Response of topsoil Fe-bound organic carbon pool and microbial community to *Spartina alterniflora* invasion in coastal wetlands

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Abstract Wetland ecosystems have dual functions as both carbon (C) sources and C sinks, playing a vital role in the world's C budget. Wetlands are prone to suffer from plant invasions, and for example, *Spartina alterniflora*, a well-known invasive species, has rapidly expanded in coastal areas of Asia since 1979 and altered the C cycles in coastal wetland ecosystems. Fe(III) oxides are critical in the OC storage by formation of Fe-bound organic carbon (Fe-OC). But the impact of the invasion by *S. alterniflora* in coastal wetlands on the formation and stabilization of Fe-OC is poorly known. Herein, we assessed soil Fe species contents, Fe-OC pool, and the microbial communities associated with these processes in seven wetlands dominated by both native (*Kandelia obovate*, *Avicennia marina* and *Phragmites australis*) and invasive (*S. alterniflora*) plants. Our results showed that organical complexed Fe (Fe_p) concentration and Fe complexing index under *S. alterniflora* community were lower than that under native communities. Soil Fe-OC concentration and molar OC:Fe ratio decreased (35.9% and 29.3%, respectively) after *S. alterniflora* invasion in coastal wetlands, while the abundance of FeRB increased by 78.6%. The invasion of *S. alterniflora* increased soil water content while decreased bulk density, and these changes could have important effects on Fe species and FeRB abundance. Moreover, Fe-OC was positive correlated with soil organic carbon (SOC), Fe_p, and Fe(III), indicating that SOC and Fe(III) concentrations directly affect the formation of Fe-OC. FeRB reacted on the combination of Fe (hydr-) oxides and SOC to indirectly affect Fe-OC through dissimilatory Fe reduction, which further determined the stabilization and mineralization of SOC. Taken together, the invasion of *S. alterniflora* hindered the generation and accumulation of soil Fe-OC pool weakening the stabilization of SOC in coastal wetlands. Therefore, the conservation and restoration of mangroves and other coastal wetlands provide a promising strategy for SOC sequestration and climate change mitigation.

Keywords Coastal wetlands; Carbon-cycle; Fe-bound organic carbon; Plant invasion; *Spartina alterniflora*

1. Introduction

The biogeochemical cycles of iron (Fe) and organic carbon (OC) are closely linked. Given that the highly active Fe phase exists in a metastable state in the soil, its close association with OC is regarded as an extremely efficient "rust sink" and an important mechanism for the sequestration and stabilization of OC in sediments and soils (Lalonde et al., 2012; Jeewani et al., 2021), as the Fe-OC formed is relatively stable and difficult to degrade (Chen et al., 2014; Lavallee et al., 2020). The protective effect of Fe (hydr-) oxides on SOC operates via adsorption and co-precipitation (Eusterhues et al., 2011). Studies have demonstrated that the molar OC:Fe ratio can be used as an indicator of the type of association of Fe (hydr-) oxides with OC, in which a ratio of less than 1 indicates adsorption; however, a ratio over 6 indicates that Fe-OC complexes are being formed mainly by co-precipitation (Guggenberger and Kaiser, 2003; Wagai and Mayer, 2007; Zhao et al., 2016). Therefore, OC concentrations of Fe (hydr-) oxides formed by co-precipitation are much higher than formed by adsorption, and the formation pathway of Fe-OC is crucial for SOC sequestration.

As a transition zone buffering the interaction between terrestrial and the marine ecosystems (Bianchi et al., 2013), coastal wetlands account for only about 5-8% of the world's surface area, but SOC stocks represent 20-30% or more of the global terrestrial carbon (C) pool and plays a crucial role as "blue C" (Macreadie et al., 2019). Wetland soils are rich in Fe (hydro) oxides (Kostka and Luther, 1994) and are regarded as "hotspots" of Fe(III) reduction (Weiss et al., 2004). The Fe cycle in the soil is driven by both biological and abiotic pathways based on the redox interconversion of Fe(II) and Fe(III) (Catalano et al., 2009). When the soil is in a flooded anaerobic state, Fe-reducing bacteria use organic matter as electron donor and Fe(III) oxides as terminal electron acceptor to

reduce to Fe(II) (Lovley and Chapelle, 1995), and the contribution of the Fe reduction to OC mineralization accounts for 69-90% (Vandieken et al., 2006). Of particular concern is the vulnerability of coastal wetlands to plant invasion – exemplified by the alteration and destruction caused by *Spartina alterniflora*. Changes in the dominant species within a plant community alter ecosystem structure and matter cycling processes, which in turn will affect the soil and the soil C pool (Zhang et al., 2010). Previous studies have found that there is a great potential of *S. alterniflora* litter and roots with their low proportion of labile SOC fractions to increase the formation of SOC (Jin et al., 2017; Zhang et al., 2010). However, some studies have demonstrated that invasion by *S. alterniflora* in fact weakens the stability of wetland SOC pool and hinders the accumulation of SOC (Sun et al., 2019; Xia et al., 2021; Zhang et al., 2021). Although the effects of invasion by *S. alterniflora* on soil C pool storage and stability have been demonstrated in many previous studies, few studies have evaluated the impact of the invasion by *S. alterniflora* to Fe-OC pool and Fe-C binding mechanism in wetland soils. Fe-OC serves as 'Fe gate' to protect OC from rapid decomposition (Wang et al., 2017), and its mineralization degree is 2-3 times lower than that of unconjugated OC, while the average residence time is 6-15 times higher (Eusterhues et al., 2014). Consequently, it is imperative to better understand how Fe-OC pool responds to the invasion by *S. alterniflora*.

Herein, in the present study, we aimed to explore: 1) Does the *S. alterniflora* invasion affect Fe species contents in coastal wetlands? 2) How does the response of Fe-OC pool and its combination relationship to *S. alterniflora* invasion? 3) What is the effect of *S. alterniflora* invasion on soil Fe-reducing bacteria community? To answer these questions, we investigated and compared soil Fe

species contents, Fe-OC pool, and the microbial communities associated with these processes under native and invasion species in coastal wetlands of South-east Asia. In addition, the variation of soil physiochemical properties before and after *S. alterniflora* invasion were measured to examine the potential effect on Fe-OC. The answers to these questions can help better understand the effect of invasion by *S. alterniflora* on the Fe-OC formation mechanism, and the important role played by coastal wetlands in Fe-C biogeochemical cycles.

2. Materials and methods

2.1. Site description and sample collection

As a means of studying the vast areas that *S. alterniflora* has invaded, rather than focus on a single coastal or estuary wetland, we selected nine locations with a total of 27 paired samples (native and invasive) from the seven independent typical coastal wetlands in the subtropical and tropical climate regions of South-east Asia, including Chongming Island (CI), Hengsha Island (HI), Minjiang River Estuary (ME1 and ME2), Jiulong River Estuary (JE), Zhangjiang River Estuary (ZE1 and ZE2), Zhanjiang (ZJ), and Beihai (BH). Each wetland had two dominant plant community types, one of which was dominated by native species including *Kandelia obovate* (*K. obovate*, mangrove), *Avicennia marina* (*A. marina*, mangrove), and *Phragmite australis* (*P. australis*, grass), and the other by the invasive species *S. alterniflora*. The native species of ME1, JE, and ZE1were *K. obovate*; the native species of ME2, CI, and HI were *A. marina* and that of BH, ZE2, and ZJ were *P. australis*. Details of the sampling sites are showed in Table S1.

We randomly selected three independent replicate plots for each community type at low tide and collected the upper 0-10 cm of soils during the main growth season in June to August 2015 (Wang et al., 2018). As a result, a total of 54 soil samples (nine sampling sites × two community types × three replicate plots) were collected and stored in a portable refrigerator before transportation to the laboratory. Before grinding and analysis, visible root residues and stones were removed from the soil samples. The treated soil samples were stored at 4 ℃ for chemical analysis and -20 ℃ for microbiological analysis. The physicochemical properties of the soils dominated by native and invasive vegetation have been reported previously by Wang (2018) and are given in Table S2.

2.2. Soil analysis

Soil pH was measured using a pH meter (IQ Scientific Instruments, Carlsbad, USA), salinity was characterized by electrical conductivity (EC) using a 2265FS EC meter (Spectrum Technologies Inc., Paxinos, USA), water content (WC) was measured as the difference in soil mass before and after drying at 105 °C to a constant weight, and bulk density (BD) was measured using the ring-knife method (Lu, 2000). Total nitrogen (TN) and SOC concentrations were determined using a Vario EL III Elemental Analyzer (Elementar Scientific Instruments, Hanau, Germany). Dissolved organic carbon (DOC) was extracted at a soil-to-deionized water ratio of 1:5 (m/v) and measured with a TOC-V_{CPH} analyzer (Shimadzu Scientific Instruments, Kyoto, Japan). Microbial biomass carbon (MBC) was assayed by chloroform fumigation-extraction and measured using a TOC-V_{CPH} analyzer (Lu, 2000).

Soil Fe(II) concentrations were determined by digesting fresh soils in a 1-M HCl solution for 12-h extraction, and HCl-extracted total Fe (HCl-Fe_t) was analyzed by the same procedure with the

exception that the extractant was 0.25-M hydroxylamine hydrochloride, and Fe(III) concentrations were calculated by subtracting Fe(II) from HCl-Fe_t (Knorr and Blodau, 2009). Total Fe oxyhydroxides (Fe_d) refers to Fe forms that do not belong to phyllosilicates and mainly correspond to Fe (hydr-) oxides and water compounds in the soil. The poorly crystalline Fe oxyhydroxides (Fe_o) are the most active part of the Fe_d oxides, and have a large specific surface area and high chemical activity. Organical complexed Fe (Fe_p) is an organic Fe compound formed by chelation of Fe by organic matter. Fe_d, Fe_o, and Fe_p were extracted using dithionte-citrate-bicarbonate (DCB), ammonium oxalate buffer, and sodium-pyrophosphate solutions, respectively (Duan et al., 2020). All Fe species were detected using the 1,10-phenanthroline colorimetric method and expressed as g kg^{-1} soil. The activation and complexing indices of Fe were calculated according to the following formulas (Zou et al., 2008; Liu et al., 2019):

$$\text{Fe activation index (\%)} = Fe_o / Fe_d \times 100\%$$

$$\text{Fe complexing index (\%)} = Fe_p / Fe_d \times 100\%$$

Soil Fe_d were extracted using a DCB reduction solution (containing 0.27-M trisodium citrate and 0.11-M sodium bicarbonate), and the OC released during the extraction process was Fe-OC (Lalonde et al., 2012). The release of water-soluble OC was corrected with a mixed sodium chloride solution (containing 1.60-M sodium chloride and 0.11-M sodium bicarbonate) as a control at an equivalent ionic strength (Lalonde et al., 2012; Zhao et al., 2016). Fe-OC, the percentage of Fe-OC in SOC ($f_{Fe\text{-}OC}$), and the molar OC:Fe ratios were calculated according to the following formulas, respectively (Duan et al., 2020):

$$\text{Fe-OC} = OC_{NaCl} - OC_{DCB}$$

$$f_{Fe\text{-}OC} = \text{Fe-OC} / \text{SOC} \times 100\%$$

$$\text{Molar OC:Fe ratios} = (\text{Fe-OC} / M_c) / (m_{Fed} / M_{Fe}) \quad (170)$$

where OC_{NaCl} and OC_{DCB} correspond to the OC contents of NaCl- and DCB-extracted soil residues, respectively, and M_c and M_{Fe} refer to the molar mass of C and Fe, respectively.

2.3. Microbial measurement

Fe-reducing bacteria (FeRB) abundance was measured by the most probable number (MPN) method according to Lin (2022). Genomic FeRB DNA of soil samples was extracted by the cetyl trimethyl ammonium bromide (CTAB) method (Abdel-Latif and Osman, 2017; Prakash et al., 2019). Its purity and concentration were analyzed using an ultra-micro spectrophotometer (NanoDrop2000, Thermo Fisher Scientific Company, USA), and the quality of the extracted DNA was determined with a 1% agarose gel electrophoresis. Then, the extracted DNA was diluted with sterile water to 1 ng μL^{-1} and used as template for PCR amplification with the specific primers for the 16S V3-V4 region (341F: CCT AYG GGR BGC ASC AG; and 806R: GGA CTA CNN GGG TAT CTA AT) and high-fidelity enzymes (Lin et al., 2022). Amplified PCR products were purified using a PCR clean-up kit (Thermo Scientific, Shanghai, China) and stored at -20 °C prior to analysis. The Ion Plus Fragment Library Kit 48 rxns from Thermofisher was used for library construction. After the constructed library passed Qubit quantification and library detection, Thermofisher's Life Ion S5™ was used for on-machine sequencing.

2.4. Statistical analysis

Datasets were checked for normality before analysis and the results were expressed as the mean ± SE. A one-way ANOVA was used to compare the differences in soil Fe (Fe_d, Fe_o, Fe_p) content of vegetation types at particular sites or between single plant communities at different sites. SPSS

191 software version 23.0 for Windows (IBM, Armonk, NY, USA) was used to compare variation in
192 organic C fractions (SOC, DOC, MBC), Fe phases (Fe(II), Fe(III), HCl-Fe_t) and Fe-OC parameters
193 at native and invasive species. A two-way ANOVA was employed to compare differences in
194 physicochemical properties depending on sites and plant communities, as well as in their interactions
195 (site × plant community). Pearson correlation was used to analyze the relationships between soil
196 physicochemical properties (pH, WC, BD, EC, TN), organic C fractions (SOC, DOC, MBC, Fe-OC,
197 and $f_{Fe\text{-}OC}$), and Fe (hydr-) oxides fractions (Fe(II), Fe(III), Fe_d, Fe_o, Fe_p) at native and invasive
198 communities. The origin 2018 was used to construct box plot and bar charts. The *ggplot2* package in
199 R software version 2.15.3 (R core team, 2018) was used to construct a bubble map of the relative
200 abundances of FeRB. All the above-mentioned analyses had a significance level of $\alpha = 0.05$.

201 **3. Results**

202 *3.1. Physicochemical characteristics of soils dominated by native and invasive vegetation*

203 The soil physicalchemical properties changed after *S. alterniflora* invasion. Soil pH increased from
204 6.41 ± 0.22 to 6.60 ± 0.22, and water content increased from 46.57 ± 1.41% to 53.71 ± 2.22%, while
205 bulk density decreased from 0.82 ± 0.04 to 0.72 ± 0.04 g cm^{-3} (Table S2). Soil salinity and TN content
206 changed significantly after the invasion of *S. alterniflora*, ranging from 2.91 ± 0.38 to 3.75 ± 0.56 mS
207 cm^{-1} and 1.83 ± 0.11 to 1.66 ± 0.07 g kg^{-1}, respectively (Table S2).

208 The invasion of *S. alterniflora* tended to decrease SOC content of up to 30.6% (p<0.05), being
209 significant in Jiulong Estuary (Table 1). Meanwhile, the invasion caused 50.0% and 14.3% increase
210 in soil DOC content, respectively, in Zhangjiang Estuary and Chongming Island, while DOC

decreased by 27.7% in Zhanjiang (p<0.05). Concerning Fe, Fe(II) content increased from 1.92 ± 0.17 to 2.76 ± 0.39 g kg^{-1}, and Fe(III) content decreased from 13.10 ± 1.80 to 12.62 ± 1.88 g kg^{-1} (Table 1). Fe(III):Fe(II) ratios generally decreased from 7.83 ± 1.52 to 5.32 ± 0.86, indicating that the invasion of *S. alterniflora* resulted in Fe(III) loss and Fe(II) increase. Plant community significantly affected SOC, DOC, Fe(II), HCl-Fe_t, and Fe(III)/Fe(II) (p<0.05). The site and the interactive effects of site and plant community on SOC, DOC, MBC, Fe(II), Fe(III), HCl-Fe_t, and Fe(III)/Fe(II) (p<0.05) were significant (Table S3).

3.2. Fe species in soils with native and invasive vegetation

Site and plant community as well as their interaction had significant effects on soil Fe_d, Fe_p, and Fe_o concentrations (p<0.001). Fe_d and Fe_o concentrations decreased significantly with the invasion of *S. alterniflora* in Jiulong Estuary, which decreased by 26.7% and 19.6% (p<0.05), respectively, and Fe_p and Fe_o concentrations decreased significantly by 37.9% and 7.8% (p<0.05) in Zhangjiang Estuary, respectively (Fig. 1). And Fe_p concentration decreased by 56.7% after *S. alterniflora* invasion in Zhanjiang. Fe (hydr-) oxides concentrations of Minjiang Estuary were significantly higher than those of Chongming Island and Hengsha Island (p<0.05), which holds true also after the invasion by *S. alterniflora* into this vegetation community (Fig. 1).

In general, the average soil Fe_d, Fe_p, and Fe_o concentrations under *S. alterniflora* community were lower than that under native communities. An exception is Fe_o concentrations in the *P. australis* community (4.70 to 8.28 g kg^{-1}) increased slightly after the *S. alterniflora* invasion, ranging from 5.15 to 8.67 g kg^{-1} (Fig. 1).

3.3. Fe-OC in soils dominated by native and invasive vegetation

Soil Fe-OC, molar OC:Fe ratios, and the Fe complexing index decreased after the invasion by *S. alterniflora* regardless of the native vegetation type, which decreased by 35.9%, 29.3% and 7.4%, respectively (Fig. 2a, c, and e). The strongest decrease in Fe-OC (40.3%; $p<0.05$) was observed after *S. alterniflora* invasion into the *K. obovate* community (Fig. 2a). Soil $f_{Fe\text{-}OC}$ in the native *K. obovate* and *P. australis* communities all decreased after *S. alterniflora* invasion, which decreased by 14.8% and 11.0%, respectively. (Fig. 2b). Molar OC:Fe ratios in soils under native and invasive vegetation types were less than 1; the responses to *S. alterniflora* invasion in the *K. obovate* and *P. australis* communities were the most significant, and the molar OC:Fe ratios decreased by 37.3% and 36.0% ($p<0.05$), respectively (Fig. 2c).

3.4. Relationships between C and Fe

Correlation analysis showed that SOC content was positively correlated with Fe_d, Fe_o, and Fe(III)/Fe(II) ($p<0.01$). Fe_d, Fe_p, Fe_o, Fe(III) and SOC content all had significant correlations with Fe-OC ($p<0.05$). Among the soil chemical factors, pH was positively correlated with DOC content, but negatively correlated with Fe_p ($p<0.05$). Soil salinity was positively correlated with MBC content and $f_{Fe\text{-}OC}$, but negatively correlated with SOC content ($p<0.05$). Soil bulk density was negatively correlated to SOC, Fe(II), Fe(III), Fe_d, Fe_o, and Fe-OC content ($p<0.05$, Fig. S1).

3.5. Microorganisms in soils dominated by native and invasive vegetation

The abundance of FeRB before the invasion ranged from 7.96×10^3 to 5.86×10^4 MPN g^{-1}. After invasion, the abundance varied from 6.64×10^3 to 3.79×10^5 MPN g^{-1} in these coastal wetlands (Fig.

3a). The invasive of *S. alterniflora* drastically increased the abundance of FeRB by about 7 folds than that under *K. obovate* community in Minjiang Estuary (p<0.05). Meanwhile, the abundance increased by 61.3% after *S. alterniflora* invasion into *P. australis* community in Minjiang Estuary. In contrast, the abundance of FeRB in Zhangjiang Estuary decreased by 20.5% after *S. alterniflora* invasion (Fig. 3a). Overall, the invasive of *S. alterniflora* resulted in significant increase in the abundance of FeRB, which increased by 78.6% in average (p<0.05; Fig. 3b). The predominant species (to genus level) of FeRB included *Thiobacillus*, *Geobacter*, *Desulfobulbus*, *Anaeromyxobacter* and *Geothrix* (Fig. 3c). The invasion of *S. alterniflora* not only led to the disappearance of original bacterial genera, but also provoked the appearance of novel ones, particularly the relative abundance of *Thiobacillus*, *Desulfobulbus* and *Bacteroides* changed significantly.

4. Discussion

4.1. Effects of the invasion by S. alterniflora on Fe species

Many reports have indicated that invasive plants are able to modify soil physicochemical properties (Yang et al., 2016; Xie et al., 2019; Li et al., 2020; Lin et al., 2021), which also leads to the migration and transformation of sensitive Fe (hydr-) oxides due to the influence of external environment. Fe(III)/Fe(II) ratio could indicate the presence of redox conditions in wetland soils (Doran et al., 2006; Duan et al., 2020). Our research pointed out that the invasion of *S. alterniflora* resulted in the loss of Fe(III) and the increase of Fe(II), and observed the Fe(III)/Fe(II) ratio in soils decreased from 7.83 to 5.32, indicating that the invasion of *S. alterniflora* created anaerobic conditions for soil Fe (hydr-) oxides, which transformed the soil from an oxidized to a reduced state. Wang (2013) also had the same result. This is mainly because that *S. alterniflora* has the ability to promote siltation, with freshly

sedimented soils having a looser structure, lower bulk density, and higher water content (Craft, 2007; Yang et al., 2016), which is consistent with our results. Meanwhile, the increase of water content expands the range of anaerobic environment and stimulates the activity of anaerobic microorganisms (Yan et al., 2015), thus promoting the accumulation of Fe(II). In the present study, we did not install the in-situ equipment to directly measure O_2 concentration and redox potential, but we will further strengthen our conclusions in the future studies.

Soil Fe_p concentration and Fe complexing index under *S. alterniflora* community were lower than that under native communities, and the correlation analysis showed that Fe_p was positively correlated with Fe-OC ($p<0.05$). Therefore, the change of Fe_p was consistently associated to the change of organic matter content. Nierop (2002) and C (1968) also came to the same conclusion. This is mainly because Fe_p is a Fe (hydr-) oxides in soil that combines with soil humus (Chi et al., 2021), and mangrove wetland is one of the most productive environments in the world, with high C storage per unit area (Bouillon et al., 2008). Meanwhile, the decomposition rate of litter in *K. obovate* community was faster than that in *S. alterniflora*, and the turnover time was shorter (Chen et al., 2017). In this process, litter is decomposed by microorganisms to produce a large amount of humus as organic C input into the soil (Berg, 2018). Thus the substitution of *S. alterniflora* profoundly affected C storage function of soil and Fe (hydr-) oxides content in wetland ecosystem (Yu et al., 2015). In addition, our correlation analysis indicated that Fe_o was positively correlated with SOC. The reason could be that Fe_o is easier to change its valence compared with other forms of Fe, and Fe(II) has weak adsorption capacity for SOC, and SOC will be dissolved in the process of Fe reduction, thus affecting the mineralization of SOC (Benbi and Khosa, 2014; Kleber et al., 2015).

4.2. Effects of the invasion by S. alterniflora on Fe-OC pool

The study found that the soil C storage of global mangrove wetlands is more than five times that of the aboveground part, while 49-98% of the C is stored in underground sediments at depths of 0.5-3 m (Donato et al., 2011). The organic C storage in soils depends on its input by photoautotrophic organics, mainly plants, and the decomposition by heterotrophic organisms. Our research found that the substitution of *S. alterniflora* for native communities led to a negative trend in SOC content, which, for example, significantly decreased by 30.6% in Jiulong Estuary. The size of the storage capacity was affected by many factors including climate, vegetation, soil physicochemical properties, and geographical environment, and the interaction of these factors greatly influences the dynamic changes occurring in the SOC pool (Mcleod et al., 2011). The alteration of native community by plant invasion can dramatically influence net ecosystem production and soil nutrients, thereby profoundly altering the processes of organic matter formation and turnover (Feng et al., 2017; Yang et al., 2016). Yu (2015) and Shao (2011) reported that the large-scale invasion and spread of *S. alterniflora* had a significant impact on the distribution of biological elements in subtropical coastal wetlands, and the disappearance of a large number of mangroves would significantly reduce the C storage function of soil in these wetland ecosystems, which is consistent with the results of our study. The reason could be that *S. alterniflora* spreads extensively into the growing space of mangroves and other associated plants thanks to its excellent fecundity, and its inhibition on the regeneration of mangrove seedlings also help to reduce the C pool of plants and hinder the expansion of C storage in mangrove ecosystems (Feng et al., 2017; Zhang et al., 2012).

Our research pointed out that Fe-OC was significantly positive correlated with SOC, Fe_p, Fe_d, Fe_o and Fe(III), indicating that SOC and Fe(III) concentrations directly affect the formation of Fe-OC, and similar results have been found in other freshwater wetlands (Duan et al., 2020). Compared with Fe(II), SOC has a great affinity for Fe(III) (Han et al., 2016), which could provide a critical co-precipitate preservation mechanism for SOC to promote its stabilization (Wang et al., 2017). Among them, Fe_p is combines with soil humus and is a stable Fe pool, while Fe_o is a part of Fe_d, which generally has a higher SOC sorption capacity and in turn preserve SOC (Liu et al., 2019). However, the invasion by *S. alterniflora* of native vegetation communities reduced SOC and Fe(III) concentrations, which contributed to the decrease of Fe-OC concentration(Chen et al., 2017). In addition, mangrove plants are rich in sulfur and tannins, and acid-causing substances including H_2S are produced after the decomposition of plant litter, and reduce soil pH (Huang et al., 2022), but if the original habitat of the soil is changed by *S. alterniflora*, there is an increase in soil pH. Zhao (2016) also demonstrated that the Fe-OC association could be influenced by the soil pH, which affects the mineral phase and surface charge of Fe (hydr-) oxides, as well as their interactions with OC. In the Pearson correlation analysis, it was found that there was no significant negative correlation between Fe-OC and pH, but Fe_p was negatively correlated with pH, indicating that the change in soil pH value would also affect the sequestration of soil Fe-OC pool. Eusterhues (2011) showed that the protective effect of Fe (hydr-) oxides on SOC is performed mainly through adsorption and co-precipitation. Therefore, the molar OC:Fe ratio was thought to reflect the binding mechanism of SOC and Fe (hydr-) oxides (Coward et al., 2018). We found that the molar OC:Fe ratio of native soil decreased significantly after invasion by *S. alterniflora*, but molar OC:Fe ratios of soil before and after the invasion were less than 1, thereby indicating that Fe-OC was mainly formed by surface adsorption

335 and the co-precipitation method contributed little to its formation. This may be due to lower pH values
336 favoring the complexation and precipitation of Fe with OC, while higher pH values favor sorptive
337 interactions between Fe minerals and OC (Tipping et al., 2002), but organic matter formed by
338 precipitated complexes is more stable than that formed by adsorbed complexes. Therefore, the
339 restoration of mangrove vegetation aimed at halting further invasion by *S. alterniflora* will have a
340 significant effect on the sequestration of soil Fe-OC pool. Our research focuses on the topsoil，but
341 Rattan (2015) showed that soils hold C not only close soil to the soil surface but also to the full soil
342 profile depth. While SOC concentrations in topsoil is generally higher than those at deeper depth,
343 subsoil may be very far from being saturated with SOC, and also showed a pattern of increasing
344 variability with depth (Syswerda et al., 2011). This is because that root distributions affected the
345 vertical placement of C in the soil (Hou et al.,2019). Therefore, the deeper soil horizons are a critical
346 part of adequate ecosystem analysis. In future studies, we will also focus on C pool in deep soil.

347 *4.3. Effects of microorganisms on coupled relationships between C and Fe*

348 The invasion of *S. alterniflora* has been reported to have a significant influence on the relative
349 abundance and composition of microbial communities, which is likely caused by *S. alterniflora*
350 altering soil physiochemical properties of the original vegetation (Sun et al., 2019; Cao et al., 2021).
351 In our studied coastal wetlands, we observed that the invasive of native communities by *S. alterniflora*
352 resulted in large increase in the abundance of FeRB (except Zhangjiang Estuary), which may be due
353 to the fact that most FeRB are anaerobic or facultative anaerobic bacteria, soil water content increased
354 and bulk density decreased after *S. alterniflora* invasion, creating a favorable microenvironment for
355 the growth of FeRB.

The coupling relationship between C and Fe (hydr-) oxides in soils is mainly affected by the solid-phase characteristics of Fe (hydr-) oxides (i.e. size, structure, and surface area) and its chemical reactivity (free, amorphous, or complex states), and is closely related to the role of microorganisms in the redox process (Dubinsky et al., 2010; Liu et al., 2019). Our results showed that the predominant species of FeRB included *Thiobacillus*, *Geobacter*, *Desulfobulbus*, *Anaeromyxobacter* and *Geothrix*. *Geobacter*, *Anaeromyxobacter* and *Geothrix* act as Fe respiration reducers, which use organic matter as electron donor and Fe(III) oxides as terminal electron acceptor (Lovley and Chapelle, 1995), linking the reduction of Fe (hydr-) oxides to the oxidation (mineralization) of organic matter (Weber et al., 2006). Therefore, the contents of Fe (hydr-) oxides and organic matter certainly affected FeRB community, and FeRB reacted on the combination of Fe (hydr-) oxides and SOC to indirectly decrease Fe-OC through dissimilatory Fe reduction, which further determined the stabilization and mineralization of SOC (Jeewani et al., 2021).

5. Conclusions

In summary, our study demonstrated that the invasion of *S. alterniflora* had significant impacts on the coupled biogeochemical cycles of Fe-C from the perspective of chemical and microbial mechanisms. The dual effects of Fe (hydr-) oxides on the preservation of OC and microbial mineralization on the loss of OC should be taken into consideration for OC sequestration function of wetland soils. Our results showed that Fe_p concentration and Fe complexing index under *S. alterniflora* community were lower than that under native communities. Soil Fe-OC concentration and molar OC:Fe ratio decreased (35.9% and 29.3%, respectively) after *S. alterniflora* invasion in

coastal wetlands, while the abundance of FeRB increased by 78.6%. The invasion of *S. alterniflora* increased soil water content while decreased bulk density, and these changes could have important effects on Fe species and FeRB abundance. In addition, Fe-OC was positive correlated with SOC, Fe_p, and Fe(III), indicating that SOC and Fe(III) concentrations directly affect the formation of Fe-OC. FeRB reacted on the combination of Fe (hydr-) oxides and SOC to indirectly affect Fe-OC through dissimilatory Fe reduction, which further determined the stabilization and mineralization of SOC. Taken together, the invasion of *S. alterniflora* hindered the generation and accumulation of soil Fe-OC pool weakening the stabilization of SOC in coastal wetlands, thus mangrove wetlands must be protected and repaired to prevent and control the spread of *S. alterniflora*.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1. Soil physicochemical properties of native and invasive plant communities in coastal wetlands (n=3).

Sampling sites	Plant species	Vegetation types	SOC (g kg^{-1})	DOC (g kg^{-1})	MBC (g kg^{-1})	Fe(II) (g kg^{-1})	Fe(III) (g kg^{-1})	HCl-Fe$_t$ (g kg^{-1})	Fe(III)/Fe(II)
Minjiang River Estuary	Native	*K. obovate*	27.04 ± 0.56a	0.08 ± 0.00a	0.40 ± 0.10a	**1.27 ± 0.52b**	**21.82 ± 4.55a**	23.09 ± 3.10a	**21.27 ± 5.50a**
	Invasive	*S. alterniflora*	20.13 ± 1.95a	0.08 ± 0.00a	0.24 ± 0.04a	**5.28 ± 0.66a**	**12.00 ± 2.56b**	17.28 ± 1.42a	**2.36 ± 0.42b**
Jiulong River Estuary	Native	*K. obovate*	**20.03 ± 0.99a**	0.08 ± 0.00a	0.23 ± 0.00a	**1.73 ± 0.22b**	**12.55 ± 6.64b**	**14.28 ± 3.99b**	7.09 ± 1.56a
	Invasive	*S. alterniflora*	**13.91 ± 0.44b**	0.08 ± 0.01a	0.44 ± 0.08a	**4.38 ± 0.24a**	**23.53 ± 3.54a**	**27.91 ± 2.27a**	5.36 ± 0.19a
Zhangjiang River Estuary	Native	*K. obovate*	24.16 ± 0.58a	**0.04 ± 0.01b**	0.54 ± 0.11a	1.73 ± 0.09a	9.52 ± 7.58a	11.25 ± 4.45a	5.33 ± 2.22a
	Invasive	*S. alterniflora*	23.86 ± 0.64a	**0.08 ± 0.01a**	0.58 ± 0.07a	1.66 ± 0.15a	9.34 ± 3.91a	11.00 ± 2.37a	5.55 ± 0.99a
Beihai	Native	*A. marina*	10.06 ± 0.37a	0.06 ± 0.01a	0.36 ± 0.02a	0.71 ± 0.05a	0.78 ± 0.31a	1.49 ± 0.14a	1.12 ±0.30a
	Invasive	*S. alterniflora*	13.38 ± 0.15a	0.07 ± 0.00a	0.45 ± 0.01a	0.97 ± 0.55a	0.59 ± 0.08a	1.56 ± 0.51a	0.62 ± 0.08a
Zhangjiang River Estuary	Native	*A. marina*	19.63 ± 0.35a	0.07 ± 0.00a	0.46 ± 0.02a	1.74 ± 0.20a	4.04 ± 1.23a	5.78 ± 0.90a	2.29 ± 0.17a
	Invasive	*S. alterniflora*	20.61 ± 0.62a	0.07 ± 0.01a	0.43 ± 0.06a	1.55 ± 0.09a	6.81 ± 0.38a	8.36 ± 0.25a	4.42 ± 0.27a
Zhanjiang	Native	*A. marina*	17.87 ± 0.17a	**0.11 ± 0.01a**	0.87 ± 0.04a	2.50 ± 0.17a	**27.19 ± 7.75a**	**29.69 ± 4.64a**	10.75 ± 1.20a
	Invasive	*S. alterniflora*	17.03 ± 0.19a	**0.08 ± 0.01b**	0.71 ± 0.07a	2.55 ± 0.76a	**11.00 ± 5.13b**	**13.55 ± 3.42b**	4.60 ± 1.35a
Minjiang River Estuary	Native	*P. australis*	28.86 ± 1.87a	0.07 ± 0.00a	0.20 ± 0.04a	**2.77 ± 1.04a**	18.08 ± 6.60a	**20.85 ± 2.84a**	12.54 ± 8.52a
	Invasive	*S. alterniflora*	24.50 ± 2.91a	0.07 ± 0.01a	0.28 ± 0.03a	**0.93 ± 0.25b**	12.42 ± 1.62a	**13.35 ± 0.85b**	15.37 ± 4.06a
Chongming Island	Native	*P. australis*	20.40 ± 0.16a	**0.18 ± 0.00b**	0.39 ± 0.00a	**2.48 ± 0.24b**	**17.12 ± 2.46b**	**19.60 ± 1.38b**	7.06 ± 0.99a
	Invasive	*S. alterniflora*	24.15 ± 0.62a	**0.21 ± 0.00a**	0.44 ± 0.01a	**6.31 ± 0.32a**	**32.70 ± 4.00a**	**39.01 ± 2.17a**	5.23 ± 0.53a
Hengsha Island	Native	*P. australis*	19.94 ± 0.26a	0.17 ± 0.01a	0.42 ± 0.02a	**2.34 ± 0.17a**	6.84 ± 2.60a	9.18 ± 1.33a	3.04 ± 0.81a
	Invasive	*S. alterniflora*	19.49 ± 0.17a	0.16 ± 0.01a	0.43 ± 0.01a	**1.17 ± 0.04b**	5.15 ± 0.47a	6.31 ± 0.28a	4.41 ± 0.27a
Combined	Native		20.89 ± 1.04a	0.09 ± 0.01a	0.43 ± 0.04a	1.92 ± 0.17a	13.10 ± 1.80a	15.02 ± 1.86a	7.83 ± 1.52a
	Invasive		19.67 ± 0.85a	0.10 ± 0.01a	0.45 ± 0.03a	2.76 ± 0.39a	12.62 ± 1.88a	15.37 ± 2.20a	5.32 ± 0.86a

SOC, soil organic carbon; DOC, dissolved organic carbon; MBC, microbial biomass carbon; HCl-Fe_t, HCl-extracted total Fe. Different letters indicate the differences between soils of native and invasive community within each site at $p<0.05$. Data are given as the mean ± standard error. Bold letters denote significant differences at $p<0.05$.

Figure captions

Fig. 1. Comparisons of Fe species (a, b, c, respectively; expressed as the mean ± S.E., n=3) and combined concentrations (d, e, f, respectively; expressed as the mean ± S.E., n=9) between native and invasive vegetation types in the coastal wetlands. The effects of site and plant community on total Fe oxyhydroxides (Fe_d), organical complexed Fe (Fe_p), and poorly crystalline Fe oxyhydroxides (Fe_o) concentrations were analyzed using a two-way ANOVA. Different uppercase letters indicate differences between sites within one plant community; lowercase letters indicate differences between plant communities within one site (p<0.05). ME1, Minjiang River Estuary 1; ME2, Minjiang River Estuary 2; JE, Jiulong River Estuary; ZE1, Zhangjiang River Estuary 1; ZE2, Zhangjiang River Estuary 2; BH, Beihai; ZJ, Zhanjiang; CI, Chongming Island; HI, Hengsha Island.

Fig. 2. Comparisons between (a) Fe-OC, (b) $f_{Fe\text{-}OC}$ (i.e. ratio of Fe-OC to SOC), (c) molar OC:Fe ratios, (d) Fe activation index, (e) Fe complexing index, and (d) DOC/Fe(III) (i.e. ratio of DOC to Fe(III)) in native and invasive vegetation types of coastal wetlands. Significant differences between plant communities are indicated by *p<0.05, **p<0.01. Data are shown as the mean ± standard error (n=9).

Fig. 3. Abundances of FeRB measured from individual wetlands (a), all wetlands (b) and bubble map at genus level (c) of the native and invasive vegetation types in Minjiang River Estuary (ME1, ME2) and Zhangjiang River Estuary (ZE). Lowercase letters indicate differences between plant communities at one site (p<0.05). Bubble sizes represented the relative abundance of bacteria.

Fig. 4. Schematic representation of coupled relationships among SOC, key Fe (hydr-) oxides, Fe-OC and FeRB in responses to the invasion by *Spartina alterniflora* in coastal wetlands.

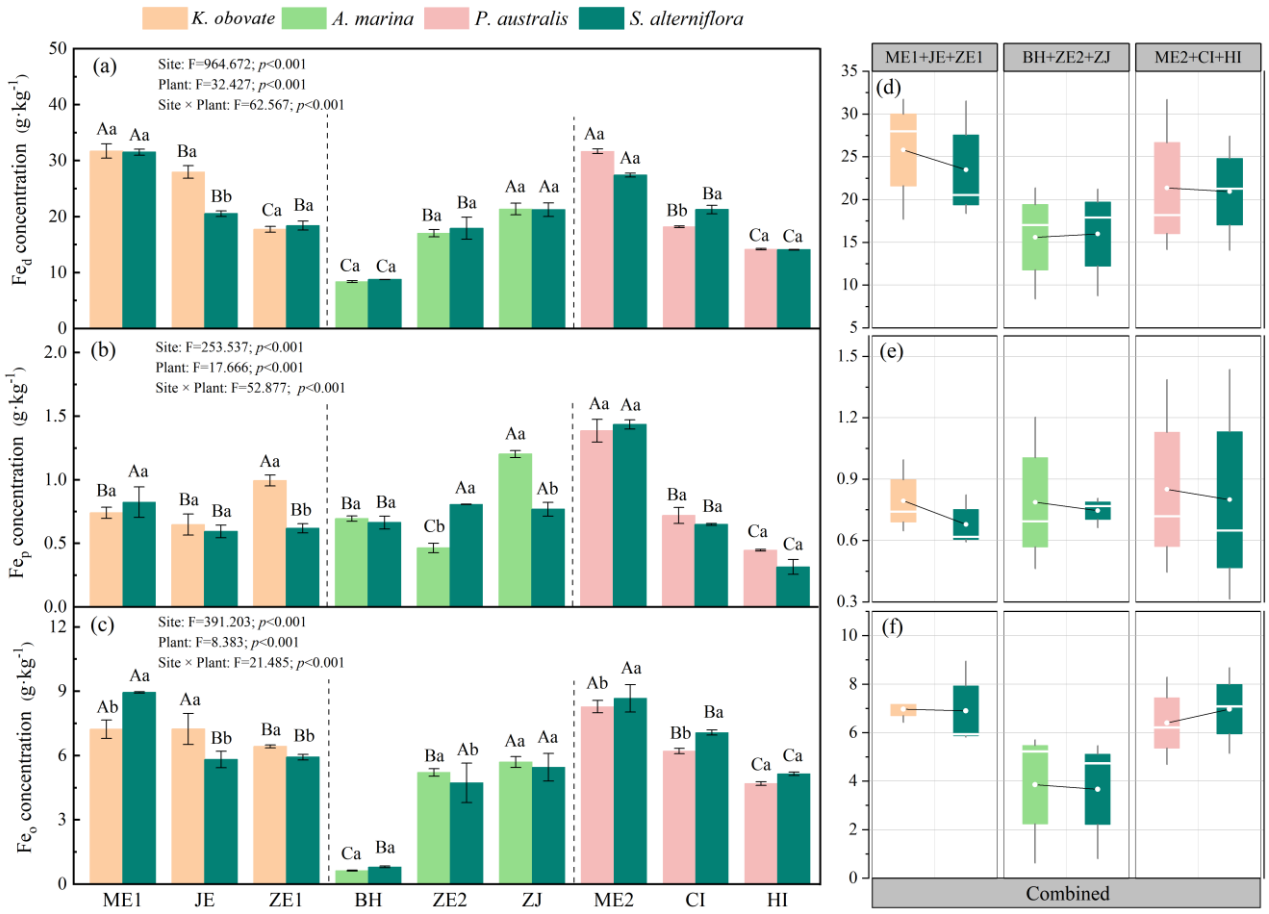

K. obovate
A. marina
P. australis
S. alterniflora
(a)
Site: F=964.672; p<0.001
Plant: F=32.427; p<0.001
Site × Plant: F=62.567; p<0.001
Fe_d concentration (g·kg⁻¹)
(b)
Site: F=253.537; p<0.001
Plant: F=17.666; p<0.001
Site × Plant: F=52.877; p<0.001
Fe_p concentration (g·kg⁻¹)
(c)
Site: F=391.203; p<0.001
Plant: F=8.383; p<0.001
Site × Plant: F=21.485; p<0.001
Fe_o concentration (g·kg⁻¹)
ME1
JE
ZE1
BH
ZE2
ZJ
ME2
CI
HI
ME1+JE+ZE1
BH+ZE2+ZJ
ME2+CI+HI
(d)
(e)
(f)
Combined

Fig. 1.

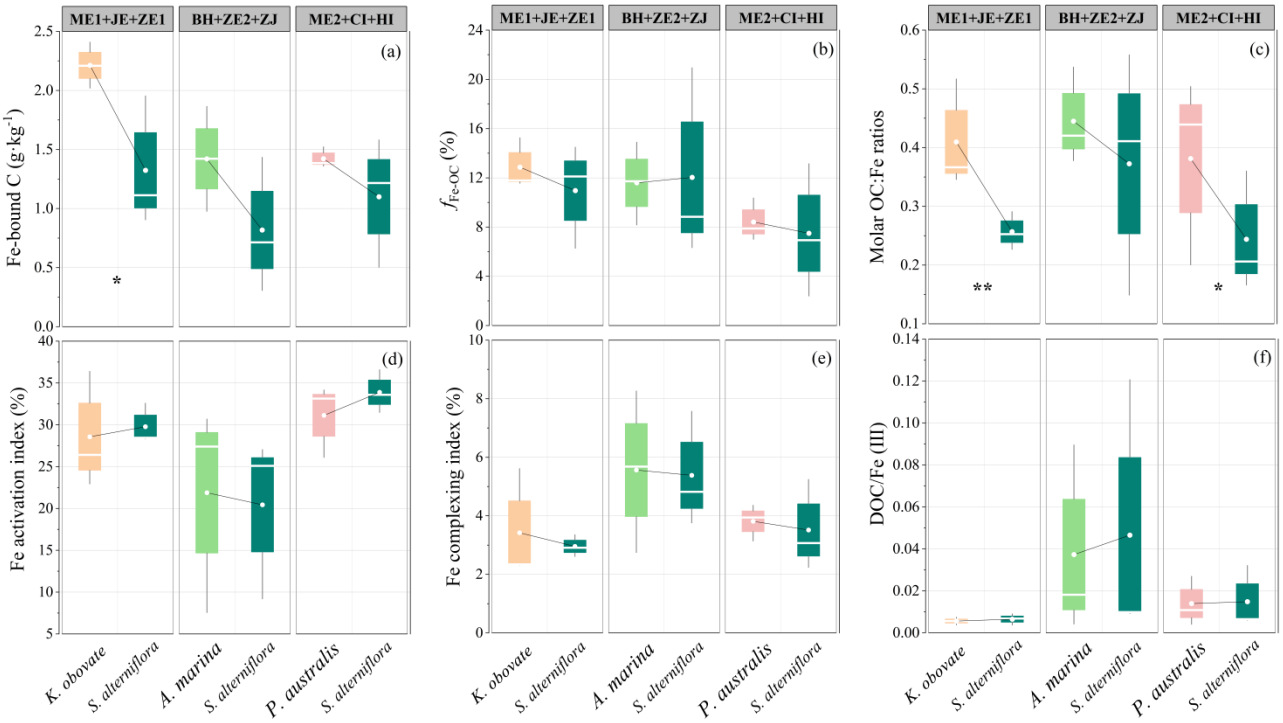

ME1+JE+ZE1
BH+ZE2+ZJ
ME2+CI+HI
(a)
(b)
(c)
(d)
(e)
(f)
Fe-bound C (g·kg⁻¹)
$f_{Fe\text{-}OC}$ (%)
Molar OC:Fe ratios
Fe activation index (%)
Fe complexing index (%)
DOC/Fe (III)
*
**
*
K. obovate
S. alterniflora
A. marina
S. alterniflora
P. australis
S. alterniflora

Fig. 2.

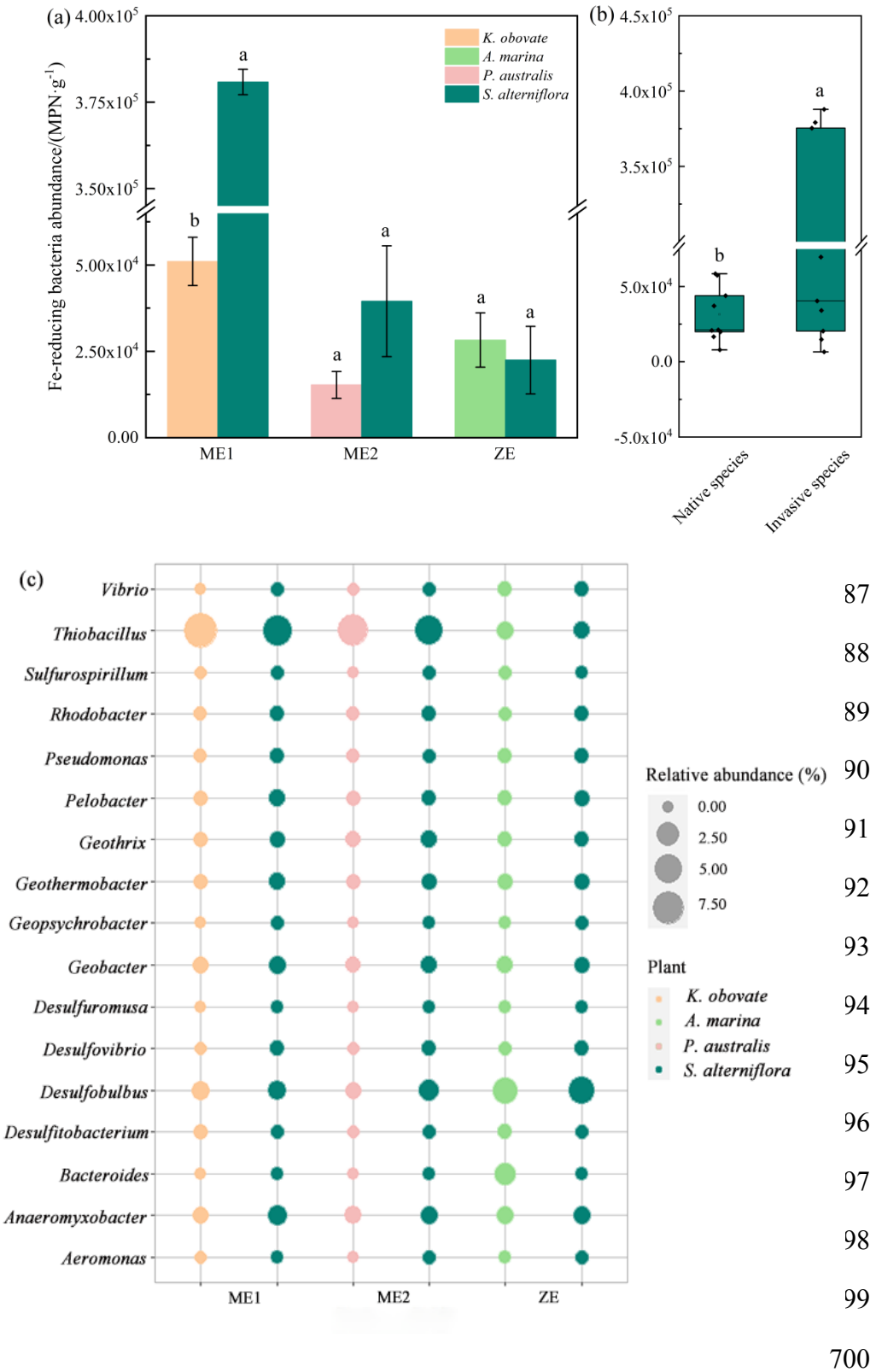
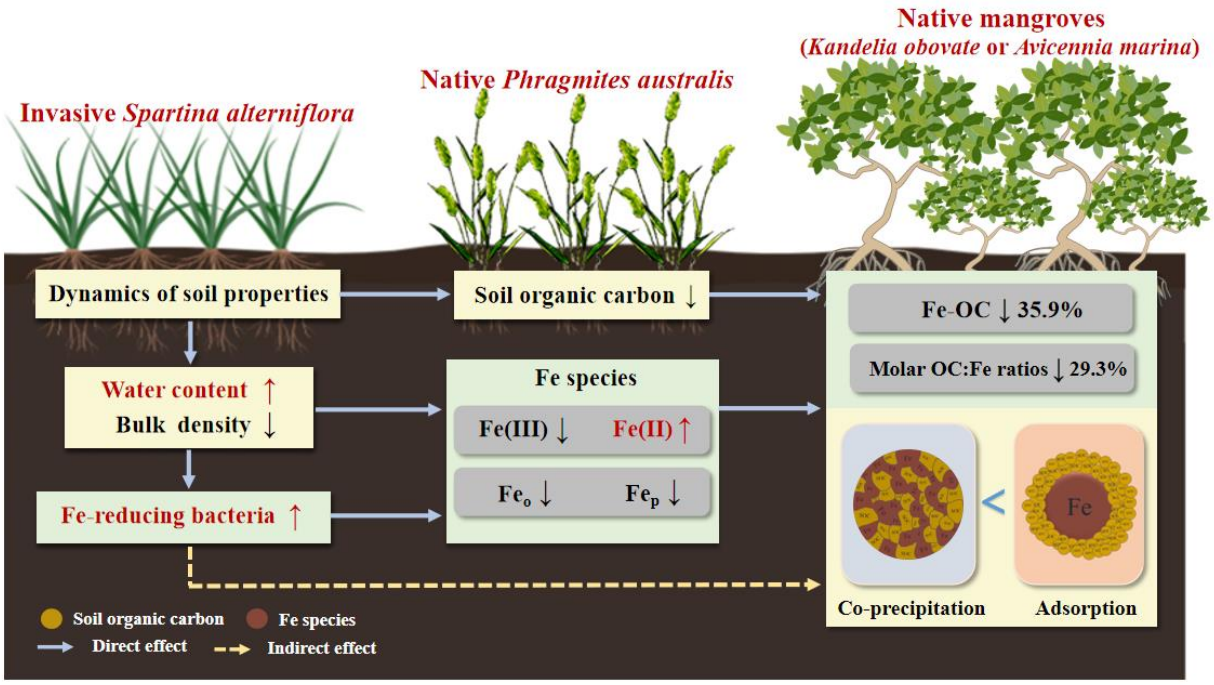

(a)
Fe-reducing bacteria abundance/(MPN·g-1)
4.00x10^5
3.75x10^5
3.50x10^5
5.00x10^4
2.50x10^4
0.00
K. obovate
A. marina
P. australis
S. alterniflora
a
b
a
a
a
a
ME1
ME2
ZE
(b)
4.5x10^5
4.0x10^5
3.5x10^5
5.0x10^4
0.0
-5.0x10^4
a
b
Native species
Invasive species
(c)
Vibrio
Thiobacillus
Sulfurospirillum
Rhodobacter
Pseudomonas
Pelobacter
Geothrix
Geothermobacter
Geopsychrobacter
Geobacter
Desulfuromusa
Desulfovibrio
Desulfobulbus
Desulfitobacterium
Bacteroides
Anaeromyxobacter
Aeromonas
ME1
ME2
ZE
Relative abundance (%)
0.00
2.50
5.00
7.50
Plant
K. obovate
A. marina
P. australis
S. alterniflora

Fig. 3.


Native mangroves
(Kandelia obovate or Avicennia marina)
Native Phragmites australis
Invasive Spartina alterniflora
Dynamics of soil properties
Soil organic carbon ↓
Water content ↑
Bulk density ↓
Fe species
Fe(III) ↓
Fe(II) ↑
Fe-reducing bacteria ↑
Fe$_o$ ↓
Fe$_p$ ↓
Fe-OC ↓ 35.9%
Molar OC:Fe ratios ↓ 29.3%
Fe
Co-precipitation
Adsorption
Soil organic carbon
Fe species
Direct effect
Indirect effect

703

704 **Fig. 4.**