



# Significant alterations in soil fungal communities along a chronosequence of *Spartina alterniflora* invasion in a Chinese Yellow Sea coastal wetland

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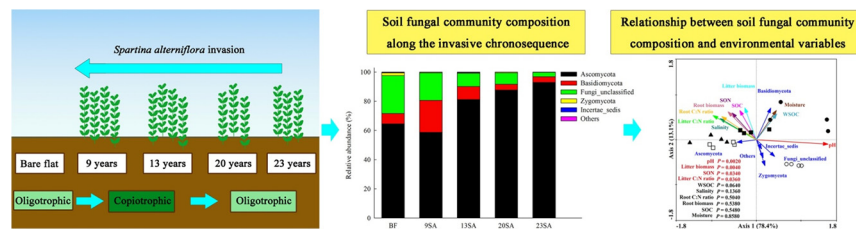
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## HIGHLIGHTS

- *S. alterniflora* invasion altered soil fungal abundance, diversity and composition.
- The fungal abundance and diversity were highest in 9-year-old *S. alterniflora* soil.
- Basidiomycota was gradually replaced by Ascomycetes along *S. alterniflora* invasion.
- *S. alterniflora* invasion greatly shifted fungal trophic modes and functional groups.
- Soil fungal communities were driven by plant and soil properties, nutrient substrate.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Plant invasion typically alters the microbial communities of soils, which affects ecosystem carbon (C) and nitrogen (N) cycles. The responses of the soil fungal communities to plant invasion along its chronosequence remain poorly understood. For this study, we investigated variations in soil fungal communities through Illumina MiSeq sequencing analyses of the fungal internal transcribed spacer (ITS) region, and quantitative polymerase chain reaction (qPCR), along a chronosequence (i.e., 9-, 13-, 20- and 23-year-old) of invasive *Spartina alterniflora*. We compared these variations with those of bare flat in a Chinese Yellow Sea coastal wetland. Our results highlighted that the abundance of soil fungi, the number of operational taxonomic units (OTUs), species richness, and Shannon diversity indices for soil fungal communities were highest in 9-year-old *S. alterniflora* soil, which gradually declined along the invasion chronosequence. The relative abundance of copiotrophic Basidiomycota revealed significant decreasing trend, while the relative abundance of oligotrophic Ascomycota gradually increased along the *S. alterniflora* invasion chronosequence. The relative abundance of soil saprotrophic fungi (e.g., undefined saprotrophs) was gradually reduced while symbiotic fungi (e.g., ectomycorrhizal fungi) and pathotrophic fungi (e.g., plant and animal pathogens) progressively increased along the *S. alterniflora* invasion chronosequence.

**Abbreviations:** ACE, Abundance-based coverage estimator; AMF, Arbuscular mycorrhizal fungi; ANOVA, Analysis of variance; C, Carbon; C:N ratio, Carbon: Nitrogen ratio; Chao1, Chao's species richness estimator; ECM, Ectomycorrhizal; ITS, Internal transcribed spacer; N, Nitrogen; NMDS, Non-metric multidimensional scaling; OTUs, Operational taxonomic units; PCoA, Principal coordinates analysis; QIIME, Quantitative insights into microbial ecology; qPCR, Quantitative polymerase chain reaction; RDA, Redundancy analysis; RDP, Ribosomal database project; Shannon, Shannon's diversity index; SOC, Soil organic carbon; SOM, Soil organic matter; SON, Soil organic nitrogen; WSOC, Water-soluble organic carbon.

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Our results suggested that *S. alterniflora* invasion significantly altered soil fungal abundance and diversity, community composition, trophic modes, and functional groups along a chronosequence, via substantially reduced soil litter inputs, and gradually decreased soil pH, moisture, and soil nutrient substrates along the invasion chronosequence, from 9 to 23 years. These changes in soil fungal communities, particularly their trophic modes and functional groups along the *S. alterniflora* invasion chronosequence could well impact the decomposition and accumulation of soil C and N, while potentially altering ecosystem C and N sinks in a Chinese Yellow Sea coastal wetland.

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## 1. Introduction

Plant invasion is an emerging driver of global change (Craig et al., 2015), which threatens natural habitats (Bazzichetto et al., 2018), alters species compositions (Carboni et al., 2018), ecosystem processes, and their functions (Craig et al., 2015; Stefanowicz et al., 2016; Carboni et al., 2018). These shifts in plant communities, induced by plant invasion, may considerably alter net primary production (Stefanowicz et al., 2016), soil nutrient inputs (e.g., invasive plant litter and exudates) and decomposition (Liao et al., 2007), as well as soil characteristics (Stefanowicz et al., 2016). These changes further modify nutrient fluxes and biogeochemical cycles (Turpin-Jelfs et al., 2019), particularly ecosystem carbon (C) and nitrogen (N) cycles (Craig et al., 2015; Lee et al., 2018). Meanwhile, soil microbes play a critical role in the regulation of ecosystem C and N cycles through the decomposition of various organic detritus, while controlling soil organic matter (SOM) turnover and formation (Morris and Blackwood, 2015; Yang et al., 2016). Although the impacts of plant invasion on ecosystem C and N cycles have been extensively reported (Liao et al., 2008; Wolkovich et al., 2010; Lee et al., 2018), the elucidation of soil microbial communities responses to plant invasion remains limited (Lazzaro et al., 2018).

Plant invasion has been demonstrated to alter the abundance and diversity of plant and animal communities (Stefanowicz et al., 2019), as well as soil nutrient substrates (Yang et al., 2016). Further, it eventually modifies the biomass, composition, and structures of soil microbial communities (Stefanowicz et al., 2019). Recently, the responses of soil bacterial communities to plant invasion have been extensively documented (Piper et al., 2015; Rodríguez-Caballero et al., 2017; Xiang et al., 2018). In contrast, the impacts of plant invasion on soil fungal communities have not been well addressed (Li et al., 2017; Phillips et al., 2019). Soil fungi are among the most abundant and diverse taxonomic groups on the earth (Egidi et al., 2019), and comprise diverse groups of eukaryotic microorganisms that form essential component of soil microbial communities (Mickan et al., 2017). Soil fungal communities play a crucial role in the decomposition of plant litter in soils due to their robust capacities to decompose recalcitrant organic materials (e.g., lignin and cellulose) (Matsuoka et al., 2018). Moreover, soil C cycle and SOM decomposition rate are driven to a great extent by soil fungal communities, as fungi are key facilitators in the decomposition of organic matter (Mäkipää et al., 2017). Additionally, previous studies have demonstrated that soil fungal communities are involved in the regulation of the soil N cycle, on account of their ability to adapt to a wide variety of microsites, and the secretion of exoenzymes that depolymerize N-containing compounds (Nemergut et al., 2008; Li et al., 2017). Thus, a comprehensive evaluation of the impacts of invasive plant species on soil fungal communities have vital implications for understanding their influences on C and N cycles in ecosystems.

Soil fungal communities are significantly affected by biotic (e.g., plant community) and abiotic (e.g., soil microclimate, physicochemical property, and nutrient substrate) factors (Peay et al., 2013; Leff et al., 2015; Bachelot et al., 2016). Soil fungi predominantly decompose recalcitrant plant materials (Collins et al., 2018). Accordingly, plant communities directly affect soil fungal communities by modifying the quality (e.g., litter and root C:N ratios) and quantity of plant litter that

is added to the soil (Bachelot et al., 2016). The physicochemical properties of soils (e.g., soil pH, and moisture) have been implicated as one of the essential driving factors in soil fungal communities (Leff et al., 2015). For instance, soil pH is considered to be one of the most vital influential predictors of soil fungal communities (Geml et al., 2014; Hu et al., 2017). Maestre et al. (2015) observed that the abundance and diversity of soil fungi decrease with a decreasing soil pH. Further, the quality and quantity of soil nutrient substrates have been reported to modify soil fungal communities (Lauber et al., 2008; Peay et al., 2013), as most soil fungi are saprophytes (Zimudzi et al., 2018). Geml et al. (2014) revealed that the soil fungal communities were closely associated with soil organic carbon (SOC), N content, and C:N ratio. Previous studies revealed that invasive plant species greatly modified the characteristics of plant litter (Liao et al., 2007), soil physicochemical properties (Yang et al., 2016, 2019), and soil organic C and N sequestration (Wolkovich et al., 2010; Lee et al., 2018). The identification of biotic and abiotic factors that drive variations in soil fungal communities will help better understand the influential mechanisms of invasive plants on the abundance of soil fungi, as well as their diversity, community composition, trophic modes, and functional groups.

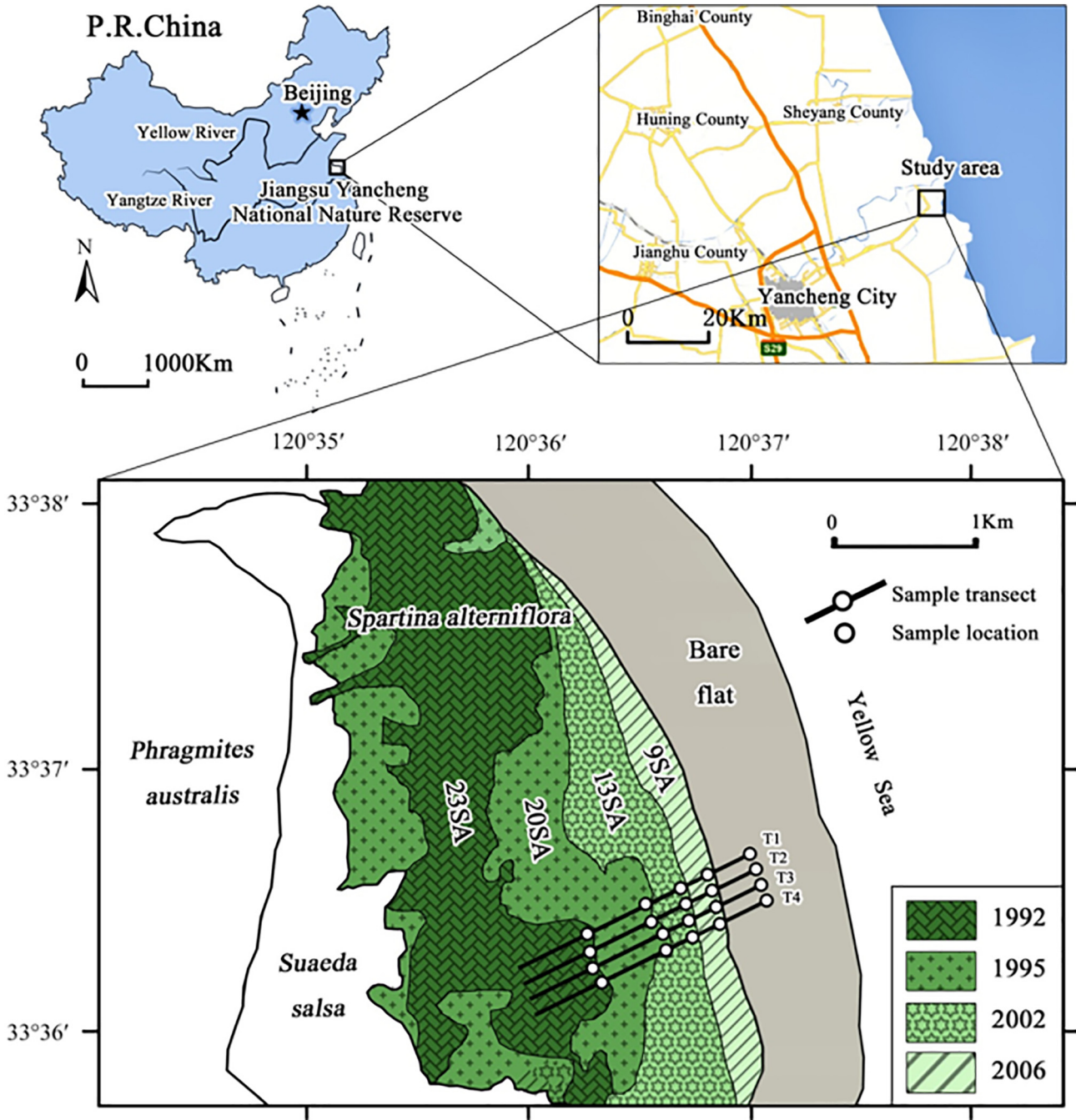
*Spartina alterniflora* (a perennial grass), which is native to North America, was introduced to China in 1979 to accelerate sedimentation and the stabilization of tidal flat lands. Subsequently, *S. alterniflora* rapidly expanded across the eastern coast of China, from Tianjin (in the north) to Beihai (in the south), by invading bare flat and/or by replacing native plants (e.g., *Suaeda salsa*, *Phragmites australis*, and *Scirpus mariqueter*) (Liao et al., 2007; Yang et al., 2016). It has since become one of the dominant plants in China's coastal wetlands (Yang et al., 2017). The Jiangsu coast has the greatest *S. alterniflora* distribution area in China (Yang et al., 2015). *S. alterniflora* has been demonstrated to have unique ecophysiological properties relative to native plants, such as higher growth rate, greater net primary production, and higher salt tolerance (Liao et al., 2007). Moreover, earlier studies revealed that *S. alterniflora* invasion distinctly altered organic C and N accumulation and turnover in soils (Liao et al., 2007; Yang et al., 2015, 2017), as well as their soil physicochemical properties (Yang et al., 2016). It was reported that *S. alterniflora* invasion altered the soil microbial communities (Yang et al., 2016), particularly the bacterial communities (Gao et al., 2019; Yang et al., 2019), including several specific soil microbial taxa associated with nitrification (Xia et al., 2015). However, the impacts of *S. alterniflora* invasion on fungal abundance, diversity, community composition, trophic modes, and functional groups, along the invasion chronosequence, from short- to long-term, remain unclear. We hypothesized that *S. alterniflora* invasion altered soil fungal abundance, diversity, community composition, trophic modes, and functional groups by changing the quantity and/or quality of plant litter returning to the soil, as well as soil physicochemical properties and nutrition substrates along the invasion chronosequence. To test this hypothesis, we employed Illumina MiSeq sequencing of the fungal internal transcribed spacer (ITS) region and quantitative polymerase chain reaction (qPCR), to analyze variations in soil fungal abundance, diversity, and community composition. Soil fungal trophic modes and functional groups were inferred using the FUNGuild database (Nguyen et al., 2016). Soil pH, moisture, salinity, SOC, water-soluble organic carbon (WSOC), soil

organic nitrogen (SON) concentrations, litter C:N ratio, root C:N ratio, litter, and root biomass were measured in 9-, 13-, 20-, and 23-year-old *S. alterniflora* communities, and an adjacent bare flat in a Chinese Yellow Sea coastal wetland. The objectives of this study were to: (1) investigate the effects on the abundance, diversity, and community composition of soil fungal communities via the *S. alterniflora* invasion chronosequence, (2) evaluate alterations in soil fungal trophic modes and functional groups along the *S. alterniflora* invasion chronosequence, (3) identify the most significant driving factors that initiated these changes in the fungal communities along the *S. alterniflora* invasion chronosequence, and (4) explore how these shifts in soil fungal communities were involved in the regulation of ecosystem C and N cycles.

## 2. Materials and methods

### 2.1. Study area and soil sampling

The current study was carried out in the core region of the Jiangsu Yancheng Wetland National Nature Reserve for Rare Birds, China (32° 36' 51"–34° 28' 32" N and 119° 51' 25"–121° 5' 47" E; Fig. 1). The local climate is a typical monsoonal climate, characterized by a mean annual temperature of 13.6 °C, and a mean annual precipitation of 1024 mm. This reserve comprises the world's largest winter habitat for *Grus japonensis*, and plays a critical role in biodiversity and natural wetland conservation (Yang et al., 2017). For the purposes of accelerating siltation and the stabilization of tidal flat lands, *S. alterniflora* was



**Fig. 1.** Location of sampling sites in a Chinese Yellow Sea coastal wetland. *S. alterniflora* occupied the bare flat, and 9SA, 13SA, 20SA, and 23SA represent the communities of *S. alterniflora* that replaced the bare flat in 2006, 2002, 1995, and 1992, respectively. T1–4 = Transects 1–4; 9SA = 9-year-old *S. alterniflora*; 13SA = 13-year-old *S. alterniflora*; 20SA = 20-year-old *S. alterniflora*; 23SA = 23-year-old *S. alterniflora*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

introduced to the reserve in 1983, which rapidly expanded to form a large area of *S. alterniflora* salt marshes (Yang et al., 2017). Bare flat and *S. alterniflora* salt marshes are located in the lower and middle regions of the intertidal zone, respectively (Yuan et al., 2015). The seaward *S. alterniflora* region is bare flat which was devoid of vegetation cover prior *S. alterniflora* invasion (Yang et al., 2015, 2017). *S. salsa* and *P. australis* are the most prominent native plant communities in this reserve (Fig. 1).

The sampling region, with its different *S. alterniflora* invasion times, was identified based on Thematic Mapper satellite images analyses and historical records in Sheyang County, Jiangsu, China (Fig. 1). This seaward to landward chronosequence contained bare flat (the control), and invasive *S. alterniflora* communities that were introduced to the bare flat in 2006 (i.e., 9-year-old *S. alterniflora*), 2002 (i.e., 13-year-old *S. alterniflora*), 1995 (i.e., 20-year-old *S. alterniflora*), and 1992 (i.e., 23-year-old *S. alterniflora*), respectively (Fig. 1). In December 2015, four parallel transects were selected along the chronosequence (T1–T4; Fig. 1). Each transect was approximately 2 km long, and the distance between the adjacent transects was approximately 200 m. In every transect, there were five locations, which included the bare flat, 9-, 13-, 20-, and 23-year-old *S. alterniflora* communities (Fig. 1). Three 2 × 2 m plots were randomly established within each transect location. Apart from the *S. alterniflora* cover, no other plants were distributed in any of the *S. alterniflora* community plots. Three soil cores (5 cm diameter × 30 cm depth) were randomly extracted from each plot. Subsequently, the soil samples from each transect location were thoroughly mixed to yield a final soil sample, which resulted in a total of 20 samples (4 replications × 5 communities). Three 50 cm × 50 cm quadrants were established to collect litter materials, and three soil blocks (15 cm length × 15 cm width × 30 cm depth) were extracted to collect root materials from every community in each transect.

## 2.2. Soil and plant characteristics analysis

Each root-sample block was repeatedly flushed with water through a 0.15 mm sieve, and the roots that finally remained in the sieve were collected (Yang et al., 2017). All litter and root materials were carefully cleaned and oven-dried at 65 °C to a constant weight, for measuring the litter and root biomass. Following the removal of visible plant litter and stones, the soil samples were segmented into four subsamples after thorough mixing. The first soil subsample was introduced into an aluminum box and oven-dried at 105 °C for 24 h to measure the soil moisture content (Yang et al., 2019). The second soil subsample was air-dried and passed through a 1 mm sieve for the determination of soil pH, salinity, SOC, and SON. The third soil subsample was passed through a 2 mm sieve and stored at 4 °C to quantify the WSOC concentration. The fourth soil subsample was passed through a 2 mm sieve and immediately stored at –80 °C for use in molecular analyses. The soil pH was determined using a pH meter (dry soil: water = 1: 2), whereas the soil salinity was measured using an electric conductivity meter (dry soil: water = 1: 5). The SOC and SON were measured using a Vario Micro CHNS analyzer (Elementar Analysensysteme GmbH, Germany). Prior to the determination of SOC and SON, dried soil samples were decarbonized using 1 M HCl at room temperature for 24 h. The WSOC analyses followed the procedure outlined in our previous study (Yang et al., 2016).

## 2.3. DNA extraction and qPCR analysis

DNA from the frozen soil samples (equivalent of 0.5 g dry weight of soil) were extracted in accordance with manufacturer's protocols using the PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA). The ITS1F primer (5S-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 primer (5'-GCTGCGTTCATCGATGC-3') were used for fungal ITS region amplification to determine the abundance of soil fungi (Gardes and Bruns, 1993). The DNA template was diluted five times

prior to amplification. The ITS gene was amplified using an ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA) with a program that provided an initial denaturing step at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s, and finally 60 °C for 1 min. The total qPCR reaction contained 12.5 μL of SYBR Green qPCR Master Mix (2×), 2 μL of the DNA template, 0.5 μL each of 10 μM forward and reverse primers, and 9.5 μL of ddH<sub>2</sub>O, in a 25 μL final volume reaction. All real-time PCR reactions were run in triplicate on the DNA extracted from each soil sample. The ITS gene copy number (A) was calculated per gram of dry soil using the following formula (Sun et al., 2015):

$$A = \frac{\left(\frac{X}{n}\right) \times C \times V}{0.5 \times (1-M)} \quad (1)$$

where X is the copy number of ITS gene detected by qPCR; n is the amount (ng) of DNA used as template in amplification reactions; C is the concentration of extracted DNA (ng μL<sup>-1</sup>); V is the volume (μL) of extracted DNA; 0.5 is the amount (g) of soil used for DNA extraction; and M is soil moisture (%).

## 2.4. Illumina MiSeq high-throughput sequencing

The set of primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (5'-GCTGCGTTCATCGATGC-3') were used to amplify the ITS region of soil fungal DNA (Gardes and Bruns, 1993). Both forward and reverse primers had 6-bp barcodes that were unique to each sample, which were employed to permit sample multiplexing. The PCR mixture consisted of 2 μL of 2.5 mM dNTPs, 0.4 μL of FastPfu Polymerase, 0.4 μL of 5× FastPfu Buffer, 0.8 μL each of the forward and reverse primers (5 μM), 0.2 μL of bovine serum albumin, 10 ng of soil DNA, and sterile deionized H<sub>2</sub>O in a final 20 μL volume reaction. The PCR reactions were conducted using an ABI GeneAmp® 9700 PCR System (Applied Biosystems, Foster City, USA), and the following program: 95 °C for 3 min, with amplification that proceeded for 35 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, followed by a final extension of 10 min at 72 °C. Following amplification, the PCR products were separated by 2% agarose gel electrophoresis and purified using an AxyPrep DNA Gel Extraction kit (Axygen, Union City, CA, USA) following the manufacturer's instructions. The PCR products were subsequently quantified using QuantiFluor™-ST (Promega, Madison, WI, USA). Purified amplicons were pooled at an equimolar volume and paired-end sequenced (2 × 250) using an Illumina MiSeq PE250 sequencing machine, following standard protocols (Majorbio Bio-pharm Technology Co., Ltd., Shanghai, China).

## 2.5. Sequence data processing

Sequences from the Illumina MiSeq platform were processed using the Quantitative Insights Into Microbial Ecology (QIIME) (version 1.8.0) software package (Caporaso et al., 2010). Raw FASTQ files were demultiplexed, quality filtered using Trimmomatic (version 0.32, Bolger et al., 2014), and merged using FLASH under the following standards: (a) low quality regions of sequence reads, i.e., an average quality value of <20 over a 50 bp sliding window, and sequences containing homopolymer regions (>6 bp) were removed from the paired-end sequence read files (Bolger et al., 2014); (b) The primers were closely matched allowing two mismatches of nucleotide sequences, and reads containing ambiguous bases were eliminated; (c) Sequences with overlaps longer than 10 bp were merged on the basis of their overlap sequence. A total of 1,230,117 reads were obtained from the 20 soil samples using Illumina MiSeq sequencing. To obtain an equivalent sequencing depth for downstream analyses, the minimum number of reads (i.e., 42,865) in all subsets from each sample was randomly selected, using the "sub.sample" function in the Mothur program (version 1.30.2), which finally yielded 857,300 reads from the 20 soil samples.

This subsampling process standardized the library size across the samples and mitigated the issue associated with variable library sizes (i.e., very different numbers of sequences across samples), which are a reflection of the differential efficacy of the sequencing process, rather than true biological variation (Weiss et al., 2017). All these normalized (i.e., subsampled) reads were exported for downstream analyses. The subsampled sequences were grouped by operational taxonomic units (OTUs) at 97% similarity levels following the removal of singletons and doubletons using UPARSE (version 7.1, Edgar, 2013). A total of 857,300 reads, representing 6282 OTUs, remained in the 20 soil samples. The fungal diversity of the soil was evaluated based on the numbers of OTUs, Chao's species richness estimator (Chao1), abundance-based coverage estimator (ACE), and Shannon's diversity index (Shannon), using the Mothur program (version 1.30.2, Schloss et al., 2009). The taxonomic classification to phylum and class levels was carried out using the Ribosomal Database Project (RDP) classifier (version 2.2, Wang et al., 2007). Afterwards, the tags were compared to the UNITE ITS Database (version 7.0, Abarenkov et al., 2010) for the detection of chimeric sequences. The relative abundance of each phylum and class was calculated by comparing the number of sequences classified in each phylum, and class to the total number of rDNA gene sequences detected per sample. To investigate the function of soil fungal communities, the fungal OTUs were transformed to text formatting, and the text was uploaded to FUNGuild v1.0: Taxonomic Function (<http://www.stbates.org/guilds/app.php>) (Nguyen et al., 2016), wherein we assigned fungal OTUs to specific trophic modes, subdivided them by fungal functional guilds, and compared the relative sequence abundance of the fungal trophic modes and dominant functional groups between the communities. Three confidence ranks, namely, "possible", "probable", and "highly probable" were evaluated according to comparisons in the fungal database, which indicated the possibility of assumed guilds. Only assignments with confidence levels of "highly probable" or "probable" were included in these analyses (Looby and Treseder, 2018).

## 2.6. Statistical analyses

One-way analysis of variance (ANOVA) was employed to evaluate the effects of *S. alterniflora* invasion times on soil and plant properties, fungal abundance based on ITS gene copy number, the number of OTUs, fungal community richness and diversity indices, and the relative abundance of dominant fungal phylum, class, fungal trophic modes, and functional groups, using SPSS 24 statistical software. Significant differences between the group means were evaluated with Tukey's honest significant difference test at  $P < 0.05$ . Principal coordinate analysis (PCoA) of the OTUs data was performed for  $\beta$ -diversity analyses based on the Bray–Curtis dissimilarity matrix, using the "prcomp" function in the "stats" package, and "plot" function in the "graphics" package in R software (version 3.2.2) (McMurdie and Holmes, 2013). The relationships between the soil fungal community compositions, at both phylum and class levels with soil and plant properties, were analyzed with redundancy analysis (RDA) using CANOCO 4.5 software. The statistical significance of the RDA was tested using Monte Carlo permutation tests (499 permutations;  $P < 0.05$ ). Pearson's correlation analyses of the soil and plant properties were performed to correlate soil fungal abundance, diversity, the relative abundance of the dominant fungal phyla, classes, fungal trophic modes, and functional groups. Linear regression analysis was performed to determine the relationship between litter biomass with soil moisture.

## 3. Results

### 3.1. Soil and plant characteristics

The soil moisture and WSOC concentrations along the *S. alterniflora* invasion chronosequence was highest at 9 years, followed by 13, 20, and 23 years (Table S1). The soil pH was highest in the bare flat, while

it gradually decreased along the *S. alterniflora* invasion chronosequence (Table S1). The salinity in *S. alterniflora* soils was significantly higher than that in the bare flat (Table S1). The SOC concentration was highest in 9- and 13-year-old *S. alterniflora* soils, followed by 20- and 23-year-old *S. alterniflora* soils, in contrast to the bare flat (Table S1). The SON concentration in the 9-, 13- and 20-year-old *S. alterniflora* soils was significantly higher than that in the 23-year-old *S. alterniflora* and bare flat soils (Table S1). The litter biomass along the *S. alterniflora* invasion chronosequence was maximal at 9 years, followed by 13, 20, and 23 years, whereas root biomass in *S. alterniflora* communities was not altered over time since invasion (Table S1). The C:N ratios of the litter and roots of *S. alterniflora* communities showed no statistical differences along the invasion chronosequence (Table S1).

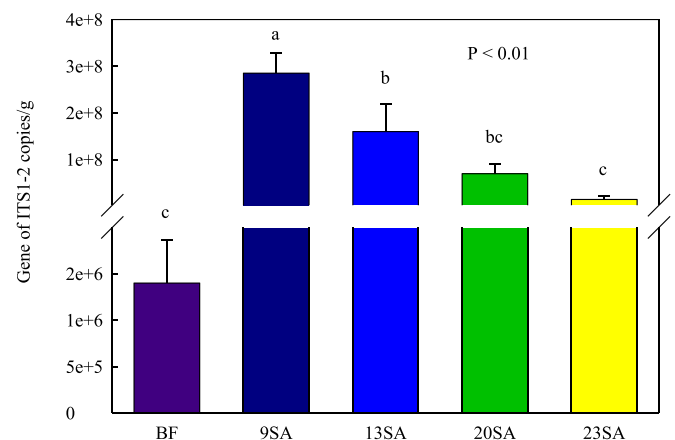
### 3.2. Soil fungal community abundance and diversity

The soil fungal abundance was evaluated through the qPCR amplification of the fungal ITS region. The ITS gene copy numbers were  $1.40 \times 10^6$  copies/g for bare flat,  $2.85 \times 10^8$  copies/g for 9-year-old *S. alterniflora* soil,  $1.60 \times 10^8$  copies/g for 13-year-old *S. alterniflora* soil,  $6.94 \times 10^7$  copies/g for 20-year-old *S. alterniflora* soil, and  $1.43 \times 10^7$  copies/g for 23-year-old *S. alterniflora* soil (Fig. 2). Total fungal abundance in *S. alterniflora* soils increased from 9 to 203 fold in contrast to bare flat (Fig. 2). Total fungal abundance in the 9-year-old *S. alterniflora* soil was significantly higher than that in the 13-, 20- and 23-year-old *S. alterniflora* and bare flat soils (Fig. 2).

The number of OTUs and richness indices (i.e., ACE and Chao1) were highest in the 9-year-old *S. alterniflora* soil, followed by that of the 13-, 20- and 23-year-old *S. alterniflora* and bare flat soils (Table 1). The Shannon diversity index between the communities ranged from 2.89 to 4.42, which was highest in the 9-year-old *S. alterniflora* soil, and progressively decreased along the invasion chronosequence (Table 1). Further, the coverage of each sample ranged from 99.87 to 99.96% between the communities, with the highest coverage being observed in the 20- and 23-year-old *S. alterniflora* soils (Table 1).

### 3.3. Taxonomic classification of soil fungal communities

Ascomycota, Basidiomycota, and unclassified fungi (Fungi\_unclassified) were the predominant fungal phyla, where their relative abundances ranged from 58.70%–92.97%, 3.86%–21.89%, and 2.98%–26.05%, respectively, in all of the soil samples (Table 2). The Zygomycota and Incertae\_sedis were minor phyla, with relative



**Fig. 2.** Total fungal abundance was indicated by the fungal ITS gene copies per gram of soil (mean  $\pm$  SE,  $n = 4$ ) in bare flat and different invasion times of *S. alterniflora* soils (0–30 cm depth) obtained for clustering at 97% identity. Different letters over the bars indicate statistically significant differences at  $\alpha = 0.05$  level between the *S. alterniflora* invasion chronosequence, using Tukey's honestly significant difference test. BF = bare flat. See Fig. 1 for abbreviations.

**Table 1**  
Number of sequences analyzed, observed fungal community richness and diversity indexes (mean  $\pm$  SE, n = 4) in bare flat and different invasion times of *S. alterniflora* soils (0–30 cm depth) obtained for clustering at 97 % identity.

Characteristics	Bare flat	<i>Spartina alterniflora</i>				Source of variation Invasion times
		9 years	13 years	20 years	23 years	
Reads	42865	42865	42865	42865	42865	
OTU richness	205 $\pm$ 13 <sup>b</sup>	435 $\pm$ 53 <sup>a</sup>	306 $\pm$ 25 <sup>b</sup>	233 $\pm$ 40 <sup>b</sup>	225 $\pm$ 16 <sup>b</sup>	**
Richness (ACE)	226 $\pm$ 12 <sup>c</sup>	471 $\pm$ 60 <sup>a</sup>	360 $\pm$ 25 <sup>b</sup>	245 $\pm$ 43 <sup>c</sup>	238 $\pm$ 16 <sup>c</sup>	**
Richness (Chao1)	226 $\pm$ 12 <sup>c</sup>	479 $\pm$ 61 <sup>a</sup>	351 $\pm$ 27 <sup>b</sup>	246 $\pm$ 43 <sup>bc</sup>	242 $\pm$ 16 <sup>bc</sup>	*
Diversity (Shannon)	2.86 $\pm$ 0.05 <sup>d</sup>	4.42 $\pm$ 0.07 <sup>a</sup>	3.62 $\pm$ 0.22 <sup>b</sup>	3.23 $\pm$ 0.08 <sup>c</sup>	3.20 $\pm$ 0.07 <sup>cd</sup>	**
Coverage (%)	99.87 $\pm$ 0.03 <sup>b</sup>	99.87 $\pm$ 0.03 <sup>b</sup>	99.87 $\pm$ 0.02 <sup>b</sup>	99.96 $\pm$ 0.02 <sup>a</sup>	99.95 $\pm$ 0.02 <sup>a</sup>	*

\*  $P < 0.05$ ; \*\*  $P < 0.01$  (One-way ANOVA,  $df_1 = 4$ ,  $df_2 = 15$ ). Different superscript lower case letters indicate statistically significant differences at  $\alpha = 0.05$  level between the *S. alterniflora* invasion chronosequence, using Tukey's honestly significant difference test. Reads are the high-quality sequences following filtering and normalization. The richness estimators, diversity indices and coverage were calculated using the Mothur program. OTU richness: the total number of measured operational taxonomic units (OTUs).

abundances ranging from 0.06%–1.77%, and from 0.04%–0.34%, respectively, in all of the soil samples (Table 2). Ascomycota was the most dominant fungal phylum across all communities (Table 2). The relative abundance of Ascomycota was highest in the 23-year-old *S. alterniflora* soil, followed by the 20- and 13-year-old *S. alterniflora* soils, in contrast to the 9-year-old *S. alterniflora* and bare flat soils (Table 2). The relative abundance of Basidiomycota gradually declined with increasing time since invasion (Table 2). The relative abundance of Fungi\_unclassified in the bare flat land and 9-year-old *S. alterniflora* soils was significantly higher than that in the 13-, 20-, and 23-year-old *S. alterniflora* soils (Table 2). The relative abundance of Zygomycota in the bare flat was significantly higher than that in *S. alterniflora* soils, which did not significantly change between the different invasion times of *S. alterniflora* soils (Table 2).

Further taxonomical classification at the class level revealed that high levels of fungi belonging to Dothideomycetes, Sordariomycetes, Ascomycota\_unclassified, Fungi\_unclassified, Tremellomycetes, Eurotiomycetes, and Agaricomycetes occurred in all the soil samples (Fig. 3). The Cystobasidiomycetes, Wallemiomycetes, and Microbotryomycetes were minor classes between all soil samples (Fig. 3). Dothideomycetes, Sordariomycetes, Ascomycota\_unclassified were the most dominant classes in *S. alterniflora* soils (Fig. 3a, b, and c). The relative abundance of Dothideomycetes ranged from 21.61%–38.44%, which progressively decreased along the invasion chronosequence (Fig. 3a). The relative abundance of Tremellomycetes was highest in the 9-year-old *S. alterniflora* soil (Fig. 3e), whereas the relative abundance of Eurotiomycetes was highest in the 23-year-old *S. alterniflora* and bare flat soils (Fig. 3f). The relative abundance of Agaricomycetes in the 9- and 13-year-old *S. alterniflora* soils was significantly lower than that in the bare flat, 20- and 23-year-old *S. alterniflora* soils (Fig. 3h). The relative abundances of Basidiomycota\_unclassified, Cystobasidiomycetes, and Microbotryomycetes was highest in the 9-year-old *S. alterniflora* soil, which progressively declined with increasing time since invasion (Fig. 3j, k and m). The relative abundances of Sordariomycetes, Leotiomycetes, and Saccharomycetes showed no significant differences along the *S. alterniflora* invasion chronosequence (Fig. 3b, g, and i).

**Table 2**  
Relative abundance (% of individual taxonomic group) of the dominant fungal phyla (mean  $\pm$  SE, n = 4) present in the microbial community in bare flat and different invasion times of *S. alterniflora* soils (0–30 cm depth) in a Chinese Yellow Sea coastal wetland.

Phylum	Bare flat	<i>Spartina alterniflora</i>				Source of variation Invasion times
		9 years	13 years	20 years	23 years	
Ascomycota	64.55 $\pm$ 2.04 <sup>c</sup>	58.70 $\pm$ 6.11 <sup>c</sup>	81.25 $\pm$ 3.29 <sup>b</sup>	87.73 $\pm$ 1.02 <sup>ab</sup>	92.97 $\pm$ 1.46 <sup>a</sup>	**
Basidiomycota	7.03 $\pm$ 0.58 <sup>bc</sup>	21.89 $\pm$ 2.24 <sup>a</sup>	8.90 $\pm$ 0.55 <sup>b</sup>	3.94 $\pm$ 0.53 <sup>c</sup>	3.86 $\pm$ 0.61 <sup>c</sup>	**
Fungi_unclassified	26.05 $\pm$ 1.58 <sup>a</sup>	18.83 $\pm$ 4.64 <sup>a</sup>	8.97 $\pm$ 2.38 <sup>b</sup>	7.73 $\pm$ 0.81 <sup>b</sup>	2.98 $\pm$ 0.83 <sup>b</sup>	**
Zygomycota	1.77 $\pm$ 0.75 <sup>a</sup>	0.45 $\pm$ 0.36 <sup>b</sup>	0.46 $\pm$ 0.23 <sup>b</sup>	0.56 $\pm$ 0.19 <sup>b</sup>	0.06 $\pm$ 0.03 <sup>b</sup>	n.s.
Incertae_sedis	0.33 $\pm$ 0.26 <sup>a</sup>	0.08 $\pm$ 0.08 <sup>a</sup>	0.34 $\pm$ 0.12 <sup>a</sup>	0.04 $\pm$ 0.03 <sup>a</sup>	0.10 $\pm$ 0.04 <sup>a</sup>	n.s.
Others	0.27 $\pm$ 0.16 <sup>a</sup>	0.05 $\pm$ 0.02 <sup>ab</sup>	0.08 $\pm$ 0.07 <sup>ab</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	0.03 $\pm$ 0.02 <sup>ab</sup>	n.s.

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; n.s.: not significant (One-way ANOVA,  $df_1 = 4$ ,  $df_2 = 15$ ). Different letters indicate statistically significant differences at  $\alpha = 0.05$  level between the *S. alterniflora* invasion chronosequence, using Tukey's honestly significant difference test.

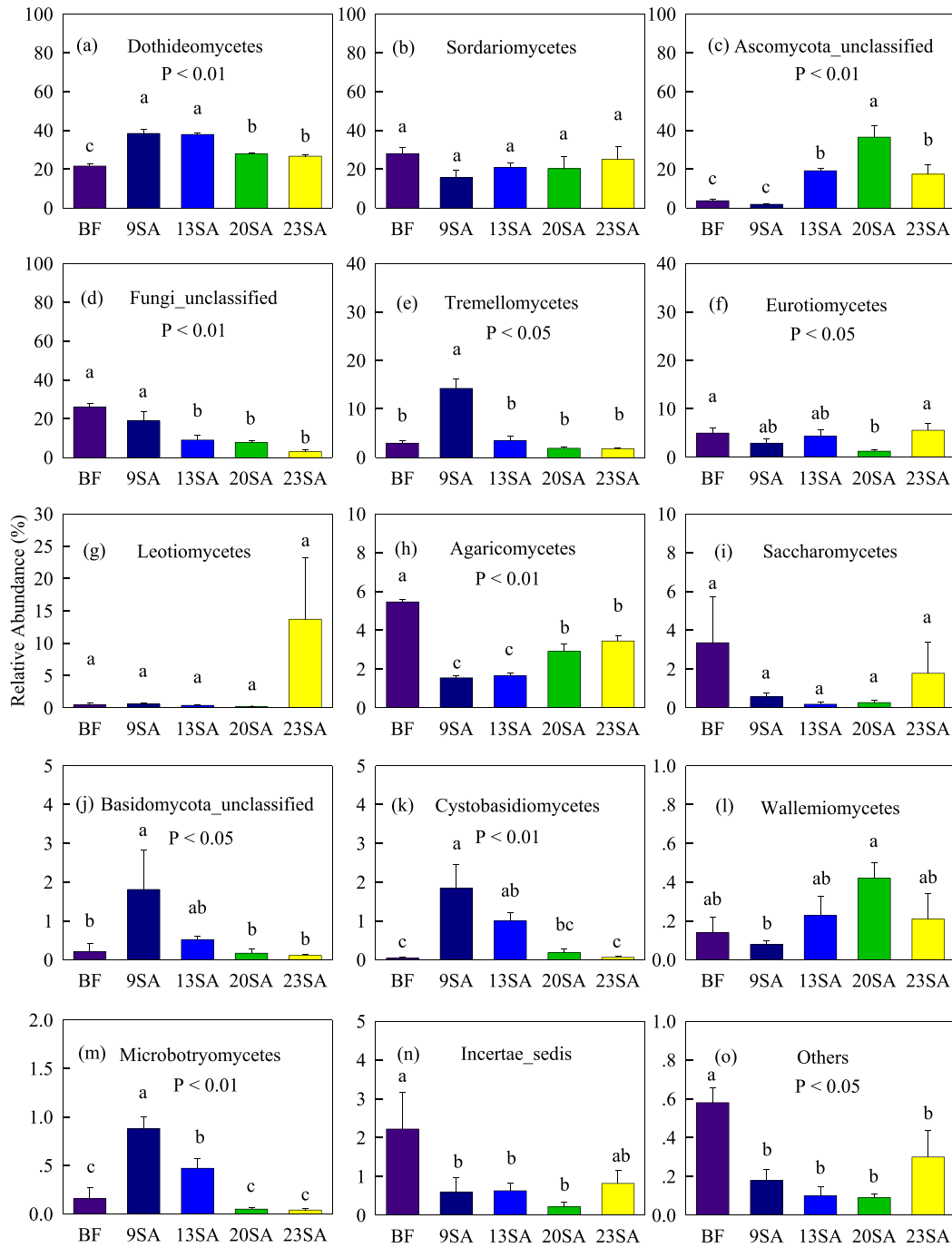
### 3.4. Fungal trophic modes and functional groups

The fungal trophic modes and functional groups (guilds) of the OTUs were inferred using FUNGuild. Regarding the trophic modes of the fungal communities (Table 3), only 36.93%, 57.65%, 38.46%, 24.53%, 31.93% of the OTUs from the bare flat, 9-, 13-, 20-, and 23-year-old *S. alterniflora* soils, respectively, were assigned to seven trophic modes, while the remainder were undefined fungi (Table 3). The relative abundance of symbiotroph was highest in the bare flat, followed by the 20- and 23-year-old *S. alterniflora* soils, as compared with 9- and 13-year-old *S. alterniflora* soils (Table 3). The relative abundance of saprotroph in the 9- and 13-year-old *S. alterniflora* soils was significantly higher than that in bare flat, 20-, and 23-year-old *S. alterniflora* soils (Table 3). The relative abundance of pathotroph in the bare flat, 20-, and 23-year-old *S. alterniflora* soils was significantly higher than that in the 9- and 13-year-old *S. alterniflora* soils (Table 3). The highest relative abundance of pathotroph-saprotroph was observed in the 9-year-old *S. alterniflora* soils (Table 3).

For fungal function, 12 fungal functional guilds, i.e., ectomycorrhizal (ECM), undefined saprotroph, dung saprotroph, dung saprotroph-plant saprotroph, dung saprotroph-plant saprotroph-wood saprotroph, dung saprotroph-soil saprotroph, dung saprotroph-undefined saprotroph, undefined saprotroph-wood saprotroph, wood saprotroph, plant pathogen, animal pathogen, and others were detected from symbiotroph, saprotroph, and pathotroph trophic groups (Table S2). The 9- and 13-year-old *S. alterniflora* soils hosted the lowest relative abundance of ECM fungi and animal pathogen between the communities (Table S2). The relative abundance of undefined saprotroph fungi was highest in the 9-year-old *S. alterniflora* soil, which progressively declined with increasing time since invasion (Table S2). Conversely, the relative abundance of plant pathogen was lowest in the 9-year-old *S. alterniflora* soil, which gradually increased along the invasion chronosequence (Table S2).

### 3.5. Beta diversity of soil fungal communities

PCoA analyses were employed to analyze beta diversity, and to identify the differences in the soil fungal community compositions between



**Fig. 3.** Relative abundance (% of individual taxonomic group) of the dominant fungal class (mean ± SE, n = 4) present in the microbial community in bare flat and different invasion times of *S. alterniflora* soils (0–30 cm depth). Different letters indicate statistically significant differences at  $\alpha = 0.05$  level between the *S. alterniflora* invasion chronosequence, using Tukey's honestly significant difference test. See Figs. 1 and 2 for abbreviations.

the communities (Fig. 4). At the OTU level, PCoA analyses indicated that the different soil locations from the bare flat were clustered together, away from the *S. alterniflora* soils (Fig. 4). The different soil locations of the 9-, 13-, 20-, and 23-year-old *S. alterniflora* were close together, which indicated that the fungal community composition of *S. alterniflora* soils at different invasion times were more similar to each other than to those in the bare flat soil (Fig. 4).

**3.6. Important environmental variables for soil fungal communities**

Ten environmental variables (soil pH, moisture, salinity, SOC, SON, WSOC, litter biomass, root biomass, litter C:N ratio, and root C:N ratio)

explained 91.6% and 72.9% of the total changes in the soil fungal community composition, at the phylum and class levels, respectively (Fig. 5). The results of Monte Carlo permutation tests ( $P < 0.05$ ) indicated that variations in the soil fungal community composition at the phylum level were highly related to soil pH ( $F = 28.42, P = 0.0020$ ), litter biomass ( $F = 8.57, P = 0.0040$ ), SON ( $F = 4.63, P = 0.0340$ ), and litter C:N ratio ( $F = 4.51, P = 0.0360$ ) (Fig. 5a), while changes in the soil fungal community composition at the class level were intimately associated with soil pH ( $F = 6.79, P = 0.0020$ ), root biomass ( $F = 4.85, P = 0.0040$ ), and soil moisture ( $F = 3.64, P = 0.0120$ ) (Fig. 5b). The most significant variations, at 78.4% and 37.5%, were explained by the total variation in the soil fungal community composition in Axis 1. Axis 2

**Table 3**  
Relative abundance (% sequences) of the corresponding fungal trophic mode (mean  $\pm$  SE, n = 4) in bare flat and different invasion times of *S. alterniflora* soils (0–30 cm depth) in a Chinese Yellow Sea coastal wetland, inferred by FUNGuild.

Trophic mode	Bare flat	<i>Spartina alterniflora</i>				Source of variation
		9 years	13 years	20 years	23 years	Invasion times
Symbiotroph	4.72 $\pm$ 0.35 <sup>a</sup>	1.11 $\pm$ 0.11 <sup>c</sup>	1.41 $\pm$ 0.17 <sup>c</sup>	2.59 $\pm$ 0.12 <sup>b</sup>	3.04 $\pm$ 0.13 <sup>b</sup>	**
Saprotroph	3.76 $\pm$ 0.38 <sup>c</sup>	13.62 $\pm$ 1.00 <sup>a</sup>	12.06 $\pm$ 0.52 <sup>a</sup>	8.49 $\pm$ 0.54 <sup>b</sup>	7.70 $\pm$ 0.47 <sup>b</sup>	**
Pathotroph	9.46 $\pm$ 0.67 <sup>a</sup>	5.83 $\pm$ 0.40 <sup>b</sup>	6.39 $\pm$ 0.32 <sup>b</sup>	8.07 $\pm$ 0.40 <sup>a</sup>	8.98 $\pm$ 0.48 <sup>a</sup>	**
Pathotroph-Saprotroph	3.74 $\pm$ 1.39 <sup>c</sup>	20.76 $\pm$ 5.73 <sup>a</sup>	15.65 $\pm$ 5.67 <sup>ab</sup>	1.90 $\pm$ 0.31 <sup>c</sup>	6.09 $\pm$ 1.66 <sup>bc</sup>	**
Pathotroph-Symbiotroph	0.50 $\pm$ 0.14 <sup>d</sup>	0.57 $\pm$ 0.23 <sup>a</sup>	0.04 $\pm$ 0.01 <sup>b</sup>	0.05 $\pm$ 0.03 <sup>b</sup>	0.08 $\pm$ 0.06 <sup>b</sup>	*
Saprotroph-Symbiotroph	1.43 $\pm$ 0.65 <sup>a</sup>	0.34 $\pm$ 0.19 <sup>a</sup>	0.52 $\pm$ 0.17 <sup>a</sup>	0.46 $\pm$ 0.12 <sup>a</sup>	0.63 $\pm$ 0.55 <sup>a</sup>	n.s.
Pathotroph-Saprotroph-Symbiotroph	13.32 $\pm$ 2.51 <sup>a</sup>	15.42 $\pm$ 3.14 <sup>a</sup>	2.39 $\pm$ 0.61 <sup>b</sup>	2.97 $\pm$ 1.06 <sup>b</sup>	5.41 $\pm$ 1.89 <sup>b</sup>	*
Undefined fungi	63.07 $\pm$ 3.97 <sup>ab</sup>	42.35 $\pm$ 5.00 <sup>c</sup>	61.54 $\pm$ 6.53 <sup>b</sup>	75.47 $\pm$ 0.61 <sup>a</sup>	68.07 $\pm$ 2.10 <sup>ab</sup>	*

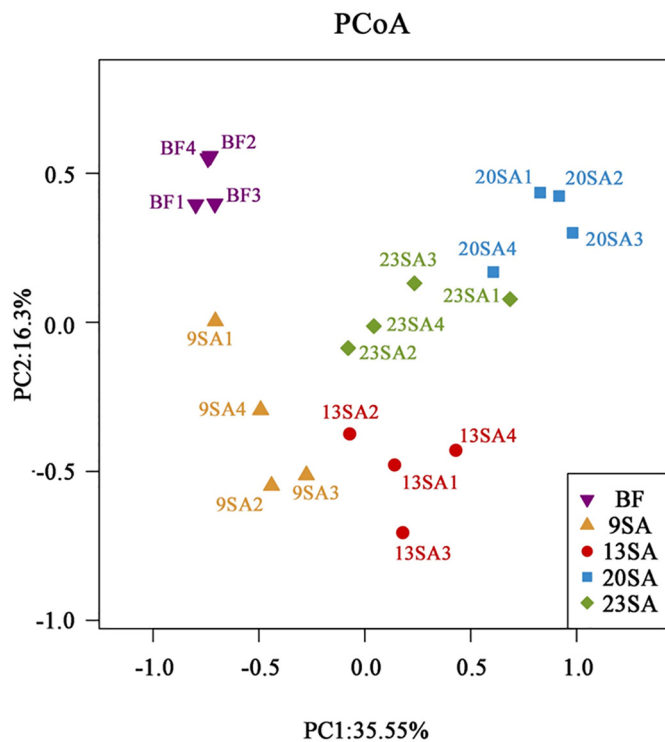
\* P < 0.05; \*\* P < 0.01; n.s.: not significant (One-way ANOVA, df1 = 4, df2 = 15). Different letters indicate statistically significant differences at  $\alpha = 0.05$  level between the *S. alterniflora* invasion chronosequence, using Tukey's honestly significant difference test.

explained 13.1% and 16.1% of the total variations of the soil fungal community composition at phylum and class levels, respectively (Fig. 5). Pearson's correlation analysis revealed that the variations in fungal community abundance, the number of OTUs, and richness indices (Ace and Chao1) of the soil fungal communities were closely related to SOC, WSOC, SON, litter biomass, root biomass, and soil moisture (Table 4). The diversity (Shannon) of soil fungal communities, and the relative abundance of Dothideomycetes were positively correlated with soil moisture, SOC, WSOC, SON, litter biomass, root biomass, litter C:N ratio, and root C:N ratio (Table 4). The relative abundance of Basidiomycota was highly associated with WSOC, litter biomass, soil moisture, and pH (Table 4). The relative abundance of Cystobasidiomycetes was positively correlated with SOC, WSOC, SON, litter biomass, root biomass, and soil moisture (Table 4). The relative abundance of Microbotryomycetes was closely related to SOC, WSOC, litter biomass, soil moisture, and pH (Table 4). The relative abundances of saprotroph and undefined saprotroph had positive correlations with SOC, SON, WSOC, litter biomass, root biomass, litter C:N ratio, root C:N ratio, and soil moisture (Table S3). Further, the relative abundance of

symbiotroph, pathotroph, ECM fungi, plant pathogen, and animal pathogen showed the complete opposite trend (Table S3). The linear regression analysis indicated that litter biomass was significantly positively correlated with soil moisture (Table S4).

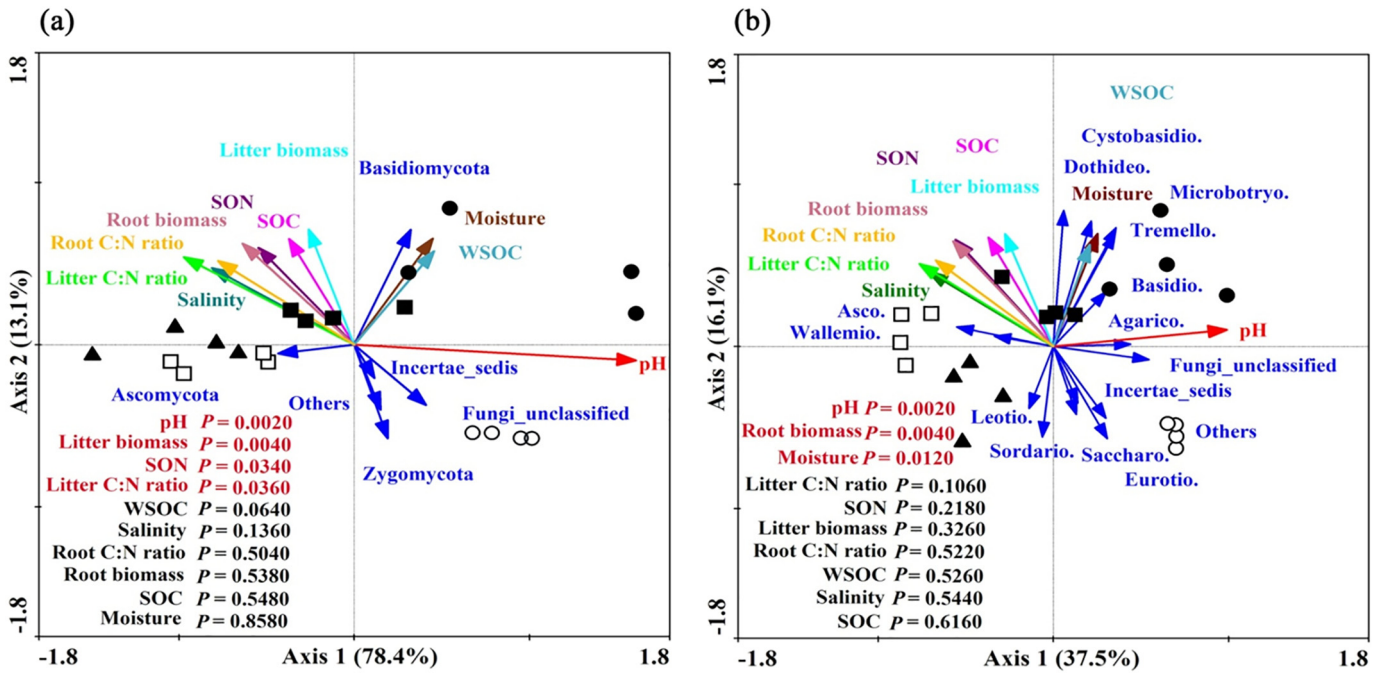
#### 4. Discussion

*Spartina alterniflora* invasion significantly altered soil fungal abundance and diversity along the invasion chronosequence in a Chinese Yellow Sea coastal wetland (Fig. 2 and Table 1). Our results revealed that 9-year-old *S. alterniflora* soil possessed the highest abundance and diversity of fungal communities (Fig. 2 and Table 1). These results further demonstrated that invasive plant species can alter the abundance and diversity of soil fungal communities (Collins et al., 2018; Gaggini et al., 2018). Bachelot et al. (2016) reported that changes in plant community directly impacted soil fungal communities by modifying the quality and quantity of plant litter that enters the soil. It was demonstrated that soil fungi predominantly degraded recalcitrant substrates, such as lignin and hemi-cellulose in plant litter into smaller molecules (Prewitt et al., 2014; Voříšková et al., 2014). Previous studies revealed that *S. alterniflora* plant materials inherently contained higher levels of refractory compounds (e.g., higher concentrations of lignin, hemi-cellulose, litter, and root C:N ratios) in contrast to native *S. salsa*, and *P. australis* communities, which have slower decay rates (Yang et al., 2009; Yang et al., 2015). In this study, although root biomass, litter and root C:N ratios in *S. alterniflora* communities were not significantly different along the invasion chronosequence, the litter biomass was highest in the 9-year-old *S. alterniflora* community, which gradually decreased over time since invasion (Table S1). Previous studies reported that the total and aboveground *S. alterniflora* biomass increased with higher soil moisture, whereas low soil moisture reduced the *S. alterniflora* survival rate and inhibited its growth due to the limited access to water (He et al., 2009). In this study, the highest litter biomass was found in the 9-year-old *S. alterniflora* community, which was likely associated with the highest soil moisture level (Table S1; He et al., 2009). This inference was supported by the result of our linear regression analysis, which showed that litter biomass was significantly positively correlated with soil moisture (Table S4). Thus, the highest fungal abundance and diversity, found in the 9-year-old *S. alterniflora* soil, was primarily attributed to the greatest quantity of low-quality *S. alterniflora*'s litter returning to the soil (Tables 1 and S1; Fig. 2; Yang et al., 2015). Additionally, soil physicochemical properties have been implicated as one of the crucial factors that influence fungal abundance and diversity (Leff et al., 2015). Maestre et al. (2015) reported that soil fungal abundance and diversity decreased following an upsurge in aridity. In this study, soil moisture was highest in the 9-year-old *S. alterniflora* soil, which gradually declined along the invasion chronosequence (Table S1), where the abundance and diversity of fungi were intimately related to soil moisture (Table 4). We reasoned that the highest fungal abundance and diversity in the 9-year-old



**Fig. 4.** (a) Principal coordinates analysis (PCoA) and (b) non-metric multidimensional scaling (NMDS) analysis of OTU data using the R Package. See Figs. 1 and 2 for abbreviations.





**Fig. 5.** Redundancy analysis (RDA) diagram illustrating the relationship between the soil fungal community composition at the phyla-level (a) and class-level (b) from different sampling sites and environmental variables. The explanatory variables are showed by different arrows: soil fungal community composition by blue solid arrows: Dothideomycetes (Dothideo.); Sordariomycetes (Sordario.); Ascomycota\_unclassified (Asco.); Eurotiomycetes (Eurotio.); Saccharomycetes (Saccharo.); Leotiomycetes (Leotio.); Tremellomycetes (Tremello.); Agaricomycetes (Agarico.); Wallemiomycetes (Wallemio.); Microbotryomycetes (Microbotryo.); Basidiomycota\_unclassified (Basidio.); Cystobasidiomycetes (Cystobasidio.); Fungi\_unclassified; Incertae\_sedis; other; and the environmental variables by colored arrows: soil moisture, pH, salinity, soil organic carbon (SOC), soil water-soluble organic carbon (WSOC), soil organic nitrogen (SON), litter biomass and root biomass. Open circles represent bare flat soil, filled circles represent 9SA soil, filled squares represent 13SA soil, open squares represent 20SA soil, filled triangles represent 23SA soil. (For interpretation of color in this figure legend, the reader is referred to the web version of this article.)

*S. alterniflora* soil was partially induced by the highest soil moisture level (Tables 1 and S1; Fig. 2). Generally, soil nutrient substrates are thought to affect fungal abundance and diversity (Lauber et al., 2008; Peay et al., 2013), due to myriad soil fungal species that exhibit saprophytic behaviors (Zimudzi et al., 2018). For this study, although Pearson's correlation analysis indicated that soil fungal abundance and diversity were positively correlated with SOC, WSOC, and SON (Table 4), the link between fungal abundance and diversity with SOC and SON was somewhat weak, as the SOC and SON showed no significant difference between the 9- and 13-year-old *S. alterniflora* soils (Table S1). It was only

WSOC that gradually decreased along the *S. alterniflora* invasion from 9 to 23 years (Table S1). We deduced that gradually decreasing soil fungal abundance and diversity along the *S. alterniflora* invasion chronosequence was the result of progressively lowering litter biomass, soil moisture, and WSOC (Tables 1 and S1; Fig. 2).

Invasive *S. alterniflora* shifted the composition of soil fungal communities along the invasion chronosequence (Table 2; Fig. 3). PCoA analyses revealed that at all soil locations with different invasion intervals of *S. alterniflora* communities were clustered together, away from bare flat soil (Fig. 4). This indicated that *S. alterniflora* soils possessed a unique

**Table 4**

Pearson correlations coefficients between soil fungal communities and soil and plant characteristics (n = 4) between plant communities.

	Moisture	pH	Salinity	SOC	WSOC	SON	LB	RB	Litter C:N ratio	Root C:N ratio
Gene of ITS1-2 copies/g	0.841**	0.293	0.188	0.545*	0.866**	0.484*	0.653**	0.478*	0.433	0.472*
OTU richness	0.785**	0.272	0.356	0.570**	0.631**	0.522*	0.652**	0.474*	0.398	0.321
Richness (Ace)	0.824**	0.301	0.327	0.583**	0.618**	0.521*	0.648**	0.468*	0.382	0.324
Richness (Chao1)	0.806**	0.295	0.339	0.577**	0.614**	0.514*	0.647**	0.465*	0.378	0.313
Diversity (Shannon)	0.895**	0.184	0.306	0.623**	0.902**	0.553*	0.769**	0.579**	0.505*	0.530*
Ascomycota	-0.274	-0.845**	0.405	0.161	-0.270	0.255	0.098	0.304	0.484*	0.383
Basidiomycota	0.732**	0.541*	0.090	0.414	0.667**	0.300	0.509*	0.287	0.088	0.140
Fungi_unclassified	-0.082	0.836**	-0.622**	-0.489*	-0.042	-0.552*	-0.458*	-0.604**	-0.731**	-0.626**
Zygomycota	-0.360	0.491*	-0.570**	-0.552*	-0.243	-0.513*	-0.560*	-0.623**	-0.622**	-0.535**
Dothideomycetes	0.943**	0.076	0.424	0.808**	0.781**	0.727**	0.846**	0.731**	0.606**	0.617**
Sordariomycetes	-0.481*	-0.067	-0.321	-0.486*	-0.453*	-0.398	-0.470*	-0.422	-0.290	-0.356
Ascomycota_unclassified	-0.171	-0.523*	0.479*	0.235	-0.119	0.404	0.170	0.386	0.486*	0.432
Tremellomycetes	0.685**	0.458**	0.148	0.424	0.622**	0.330	0.525*	0.333	0.147	0.157
Eurotiomycetes	-0.186	-0.132	-0.248	-0.184	-0.285	-0.323	-0.239	-0.239	-0.336	-0.292
Leotiomycetes	-0.191	-0.512*	0.115	0.022	-0.135	-0.091	-0.016	0.000	0.115	0.112
Saccharomycetes	-0.312	0.075	-0.283	-0.435	-0.341	-0.423	-0.411	-0.370	-0.418	-0.483*
Basidiomycota_unclassified	0.426	0.249	-0.023	0.204	0.761**	0.145	0.304	0.167	0.090	0.209
Agaricomycetes	0.230	0.559*	-0.414	-0.172	-0.023	-0.284	-0.099	-0.314	-0.418	-0.338
Cystobasidiomycetes	0.749**	0.336	0.407	0.645**	0.615**	0.548*	0.594**	0.479*	0.305	0.354
Wallemiomycetes	-0.205	-0.283	0.306	0.079	-0.131	0.230	0.031	0.183	0.218	0.221
Microbotryomycetes	0.757**	0.488*	0.184	0.532*	0.678**	0.401	0.536*	0.367	0.149	0.236

\* P < 0.05, \*\* P < 0.01 (Pearson's correlation coefficient test). SOC: soil organic carbon; WSOC: soil water-soluble organic carbon; SON: soil organic nitrogen; LB: litter biomass; RB: root biomass. See Table 1 for abbreviations.

fungal community composition which had more similarities between *S. alterniflora* invasion intervals, relative to the bare flat (Fig. 4). In this study, RDA analyses clearly revealed that the variations in soil fungal community compositions were the most intimately associated with soil pH (Fig. 5), which further demonstrated that soil pH played a vital role in shifting the fungal community compositions of soils (Lauber et al., 2008; Rousk et al., 2010; Geml et al., 2014). Aside from soil pH, fungal community compositions were also positively correlated with soil moisture, litter, and root biomass, litter C:N ratio, and SON (Fig. 5). This result was supported by earlier studies which showed that plant biomass is a core factor for the determination of soil fungal community composition (Bachelot et al., 2016). Additionally, Jirout et al. (2011) documented that changes in soil fungal community composition were more closely associated with soil nutrient status (e.g., SOC), rather than soil pH. Interestingly, variations in soil fungal community composition were not greatly affected by SOC in this study (Fig. 5).

Ascomycota is the most ubiquitous and diverse phylum of eukaryotes, and a few Ascomycota taxa dominate soil fungal communities globally (Egidi et al., 2019). Ascomycota is considered to be one of the most vital decomposer present in the soil, which is involved in critical degradation activities (Riley et al., 2014). Basidiomycota is a critical component of the overall saprotrophic functional group (Morrison et al., 2016), which is involved in decomposing recalcitrant lignified plant materials (Lauber et al., 2008). In this study, there was a clear shift at the phylum level, whereby the relative abundance of Basidiomycota progressively decreased, while the relative abundance of Ascomycota constantly increased, i.e., Basidiomycota was gradually replaced by Ascomycota along the *S. alterniflora* invasion chronosequence (Table 2). Eventually, the fungal communities in the 23-year-old *S. alterniflora* soil transitioned to a community that was absolutely dominated by oligotrophic Ascomycota (Table 2). This finding was supported by previous studies, which reported that Ascomycota is an oligotrophic fungal group (Clemmensen et al., 2015) that flourishes in environments with scarce resource availability (e.g., C or nutrients) (Chen et al., 2017). In contrast, Basidiomycota has a preference for high fertility ecosystems, which is progressively replaced by Ascomycetes in low fertility ecosystems (Sterkenburg et al., 2015). Accordingly, Basidiomycota was gradually replaced by Ascomycota along the *S. alterniflora* invasion chronosequence had been largely driven by progressively declining litter biomass and WSOC (Tables 2 and S1), which restricted the growth of copiotrophic Basidiomycota, but was favored by oligotrophic Ascomycota (Cho et al., 2017). Further, previous studies demonstrated that most fungi prefer slightly acidic soil environments, particularly Ascomycota, and their abundance would increase in more acidic environments (Walker and White, 2011; Sterkenburg et al., 2015). Pearson's correlation analysis indicated that the relative abundance of Ascomycota was negatively correlated with soil pH (Table 4). Thus, the gradually increased relative abundance of Ascomycota may have been partially attributed to gradually decreased soil pH along the *S. alterniflora* invasion chronosequence (Tables 2 and S1). At the class level of Ascomycota, the 9- and 13-year-old *S. alterniflora* soils exhibited higher relative abundance of Dothideomycetes compared to 20- and 23-year-old *S. alterniflora* soils (Fig. 3a). Dothideomycetes was reported to decompose refractory plant materials such as lignin, cellulose, and chitin (Guan et al., 2018). We reasoned that a greatly elevated relative abundance of Dothideomycetes in the 9- and 13-year-old *S. alterniflora* soils would accelerate the decomposition of recalcitrant *S. alterniflora* materials, while enhancing SOC sequestration in the 9- and 13-year-old *S. alterniflora* soils (Table S1; Fig. 3a).

In soil ecosystems, fungi consist of phylogenetically and functionally diverse communities that are comprised of myriad ecological guilds (Nguyen et al., 2016). We found that *S. alterniflora* invasion altered the fungal trophic modes and functional groups in soils along the invasion chronosequence (Tables 3 and S2), which were inferred by FUNGuild (Nguyen et al., 2016). Specifically, saprotrophic fungi were most

enriched in the 9- and 13-year-old *S. alterniflora* soils, gradually decreased with longer time since invasion (Table 3), and primarily derived from the contribution of undefined saprotroph (Table S2). Saprotrophic fungi grow throughout the soil–litter interface, serve as the primary agents of plant litter decomposition (Crowther et al., 2012), and are vital regulators of nutrient (e.g., C and N) cycling in terrestrial ecosystems (Crowther et al., 2012; Schmidt et al., 2019). Our previous study revealed that the soil C decay rate gradually decreased along the *S. alterniflora* invasion chronosequence (Yang et al., 2017). Thus, we inferred that constantly reduced saprotrophic fungi would decrease the decomposition of recalcitrant *S. alterniflora* litter, roots and old soil C along the *S. alterniflora* invasion chronosequence, which eventually affected soil organic C and N turnover and accumulation (Tables 3 and S1; Yang et al., 2017).

It was observed that symbiotic fungi in the bare flat and *S. alterniflora* soils were almost entirely derived from ECM fungi (Table S2). Soil arbuscular mycorrhizal fungi (AMF; i.e., phylum Glomeromycota), endophytic fungi, and lichenized fungi have not emerged in bare flat and *S. alterniflora* soils (Table S2). *S. alterniflora* was reported to be without AMF (Van Duin et al., 1989; Hoefnagels et al., 1993; Burke et al., 2003), and our results further confirmed that *S. alterniflora* was hard to be infected by AMF (Table S2). Previous studies revealed that *S. alterniflora* is a non-mycorrhizal species (Burke et al., 2003; Eberl, 2011), and contained mycorrhizal colonization of <10% (Liang et al., 2016). These reports were further evidenced by our results, which showed that the relative abundance of ECM fungi in *S. alterniflora* soils ranged from 1.11%–3.01% (Table S2). Liang et al. (2016) reported that the arrival of invasive *S. alterniflora* strongly inhibited soil mycorrhizal fungi in native plants *P. australis* and *S. mariqueter*, and model plants *Lolium perenne* L. and *Trifolium repens*. In this study, the relative abundance of ECM fungi in *S. alterniflora* soils was significantly lower than that in bare flat (Table S2), which was likely because *S. alterniflora* invaded the bare flat, and greatly interfered with the growth of ECM fungi in *S. alterniflora* soils (Liang et al., 2016). Generally, ECM fungi can obtain photosynthetic sugars from host plants, which in turn promote the establishment and performance of host plants by providing available nutrients and enhancing water absorption (Shah et al., 2016; Mucha et al., 2018). In this study, the relative abundance of symbiotic fungi (i.e., ECM fungi) gradually increased along the *S. alterniflora* invasion chronosequence (Tables 3 and S2), which was conducive to the growth of 20- and 23-year-old *S. alterniflora* communities in oligotrophic and low moisture environment (Table S1; Mucha et al., 2018). Chen et al. (2019) reported that ECM fungi were negatively correlated with soil nutrient content, and soil moisture. We deduced that gradually increased symbiotic fungi (i.e., ECM fungi) along the *S. alterniflora* invasion chronosequence (Tables 3 and S2) was primarily driven by progressively reduced soil nutrient substrate levels (e.g., WSOC) and soil moisture (Tables S1 and S3).

Generally, pathotrophic fungi can obtain organic C by attacking host plants (Wutkowska et al., 2019). Our results revealed that the relative abundance of pathotrophic fungi (e.g., plant and animal pathogens) gradually increased along the *S. alterniflora* invasion chronosequence (Tables 3 and S2), which was negatively associated with litter and root biomass, soil moisture, and nutrition substrates (Table S3; Chen et al., 2019). Yao et al. (2010) reported that *S. alterniflora* invasion in the coastal wetlands of Eastern China displayed a trend of population decline following 16 years of invasion (e.g., decreased plant height, biomass, and density), which may have been caused by variations in environmental factors (e.g., tidal influx rate, soil microbes, and physicochemical properties) (Yao et al., 2010; Yang et al., 2017). In this study, gradually increased soil pathotrophic fungi (e.g., plant and animal pathogens) along the *S. alterniflora* invasion chronosequence (Tables 3 and S2) may have been one of the reasons behind the population decline in the late stage of *S. alterniflora* invasion due to additional pathotrophic fungi attacking the *S. alterniflora* community (Wutkowska et al., 2019).

## 5. Conclusions

This study highlighted the variations in soil fungal communities following the conversion of bare flat into *S. alterniflora* salt marshes along the invasion chronosequence in a Chinese Yellow Sea coastal wetland. The abundance and diversity of the soil fungal communities were highest in the 9-year-old *S. alterniflora* soil and showed a decreasing trend along the invasion chronosequence. *S. alterniflora* invasion greatly modified the composition of soil fungal communities. There was a clear shift, which revealed that the relative abundance of copiotrophic Basidiomycota gradually decreased and was replaced by oligotrophic Ascomycota along the *S. alterniflora* invasion chronosequence. Moreover, trophic modes and functional groups in the soil fungal communities were altered, and specifically, soil saprotrophic fungi gradually decreased, while symbiotic and pathotrophic fungi progressively increased along the *S. alterniflora* invasion chronosequence. These changes in soil fungal communities along the *S. alterniflora* invasion chronosequence were likely significantly driven by variations in plant litter biomass, soil physicochemical properties (e.g., soil pH and moisture), and soil nutrition substrates. Alterations in the soil fungal communities along the *S. alterniflora* invasion chronosequence ultimately contributed to soil C and N turnover and accumulation. This study represents a significant step forward in our understanding of the variations and driving patterns of soil fungal communities following plant invasion from short-term to long-term, and it provides valuable insights into the mechanisms that underlie the influences of plant invasion on C and N cycles in ecosystems.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2019.07.354>.

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