of the inshore site was high. Soil samples were collected in August and November of 2018 and in January and April of 2019 and represented different temperature and humidity features in the four seasons of spring, summer, autumn, and winter. Each sampling site was randomly sampled three times over a distance of no less than 10 meters. The stem height and density of the vegetation were measured in situ. Total above-ground plant biomass was measured by drying plant samples until constant weight at 65°C in an oven.

Soil cores were collected from depths of 0 to 100 cm by using a columnar sampler. The soil samples were kept in ziplock bags and stored at 4°C. The samples used for further analyses of sulfide and DNA extraction were stored at -20°C. The soil moisture content was determined by drying at 105°C in an oven until constant weight. The samples for other physicochemical analyses were air-dried, ground, and passed through a 0.15 mm sieve. Soil pH (1 : 2.5) was measured in the bulk soil with a pH meter (PHS-25, China), and soil salinity (1:5) was measured with a conductivity meter (Orion 145A+, ThermoFisher Scientific, FL, USA). The soil organic carbon (SOC) content was measured with a potassium dichromate oxidation method (Wang et al. 2019). The soil sulfate content was measured using a barium sulfate turbidimetry method (Chen et al. 2018). Soil total phosphorus (TP) and total iron (Fe) levels were determined by ICP-AES (Teledyne Leeman Labs, Hudson, NH, USA) after soil digestion. Soil sulfide contents were determined as described by Niu et al. (2018). The soil sulfide contents were determined using the methylene blue spectrophotometry method (HJ883-2017) (Luo et al. 2018).

2.3. Soil functional bacterial measurements

The total DNA in a representative bulk soil sample from the *S. mariqueter*, *S. alterniflora*, and mudflat sites was extracted using a MetaVx™ according to the manufacturer's protocol (GENEWIZ, Inc., South Plainfield, NJ, USA). The quality of the extracted DNA was assayed using a Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA) to determine DNA sample concentrations, and a series of PCR primers were designed to amplify two highly variable regions (V3 and V4) of 16S rDNA using 30–50 ng of DNA as a template. The V3 and V4 regions were amplified using an upstream primer with the sequence 'CCTACGGRRBGCASCAGKVRVGAAT' and a downstream primer with the sequence 'GGACTACNVGGGTWTCTAATCC'. Additionally, a linker with an index was added to the end of the PCR product of 16S rDNA by PCR for NGS. Library quality was determined using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA), and library concentrations were determined with a Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA). After the DNA libraries were mixed, 2x300 bp paired-end (PE) sequencing was performed according to the Illumina MiSeq (Illumina, San Diego, CA, USA) instrument

instruction manual, and sequence information was read by MiSeq Control Software (MCS), which was included with the MiSeq device. For the high-throughput sequencing results, we referred to the classification method of Meyer et al. (2016). We divided the sulfur-related microbial communities in the high-throughput sequencing results into two categories: SRB and SOB, which can respectively reduce sulfate and oxidize sulfide. The quantitative microbial element cycling (QMEC), a high-throughput quantitative-PCR-based chip, was used to obtain the specific functional genes, including sulfur-reducing (*dsrA* and *dsrB*) and sulfur-oxidizing (*soxY* and *yedZ*) functional genes, for the S cycle (Zheng et al. 2018).

2.4. Seed germination under different sulfide levels

A lab-scale seed germination experiment with both *S. alterniflora* and *S. maritima* seeds was carried out to test their tolerance and survivability of sulfide under different sulfide levels. *S. alterniflora* and *S. maritima* seeds were collected from sampling sites in the Jiuduansha Wetland in autumn 2018. The seeds were soaked in 5% sodium chloride solution at 4°C to preserve the vigor of seeds (Vertucci and Roos 1990). The experiment was conducted in the phytotron of the School of Environmental and Chemical Engineering, Shanghai University, in 2019, and the temperature was controlled at 28°C (day) / 20°C (night) (Ricketts et al. 2018), the relative humidity was 80%, and the daily light duration was 12 h.

Twenty seeds of *S. alterniflora* and *S. maritima* were disinfected with 0.1% sodium hypochlorite solution and rinsed with distilled water. Then the seeds were placed in Petri dishes that were cleaned and disinfected in advance. Three replications were conducted for each treatment. The dishes contained the same amount of washed and disinfected silica sand, and 10 mL of sodium sulfide (Na₂S) solution from a gradient (0, 10, 20, 50, 100 and 200 mmol/L) was added, because 200 mmol/L Na₂S was reported to kill *S. alterniflora* (Shen et al. 2011). After silica sand and Na₂S solution being thoroughly mixed, 5mL of distilled water was added, while 15 mL of distilled water was added to the control group. The weight of each Petri dish was then recorded. The Petri dishes were placed in a phytotron, and distilled water was added every 24 hours to reach the original weight. The plant biomass, root length, shoot lengths were weighed and measured with electronic balance and rulers after 7 days of seed culture.

2.5. Data analysis

The stem height, stem density, and above-ground biomass of *S. alterniflora* and *S. maritima* were calculated by averaging morphological data of four seasons in field investigation. The soil water content, pH, and salinity in five soil layers (0–20 cm, 20–40 cm, 40–60 cm, 60–80 cm, and 80–100 cm) in the range of 0–100 cm were calculated by averaging the values of four seasons. The soil sulfide and sulfate concentrations of different seasons were calculated by averaging the data in five depths, and significant differences between four seasons were analyzed using analysis of variance. The annual average sulfide, sulfate, SOC, TN, TP, and total iron concentrations in soil were calculated by averaging all data in five depths and four seasons. Statistically significant differences between vegetation types were analyzed using one-way analysis of variance and Tukey's range test at 5% significance level (Dat et al. 2000). All reported differences were statistically significant at a $P < 0.05$ level. The above statistical analyses of the

data were performed using SPSS 18.0 (SPSS for Windows, SPSS, Inc.), and diagrams were created with OriginPro 8.5. The relationships between sulfide and soil parameters (water content, SOC, TN, TP, Fe, sulfide, sulfate) were analyzed using principal component analysis (PCA) using Origin 8.5 software and Pearson correlation analysis using SPSS 18.0. A principal coordinates analysis (PcoA) was also applied, to explore the difference of bacterial communities among the soils collected from different sites.

3. Results

3.1. Vegetation and soil properties

As shown in Table 1, the plant stem height and above-ground biomass of *S. alterniflora* were 8 and 3 times higher than those of *S. marigueter*, respectively. In contrast, the stem density of *S. alterniflora* was significantly lower than that of *S. marigueter*. Both soil moisture content and salinity in *S. alterniflora* invaded area were higher than those in *S. marigueter* and mudflat soils. In contrast, the *S. alterniflora* invaded area's soil pH was lower than that in the mudflat soil.

Table 1

Vegetation and soil properties of three sampling sites (Mudflat (MF); *S. mariqueter* (SM); *S. alterniflora* (SA)). The data are expressed as means $\pm$ SDs, which are the annual averages

Sample Site	Depth (cm)	Stem height(cm)	Stem density (individual plants/m ²)	Above-ground plant biomass(g/m ²)	Water content (%wt.)	pH (0-100cm)	Salinity (0-100cm)
MF	0-20	00.00 $\pm$ 00.00 c	00.00 $\pm$ 00.00 c	00.00 $\pm$ 00.00 b	27.45 $\pm$ 1.55	8.67 $\pm$ 0.38	0.25 $\pm$ 0.09
	20-40				26.00 $\pm$ 2.17	8.67 $\pm$ 0.32	0.25 $\pm$ 0.11
	40-60				25.83 $\pm$ 2.06	8.68 $\pm$ 0.34	0.28 $\pm$ 0.11
	60-80				26.53 $\pm$ 1.52	8.57 $\pm$ 0.36	0.23 $\pm$ 0.08
	80-100				26.17 $\pm$ 1.60	8.60 $\pm$ 0.40	0.28 $\pm$ 0.11
SM	0-20	20.80 $\pm$ 11.83 b	757.33 $\pm$ 243.65 a	501.00 $\pm$ 128.12 a	25.56 $\pm$ 0.24	8.71 $\pm$ 0.39	0.33 $\pm$ 0.08
	20-40				26.99 $\pm$ 1.25	8.40 $\pm$ 0.42	0.38 $\pm$ 0.15
	40-60				26.80 $\pm$ 1.03	8.62 $\pm$ 0.38	0.38 $\pm$ 0.11
	60-80				26.50 $\pm$ 0.75	8.67 $\pm$ 0.35	0.33 $\pm$ 0.11
	80-100				27.12 $\pm$ 0.85	8.72 $\pm$ 0.36	0.33 $\pm$ 0.13
SA	0-20	165.50 $\pm$ 70.71 a	278.67 $\pm$ 127.58 b	1672.25 $\pm$ 950.60 a	28.93 $\pm$ 1.06	8.57 $\pm$ 0.24	0.35 $\pm$ 0.15
	20-40				26.77 $\pm$ 1.35	8.60 $\pm$ 0.29	0.35 $\pm$ 0.11
	40-60				27.67 $\pm$ 0.46	8.52 $\pm$ 0.25	0.38 $\pm$ 0.11
	60-80				27.91 $\pm$ 1.28	8.55 $\pm$ 0.33	0.30 $\pm$ 0.12
	80-100				27.47 $\pm$ 2.13	8.52 $\pm$ 0.32	0.33 $\pm$ 0.08

The sulfide concentrations of soils from the mudflat were generally the lowest, with an average value of 22.10 ± 19.87 mg/kg. The sulfide concentrations in *S. mariqueter* soil were higher than those in the mudflat soil, with an average value of 79.65 ± 65.53 mg/kg. The highest sulfide concentration was

observed in *S. alterniflora* soil (210.97 ± 133.41 mg/kg), which was 9.56 and 2.65 times higher than that in the mudflat and *S. mariqueter* soils, respectively (Fig. 2A). The soil sulfide concentrations were generally higher in spring and summer and relatively lower in the rest of the year regardless of vegetation type (Table 2). The annual average sulfate content in the soils was not significantly different among soils covered by *S. alterniflora* and the native species *S. mariqueter* and in the nearby bare mudflat. The sulfate content in *S. mariqueter* soil was 295.74 ± 108.80 mg/kg, while that in *S. alterniflora* was 268.75 ± 129.61 mg/kg (Fig. 2B). The sulfate content in *S. mariqueter* soil (340.44 ± 28.04 mg/kg) in autumn was higher than in *S. alterniflora* soil (Table 2). In spring, the sulfate content in *S. alterniflora* soil (458.88 ± 64.43 mg/kg) was higher than that in *S. mariqueter* soil (425.66 ± 82.64 mg/kg) and mudflat soil (320.51 ± 115.1 mg/kg). Among soils covered by *S. alterniflora* and *S. mariqueter* and in the nearby bare mudflat, the sulfate contents were generally higher in spring and summer (Table 2).

Table 2

Concentrations of sulfide (mg/kg) and sulfate (mg/kg) in the soil of mudflat (MF), *S. mariqueter* (SM), *S. alterniflora* (SA) of four different seasons. Different letters indicate significant differences ($P < 0.05$) between soil sulfide and sulfate concentrations in different seasons

Locations	Sulfide/Sulfate	Spring	Summer	Autumn	Winter
MF	Sulfide (mg/kg)	25.98 ± 24.16 b	44.62 ± 13.39 a	7.26 ± 1.77 c	10.56 ± 4.04 bc
	Sulfate (mg/kg)	320.51 ± 115.10 a	253.27 ± 5.49 b	242.51 ± 25.82 b	140.43 ± 34.11 c
SM	Sulfide (mg/kg)	114.27 ± 97.17 a	115.13 ± 43.48 a	38.58 ± 37.21 b	50.62 ± 38.07 b
	Sulfate (mg/kg)	425.66 ± 82.64 a	265.56 ± 14.70 c	340.44 ± 28.04 b	151.32 ± 23.40 d
SA	Sulfide (mg/kg)	218.19 ± 157.89 b	351.07 ± 84.80 a	102.21 ± 75.54 c	172.4 ± 74.01 bc
	Sulfate (mg/kg)	458.88 ± 64.43 a	277.62 ± 22.18 b	210.89 ± 20.44 c	127.63 ± 67.56 d

The SOC content in *S. alterniflora* soil was 6.77 ± 1.94 mg/g, which was much higher than that in *S. mariqueter* soil (3.68 ± 1.43 mg/g) and mudflat soil (3.08 ± 1.49 mg/g) (Fig. 2C). The TN content for *S. alterniflora* soil was 286.65 ± 64.56 mg/kg, which was higher than that for *S. mariqueter* (155.57 ± 36.72 mg/kg) and mudflat soils (137.64 ± 31.42 mg/kg) (Fig. 2D). The TP content for *S. alterniflora* soil was 0.47 ± 0.13 mg/g, which was lower than that for the mudflat (0.52 ± 0.08 mg/g) and *S. mariqueter* (0.55 ± 0.08 mg/g) soils (Fig. 2E). The total iron content of *S. alterniflora* soil was 20.72 ± 2.68 mg/g, and the total iron content of *S. alterniflora* soil was slightly higher than that of the mudflat (19.95 ± 1.84 mg/g) and *S. mariqueter* (19.93 ± 2.77 mg/g) soils (Fig. 2F).

3.2. Relationship between sulfide, sulfate, and environmental factors

In the mudflat soil, the sulfide content was substantially correlated with total iron ($r = 0.497$, $p < 0.05$) and TP ($r = 0.571$, $p < 0.01$), and sulfate was significantly correlated with TN ($r = 0.668$, $p < 0.01$). Meanwhile, the total iron was positively correlated with TP in the mudflat soil ($r = 0.761$, $p < 0.01$) (Fig. 3a). In the *S. mariqueter* soil, there was a negative relationship between sulfate and total iron ($r = -0.595$, $p < 0.01$) (Fig. 3b). In the *S. alterniflora* soil, the sulfide content exhibited a strong correlation with SOC ($r = 0.616$, $p < 0.01$) and total iron ($r = 0.472$, $p < 0.05$), and sulfate was significantly correlated with TP ($r = 0.547$, $p < 0.05$). Besides, SOC was positively correlated with total iron and TP, with coefficients of 0.546 ($p < 0.05$) and 0.468 ($p < 0.05$), respectively. The total iron in the *S. alterniflora* soil exhibited a significantly positive correlation with TP ($r = 0.578$, $p < 0.01$) (Fig. 3c).

3.3. Effects of plant invasion on S cycle-related soil functional bacteria

The PCoA mapping analysis results based on the Bray-Curtis distance matrix (Fig. 4) showed that the bacterial communities were different among the soils collected from different sites. The PC1 and PC2 axes together accounted for 52.46% of the variation. The total number of SRB measured at the family level was lower for *S. alterniflora* soil than in the other soils (Fig. 5A). The relative abundance of SRB in *S. alterniflora* soil was 7.32%, which was lower than that in *S. mariqueter* soil (14.9%) and higher than that in mudflat soil (7.28%). The main groups of SRB in *S. alterniflora* soil were *Desulfobulbaceae*, *Desulfuromonadales*, and *Desulfobacteraceae*, which accounted for 68.2% of the SRB *S. alterniflora* community, and with the invasion of *S. alterniflora*, *Nitrospiraceae* and *Desulfarculaceae* increased (Fig. 5A). The relative SOB abundances were lower for *S. alterniflora* soil (3.03%) than for *S. mariqueter* (3.75%) soil and for mudflat (4.49%) soil (Fig. 5B). The *S. alterniflora* communities mainly consisted of *Rhodobacteraceae* and *Hyphomicrobiaceae*, while *Hydrogenophilaceae* was the main SOB component in the mudflat and *S. mariqueter* soils. The QMEC test results showed that the variation trend of the relative abundance of sulfur-reducing functional genes was similar to that of the sulfide content, and the main sulfur-reducing functional gene was *dsrA*. The highest sulfur-reducing functional gene abundance, which was 1.49%, appeared in the soil of *S. alterniflora*, while in the mudflat and *S. mariqueter* soils, the abundances were 1.27% and 1.17%, respectively (Fig. 5C). The primary sulfur-oxidizing functional gene was *yedZ*. The lowest abundance of sulfur-oxidizing functional genes was observed in the soil of *S. alterniflora*, which was 0.19%, while in the mudflat and *S. mariqueter* soils, the abundances were 0.25% and 0.32%, respectively (Fig. 5D).

3.4. Effects of sulfide on the growth of *S. alterniflora* and *S. mariqueter*

After seven days of the seed germination experiment, the root length, shoot length, and biomass of *S. alterniflora* and *S. mariqueter* showed variability along the gradients of Na_2S incubations (Fig. 6). The root tissues of *S. mariqueter* ceased to grow when the Na_2S concentration reached 50 mmol/L. Although low concentrations of Na_2S had no significant effect on *S. alterniflora* root length, *S. alterniflora* root length showed a decreasing trend with the increase of Na_2S concentration. The root tissue of *S.*

alterniflora was still alive at the concentration of Na₂S was as high as 200 mmol/L (Fig. 6A). When the concentration of Na₂S reached 50mmol/L, a significant decrease in the shoot lengths of *S. mariqueter* occurred. However, with the increase of the concentration of Na₂S, there was no significant effect on the shoot lengths of *S. alterniflora* increased (Fig. 6B). Na₂S concentrations over 100 mmol/L significantly reduced *S. mariqueter* plant biomass, while the biomass of *S. alterniflora* remains the same at high levels of Na₂S concentration (Fig. 6C).

4. Discussion

4.1. *S. alterniflora* might increase soil microbial biomass and activity by increasing soil SOC and TN

Our findings suggest that the *S. alterniflora* invasion leads to increased soil SOC (Fig. 2C) and TN (Fig. 2D), which might provide abundant substrate for more active microbial activity. The increase in plant biomass can promote the input of organic matter, carbon, and nitrogen, which can further change the ratio of carbon and nitrogen, forming a different environment for soil microorganisms (Hu et al. 2016). Plant invasion changes soil properties mainly through the litter and root exudates (Yan et al. 2018), and soil microbial structures were selected by the composition of plant litter. *S. alterniflora* has a more vital ability to enhance above-ground net primary productivity and preserve soil organic carbon than *S. mariqueter* due to the larger biomass of *S. alterniflora*, as above-ground plant biomass is the main input route for soil organic carbon (Carrera et al. 2009). In addition, the increase in soil SOC and TN contents arise due to the higher input of *S. alterniflora* tissue decomposition. Relatively higher nitrogen content was also found in *S. alterniflora* tissues, which may contribute to the raised soil TN (Zheng et al. 2017).

4.2. *S. alterniflora* altered soil S cycle-related microbial activities and resulted in soil sulfide accumulation

We reported that the increase in the abundance of S reduction-related functional genes (*dsrA* and *dsrB*) and the decrease in the abundance of S oxidation-related functional genes (*soxY* and *yedZ*) were consistent with the increase in phytotoxic soil sulfide after *S. alterniflora* for the first time. Sulfide is a reductive phytotoxicity in the rapid redox reactions with sulfate under tidal conditions in coastal wetlands (Lamers et al. 2013). According to our investigation, the invasion of *S. alterniflora* changes the community structure and living environment of soil microorganisms into a more suitable condition for sulfide production and accumulation. The differences between the horizontal and vertical projections of PCoA in the two sets of comparisons of *S. alterniflora* and *S. mariqueter* implied that these two plants possessed different mechanisms for changing the microbial community structure in soil (Fig. 4).

The composition and structure of S-associated bacterial communities in the soil were altered after *S. alterniflora* invasion (Fig. 5A). *Desulfobulbaceae*, *Desulfuromonadales*, and *Desulfobacteraceae* are strictly anaerobic SRB that can transform sulfates into sulfides in a flooded environment and play a central role in methane formation and dissimilatory sulfate reduction (Kuever 2014). The lower relative

abundance of *Hydrogenophilaceae* in the soil of *S. alterniflora* (Fig. 5B), which is a type of SOB, might lead to the oxidation of reductive soil sulfide being inhibited and further contributed to the accumulation of soil sulfide (Fig. 2A). *DsrA* and *dsrB* are functional genes involved in the S cycle; they catalyze the reduction of sulfite (SO_3^{2-}) to sulfide (S^{2-}) in the S cycle (Muyzer and Stams 2008). We found that the invasion of *S. alterniflora* promoted the microbial sulfur reduction process by increasing the relative abundance of the *dsrB* functional gene in the soil and changing their community structure (Fig. 5C), but insignificant change was found in the relative abundance of *dsrA*, thereby led to the increase of soil sulfide content (Fig. 2A). The invasion of *S. alterniflora* resulted in a decrease in the relative abundance of the SOB in the soil (Fig. 5D), which reduced the oxidation of sulfur compounds and led to the accumulation of sulfide compounds (Fig. 2A). The SOB is composed of autotrophs and facultative autotrophs (Mattes et al. 2013), which can consume H_2S and produce oxidized S compounds such as sulfides, elemental sulfur, sulfites, and thiosulfates (Meyer et al. 2016). Therefore, the increasing SOC after *S. alterniflora* invasion cannot accelerate the growth of SOB. *Sox* family can ultimately reduce sulfide compounds to sulfate (Friedrich et al. 2001). The *sox* multienzyme system is considered to be the basic and original molecular mechanism of S oxidation (Ghosh and Dam 2009); *yedZ* is considered to have a sulfite oxidation function (Brokx et al. 2005; Zheng et al. 2018).

In 2017, our team found an increase in the copy numbers of the *dsrB* functional gene in SRB after *S. alterniflora* invasion in Dongtan Saltmarsh located in Chongming Island (Zheng et al. 2017), but no significant variation in *soxB* was observed. It might because the sampling sites of *S. alterniflora* were invaded for over 20 years with stable soil physicochemical properties and microbial structure. Previous studies have found the *dsrAB* coded by the *dsr* genes in certain strains of SOB, it may lead to the insignificant variation in *sox* observed with the offset by increasing *dsrAB* (Loy et al. 2009). In the researches of Cui and Zeleke, a higher abundance of *dsrB* genes was observed in the soil of *S. alterniflora* than the native species *Suaeda salsa* (*S. salsa*) and *P. australis* in the Dongtan Wetland in the Yangtze River estuary and Yancheng Nature Reserve, which consisted with our findings (Zeleke et al. 2013; Cui et al. 2017).

4.3. *S. alterniflora* invade successfully with its high tolerance to high sulfide concentration

A higher tolerance to sulfide was revealed in *S. alterniflora*, since the decrease in *S. alterniflora* biomass was much smaller than that of *S. mariqueter* biomass (Fig. 6A) and the negative effect on the growth of *S. alterniflora* roots and shoots was significantly weaker than that on the growth of *S. mariqueter* roots and shoots (Fig. 6B, 6C), according to our lab experiment. The phytotoxicity of sulfide mainly influences the growth of roots suppressing water and nutrients absorption from the soil, which was consistent with published results that the toxicity of sulfide to plants mainly affects roots by preventing the roots from transporting energy needed for plant growth (Raven and Scrimgeour 1997).

Our findings confirmed that *S. alterniflora* is a high-sulfur-tolerant plant with a variety of mechanisms to resist sulfide toxicity, such as secretion of oxygen from roots, oxidation of sulfides in tissues (Carlson and

Forrest 1982; Martin et al. 2019) and formation of insoluble iron sulfide metals (Marbà et al. 2007). More developed root tissues in *S. alterniflora* than in the native species *P. australis* and *S. mariqueter* also lead to more resistant vitality of *S. alterniflora* under high sulfide conditions (Stribling 1997; Seliskar et al. 2004). Iron can form FeS and FeS₂ with S to reduce sulfide toxicity (Van Der Welle et al. 2006; Marbà et al. 2007; Sjøgaard et al. 2017). Thus, the accumulation of high levels of iron in the soil of *S. alterniflora* might be one of the mechanisms by which *S. alterniflora* resists the toxicity of sulfides. Simkin et al. (2013) proved that sulfide in pore water helps explain the reduction in plant species diversity and the reduction in coverage of many plant species. However, we did not observe significant variation in soil Fe content.

Compared with the *S. mariqueter* and mudflat soils, *S. alterniflora* soil showed the shortest anaerobic reduction environment and shortest flooding time caused by its location in the most inland area. Under such flooding conditions, the highest sulfide content was still found in the soils of *S. alterniflora*. Thus, native species lacking sulfide tolerance capacities could be disadvantaged in the competition with *S. alterniflora* due to the high soil sulfide content derived from soil S-cycle microbial changes after *S. alterniflora* invasion was more lethal to the growth of local species.

5. Conclusions

We found that *S. alterniflora* invasion significantly altered the physicochemical properties of the soil and sulfur-related bacterial community. First, the contents of SOC and TN in soil increased with the invasion of *S. alterniflora*, which could provide more substrates for sulfur-reducing bacteria to cause sulfide accumulation in the soil. Second, the abundance of sulfur-oxidizing functional genes decreased after the invasion of *S. alterniflora* and thus reduced the consumption of sulfide, possibly through root exudates and litter pathways. Third, the phytotoxicity of the increased soil sulfide levels caused much more lethal damage to *S. mariqueter* than to *S. alterniflora*, so that *S. alterniflora* could survive in a high-sulfide environment while *S. mariqueter* could not. Our findings provide a new perspective on how *S. alterniflora* is able to rapidly replace the native species *S. mariqueter* in the Jiuduansha Wetland.

Declarations

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Figures

Figure 1

Sampling locations in the Jiuduansha Wetland National Nature Reserve (Mudflat (MF); *S. mariqueter* (SM); *S. alterniflora* (SA)) Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Soil sulfide concentrations (A), sulfate concentrations (B), soil organic carbon (C), total nitrogen (D), total phosphorous (E), and total iron (F) of three sampling sites (Mudflat (MF); *S. mariqueuer* (SM); *S. alterniflora* (SA)). The boxes display the upper and lower quartiles of the data, and whiskers represent the standard deviation above and below the mean of the data. Different letters indicate significant differences between three sampling sites at a $p < 0.05$ level

Figure 3

Correlation of soil sulfate, sulfide and environmental factors in (a) mudflats, (b) *S. mariqueter*, (c) *S. alterniflora* by PCA

Figure 4

Principal coordinates analysis (PcoA) based on the Bray-Curtis distance matrix of microbial populations in the soils collected from different sites (Mudflat (MF); *S. mariqueter* (SM); *S. alterniflora* (SA))

Figure 6

(A) Root lengths, (B) shoot lengths, and (C) biomass of seed germination of *S. alterniflora* (SA) and *S. mariqueter* (SM) under different concentrations of Na_2S . Lowercase letters and uppercase letters respectively indicate significant differences ($P < 0.05$) of *S. alterniflora* and *S. mariqueter* between means of different stands