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REGULAR ARTICLE

Soil fungal communities vary with invasion by the exotic *Spartina alternifolia* Loisel. in coastal salt marshes of eastern China

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Abstract

Aims Soil fungal communities play a critical role in ecosystem carbon (C) and nitrogen (N) cycling. Although the effect of plant invasions on ecosystem C and N cycling is well established, its impact on soil fungal communities is not fully understood. The objective of this study was therefore to understand the variations in soil fungal communities as affected by plant invasion, and the mechanisms that drive these changes.

Methods We examined the impacts of invasive *Spartina alternifolia* Loisel. (SA) on soil fungal abundance, diversity, community composition, trophic modes and functional groups in comparison with bare flat (BF) and native *Suaeda salsa* (Linn.) Pall. (SS), *Scirpus mariqueter* Tang et Wang (SM), and *Phragmites australis* (Cav.) Trin. ex Steud. (PA) communities in coastal salt marshes of eastern China, based on analyses of the quantitative polymerase chain reaction (qPCR) and Illumina MiSeq DNA sequences of fungal internal transcribed spacer (ITS) region.

Results SA invasion increased the soil fungal abundance and diversity compared to BF, SS, SM, and PA soils. The increased soil fungal abundance and diversity were highly related to soil organic carbon (C) and nitrogen (N), water-soluble organic carbon (WSOC), litter C:N ratio, and root C:N ratio. Soil fungal community composition was shifted following SA invasion. Specifically, SA invasion significantly enhanced the relative abundance of Basidiomycota, and reduced the relative abundance of Ascomycota compared with BF, SS, SM, and PA soils. Additionally, SA invasion changed soil fungal trophic modes and functional groups. The relative abundance of saprotrophic fungi significantly increased, while the relative abundances of symbiotic and pathotrophic fungi decreased following SA invasion.

Conclusions Our data revealed that SA invasion altered soil fungal abundance, diversity, community composition, trophic modes and functional groups, which were primarily driven by the quality and quantity of plant residues, soil nutrition substrates, as well as soil physicochemical properties. The changes in soil fungal

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communities, especially their trophic modes and functional groups following SA invasion would greatly affect soil C and N decomposition and accumulation with potential feedback on climate change.

Keywords Coastal wetland · Fungal functional groups · Illumina MiSeq DNA sequencing · Plant invasions · Soil carbon and nitrogen sequestration · Soil fungal community composition

Abbreviations

ACE	Abundance-based coverage estimator
AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
BF	Bare flat
C	Carbon
Chao1	Chao's species richness estimator
C:N ratio	Carbon: Nitrogen ratio
ITS	Internal transcribed spacer
N	Nitrogen
OTUs	Operational taxonomic units
PA	*Phragmites australis* (Cav.) Trin. ex Steud.
PCoA	Principal coordinates analysis
QIIME	Quantitative insights into microbial ecology
qPCR	Quantitative polymerase chain reaction
RDA	Redundancy analysis
RDP	Ribosomal database project
SA	*Spartina alternifolia* Loisel.
Shannon	Shannon's diversity index
SM	*Scirpus mariqueter* Tang et Wang
SOC	Soil organic carbon
SOM	Soil organic matter
SON	Soil organic nitrogen
SS	*Suaeda salsa* (Linn.) Pall.
WSOC	Water-soluble organic carbon

Introduction

Invasive plant species occupy many ecosystems worldwide, and have been documented to change ecosystem structure through competition with, and substitution of native species (Bray et al. 2017). This results in changes to ecosystem processes and functions (Vilà et al. 2011). Numerous studies have reported that invasive plants can change local ecosystem carbon (C) and nitrogen (N) cycling (Liao et al. 2008; Castro-Díez et al. 2014; Lee et al. 2017). Soil microorganisms play essential roles in mediating ecosystem C and N cycling through the decomposition of plant residues and soil organic matter (SOM) (Hargreaves and Hofmockel 2014). Soil fungi are ecologically and functionally diverse kingdom of eukaryotic organisms (Khomich et al. 2017), which have the capacity to decompose numerous complex and recalcitrant compounds found in plant-derived organic matter (e.g., cellulose and lignin) compared with bacteria (Matsuoka et al. 2018). Thus, estimating the variations in soil fungal communities as a result of plant invasions is important to improve our understanding of the mechanisms by which invasive plants influence ecosystem C and N cycling. Recently, the responses of soil bacterial communities to invasive plants have been well documented (Piper et al. 2015; Rodríguez-Caballero et al. 2017; Yang et al. 2019), while the impacts of invasive plants on soil fungal communities are still poorly understood (Zubek et al. 2016; Gaggini et al. 2018).

Below-ground fungal communities consist of soil fungal communities and root-associated fungal communities (Li et al. 2018). Root-associated fungi are largely determined by the host plant community, which provide critical nutrients and energy to root-associated fungi (Li et al. 2018). In contrast, soil fungi are primarily dependent on the nutrient composition of the soil (Kim et al. 2015). These two fungal communities are closely related to plant communities at a finite scale, and plant communities are considered to be important driver in the variations of underground fungal communities (Li et al. 2018). Fungi can decompose recalcitrant plant materials (Collins et al. 2018), and consequently they have been reported to be directly affected by the plant residues characteristics (e.g., litter and root C:N ratios) and/or by the concentrations of secondary metabolites (Janoušková et al. 2018). Invasive plants have been reported to alter the composition or types of plant communities (Aragón et al. 2014), as well as litter and of root characteristics (Bray et al. 2017), and these changes may affect soil fungal communities.

Soil fungal communities are driven by a series of abiotic factors, especially soil nutrient conditions (Li et al. 2018) and local environmental factors (Wang et al. 2014; Zhang et al. 2018). Soil fungal communities are highly associated with soil nutrient conditions, since many soil fungi are saprotrophic (Read and Perez-Moreno 2003; Li et al. 2018). Liu et al. (2015) reported that biogeographical distribution of soil fungal communities was determined by the soil C concentration. In

addition, changes in the soil organic carbon (SOC) and soil C:N ratio, as well as the concentration and availability of soil N, have been demonstrated to greatly affect soil fungal communities (Stegen et al. 2013; Wang et al. 2014). Local environmental factors (Zhang et al. 2018), such as soil temperature (He et al. 2016), soil moisture (Galand et al. 2009; Wang et al. 2014), and soil pH (Rousk et al. 2011; Wang et al. 2014), are also considered to be essential driving factors for soil fungal communities. Previous studies have demonstrated that invasive plants altered soil organic C and N levels (Castro-Díez et al. 2014; Craig et al. 2015) by changing quality and quantity of plant residues input into invaded soil, and that they modified soil physicochemical properties (e.g., soil pH, moisture, salinity, and bulk density) (Yang et al. 2013). The identification of abiotic factors that drive changes in soil fungal communities may assist our understanding of the way in which invasive plants influence soil fungal abundance, diversity, community composition, trophic modes and functional groups.

Spartina alternifolia Loisel. (SA) is a rhizomatous perennial deciduous herb, and was introduced from North America to China in 1979 for use in coastal protection schemes (An et al. 2007). SA is highly invasive in the coastal regions of China. It has rapidly expanded to every coastal province of China, and now covers approximately 112,000 ha (An et al. 2007). The coastal regions of Jiangsu Province is the largest SA distribution region in China (Yang et al. 2013). *Suaeda salsa* (Linn.) Pall. (SS), *Scirpus mariqueter* Tang et Wang (SM), and *Phragmites australis* (Cav.) Trin. ex Steud. (PA) are the most common and representative native herbaceous plants found in coastal regions of Jiangsu Province (Yang et al. 2013). SA has spread rapidly by occupying bare flat (BF) and/or by replacing SS, SM, and PA to become the most dominant plant community in the coastal regions of Jiangsu Province, eastern China (An et al. 2007). Previous studies have determined that SA has a greater net photosynthetic rate and primary production than native plants (Liao et al. 2007). SA has therefore altered soil organic C and N stocks and turnover (Yang et al. 2013, 2015; He et al. 2019). SA has also changed soil microbial community (Yang et al. 2016), particularly the soil bacterial community (Yang et al. 2019). However, the impacts of SA invasion on the soil fungal abundance, diversity, community composition, trophic modes and functional groups in coastal salt marshes of eastern China are not yet to estimate. We hypothesized that SA invasion changes soil fungal abundance, diversity, community composition, trophic modes and functional groups by modifying plant residues characteristics, soil nutrient substrates, and physicochemical properties. To test this hypothesis, we used quantitative polymerase chainreaction (qPCR) and Illumina MiSeq sequencing of fungal internal transcribed spacer (ITS) region to analyze the changes in soil fungal abundance, diversity, and community composition. The FUNGuild database was applied to identify soil fungal trophic modes and functional groups. We also measured soil pH, moisture, salinity, SOC, water-soluble organic carbon (WSOC), soil organic nitrogen (SON) concentrations, litter C:N ratio, and root C:N ratio to find the determining factors for soil fungal communities in a SA community. We also compared these factors to adjacent BF, and to native SS, SM, and PA communities in coastal salt marshes of eastern China.

Materials and methods

Experimental area

The current study was undertaken 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) (Yang et al. 2016). The mean annual temperature in this reserve is 13.6 °C, and the mean annual precipitation is 1024 mm. Given its utility for siltation promotion and beach protection, SA has spread rapidly since 1983, and a large SA community has developed in this reserve (Yang et al. 2016). BF and SA communities are situated in low and middle areas of the intertidal zone, respectively (Yuan et al. 2015). The seaward SA region is BF that was without vegetation before the SA invasion (Yang et al. 2013, 2016). SS, SM, and PA are native halophytes that are found in the reserve; the SS and SM communities are in the irregularly flooded high intertidal zones, and the PA community is in the scarcely flooded supralittoral zone (Yuan et al. 2015). These communities are independent and do not overlap with each other.

Field sampling and physicochemical analysis

Four parallel transects were selected in December 2015 along a vegetation succession gradient from sea to land. Each transect was approximately 5 km long, and the

transects were adjacent to each other and had a width of 50 m. They included BF (i.e., control with no vegetation coverage), SA, SS, SM, and PA communities from sea to land in the transects. From analysis of Thematic Mapper satellite images, we determined that the SA community in the sample transects had invaded by occupying BF for nine years. Three 2 m × 2 m plots were randomly established within each site (BF, SA, SS, SM, and PA communities) of each transect. Three soil cores (5 cm diameter × 30 cm depth) were randomly collected from each plot. Soil samples from each transect location were evenly mixed to yield a final soil sample, resulting in a total of 20 samples (4 replications × 5 communities). We randomly established three 50 cm × 50 cm squares to collect all litter materials, and dug three soil blocks (15 cm long × 15 cm wide × 30 cm deep) to collect roots from all the communities in each transect.

Each root-sampling block was filtered through a 0.15 mm sieve and repeatedly flushed with water; the roots remaining in the sieve were collected. Litter and root materials were cleaned and dried in an oven at 65 °C for 48 h until a constant weight was achieved to determine their C:N ratios. Soil was oven-dried at 105 °C for over 24 h until a constant weight was achieved to measure the moisture content. Soil fauna, rocks, and organic debris in soil samples were removed, then soil samples were thoroughly mixed and divided into three subsamples. The first soil subsample was air-dried and passed through a 1 mm sieve, and then used for the measurement of soil salinity, pH, SOC, and SON. The second soil subsample was passed through a 2 mm sieve and preserved at 4 °C, and then used for the determination of WSOC concentration. The third soil subsample was passed through a 2 mm sieve, and conserved at −80 °C for use in molecular analyses. Soil pH was measured from the supernatant with a 1:2.5 soil to water ratio. Soil salinity was measured from the supernatant with a 1:5 soil to water ratio. SOC and SON were measured using a CHN analyzer (Elementar Analysensysteme, GmbH, vario MICRO cube, Germany). Prior to determination, 1 mol/L HCl was added to the soil subsamples to remove inorganic C and N (i.e., carbonate). The WSOC analyses followed the procedure outline in our previous study (Yang et al. 2016). C and N concentrations of litter and root were determined using a CHN analyzer (Elementar Analysensysteme, GmbH, vario MICRO cube, Germany), and litter and root C:N ratios were calculated from these data.

DNA extraction and qPCR analysis

DNA was extracted from the frozen soil samples (equivalent of 0.5 g dry weight soil) using a PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions. Soil fungal abundance was quantified using qPCR of fungal ITS region of the ribosomal RNA gene with the ITS1F primer (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2 primer (5′-GCTGCGTTCTTCATCGATGC-3′) (White et al. 1990; Gardes and Bruns 1993). Template DNA was diluted five times prior to amplification. The total reaction volume was 25 μL, which was comprised of 12.5 μL of SYBR Green qPCR Master Mix (2 ×), 2 μL of template DNA, 0.5 μL each of 10 μM forward and reverse primers, and 9.5 μL of ddH_20. The ITS gene was amplified using an ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA) with a program providing an initial denaturing step at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. All qPCR 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^{-1}); 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 (%).

Illumina MiSeq sequencing

Fungal DNA amplification of the ITS region was used to determine fungal communities, and this was performed using the ITS1F primer (5′-CTTG GTCATTTAGAGGAAGTAA-3′) and ITS2 primer (5′-GCTGCGTTCTTCATCGATGC-3′) (White et al. 1990; Gardes and Bruns 1993). Both the forward and reverse primers also had a 6-bp barcode that was unique to each sample, which were used to permit multiplexing of samples. The PCR reaction 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, and 10 ng of soil DNA; sterile, deionized H_2O was used to bring the total volume to 20 μL. 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 proceeding 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, PCR products were subjected to electrophoresis in 2% agarose gel, and were purified using an AxyPrep DNA Gel Extraction kit (Axygen, Union City, CA, USA) following the manufacturer's instructions. PCR products were then quantified using QuantiFluor™-ST (Promega, Madison, WI, USA). Purified amplicons were pooled at an equimolar volume, and high-throughput, paired-end sequencing (2 × 250) was performed on the Illumina MiSeq PE250 platform by Majorbio BioPharm Technology Co., Ltd., Shanghai, China.

Bioinformatic analysis

Sequences from the Illumina MiSeq platform were processed using the Quantitative Insights Into Microbial Ecology (QIIME) software package v. 1.8.0 (Caporaso et al. 2010). Raw FASTQ files were de-multiplexed, quality-filtered using Trimmomatic v. 0.32 (Bolger et al. 2014), and merged using FLASH with 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 an overlap longer than 10 bp were merged on the basis of their overlap sequence. The trimmed sequences were grouped into operational taxonomic units (OTUs) at 97% similarity levels using UPARSE v. 7.1 (Edgar 2013). The OTU richness (the total number of measured OTUs), abundance-based coverage estimator (ACE), Chao's species richness estimator (Chao1), and Shannon's diversity index (Shannon), and the coverage of the clone libraries were calculated using the Mothur program v. 1.30.1 (Schloss et al. 2009). The ACE and Chao1 were used to compare the species richness (Chao 1984; Chao and Lee 1992), and the ACE takes into account OTUs represented with less than ten sequences as rare species (Chao and Lee 1992; Krogius-Kurikka et al. 2009), and the Chao1 considers singletons and doubletons as rare species (Chao 1984; Krogius-Kurikka et al. 2009). The Shannon was used to compare fungal community diversity (Shannon 1948), which considers the number of species present and the abundance of each species, with the value of the indices increasing with greater diversity (Shannon 1948; Krogius-Kurikka et al. 2009). Taxonomic classification to phylum, class, and genus levels was assigned using the Ribosomal Database Project (RDP) classifier v. 2.2 (Wang et al. 2007). Afterwards, the tags were compared to UNITE ITS Database v. 7.0 (Abarenkov et al. 2010) to detect chimeric sequences. The relative abundance of each phylum, class, and genus was calculated by comparing the number of sequences classified in each phylum, class, and genus to the total number of rDNA gene sequences detected per sample. To investigate the function of soil fungi communities, the fungal OTUs were transformed into text formatting, and the text was uploaded to FUNGuild v1.0: Taxonomic Function (http://www.stbates.org/guilds/app.php) for identifying fungal trophic modes and functional guilds (Nguyen et al. 2016). Principal coordinates analysis (PCoA) of OTUs data were performed using R Package (McMurdie and Holmes 2013). The Bray-Curtis similarity index was calculated using OTU reads, and hierarchical clustering was determined from a β-diversity distance matrix using QIIME software package v. 1.8.0 (Bokulich et al. 2013).

Statistical analysis

One-way analysis of variance (ANOVA) was used to evaluate statistical significance of the effects of plant communities on soil and plant properties; fungal abundance based on fungal ITS gene copy number; relative abundances of dominant fungal phylum, class, and genus; fungal community richness and diversity indices; and OTUs using SPSS 24 statistical software. Significant differences between group means were evaluated with Tukey's honestly significant difference test $\alpha = 0.05$ level. The relationships between the soil fungal community composition, at both phylum and class levels, with soil and plant properties were analyzed with redundancy analysis (RDA) using CANOCO 4.5 software (ter Braak and Smilauer 2002). The statistical significance of the RDA was tested using Monte Carlo

permutation tests (499 permutations; $P < 0.05$). Pearson's correlation analysis was performed to correlate soil fungal abundance, diversity, and the relative abundances of the dominant fungal phyla and classes with the soil and plant properties.

Results

Soil and plant properties

Soil pH in SA soil was significantly lower than that in SS, SM, PA, and BF soils (Table 1). Soil moisture, SOC, and SON concentrations were highest in SA soil among the communities (Table 1). The soil moisture, SOC, and SON in SS and SM soils were significantly lower than those in SA soil, but were significantly higher than those in PA and BF soils (Table 1). Soil salinity was highest in SA soil, intermediate in BF soils, and lowest in SS, SM, and PA soils (Table 1). WSOC concentration in SA soil was significantly higher than that in SS, SM, PA, and BF soils (Table 1). The SA community showed higher C:N ratios of litter and root relative to SS, SM, and PA communities (Table 1).

Soil fungal abundance and community diversity

Fungal abundance was evaluated by qPCR amplification of the fungal ITS region. The fungal ITS gene copy numbers were 1.40×10^6 copies/g for BF, 2.85×10^8 copies/g for SA, 5.47×10^6 copies/g for SS, 3.56×10^7 copies/g for SM, and 4.74×10^6 copies/g for PA soils (Fig. 1). Total fungal abundance in SA soil was significantly higher than that in BA, SS, SM, and PA soils, and there were no significant differences between BA, SS, SM, and PA soils (Fig. 1).

A total of 1,220,680 reads and 6584 OTUs were obtained from 20 samples using Illumina MiSeq sequencing. To compare diversity indices, we normalized the number of sequences for each sample to 42,865 reads (Table 2), which yielded 857,300 reads and 6358 OTUs. The OTU richness, and the species richness indices (i.e., ACE and Chao1) were highest in SA soil followed by SM, SS, PA, and BF soils (Table 2). The Ascomycota phylum showed the highest OTU richness (100–202 OTUs), following by unclassified fungi (Fungi_unclassified, 55–174 OTUs), Basidiomycota (14–96 OTUs), and Zygomycota (5–8 OTUs) across all soil samples (Table S1). The number of OTUs in the Basidiomycota and Fungi_unclassified in SA soil were significantly higher than those in SS, SM, PA, and BF soils (Table S1). The diversity (Shannon) of fungal communities was highest and lowest in SA and BF soils among the communities, respectively (Table 2). The coverage of each sample ranged from 99.87 to 99.95% among the communities, and the highest coverage was found in PA soil (Table 2).

Taxonomic composition of soil fungal communities

Ascomycota, Basidiomycota, and Fungi_unclassified were the predominant fungal phyla, and their relative abundances ranged from 58.70% to 87.30%, from 7.03% to 21.89%, and from 3.37% to 26.38%, respectively, across all soil samples (Table 3). Zygomycota was the minor phylum, with relative abundances ranging from 0.45% to 1.77%, across all soil samples (Table 3). Ascomycota was the most dominant fungal phylum in all communities (Table 3). The relative abundance of Ascomycota in PA and SM soils were significantly higher than that in SA soil (Table 3). The relative abundance of Basidiomycota in SA soil was significantly higher than that in SS, SM, PA, and BF soils (Table 3). BF soil showed a higher relative abundance of Fungi_unclassified, compared with SS, SM, PA soils (Table 3). The relative abundance of Zygomycota showed no significant difference among the communities (Table 3).

Further taxonomical classification at the class level showed that high levels of fungi belonging to Dothideomycetes, Sordariomycetes, unclassified Ascomycota (Ascomycota_unclassified), Fungi_unclassified, Tremellomycetes, Eurotiomycetes, and Agaricomycetes occurred across all soil samples (Fig. 2). The relative abundance of Dothideomycetes was highest in SA soil, followed by SS, PA, BF, and SM soils (Fig. 2a). The relative abundances of Sordariomycetes and Ascomycota_unclassified were highest in PA and SM soils among the communities, respectively (Fig. 2b and c). The relative abundances of Tremellomycetes, unclassified Basidiomycota (Basidiomycota_unclassified), Cystobasidiomycetes, and Microbotryomycetes in SA soil were significantly higher than those in SS, SM, PA, and BF soils (Fig. 2). The relative abundance of Wallemiomycetes was highest in SS soil among the communities (Fig. 2). The relative abundances of Eurotiomycetes, Agaricomycetes, Saccharomycetes, and

Table 1 Soil (0–30 cm depth) and plant characteristics (mean ± SE, $n = 4$) in bare flat, *S. alterniflora*, *S. salsa*, *S. mariqueter* and *P. australis* communities in coastal salt marshes of eastern China

Characteristics	Community					Source of variation
	Bare flat	*S. alterniflora*	*S. salsa*	*S. mariqueter*	*P. australis*	
pH	8.38 ± 0.03^{b}	7.65 ± 0.04^{c}	8.96 ± 0.05^{a}	8.90 ± 0.07^{a}	9.01 ± 0.04^{a}	**
Moisture (%)	28.00 ± 1.11^{d}	83.02 ± 2.64^{a}	37.24 ± 0.79^{b}	33.60 ± 1.43^{b}	31.76 ± 0.51^{cd}	**
Salinity (%)	0.59 ± 0.01^{b}	1.73 ± 0.20^{a}	0.24 ± 0.02^{c}	0.16 ± 0.01^{c}	0.18 ± 0.02^{c}	**
SOC (g kg^{-1})	0.85 ± 0.10^{d}	14.23 ± 1.10^{a}	6.33 ± 0.71^{b}	6.77 ± 0.40^{b}	3.05 ± 0.21^{c}	***
SON (g kg^{-1})	0.08 ± 0.02^{d}	0.84 ± 0.06^{a}	0.38 ± 0.03^{b}	0.40 ± 0.03^{b}	0.21 ± 0.02^{c}	**
WSOC (mg kg^{-1})	37.78 ± 4.77^{b}	76.24 ± 7.97^{a}	26.53 ± 3.47^{b}	42.68 ± 7.85^{b}	38.91 ± 6.68^{b}	*
Litter C:N ratio	–	57.38 ± 2.55^{a}	29.90 ± 0.88^{c}	32.45 ± 1.65^{c}	50.45 ± 2.11^{b}	***
Root C:N ratio	–	47.95 ± 1.40^{a}	39.20 ± 4.77^{ab}	30.10 ± 1.55^{b}	36.30 ± 2.37^{b}	**

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (One-way ANOVA). Different superscript lower case letters indicate statistically significant differences at the $\alpha = 0.05$ level among the communities, using Tukey's honestly significant difference test. *SOC* soil organic carbon, *WSOC* soil water-soluble organic carbon, *SON* soil organic nitrogen

Leotiomycetes exhibited no significant difference among the communities (Fig. 2).

At the genus level, the relative abundances of unclassified Teratosphaeriaceae (Teratosphaeriaceae_unclassified), *Bullera*, *Phaeosphaeria*, *Sarocladium*, and unclassified Halosphaeriaceae (Halosphaeriaceae_unidentified) were highest in SA soil among the communities (Table S2). BF exhibited a higher relative abundance of *Guehomyces* compared with native communities (Table S2).

FUNGuild functional predictions

Using functional assessment from FUNGuild, we assigned fungal OTUs to specific trophic modes and then subdivided them into specific ecological guilds, and compared the relative sequence abundance of the fungal trophic modes and dominant functional groups among the communities (Fig. 3 and Table S3). Overall, a total of 37%, 58%, 39%, 28%, and 49% of OTUs from BF, SA, SS, SM, and PA soils, respectively, were identified as trophic modes with symbiotroph, saprotroph, pathotroph, pathotroph-saprotroph, pathotroph-symbiotroph, saprotroph-symbiotroph, and pathotroph-saprotroph-symbiotroph, while the remainder were undefined fungi (Fig. 3). The relative abundance of symbiotroph in BF (4.72%) and PA (4.45%) soils were significantly higher than that in SS (2.49%), SM (1.99%) and SA (1.11%) soils (Fig. 3). The relative

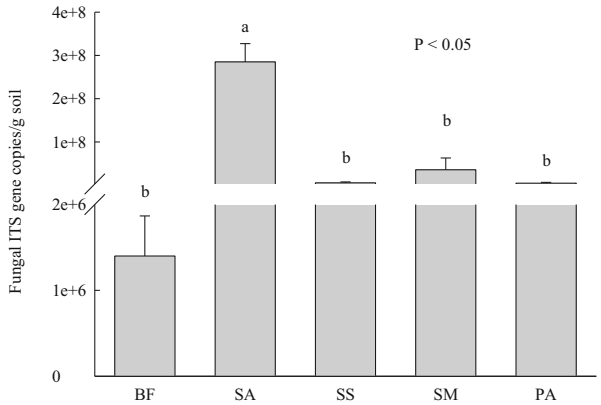

Fig. 1 The total fungal abundance was indicated by the fungal ITS gene copies per gram of soil (mean ± SE, n = 4) in bare flat, *S. alterniflora*, *S. salsa*, *S. mariqueter* and *P. australis* soils (0–30 cm soil depth). Different letters over the bars indicate statistically significant differences at $\alpha = 0.05$ level among the communities, using Tukey's honestly significant difference test. BF = bare flat; SA = *Spartina alterniflora*; SS = *Suaeda salsa*; SM = *Scirpus mariqueter*; and PA = *Phragmites australis*

Table 2 Number of sequences analyzed, observed fungal community richness and diversity indexes (mean ± SE, n = 4) of the bare flat, *S. alterniflora*, *S. salsa*, *S. mariqueter* and *P. australis* communities obtained for clustering at 97% similarity levels

Characteristics	Community					Source of variation
	Bare flat	*S. alterniflora*	*S. salsa*	*S. mariqueter*	*P. australis*	
Reads	42,865	42,865	42,865	42,865	42,865	
OTU richness	205 ± 13^{c}	435 ± 53^{a}	299 ± 32^{bc}	371 ± 45^{ab}	280 ± 16^{bc}	**
Richness (ACE)	226 ± 12^{c}	471 ± 60^{a}	318 ± 33^{bc}	424 ± 53^{ab}	308 ± 24^{bc}	**
Richness (Chao1)	226 ± 12^{c}	479 ± 61^{a}	324 ± 31^{bc}	440 ± 59^{ab}	323 ± 29^{bc}	**
Diversity (Shannon)	2.86 ± 0.05^{d}	4.42 ± 0.07^{a}	3.41 ± 0.07^{bc}	3.60 ± 0.14^{b}	3.13 ± 0.11^{cd}	***
Coverage (%)	99.87 ± 0.03^{b}	99.87 ± 0.03^{b}	99.91 ± 0.03^{ab}	99.93 ± 0.03^{ab}	99.95 ± 0.01^{a}	n.s.

** $P < 0.01$; *** $P < 0.001$; n.s.: not significant (One-way ANOVA). Different superscript lower case letters indicate statistically significant differences at the $\alpha = 0.05$ level among the communities, using Tukey's honestly significant difference test. Reads are the high-quality sequences after 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)

abundance of saprotroph in SA soil (13.62%) was significantly higher than that in SS (7.88%), SM (5.98%) and PA (4.27%) and BF (3.76%) soils (Fig. 3). The relative abundance of pathotroph was highest in PA (11.86%) soil followed by BF (9.46%), SM (7.60%), SS (7.33%), and SA (5.83%) soils (Fig. 3). SA soil showed the highest relative abundances of saprotroph and pathotroph-saprotroph, and the lowest relative abundances of symbiotroph and pathotroph among the communities (Fig. 3). PA soil exhibited higher relative abundances of pathotroph-symbiotroph, and pathotroph-saprotroph-symbiotroph compared to other communities (Fig. 3).

For fungal function, 12 fungal functional guilds (ectomycorrhizal, 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 S3). PA and BF soils showed the highest relative abundance of ectomycorrhizal fungi, and the lowest relative abundance of undefined saprotroph fungi (Table S3). SA soil contained more abundant undefined saprotroph fungi and less abundant ectomycorrhizal fungi, plant pathogen, and animal pathogen compared to other communities (Table S3). The relative abundances of plant pathogen and animal pathogen were highest in PA soil, followed by BF, SM, SS, and SA soils (Table S3).

Beta diversity analysis

PCoA and the Bray-Curtis similarity index were used to analyze beta diversity, and to identify the differences in

Table 3 The relative abundance (% of individual taxonomic group) (mean ± SE, n = 4) of the dominant fungal phyla present in the microbial community of the bare flat, *S. alterniflora*, *S. salsa*, *S. mariqueter* and *P. australis* communities

Phylum	Community					Source of variation
	Bare flat	*S. alterniflora*	*S. salsa*	*S. mariqueter*	*P. australis*	
Ascomycota	64.55 ± 2.04^{ab}	58.70 ± 6.11^{b}	73.77 ± 7.73^{ab}	82.09 ± 4.41^{a}	87.30 ± 5.04^{a}	*
Basidiomycota	7.03 ± 1.30^{b}	21.89 ± 2.24^{a}	12.86 ± 1.84^{b}	9.16 ± 2.66^{b}	8.39 ± 3.01^{b}	**
Fungi_unclassified	26.38 ± 1.45^{a}	18.92 ± 4.69^{ab}	12.77 ± 6.20^{bc}	7.93 ± 1.88^{bc}	3.37 ± 1.67^{c}	**
Zygomycota	1.77 ± 0.75^{a}	0.45 ± 0.36^{a}	0.55 ± 0.33^{a}	0.56 ± 0.18^{a}	0.93 ± 0.46^{a}	n.s.
Others	0.27 ± 0.16^{a}	0.04 ± 0.02^{a}	0.05 ± 0.02^{a}	0.26 ± 0.24^{a}	0.01 ± 0.01^{a}	n.s.

* $P < 0.05$; ** $P < 0.01$; n.s.: not significant (One-way ANOVA). Different superscript lower case letters indicate statistically significant differences at the $\alpha = 0.05$ level among the communities, using Tukey's honestly significant difference test

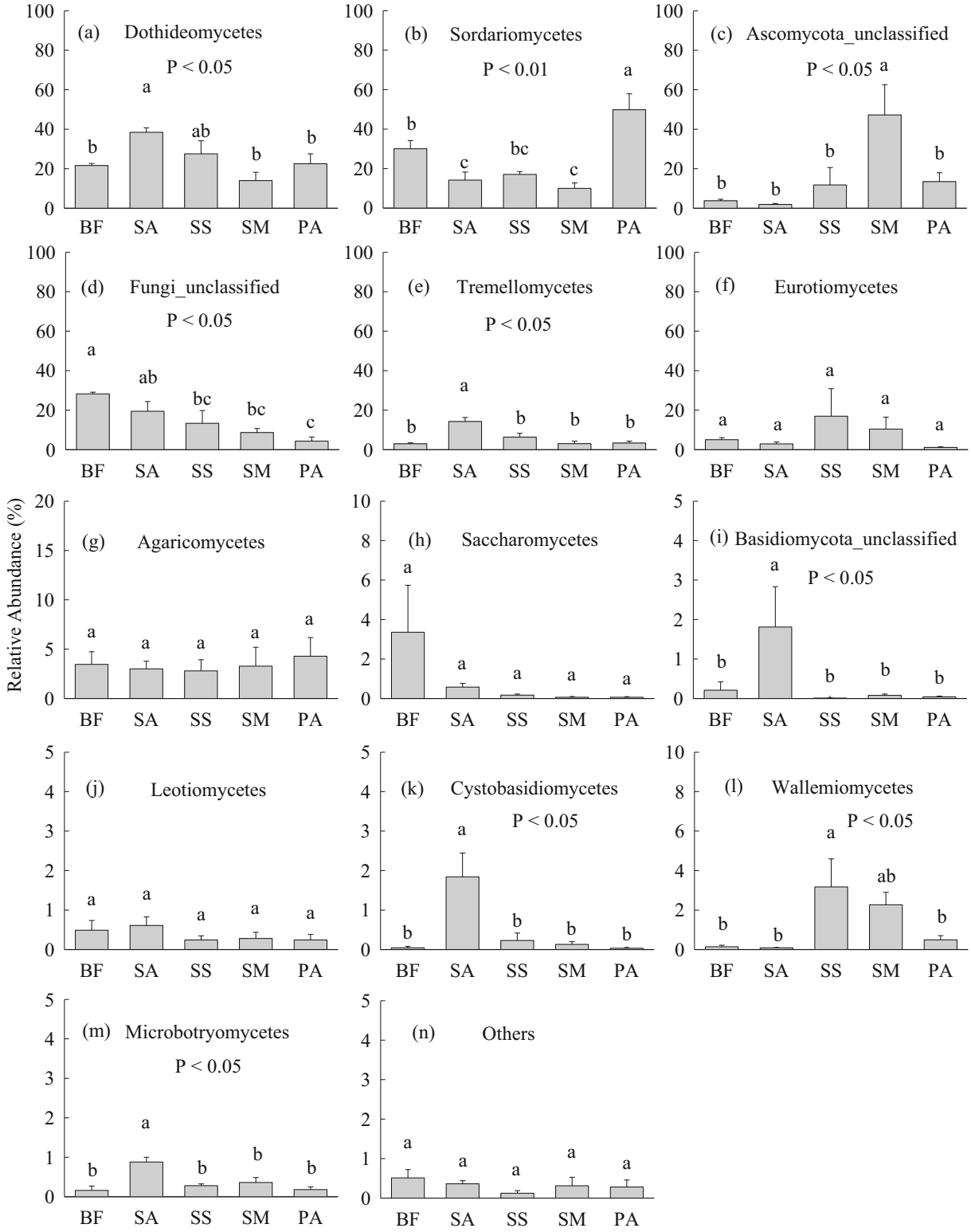

Fig. 2 The relative abundance (% of individual taxonomic group) of the dominant fungal class (mean ± SE, n = 4) present in the microbial community following *S. alterniflora* invasion in coastal salt marshes of eastern China. Different letters indicate statistically significant differences at $\alpha = 0.05$ level among the communities, using Tukey's honestly significant difference test. See Fig. 1 for abbreviations

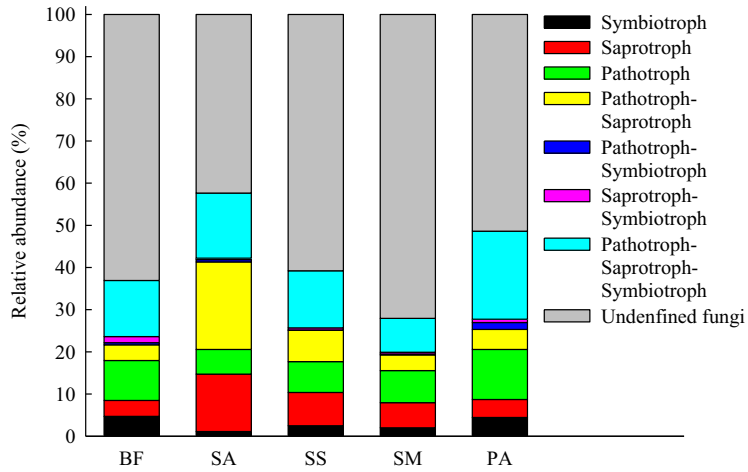

Fig. 3 The relative abundance (%, sequences) of the corresponding fungal trophic mode in bare flat, *S. alterniflora*, *S. salsa*, *S. mariqueter* and *P. australis* soils (0–30 cm soil depth), inferred by FUNGuild. See Fig. 1 for abbreviations

soil fungal community composition across five types of plant communities (Fig. 4). At the OTU level, both analyses showed that the SA soil clustered together, and were distinct from SS, SM, and PA soils. This manifested that SA soil contained unique fungal communities, compared with SS, SM, and PA soils (Fig. 4). The SS, SM, PA, and BF soils clustered closely together, indicating that their fungal community composition were similar (Fig. 4).

Linking soil fungal communities to soil and plant properties

Eight environmental variables (soil pH, moisture, salinity, SOC, SON, WSOC, litter C:N ratio, and root C:N ratio) explained 70.3% and 61.5% of total changes in the soil fungal community composition at phylum and class levels, respectively (Fig. 5). The results of Monte Carlo permutation tests ($P < 0.05$) indicated that variations in

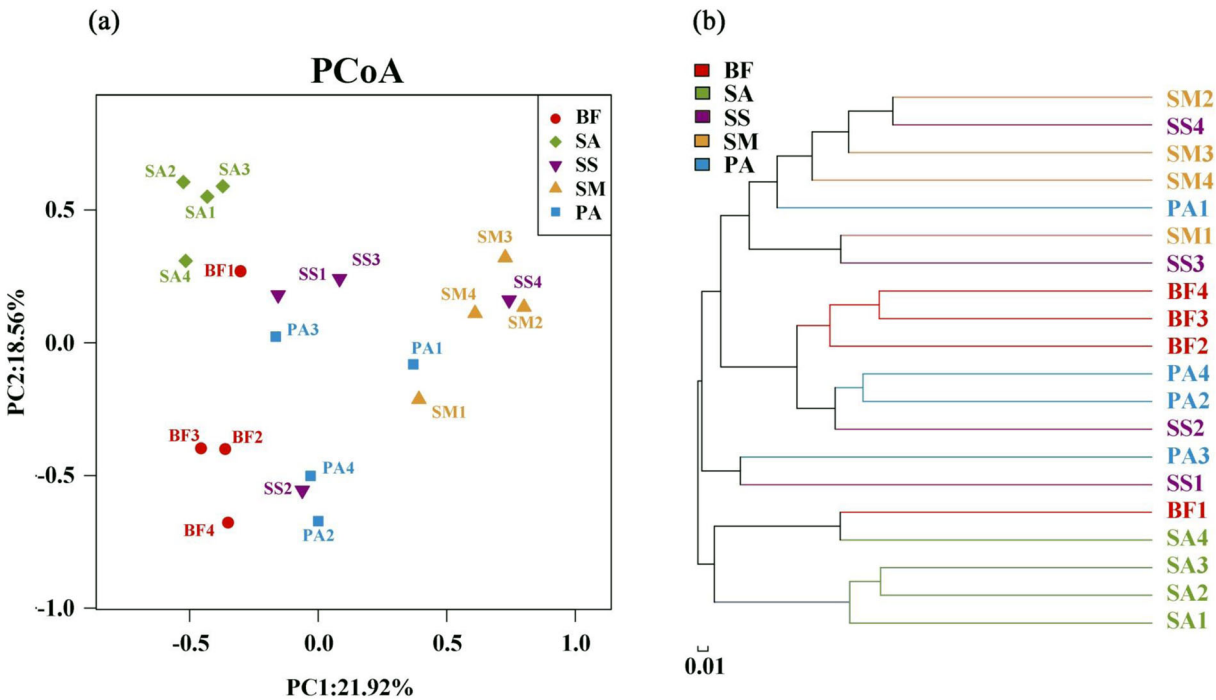

Fig. 4 **a** Principal co-ordinates analysis (PCoA) and (**b**) Clustering of samples. Bray-Curtis similarity index was calculated using the reads of OTU, and hierarchical clustering was calculated using β-diversity distance matrix using Qiime. See Fig. 1 for abbreviations

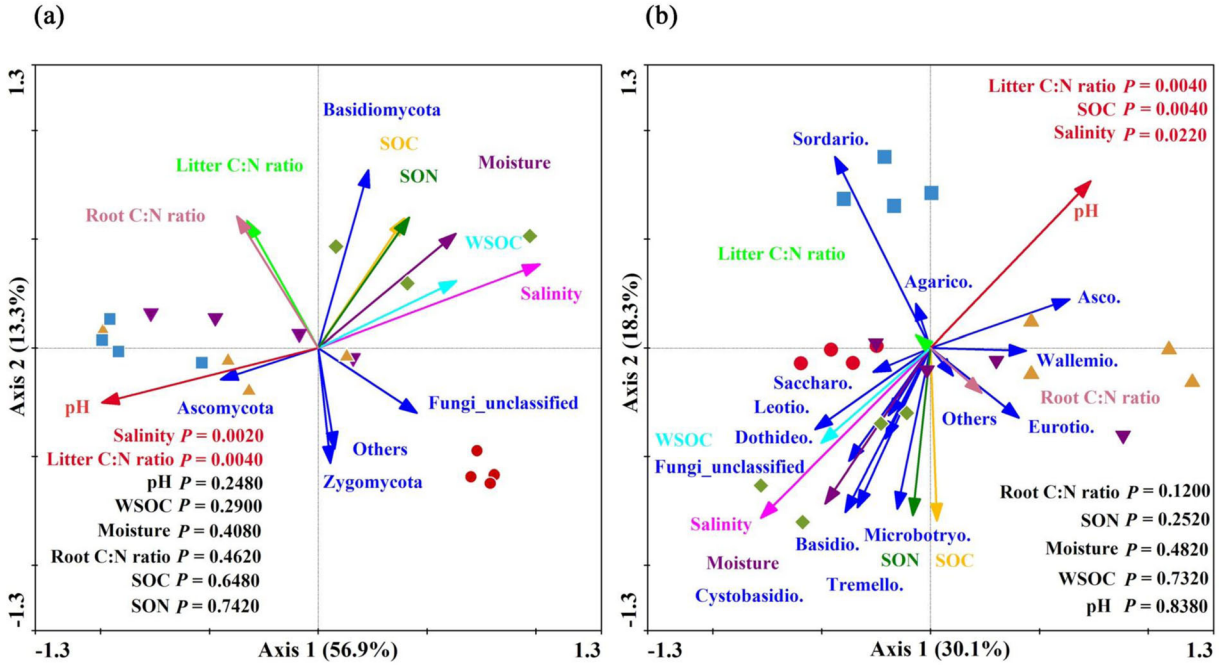

Fig. 5 Redundancy analysis (RDA) diagram illustrating the relationship between the soil fungal community composition at phylum-level (**a**) and class-level (**b**) from different sampling sites and environmental variables. The explanatory variables are showed by different arrows: soil fungal community 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; others; and the variables of soil physiochemical properties by colored arrow: soil moisture, pH, salinity, soil organic carbon (SOC), soil water-soluble organic carbon (WSOC), soil organic nitrogen (SON), litter C:N ratio and root C:N ratio. The red circles represent bare flat soil, the green diamonds represent *S. alterniflora* soil, the purple down-triangles represent *S. salsa* soil, the khaki up-triangles represent *S. mariqueter* soil, and the blue squares represent *P. australis* soil

the soil fungal community composition at phylum level were highly correlated with soil salinity ($F = 9.87$, $P = 0.0020$) and litter C:N ratio ($F = 9.89$, $P = 0.0040$) (Fig. 5a), and that changes in the soil fungal community composition at the class level were tightly correlated with SOC ($F = 4.25$, $P = 0.0040$), litter C:N ratio ($F = 3.83$, $P = 0.0040$), and soil salinity ($F = 3.82$, $P = 0.0220$) (Fig. 5b). The biggest variations, at 56.9% and 30.1%, were explained by the total variations in the soil fungal community composition in Axis 1. Axis 2 explained 13.3% and 18.3% of the total variations of the soil fungal community composition at phylum and class levels, respectively (Fig. 5). The Pearson's correlation analysis indicated that the fungal community abundance (as determined by fungal ITS gene copy number) and the relative abundances of saprotroph and undefined saprotroph were positively correlated with SOC, SON, WSOC, litter C:N ratio, root C:N ratio, and soil moisture, while negatively correlated with soil pH (Tables 4 and S4). The OTU richness, the species richness indices (i.e., ACE and Chao1), and diversity (Shannon) of the fungal communities were positively correlated with SOC, SON, litter C:N ratio, root C:N ratio, and soil moisture (Table 4). The relative abundance of Ascomycota was highly related to soil pH, and was negatively correlated with soil moisture and salinity (Table 4). The relative abundances of Basidiomycota, Tremellomycetes, Microbotryomycetes, and Cystobasidiomycetes had significantly positive correlation with soil moisture, salinity, SOC, SON, WSOC, litter C:N ratio, and/or root C:N ratio, which were negatively correlated with soil pH (Table 4). The relative abundance of Dothideomycetes was strongly associated with SOC, SON, moisture, and salinity (Table 4). The relative abundances of symbiotroph and ectomycorrhizal fungi had significantly negative relationship with SOC, SON, WSOC, litter C:N ratio, root C:N ratio, and soil moisture (Table S4). The relative

Table 4 Pearson correlations coefficients between soil fungal communities and soil and plant properties ($n = 4$) among the plant communities

		pH	Moisture	Salinity	SOC	SON	WSOC	Litter C:N ratio	Roots C:N ratio
	Fungal ITS gene copies/g	−0.817**	0.919**	0.829**	0.797**	0.805**	0.791**	0.531*	0.485*
	OTU richness	−0.350	0.664**	0.479*	0.755**	0.766**	0.417	0.597**	0.655**
	Richness (ACE)	−0.309	0.615**	0.434	0.724**	0.727**	0.394	0.566**	0.636**
	Richness (Chao1)	−0.273	0.584**	0.405	0.707**	0.710**	0.394	0.573**	0.640**
	Diversity (Shannon)	−0.610**	0.873**	0.685**	0.921**	0.922**	0.728**	0.681**	0.715**
Phylum	Ascomycota	0.629**	−0.458*	−0.662**	−0.328	−0.342	−0.426	0.097	0.120
Phylum	Basidiomycota	−0.585**	0.768**	0.734**	0.771**	0.783**	0.532*	0.532*	0.538*
Phylum	Fungi_unclassified	−0.485*	−0.164	0.447*	−0.005	0.006	0.246	−0.428	−0.461*
Phylum	Zygomycota	0.032	−0.315	−0.135	−0.435	−0.431	−0.032	−0.423	−0.462*
Class	Dothideomycetes	−0.548*	0.628**	0.588**	0.475*	0.535*	0.372	0.349	0.289
Class	Sordariomycetes	0.294	−0.348	−0.286	−0.542*	−0.496*	−0.129	0.036	−0.208
Class	Ascomycota_unclassified	0.383	−0.269	−0.417	−0.065	−0.107	−0.272	−0.007	0.043
Class	Tremellomycetes	−0.720**	0.845**	0.844**	0.830**	0.853**	0.561*	0.532*	0.493*
Class	Eurotiomycetes	0.182	−0.128	−0.187	0.079	0.024	−0.146	−0.140	0.167
Class	Agaricomycetes	0.118	−0.091	−0.069	−0.132	−0.145	−0.028	0.033	0.023
Class	Saccharomycetes	−0.206	−0.086	0.104	−0.252	−0.244	−0.077	−0.444*	−0.479*
Class	Basidiomycota_unclassified	−0.565**	0.562**	0.514*	0.433	0.437	0.772**	0.299	0.260
Class	Leotiomycetes	−0.425	0.314	0.363	0.227	0.226	0.026	−0.003	−0.009
Class	Cystobasidiomycetes	−0.729**	0.833**	0.888**	0.809**	0.833**	0.590**	0.505*	0.442
Class	Wallemiomycetes	0.469*	−0.234	−0.394	−0.034	−0.051	−0.394	−0.054	0.158
Class	Microbotryomycetes	−0.684**	0.800**	0.786**	0.849**	0.857**	0.593**	0.535*	0.490*

* $P < 0.05$; ** $P < 0.01$ (Pearson's correlation coefficient test). See Tables 1 and 2 for abbreviations

abundances of pathotroph, plant pathogen, and animal pathogen were negatively related to SOC, SON, and soil moisture (Table S4).

Discussion

SA invasion in coastal salt marshes of eastern China increased total soil fungal abundance by 7–203 fold compared with BF, SS, SM, and PA soils (Fig. 1). This was consistent with the results of our previous study, which found that SA invasion increased the soil fungal biomass relative to BF, SS, and PA soils, based on an analysis of phospholipid fatty acids (Yang et al. 2016). Previous studies have found that soil fungal abundance was positively correlated with SOM and SOC levels (Liu et al. 2015; Ding et al. 2017). Ding et al. (2017) reported that soil fungal abundance was primarily determined by the soil nutrition status, because many fungi present are saprophytic. In this study, SA soil was found to have the greatest organic C and N levels among the communities (Table 1), because of the high plant residues input and decreased decomposition rate of SOM in SA soil (Yang et al. 2013, 2015). The substantially increased total fungal abundance in SA soil is due to their high soil organic C and N levels, which are beneficial for growth and propagation of soil fungi (Table 1; Fig. 1; Ding et al. 2017). Additionally, fungi preferentially degrade recalcitrant plant-derived organic materials (e.g., ligninand hemi-cellulose) by producing extracellular enzymes, and they can do this more efficiently than soil bacteria (Strickland and Rousk 2010; Cusack et al. 2011). In the present study, litter and root C:N ratios were highest in SA community (Table 1), suggesting that SA had the lowest quality of plant residues (Yang et al. 2015). Yang et al. (2009) demonstrated that SA plant materials have more recalcitrant compounds (e.g., lignin and hemi-cellulose) that are slow to decompose (Yang et al. 2013). In addition, SA soil had a higher recalcitrance index for soil C (i.e., the proportion of recalcitrant organic C in total SOC) compared to BF soil (Yang et al. 2015). The refractory plant

materials of SA and recalcitrant SOM in SA soil, were likely beneficial to soil fungal growth (Table 1; Yang et al. 2015). The increased soil fungal abundance following SA invasion is likely the results of the higher levels of soil organic C and N, recalcitrant plant residues as well as SOM in SA soil (Fig. 1; Table 1). This hypothesis is supported by our results which show that total soil fungal abundance was strongly associated with SOC, SON, WSOC, litter C:N ratio, and root C:N ratio (Table 4).

SA invasion considerably altered soil fungal community diversity compared with BF, SS, SM, and PA soils (Table 2). The highest OTU richness was observed in SA soil, which was mainly derived from the highest OTU richness in Basidiomycota and Fungi_unclassified phyla (Table S1). Moreover, the highest species richness (i.e., ACE and Chao1), and diversity (Shannon) of the fungal communities were found in SA soil (Table 2), suggesting that SA invasion increased the richness and diversity of soil fungal communities. These results further demonstrated that plant invasions can alter soil fungal diversity (Collins et al. 2018; Gaggini et al. 2018). It has been reported that vegetation type and abiotic soil properties were considered to be vital drivers in determining soil fungal diversity (Fujimura and Egger 2012; Collins et al. 2018). Previous studies have also revealed that shifts in plant communities from herbaceous to woody species can greatly affect soil fungal diversity, since woody plants possess more recalcitrant compounds (De Boer et al. 2005; Nielsen et al. 2015). In this study, SA invasion induced substantial recalcitrant plant residues input into the soil (Table 1; Yang et al. 2009); this may partly account for the increases in fungal diversity observed in SA soil. This hypothesis was supported by the Pearson's correlation analysis which indicates that OTU richness, species richness, and diversity (Shannon) of the fungal communities were strongly correlated with litter and root C:N ratios (Table 4). In addition, soil nutrition status has been shown to be a critical factor for soil microbial community diversity (Yang et al. 2019). Sun et al. (2016) reported that soil fungal diversity was positively correlated with SOC level. Equally, our results indicated that soil fungal diversity was highly related to SOC and SON levels (Table 4). Therefore, another important reason for the increased soil fungal diversity observed following SA invasion may be the contribution of the high SOC and SON levels, which provided the rich soil nutrition substrates for the fungi.

SA invasion not only increased the soil fungal abundance and diversity (Fig. 1 and Table 2), but also altered the soil fungal community composition (Fig. 2; Tables 3 and S2). PCoA and the Bray-Curtis similarity index indicated that SA soil contained unique fungal communities compared with SS, SM, and PA soils, and that fungal community composition is more homogenous in SS, SM, PA, and BF soils (Fig. 4). In general, soil fungal community composition is affected by properties of the aboveground vegetation (Waldrop et al. 2006; He et al. 2016), soil nutrients, and soil physiochemical properties (Hinojosa et al. 2014; He et al. 2016). In our study, RDA analyses indicated that variations in soil fungal community composition at phylum and class levels were highly associated with soil salinity, litter C:N ratio, and/or SOC concentration (Fig. 5).

Ascomycota in particular was found to be the most dominant fungal taxon in all communities, with relative abundances of Ascomycota ranging from 58.70% to 87.30%, suggesting the ubiquity of this fungal group in coastal salt marshes of eastern China (Table 3). Ascomycetes have been reported to be oligotrophic fungi (Clemmensen et al. 2015; Chen et al. 2017), which have capability to tolerate stressful environments (e.g., low nutrient availability and drought stress) (Clemmensen et al. 2015; Sterkenburg et al. 2015). In this study, the relative abundance of Ascomycota was lowest in SA soil (Table 3); this may be due to the rich nutrition substrates (i.e., SOC, SON, and WSOC) found in SA soil (Table 1). Conversely, SM and PA soils showed higher relative abundance of Ascomycota (Table 3), and this was probably the results of the lower soil nutrition substrates levels and soil moisture (i.e., higher drought stress) in SM and PA soils compared to SA soil (Tables 1). The higher relative abundance of Ascomycota could obtain higher resource use efficiency, and may help SM and PA to grow better in harsh soil environments that are highly alkaline and have low nutrient levels (Tables 1 and 3; Chen et al. 2017). Additionally, Walker and White (2011) reported that most fungi prefer slightly acidic soil environments, and Ascomycota was strongly correlated with soil pH (Table 4). Thus, the low relative abundance of Ascomycota in SA soil may be partly due to the lower pH in SA soil (Tables 1 and 3; Walker and White 2011). At the class level, the relative abundances of both Dothideomycetes and Sordariomycetes counted together in BF, SA, and PA soils accounted for 51.63%, 52.59%, and 72.33% in the Ascomycota, respectively,

implying that Dothideomycetes and Sordariomycetes were the most dominant classes of Ascomycota in BF, SA, and PA soils (Fig. 2a and b).

In this study, Basidiomycota was the second largest phylum of fungi in coastal salt marshes of eastern China (Table 3). Basidiomycota is the important component of the total saprotrophic functional group, and plays vital functional roles in biomass transitions, such as the degradation of high-lignin content plant litter (Voříšková and Baldrian 2013; Guo et al. 2018). It was observed that SA invasion raised the relative abundance of Basidiomycota compared to BF, SS, SM, and PA soils (Table 3). Moreover, at the class level, the relative abundances of Cystobasidiomycetes (phylum Basidiomycota) and Microbotryomycetes (phylum Basidiomycota) were highest in SA soil (Fig. 2k and m). Our results also showed that the relative abundances of Basidiomycota, Cystobasidiomycetes and Microbotryomycetes were highly related to SOC, SON, WSOC, litter and/or root C:N ratios (Table 4). Sterkenburg et al. (2015) reported that Basidiomycota has a preference for high fertility ecosystems, but will progressively be replaced by oligotrophic Ascomycetes in the low fertility ecosystems. Thus, the increased relative abundances of Basidiomycota, Cystobasidiomycetes and Microbotryomycetes following SA invasion were likely induced by the high level of soil nutrition substrates, as well as substantial recalcitrant litter and root materials in SA community (Tables 1 and 3, and Fig. 2k and m). Siles and Margesin (2016) reported that the relative abundance of Zygomycota ranged from 5.6% to 45.1% in a forest ecosystem (Siles and Margesin 2016). Whereas, the relative abundance of Zygomycota in this study ranged from 0.45% to 1.77% across all samples (Table 3), implying that Zygomycota is a minor phylum that is uncommon in coastal salt marshes of eastern China.

FUNGuild provides information on the functional roles of fungi by parsing the provided sequences into trophic guilds (Nguyen et al. 2016). The functional annotation revealed that many OTUs were assigned to undefined fungi, similar to the results of Nguyen et al. (2016). Importantly, we found that saprotrophic fungi were most abundant in SA soil (Fig. 3), primarily derived from undefined saprotroph (Table S3). Saprotrophic fungi are identified as the most important decomposers in the soil (Schmidt et al. 2019), which are involved in organic matter decomposition, C cycling, as well as nutrient mobilization (van der Wal et al. 2013; Schmidt et al. 2019). SA invasion has been widely documented to promote soil organic C and N sequestration in coastal salt marshes of eastern China (Table 1; Yang et al. 2013, 2015; He et al. 2019). Thus, the largely increased relative abundance of saprotrophic fungi (Fig. 3), especially undefined saprotroph in SA soil would accelerate the decomposition of recalcitrant SA plant residues (Table S3; Schmidt et al. 2019), and enhance soil organic C and N accumulation following SA invasion (Tables 1; Yang et al. 2013, 2015). However, PA, BF, SM and SS soils contained less saprotrophic fungi (Fig. 3), particularly undefined saprotroph relative to SA soils (Table S3). Chen et al. (2019) reported that the relative abundance of undefined saprotroph was positively correlated with aboveground biomass, available soil N, and soil moisture, which was supported by our results showing that the relative abundances of saprotroph and undefined saprotroph were highly related to SOC, SON, WSOC, litter C:N ratio, root C:N ratio, and soil moisture (Table S4). Taken together, we reasoned that less saprotrophic fungi in PA, BF, SM and SS soils were attributed to lower soil moisture and nutrition levels, these oligotrophic environments in PA, BF, SM and SS soils limited the increase in saprotrophic fungi (Table 1; Fig. 3).

Meanwhile, the lowest and highest relative abundance of symbiotic fungi were found in SA and PA soil, respectively (Fig. 3). The symbiotic fungi in BF, SA, SS, SM, and PA soils were almost entirely derived from ectomycorrhizal fungi (Table S3). Soil arbuscular mycorrhizal fungi (AMF; i.e., phylum Glomeromycota), endophytic fungi, and lichenized fungi were not observed in all plants communities. Previous studies have reported that SA is not colonized by AMF (Daleo et al. 2008), which is a non-mycorrhizal species (McHugh and Dighton 2004), and revealed increased growth only following nutrient enrichment (Daleo et al. 2008). In this present study, AMF was not found in SA soil (Table S3), which further implied that SA was hard to infect by AMF. Interestingly, we observed that SA soil contained lowest relative abundance of ectomycorrhizal fungi (1.11%) relative to other plant communities (Table S3), rather than absolutely non-mycorrhizal species (McHugh and Dighton 2004). Thus, the lowest relative abundance of symbiotic fungi, i.e., ectomycorrhizal fungi was found in SA soil (Fig. 3 and Table S3), suggesting that SA invasion was not dependent on mycorrhizal symbiosis to obtain more nutrients. Our results exhibited that PA soil had the most

enriched symbiotic fungi (i.e., ectomycorrhizal fungi) (Fig. 3 and Table S3). Recent studies documented that ectomycorrhizal fungi rely on plant-transferred sugars as their C source and in return can acquire more N from organic compounds composing SOM, and help plants to access growth-limiting N environments (Nicolás et al. 2019; Zak et al. 2019). Chen et al. (2019) also showed that the relative abundance of ectomycorrhizal fungi had significantly negative relationship with litter, soil nutrients content, and soil moisture, which was supported by our Pearson's correlation analysis (Table S4). It was inferred that the relative abundance of symbiotic fungi (i.e., ectomycorrhizal fungi) was highest in PA soil (Fig. 3 and Table S3), which was strongly associated with the lowest levels of SOC, WSOC, SON, and soil moisture (Table S4). The most enriched symbiotic fungi in PA soil can acquire more N and P, and supply plants with essential nutrients, and assist PA community to adapt oligotrophic and harsh environment (Fig. 3, Tables 1 and S3).

Generally, pathotrophic fungi can obtain nutrients by attacking host cell (Anthony et al. 2017), but also control populations of nematodes, insects, plant or fungal pests (Schmidt et al. 2019). We found that the relative abundance of pathotrophic fungi (e.g., plant pathogen and animal pathogen) was highest in PA soil and lowest in SA soil (Fig. 3; Table S3), which was highly related to soil nutrition substrates and moisture (Table S4; Chen et al. 2019). On the whole, the trophic modes and functional groups of fungal communities were remarkably different between invasive SA soil and native BF, SS, SM, and PA soils (Fig. 3 and Table S3). Specifically, SA soil contained the most abundant saprotrophic fungi, and the minimum symbiotic and pathotrophic fungi, conversely, native plant communities showed more enriched soil symbiotic and pathotrophic fungi, and less saprotrophic fungi (Fig. 3 and Table S3). These great differences in trophic modes and functional groups of soil fungal communities can be primarily driven by soil nutrition substrates, physicochemical properties, as well as litter and root characteristics (Table S4).

Conclusions

This study highlighted the variations in soil fungal communities following SA invasion in coastal salt marshes of eastern China. Our results showed that SA invasion considerably increased soil fungal abundance and diversity compared with BF, SS, SM, and PA soils. The changes in soil fungal community composition induced by SA invasion is highly associated with soil salinity, litter C:N ratio, and/or SOC concentration. SA invasion significantly raised the relative abundance of Basidiomycota, and reduced relative abundance of Ascomycota compared to native communities. Additionally, SA invasion shifted trophic modes and functional groups in soil fungal communities. The relative abundance of saprotrophic fungi greatly increased, while the relative abundances of symbiotic and pathotrophic fungi considerably decreased following SA invasion. The alterations in trophic modes and functional groups of soil fungal communities were collectively driven by soil nutrition substrates, soil moisture, and plant residues characteristics, which potentially affected soil C and N decomposition and sequestration. Although the impacts of plant invasions on aboveground ecosystem have been widely reported and focused, the potential opportunities and/or risks of belowground ecosystem, particularly soil microbial communities as affected by plant invasions are not yet to fully estimate. This study provided new evidence that plant invasions can alter soil fungal abundance, diversity, community composition, trophic modes and functional groups, which can help us to better understand the variations and its driving patterns of soil fungal communities following plant invasions.

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