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RESEARCH ARTICLE

Continental-scale plant invasions reshuffle the soil microbiome of blue carbon ecosystems

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Abstract

Theory and experiments support that plant invasions largely impact aboveground biodiversity and function. Yet, much less is known on the influence of plant invasions on the structure and function of the soil microbiome of coastal wetlands, one of the largest major reservoirs of biodiversity and carbon on Earth. We studied the continental-scale invasion of *Spartina alterniflora* across 2451 km of Chinese coastlines as our model-system and found that *S. alterniflora* invasion can largely influence the soil microbiome (across six depths from 0 to 100 cm), compared with the most common microhabitat found before invasion (mudflats, Mud). In detail, *S. alterniflora* invasion was not only positively associated with bacterial richness but also resulted in important biotic homogenization of bacterial communities, suggesting that plant invasion can lead to important continental scale trade-offs in the soil microbiome. We found that plant invasion changed the community composition of soil bacterial communities across the soil profile. Moreover, the bacterial communities associated with *S. alterniflora* invasions where less responsive to climatic changes than those in native Mud microhabitats, suggesting that these new microbial communities might become more dominant under climate change. Plant invasion also resulted in important reductions in the complexity and stability of microbial networks, decoupling the associations between microbes and carbon pools. Taken together, our results indicated that plant invasions can largely influence the microbiome of coastal wetlands at the scale of China, representing the first

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continental-scale example on how plant invasions can reshuffle the soil microbiome, with consequences for the myriad of functions that they support.

KEYWORDS
biotic homogenization, coastal wetland, ecological networks, microbial biogeography, *S. alterniflora*, soil carbon

1 | INTRODUCTION

Coastal wetlands are considered fundamental blue carbon ecosystems, playing key roles in regulating carbon sequestration and supporting biodiversity and ecosystem productivity worldwide (Alongi, 2014; Schuerch et al., 2018). Coastal wetlands are highly vulnerable to many aspects of climate change and human activity, due to their pioneering positions in the intertidal zones (Osland et al., 2016). Among these threats, invasive plant species is one of the most important. Strikingly, although the influences of plant invasions are well described for aboveground biodiversity and functions (Chen et al., 2004; He et al., 2007), much less is known their impacts on the structure and function of the soil microbiome—the largest reservoir of biodiversity on Earth. Here, we used the invasion of *Spartina alterniflora*, native to the southeastern coastline of the United States, through the entire coastline of China as a model system to investigate the impact of plant invasions on the soil microbiome across contrasting climatic conditions.

Spartina alterniflora was first introduced to China in 1979 for ecological engineering and has expanded rapidly and extensively along most coastlines of China over the last few decades, encroaching large areas of native bare mudflats (vegetation-free, Mud hereafter) (An et al., 2007; Liu et al., 2018). *Spartina alterniflora* is a perennial herb with a well-developed root system that could reach up to 100 cm underground. The microenvironment varies greatly across different soil depths, such as oxygen status. Thus, the study of the vertical distribution of soil microorganisms before and after *S. alterniflora* invasion is important for an in-depth understanding of the ecological consequences of SA invasion. Currently, the distribution area of *S. alterniflora* in Mainland China has reached 545.80 km^2 by 2015 (Liu et al., 2018), ranging from the Liaoning province to the Guangdong province across more than 20 latitudes (Liu et al., 2018; Zuo et al., 2012). We compared the influence of *S. alterniflora* to the most common microhabitat found before invasion (i.e., Mud; Figure 1). China has approximately 7474.6 km^2 of coastal wetlands, dominated by 5379.8 km^2 of Mud microhabitats (Wang et al., 2020). By doing so, we conducted the first continental-scale example on how soil microbiome responds to plant invasions. The extensive occupation of *S. alterniflora* in coastal wetlands provides a suitable experimental platform for this study to assess the ecological impact of plant invasion on soil microbial communities within its invasive range.

In particular, we carried out a standardized field survey across the coastline of China to investigate the impacts of a model-system plant invasion on the soil microbiome. Among all soil organisms, soil bacteria are the most dominant and diverse organisms of the planet, and support multiple ecosystem functions and services such as nutrient cycling, waste decomposition and carbon sequestration. Because of this, we investigated the influence of plant invasions on the diversity, community composition, ecological networks, and function of soil bacterial microbiomes (our model organism) across China's coastline, compared with Mud microhabitats, which were the most common previously found microhabitat in these ecosystems (Figure 1).

To such an end, we conducted a block design study with 12 sites and paired *S. alterniflora* and Mud microhabitats across 20° of latitudes along the Chinese coastline. In these locations, we analyzed 407 composite soil samples from six soil depths (across from 0 to 100 cm). Standardized soil samplings including multiple soil depths at a continental scale are largely lacking in the literature. Biological homogenization associated with plant invasions has been previously observed for plant (Muthukrishnan & Larkin, 2020; Stotz et al., 2019) and animal communities (Leprieur et al., 2007; Olden & Poff, 2004). Also, recent studies have provided evidences of biological homogenization for fungi at local scale (Zhang et al., 2021) and nematode across the coastlines in China (Zhang et al., 2019) associated with plant invasions. Thus, we hypothesized that the invasion of *S. alterniflora* can result in an important biotic homogenization of bacteria by creating very similar environments associated with *S. alterniflora* microbiomes across the Chinese coast.

2 | MATERIAL AND METHODS

2.1 | Study sites

Based on the extent area of *S. alterniflora* distribution in China, a total of 12 sites were selected across more than 20 latitudes (ranging from 20.60°N to 40.80°N), including Huludao (HLD, introduced in 1980s), Tanggu (TG, introduced in 1997), Dongying (DY, introduced in 1990), Lianyungang (LYG, introduced in 1982), Yancheng (YC, introduced in 1983), Chongming (CM, introduced in 1995), Yueqing (YQ, introduced in 1983), Xiapu (XP, introduced in 1980), Yunxiao (YX, introduced in 1999), Zhuhai (ZH, introduced in 1980s), Beihai (BH, introduced in 1986), and Zhanjiang (ZJ, introduced in 1980s) sites (Figure 1a). These sites belong to temperate monsoon climate or tropical-subtropical monsoon climate, with a mean annual temperature ranging from 4.17 to 23.98°C (Figure 1a).

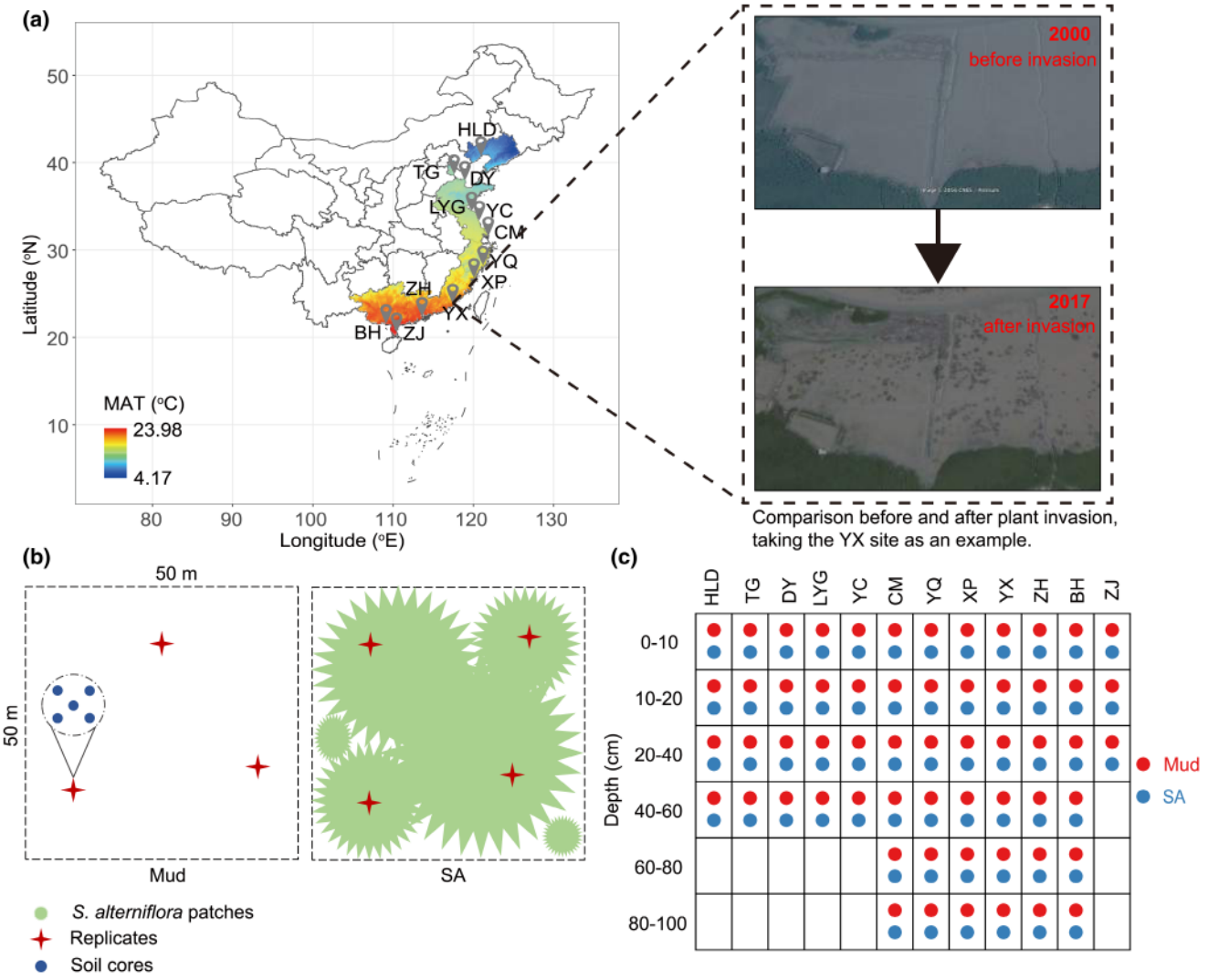


FIGURE 1 The sample sites and their corresponding MAT along the coastal wetlands in China (a). The locations of each replicate in a 50×50 m quadrat for mud and *Spartina alterniflora* habitats. Five intact soil cores were randomly collected and completely mixed as a replicate (b). Schematic diagram of the sampling depth for each sample site (c). Mud: Bare mudflat; SA: *S. alterniflora*; HLD: Huludao; TG: Tanggu; DY: Dongying; LYG: Lianyungang; YC: Yancheng; CM: Chongming; YQ: Yueqing; XP: Xiapu; YX: Yunxiao; ZH: Zhuhai; BH: Beihai; ZJ: Zhanjiang. MAT, mean annual temperature [Colour figure can be viewed at wileyonlinelibrary.com]

At each site, we sampled soils under paired individuals of Mud and *S. alterniflora* microsites (Figure 1b). Within each site, the selected Mud and *S. alterniflora* habitats are located at the comparable elevations and experience a similar tidal dynamic. All selected sample sites were in the mid-tide levels, between the neap high tide and neap low tide levels. In the present study, the soil bacteriome of Mud and *S. alterniflora* habitats were compared to reflect the ecological consequences of *S. alterniflora* invasion on the most common native habitats before invasion for two reasons: (1) Emerging evidence suggest that the expansion of *S. alterniflora* in China was mainly converted from Mud habitats; (2) The native plants of China's coastal wetlands are different from North to South, and it is impossible to find consistent native plants at such a large spatial scale. Due to this reason, Mud habitats were selected as our reference habitat to exclude the possible bias caused by native vegetations.

2.2 | Collection of soil samples and environmental variables

Soil samples were collected in October 2018 following a consistent sampling and processing scheme. Specifically, a quadrat with 50×50 m was established at each site for Mud and *S. alterniflora* habitats, respectively (Figure 1b). Within this quadrat, three replicates for Mud habitat and four replicates for SA habitat were randomly sampled, with at least 15 m away in geographical distance from each other. Then, around each replicate, five intact soil cores (~within 1 m to the centroid point) were randomly collected by using PVC pipes (5 cm diameter, 100 cm length). Here, considering the highly developed root system of SA that might alters the microbial distributions between soil depths, soil in different depths were collected up to 100 cm maximum. It is important to note that due to the differences in soil textures, individual plots could only be collected to a

maximum depth of 40–60 cm. After achieving the soil cores, they were then divided into six sections (0–10 cm, 10–20 cm, 20–40 cm, 40–60 cm, 60–80 cm, and 80–100 cm) using a stainless steel knife, referring to different soil depths (Figure 1c). The corresponding soils from all the five soil cores were completely homogenized as a replicate. Soil samples were sealed immediately after removing gravel stones and other debris. Finally, a total of 407 soil samples were obtained, of which 177 and 230 samples for Mud and *S. alterniflora* habitat, respectively (Table S1). All the soil samples were stored on ice during the transportation.

A suite of 18 environmental variables were obtained, including 11 soil properties and 7 climatic factors (Gao et al., 2022). For soil variables, soil temperature of corresponding sample was determined in-situ using a mercury thermometer. Soil redox potential (Eh, mV) and salinity/pH were measured by inserting portable probes into the corresponding depth, by using the ExStikTM RE300 and ExStikTM EC500, respectively. In the laboratory, soil water content was determined by 30°C oven-drying of 50 g fresh soil to a constant weight. The air-dried soils were ground into powder and sieved through a 2 mm mesh sieve. Soil electrical conductivity (EC) was determined at 25°C by using a conductivity meter (Leici DDS-307). Soil organic matter (SOM) was measured based on the loss on ignition at 550°C for 6 h after 105°C oven-dried (Heiri et al., 2001). Soil total carbon (TC), total nitrogen (TN), and total sulfur (TS) were determined using a Vario EL III Elemental Analyzer (Elementar), and then the carbon-to-nitrogen ratio (C/N) was calculated. For the climatic factors, the mean annual temperature (MAT), mean annual precipitation (MAP), isothermality, precipitation seasonality, and temperature seasonality were compiled from the WorldClim version 2 (https://www.worldclim.org/) at 30 arc-second resolutions. Aridity index and potential evapotranspiration (PET) were obtained from Global Aridity Index and Potential Evapotranspiration (ET0) Climate Database v2 (https://cgiarcsi.community), respectively.

2.3 | Soil DNA extraction and high-throughput sequencing

Soil DNA was extracted by using FastDNA SPIN Kit (MP Biomedicals), according to the manufacturer's instructions. Then, the quality and quantity of the extracted DNA were checked by using NanoDrop2000 spectrophotometer (NanoDrop Tech) and 1% agarose gel electrophoresis, respectively. The V4–V5 region of the bacterial *16S rRNA* gene was amplified using the primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′). PCR reactions were performed on ABI GeneAmp® 9700 (ABI). High-throughput sequencing was performed on an Illumina MiSeq PE300 platform (Illumina, Inc.). All the generated raw sequences have been deposited in the Genome Sequence Archive in National Genomics Data Center, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences, under the accession number of CRA005208 (https://bigd.big.ac.cn/gsa/browse/CRA005208).

2.4 | Bioinformatic analyses

Raw reads were processed using the amplicon sequence variants (ASV) method by Quantitative Insight into Microbial Ecology 2 (QIIME2) (Bolyen et al., 2019). Sequences with poor-quality (read length <200 bp or average quality score <25) were discarded (Ladau et al., 2018). Finally, a total of 23,508,265 sequences were obtained. The clean sequences were denoised using the DADA2 pipeline (Callahan et al., 2016), and generated a total of 49,497 ASVs. Representative sequences were used to construct the phylogenetic tree and for species annotations. SILVA database (https://www.arb-silva.de/) was applied to assign the taxonomy. All the soil samples were rarefied to 28,759 sequences (minimum) per sample for downstream analyses.

2.5 | Co-occurrence network constructions

All the 407 soil samples were used to construct the co-occurrence networks by using the *WGCNA* package (Langfelder & Horvath, 2012). Amplicon sequence variants that occur in <5 soil samples were excluded (Gao et al., 2021), leaving a total of 5134 ASVs. The pairwise Spearman correlations matrix among ASVs were calculated, and *p* values were corrected using Benjamini Hochberg's correction (Benjamini et al., 2006). Nodes with correlations >.5 and $p < .001$ were retained (Delgado-Baquerizo et al., 2018). The networks were visualized using Gephi (version 0.9.2). Then, the ecological modules within the network were identified in Gephi, and the relative abundance of each ecological module was calculated by averaging the standardized relative abundance (*Z*-score). Sub-networks were generated from the original network by preserving the presented nodes and edges of the target samples. The topological parameters of all the generated networks were calculated, including betweenness centrality (the number of times a node acts as a bridge along the shortest path between two other nodes), closeness centrality (the number of steps required to access all other nodes from a given node), clustering coefficient (a ratio of the number of links between the neighbors of a node, and the maximum number of links that could possibly exist between its neighbors) and degree (number of edges connecting a node to other nodes). The average degree (avgK) represents the average number of edges per node, was used to describe the network complexity (Yao et al., 2014). Natural connectivity provides a sensitive discrimination of network structural robustness. Thus, network robustness was estimated by removing nodes in the static network to assess how quickly the natural connectivity degraded (Peng & Wu, 2016; Wu et al., 2010).

2.6 | Statistical analyses

To explore the environmental variables affecting bacterial alpha diversity, the importance of variables was analyzed using the partial correlations method (Kim, 2015). We identified the major

environmental factors that influence the taxa's relative abundance (i.e., the top 10 phyla and core species). Multiple linear regressions were fitted separately between taxa's relative abundance and environmental variables using the lm function. Backward stepwise regression was then performed to filter variables using stepAIC in *MASS* package. For the optimal model obtained, the contribution of the main environmental variables to the total variance in taxa's relative abundance was assessed using the calc.relimp function in *relaimpo* package. A classification of random forest model was used to identify biomarkers between Mud and SA habitats on class level using the *randomForest* package (Liaw & Wiener, 2002). Then, the accuracy of the classification model was assessed by receiver operating characteristic (ROC) and the prediction of confusion matrix. Those ASVs that occurring in more than 50% samples were defined as core species for Mud and *S. alterniflora* habitat, respectively. Covariate adjusted principal coordinates analysis was used to describe pairwise dissimilarity between samples based on the weighted Unifrac distance (Shi et al., 2020). Pairwise permutational multivariate analysis of variance (PERMANOVA) was used to test the significance of differences in bacterial communities between soil samples. To better understand the biodiversity patterns and to explore their underlying mechanisms, compositional dissimilarities (BDtotal) were divided into Repl (species replacement) and RichDiff (difference in abundance) components using the *adespatial* package. Dissimilarity-overlap curve (DOC) was used to test whether the underlying ecological dynamics of microbiomes are universal across all communities or unique to individual communities (Kalyuzhny & Shnerb, 2017). We used a variety of methods to explore the role of environmental factors in influencing bacterial communities. First, the variation partitioning analysis (VPA) was performed to estimate the effects of space, depth, climate, and soil factors on bacterial community using the *vegan* package. Then, Mantel and partial Mantel tests were applied to examine the relationships between environmental variables and bacterial communities. Finally, these relationships were tested by the multiple regression on distance matrices (MRM) using the *ecodist* packages, after filtering the environmental variables with high multicollinearity (Spearman $\rho^2 > .7$) (Wang et al., 2017). Fast expectation–maximization microbial source tracking (FEAST) analysis was used to estimate the source proportion of bacteria in *S. alterniflora* soils that derived from Mud soils, and vice versa (Shenhav et al., 2019). All analyses involving R software were performed under R version 4.0.5 (R Core Team, 2018), unless otherwise stated.

The structural equation model (SEM) was used to evaluate the direct and indirect effects of biotic (i.e., module abundance, bacterial richness, network complexity) and abiotic factors (i.e., climatic factors, soil properties) on soil TC. The predicted causal relationships of the SEM were constructed based on prior knowledge (Delgado-Baquerizo et al., 2020). All the environmental variables were treated as independently observed variables in the SEM, rather than latent variable. Prior to SEM analysis, the multicollinearity of environmental variables was examined by using the *Hmisc* package, and those variables with Spearman ρ^2 greater than .7 were removed. Then, Random Forest analyses were performed to identify the key predictors that influencing soil TC by using the *rfPermute* package. A 1000 permutation was then applied to compute the null distribution and calculate the *p* values. Since most of the variables were not normally distributed, the bootstrapping method were used to evaluate the probability that the path coefficients differed from zero (Delgado-Baquerizo et al., 2017). Then, three indices were used to assess the SEM performance (Schermelleh-Engel & Moosbrugger, 2003): (1) Chi-square (χ^2) test, a good fit is defined as $0 \le \chi^2/\text{df (degree of freedom)} \le 2$, and $0.05 < p \le 1.00$; (2) Root mean square error of approximation (RMSEA), in which a good fit is defined as $0 \le \text{RMSEA} \le 0.05$; (3) Bollen-Stine bootstrap test, a good fit is defined as $0.10 < \text{Bollen-Stine bootstrap } p \le 1.00$. The standardized total effect of each predictor on soil TC was calculated. SEM analyses were performed in AMOS 21 (IBM SPSS, Inc.).

3 | RESULTS

3.1 | Plant invasions causes drastic changes in soil environments and on the biodiversity and community composition of soil bacteriomes

A total of 18 environmental variables were obtained, including 11 soil properties (i.e., pH, salinity) and 7 climatic factors (i.e., MAT, MAP) (Table S2). Results show that environmental variables varied considerably among habitats, sites, and soil depths. *S. alterniflora* soils have higher EC, salinity, TN, SOM and water content than that of Mud soils, but lower pH values. We further showed that plant invasions cause important homogenization in soil environments. In particular, we found that important soil properties such as Eh, soil temperature, TC, TS, C/N, and water content had smaller changes in environmental variability than those in Mud soils. Yet, Mud habitat and *S. alterniflora* habitat had similar levels of TC ($p = .08$) (Figure 2a). Both in the Mud and SA habitats, the soil carbon content had a distinct latitudinal distribution pattern, showing the highest at approximate 34°N regions. Not only that, compared to *S. alterniflora* habitat, the soil carbon in Mud habitat exhibited a larger increase with increasing soil depth and then a subsequent decrease (Figure 2b). Compared to Mud habitat, carbon accumulation in SA habitats was mainly between the soil surface and 60 cm below ground.

We then investigated patterns in soil microbial diversity. We used richness (observed ASVs) to present the taxonomic alpha diversity of bacteria. Richness ($p < .05$) were significantly higher in SA soils compared with Mud soils, but not for all the sites (Figure 3a, Table S3). In general, richness increased with latitude but decreased with soil depths (Figure 3b; Figure S1). We analyzed soil properties against richness for revealing the main predictors. Three-ways analyis of variances showed that richness was mainly influenced by the sample site heterogeneity ($F = 42.37$, $p < .001$) (Table S4). Partial correlations analyses suggested that

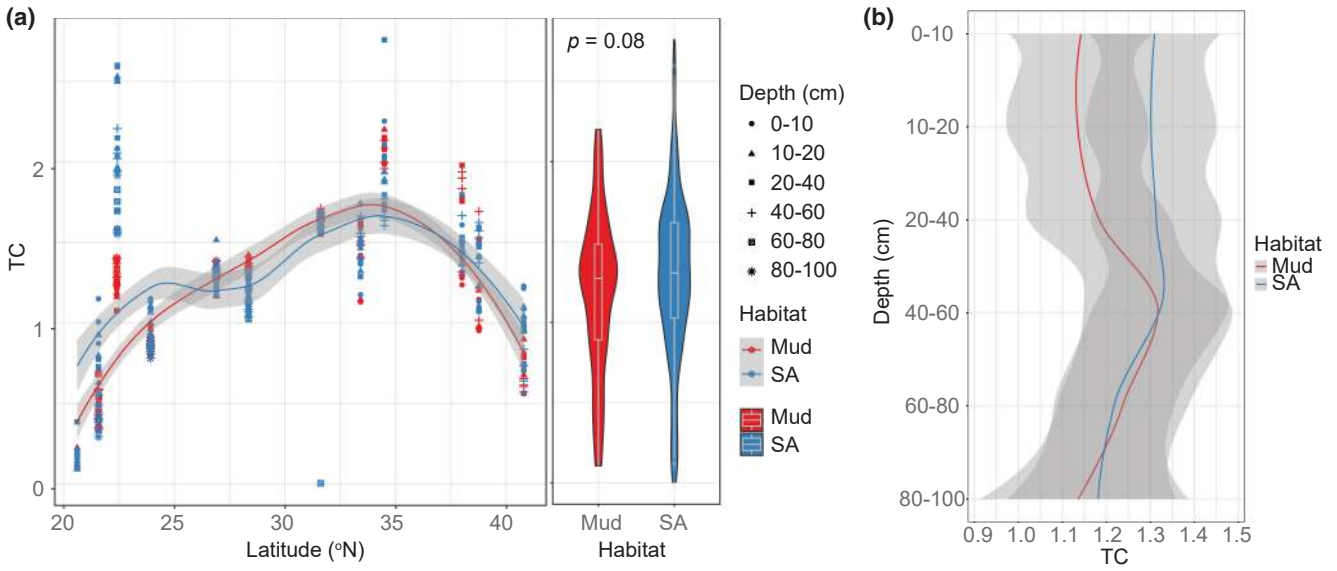


FIGURE 2 Soil TC content in coastal wetlands. The latitudinal pattern of soil TC for mud and *Spartina alterniflora* habitats respectively (a). Variation of soil TC with depth (b). Mud: Bare mudflat; SA: *S. alterniflora*. TC, total carbon [Colour figure can be viewed at wileyonlinelibrary.com]

soil properties, rather than climatic factors, were the primary factors in influencing richness (Figure 3c). Among all the environmental variables, soil temperature was found to be the best predictor (Figure S2, Table S5). Binomial regressions showed that richness decreasing when soil temperature beyond the optimum ranges (Figure 3d).

Venn diagram showing the share and unique ASVs, with 15,259 ASVs and 24,591 ASVs unique to Mud and *S. alterniflora* soils respectively (Figure S3a). A total of 60 phyla were identified from all the soil samples, and the most abundant phyla was Proteobacteria, Chloroflexi, and Epsilonbacteraeota, and their relative abundances varied considerably across different sites and depths (Figure S3b). Among the top 10 phyla, *S. alterniflora* soils have significantly ($p < .05$) higher relative abundances of Bacteroidetes, Acidobacteria, Planctomycetes, Gemmatimonadetes, Nitrospirae, and Latescibacteria than Mud soils, while have lower Epsilonbacteraeota (Figure 4a). Distinct latitudinal distributions were observed for these phyla, and the measured environmental variables could explain 21.43%–74.01% of the variations in their relative abundances (Figure 4b, Figure S4).

We identified 72 classes as the biomarkers between Mud and *S. alterniflora* soils (Figure S5a). Both the results of area under curve (AUC = 0.77) of ROC and prediction of confusion matrix ($0.21 < \text{error} < 0.22$) suggested a high accuracy of the classification model (Figure S5b, Table S6). These biomarkers included Spirochaetia, Phycisphaerae, Clostridia, and others (Figure S5c). In addition, at the 50% occurrence threshold, we identified eight and five core ASVs for Mud and SA soils respectively (Figure S6a). Among these core ASVs, 7 out of 13 ASVs were belong to class Campylobacteria, and the other 6 ASVs were belong to class Gammaproteobacteria (Table S7). Although these core ASVs were present in the majority of soil samples, their relative abundances were varied across different sites and showed a clear latitudinal pattern, which found to be mainly influenced by environmental factors (Figures S6b,c and S7).

3.2 | Biotic homogenization of soil bacterial communities by *S. alterniflora*

Our findings further revealed that plant invasions are associated with important continental-scale homogenizations of the soil bacteriomes of coastlines. First, we found significant (*pseudo-F* = 8.54, $p < .001$) differences in the soil bacterial communities between Mud and SA habitats (Figure 5a). PERMANOVA suggested that soil bacterial communities were mainly influenced by the factor of sample sites (*pseudo-F* = 23.41, $p < .001$), followed by depths (*pseudo-F* = 8.84, $p < .001$) and habitats (*pseudo-F* = 8.54, $p < .001$) (Table S8). We then showed that, in general, soil microbiomes in *S. alterniflora* microhabitat support less variability (distance of each sample to its central point) among bacterial communities than Mud soils, suggesting the biotic homogenization of bacterial community in *S. alterniflora* soils compare to Mud soils (Figure 5b; Figure S8). A significant pattern of distance decay relationship (DDR) was observed, with the slopes of DDR of the *S. alterniflora* soils higher than that of Mud soils, suggesting higher spatial turnover of bacterial community after plant invasion (Figure 5c). Beta diversity (represented by BDtotal) includes two components: species replacement (Repl) and change in abundance (RichDiff). Results showed that Repl dominated the bacterial community variation, accounting for 80.85% and 79.17% for Mud and *S. alterniflora* soils respectively (Figure S9). DOC analysis further revealed that these ecological dynamics of bacterial communities were universal across metacommunities (Figure S10a). Microorganisms could spread from one habitat to another. We used FEAST (source tracking) method to quantify this dispersal event, and found a

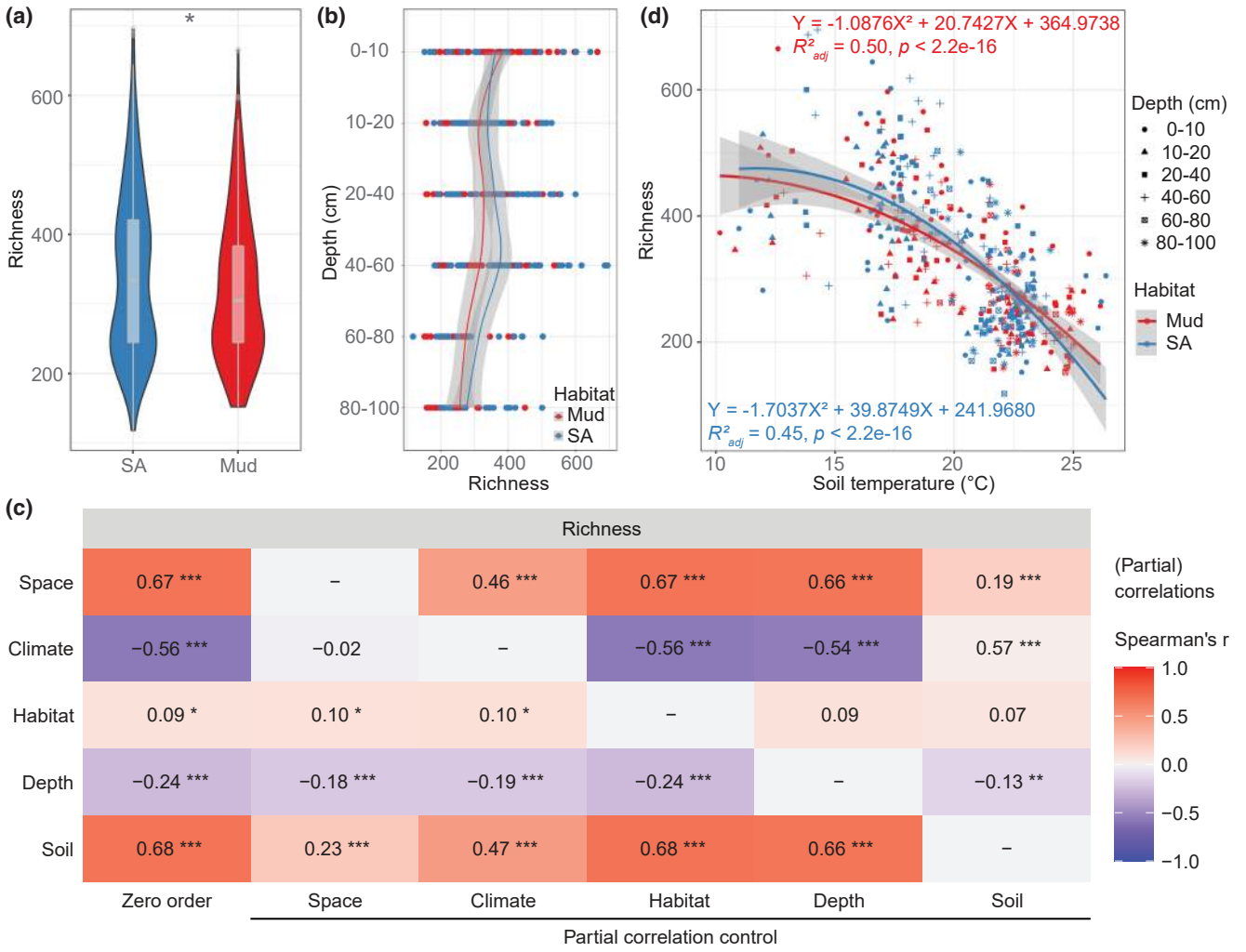


FIGURE 3 The influence of plant invasions on the response of bacterial richness to environmental variability. Difference in bacterial richness between mud and *Spartina alterniflora* habitats, and the significance was examined by Wilcoxon rank sum test (a). Variations of bacterial richness in various depths (b). Partial spearman correlations between bacterial richness and the groups of environmental variables (i.e., space, climate, habitat, depth and soil) (c). All observed variables were grouped into corresponding groups and constructed as latent variables. Latent variables were constructed using the partial least squares path modeling (PLS-PM) method. The relationships between richness and soil temperature (d). Both linear and quadratic models were used to fit these relationships, and best models were selected based on a low Akaike's information criterion (AIC) value. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$. Mud: Bare mudflat; SA: *S. alterniflora* [Colour figure can be viewed at wileyonlinelibrary.com]

higher proportion of Mud was derived from *S. alterniflora* than vice versa (Figure S10b).

We then used multiple approaches to explore the environmental variables influencing the bacterial community structure. First, partial Mantel tests demonstrated the critical role of soil properties and climatic factors in affecting bacterial communities (Table S9), which were further confirmed by the VPA analyses (Figure S11a). Then, the relationships between bacterial communities and environmental variables were tested by Mantel tests (Figure S11b, Table S10). MRM method, an extension of partial Mantel analysis which offers several advantages over partial Mantel tests, were applied to explore the key predictors (Table S11, Figure S12). MRM revealed that TS and TN were the decisive factors in influencing bacterial communities for Mud and *S. alterniflora* soils, respectively.

3.3 | *S. alterniflora* invasion reduces the complexity and robustness of co-occurrence networks

Co-occurrence networks were used to identify soil bacterial taxa organized within closely-linked ecological clusters. The network consists of 4230 nodes and 41,506 edges, with a high number of major nodes belong to phylum of Proteobacteria (51.49%), Bacteroidetes (17.02%), Chloroflexi (7.85%), and Epsilonbacteraeota (6.93%) (Figure S13a). There are many nodes clustered together (modules) in the network, of which, there are seven modules containing more than 300 nodes, namely Modules #36 (14.96%), Modules #45 (10.47%), Modules #27 (9.57%), Modules #25 (9.27%), Modules #42 (7.75%), Modules #22 (7.71%), and Modules #37 (7.66%) (Figure 6a; Figure S13b). Each module contains a wide variety of bacterial taxa, among

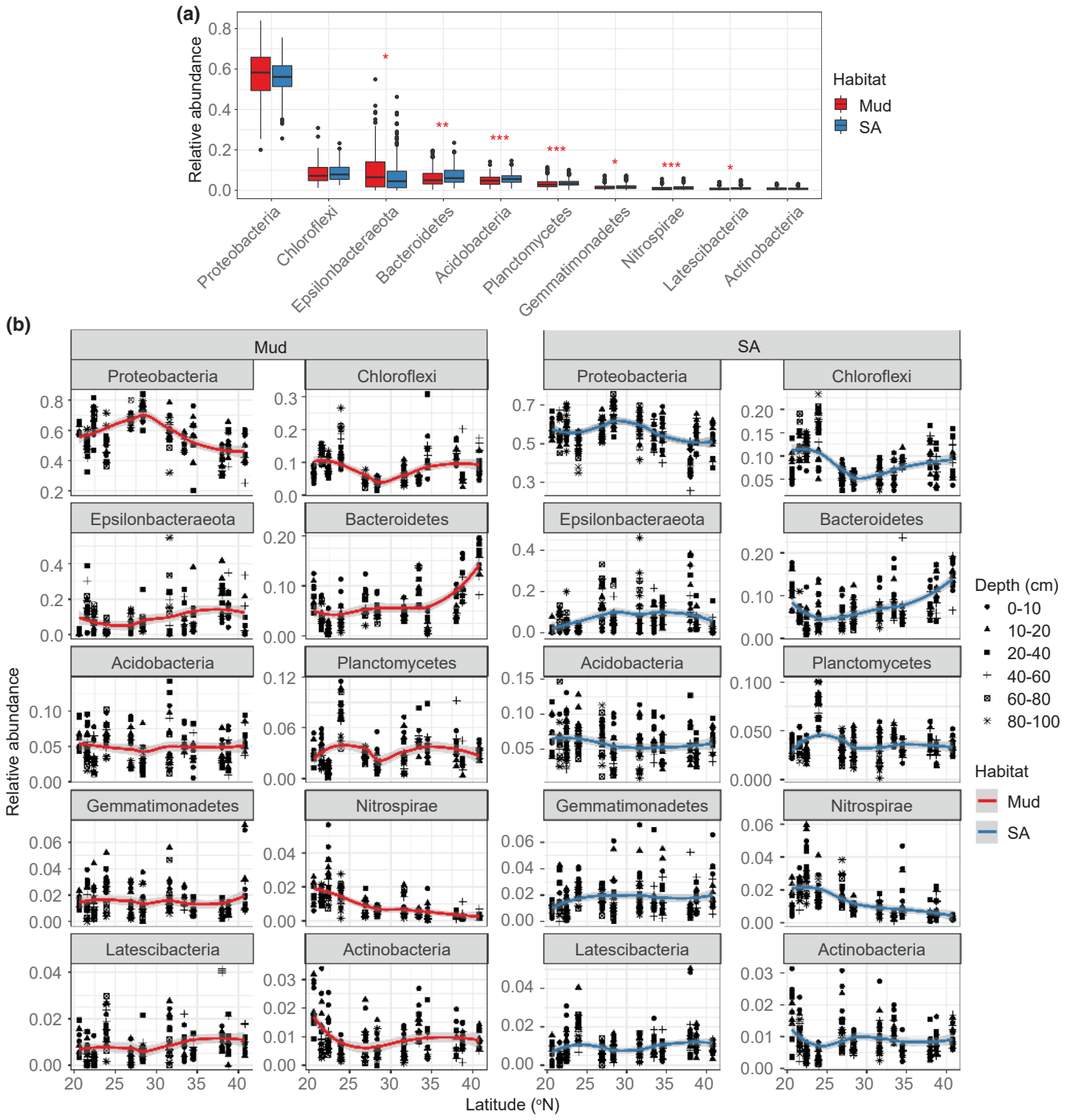


FIGURE 4 The relative abundance of the bacterial top 10 phyla. Differences in the relative abundance of the top 10 bacterial phyla between mud and *Spartina alterniflora* habitats (a). Trends in the relative abundance of the top 10 bacterial phyla with latitude (b). $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$. Mud: Bare mudflat; SA: *S. alterniflora* [Colour figure can be viewed at wileyonlinelibrary.com]

which the more abundant class groups include Campylobacteria, Deltaproteobacteria, and Bacilli (Figure 6b). The relative abundance of these modules was significantly ($p < .05$) higher in *S. alterniflora* habitats than in Mud habitats, including Modules #22, Modules #42, and Modules #45 (Figure 6c). Network properties characterize the physical structure of the network, and the Mud network has significantly ($p < .05$) higher closeness centrality (Mud: 0.20; *S. alterniflora*: 0.19) and degree (Mud: 19.27; *S. alterniflora*: 17.50) than the *S. alterniflora* network, suggesting a more complex network of Mud habitat (Figure S14a,b). In addition, the natural connectivity of the network decreased sharply as the proportion of removed nodes increases, but the slope of the decline of Mud network was lower than that of the *S. alterniflora* network, means a more stable network of the Mud network (Figure 6d). In order to understand the spatial distributions of the network, sub-networks were constructed, and results showed

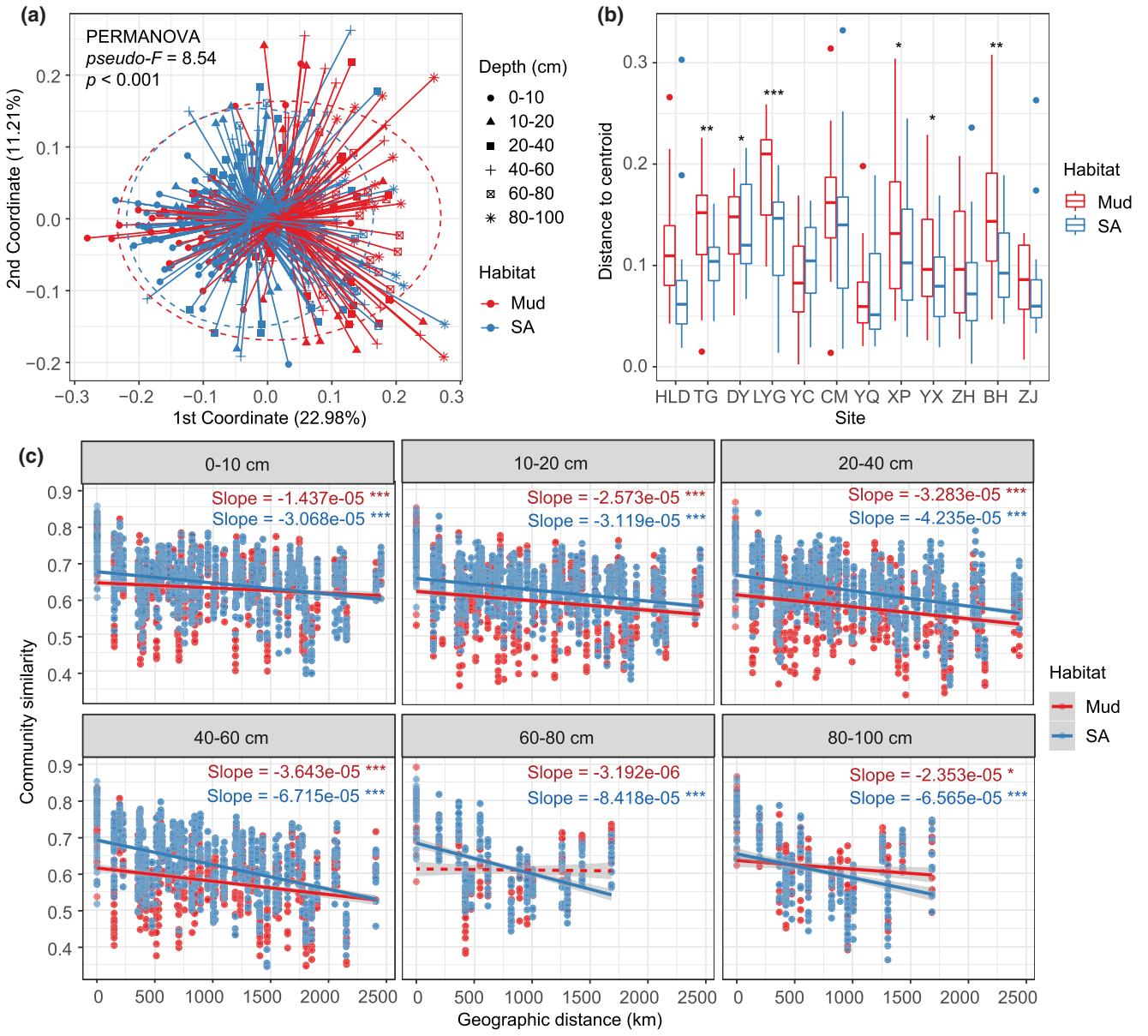


FIGURE 5 The influence of plant invasions on bacterial community composition and heterogeneity. Results of the covariate adjusted principal coordinates analysis (aPCoA) based on the weighted UniFrac distance revealing the pairwise dissimilarities between mud and *Spartina alterniflora* habitats. The significant differences were examined by pairwise permutational multivariate analysis of variance (PERMANOVA) tests (a). Distance of soil bacterial community to centroid point within each sample site (b). Distance decay relationship reveals the relationship between bacterial community similarity and geographical distance (c). The solid and dashed lines indicate significant and insignificant relationships, respectively. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$. Mud: Bare mudflat; SA: *S. alterniflora* [Colour figure can be viewed at wileyonlinelibrary.com]

that the network complexity increases with increasing latitude for both Mud and *S. alterniflora* soils (Figure 6e; Figure S14c).

3.4 | Associations between bacterial communities and soil carbon pools

Structural equation model analysis helps us to understand the relationships between soil bacterial communities and soil TC, while considering a variety of biotic and abiotic factors simultaneously. Based on the initial model (Figure S15), a Random Forest analysis was performed to identify the key environmental factors (Figure S16). SEM results showed that soil TC was regulated by a combination of environmental factors, of which, the most important were soil properties and climatic factors (Figure 7). Notably, bacterial community also played an important role in influencing TC, in detail, TC was significantly and positively correlated with avgK (path coefficient = 0.15***) and module #45 (path coefficient = 0.15***), while significantly and negatively correlated with module #36 (path coefficient = −0.35***). Not only that, the presence of aboveground plants also has a positive effect on soil TC (Figure 7a). By analyzing the total effect of each predictor, we found that ST was the most important

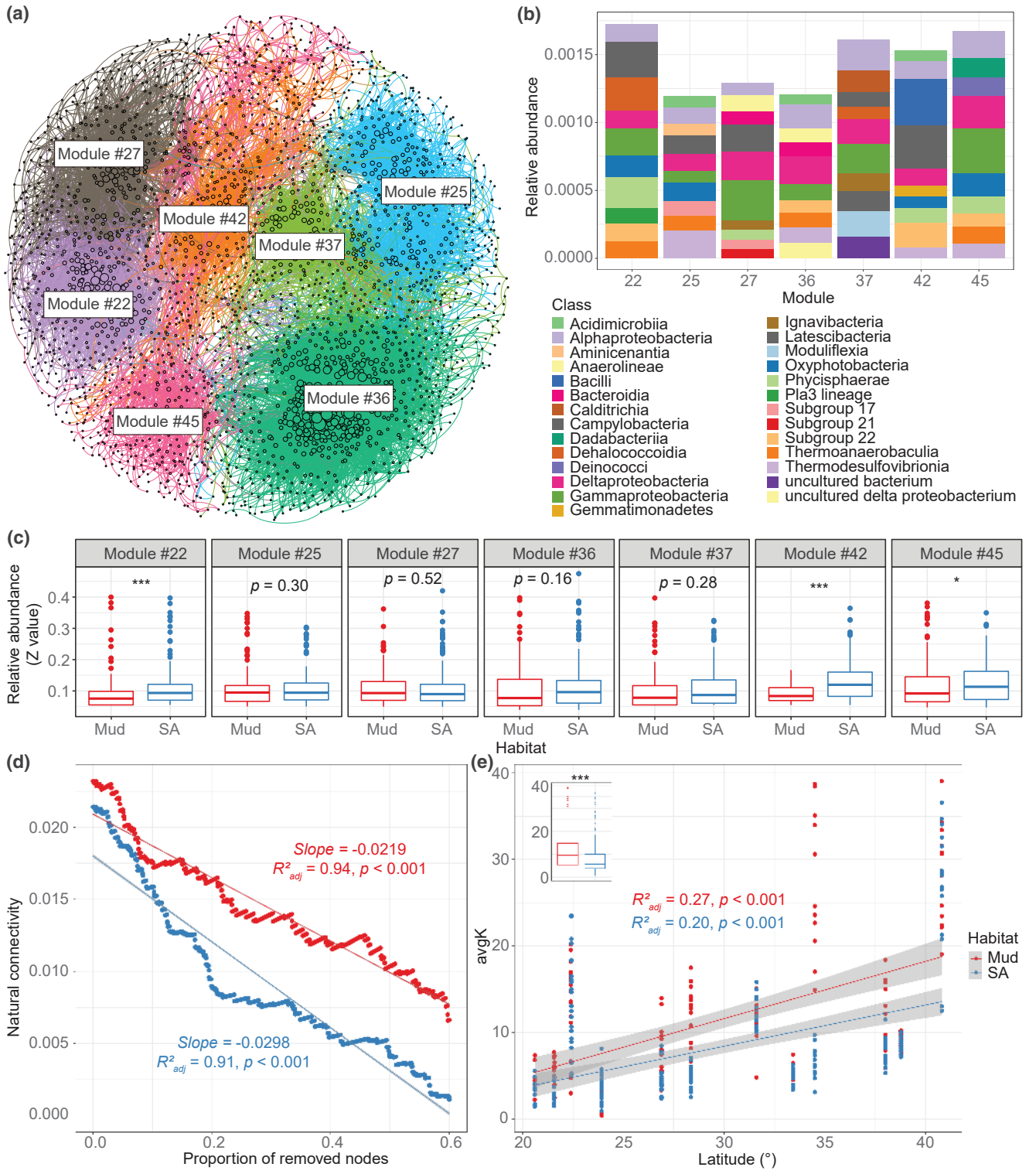


FIGURE 6 The influence of plant invasions on soil ecological networks. Modules (groups of taxa highly co-occurring with each other) with >300 taxa (nodes) within the ecological network (a). The community composition for the top microbial modules (b). The difference in the relative abundance of each ecological module between mud and *Spartina alterniflora* habitats, and significance was tested by Wilcoxon test (c). The network structural robustness assessed by the decline in natural connectivity against the removing nodes (d). The higher the slope represents the more drastic the decline in network structural robustness, in other words, the more unstable the network is. The latitudinal pattern of the network's average degree (avgK), representing the network complexity (e). *$p < .05$; ***$p < .001$. Mud: Bare mudflat; SA: *S. alterniflora* [Colour figure can be viewed at wileyonlinelibrary.com]

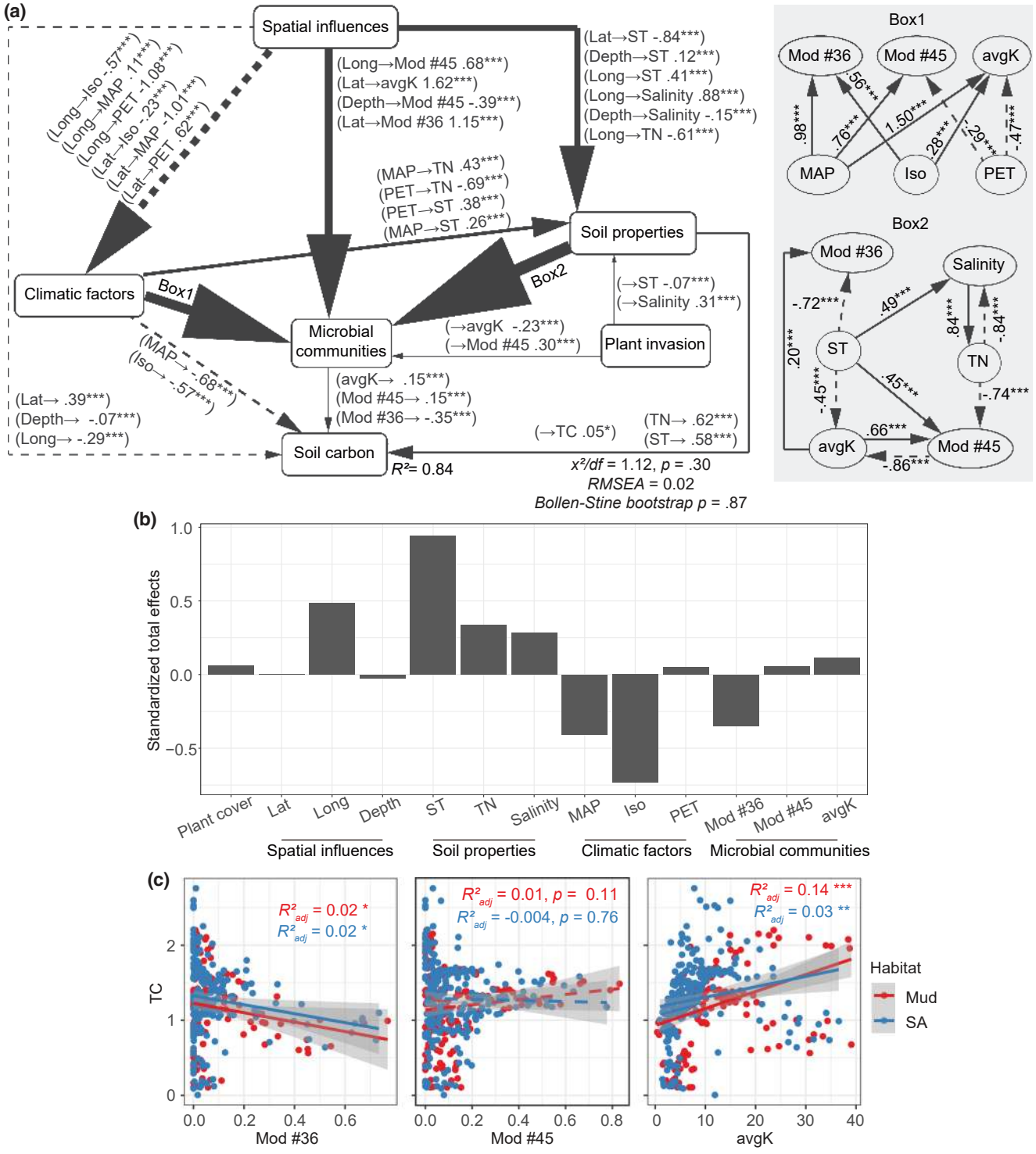


FIGURE 7 Structural equation model (SEM) revealing the direct and indirect effects of environmental variables on soil TC (a). To obtain a simplified graphic, different blocks were used to represent the various types of environmental factors, but note that all variables were treated as observed variables. Numbers within parentheses are the path coefficients and are indicative of the standardized effect size of the relationship. Solid and dashed arrows indicate positive and negative effects, respectively. R^2 value means the proportion of variance explained. The standardized total effects of each predictor attributes on soil TC (b). The relationships between soil TC and bacterial communities (mod #36, mod #45, and avgK) (c). Solid and dashed lines indicate significant and insignificant correlations, respectively. Long: longitude; Lat: latitude; Mod: module; Iso: isothermality; MAP: mean annual precipitation; PET: potential evapotranspiration; avgK: average degree; ST: soil temperature; TN: total nitrogen; TC: total carbon; Mud: Bare mudflat; SA: *S. alterniflora*. $^*p < .05$; $^{**}p < .01$; $^{***}p < .001$ [Colour figure can be viewed at wileyonlinelibrary.com]

factor in regulating soil TC (Figure 7b). From the results of the linear regression analysis, the slope of the correlation between module #45 and avgK and TC was reduced in *S. alterniflora* habitats compared to Mud habitats, implying that the role of these bacterial taxa in TC accumulation was diminished (Figure 7c). Overall, these results have revealed the strong associations between bacterial communities and soil TC, and which were reduced after plant invasion.

4 | DISCUSSION

4.1 | Continental-scale *S. alterniflora* invasion increases bacterial alpha diversity, and induces biotic homogenization of soil bacterial communities

Plant invasions are an important threat to local biodiversity conservation and ecosystem maintenance in the context of global change (Bradley et al., 2010; Theoharides & Dukes, 2007). In the last few decades, many studies have been conducted focusing on the effects of *S. alterniflora* invasion on plant communities and soil biotas in coastal wetlands (Chen & Wen, 2021; Yang et al., 2016). However, most of the previous studies focusing on soil communities have been carried out at a local scale. Here, we present the first continental-scale study investigating the impacts of plant invasions on soil microbiomes in blue carbon coastal ecosystems.

We found that *S. alterniflora* invasion significantly ($p < .05$) increased bacterial alpha diversity in Chinese coastal wetlands (Figure 3). Our results were consistent with previous local-scale studies which revealed that SA invaded into Mud soils was found to strongly increase the bacterial alpha diversity, in Fujian coastlines of southeast China (Chen & Wen, 2021; Liu et al., 2017) and in Jiangsu Yancheng Wetland National Nature Reserve (Yang et al., 2019). However, studies with inconsistent results also exist, Gao et al. (2019) found that the bacterial alpha diversity of *S. alterniflora* soils in the Zhangjiang River Estuary Mangrove National Natural Reserve was comparable to that of the Mud soils. Previous studies were done at a local scale, often impacted by local environmental contexts, and difficult to interpret at a larger spatial scale covering contrasting soil and climatic conditions. Unlike for those previous local studies, our standardized continental survey aimed to capture the environmental heterogeneity supporting the invasion of plants and their impacts on soil microbiomes. Here, we provide continental-scale evidences that plant invasions can promote soil bacterial diversity at the scale of China by introducing new species not previously present in Mud soils, because plant colonization may provide resources and ecological niches for the rare biosphere (Chen et al., 2019; Saleem et al., 2016). For instance, Venn diagrams showed the share and unique ASVs, with 15,259 ASVs and 24,591 ASVs unique to Mud and *S. alterniflora* soils respectively (Figure S3a). These results suggest that plant invasions can have a previously unreported impact on soil biodiversity, as previously found for plant and animal diversity.

We further provided unprecedented evidences that continental-scale *S. alterniflora* invasion in China can cause important biotic homogenization of soil bacterial community (Figure 5a,b; Figure S8). Biotic homogenization following biological invasions is commonly found for aboveground biodiversity (Stotz et al., 2019). For example, it has been shown in many studies that biological invasions could lead to biotic homogenization across different ecosystems, for animals and plants (Delgado-Baquerizo et al., 2021; Gossner et al., 2016; Leprieur et al., 2007; Zhang et al., 2019). However, much less studied are the impacts of plant invasions on soil microbiomes. The occurrence of biotic homogenization does not conflict with our earlier observation that *S. alterniflora* invasions increase alpha diversity, as increases in local alpha diversity are usually accomplished at the expense of beta diversity (Whittaker, 1972). Biotic homogenization is a process whereby some species in a community are replaced by other species (Olden et al., 2004; Olden & Poff, 2003). The invasion of *S. alterniflora* could lead to biotic homogenization of soil microbial community by altering soil physic-chemical properties, making them more homogenous. In addition, compared to *S. alterniflora* habitats, the dominant original native ecosystems (Mud soils) are not protected by any vegetation, and are more susceptible to environmental disturbance, which may cause greater microbial fluctuations. Not only that, but bacteria in *S. alterniflora* soils appeared to have a greater dispersal capacity than that in Mud soils, as indicated by the results of source tracking (Figure S10b), which may favor biotic homogenization within the habitats. As a result, biotic homogenization causes a narrowing of ecological niches within the community, making microbes more responsive to environmental change and thus accelerating the spatial turnover of the bacterial community (Figure 5c). However, it is currently unclear whether biotic homogenization also occurs at the genetic and functional levels following *S. alterniflora* invasion, beyond the taxonomic level. It has been shown that biotic homogenization appears to reduce the multifunctionality in forest ecosystems (Van Der Plas et al., 2016). Therefore, we speculate that SA invasion along the coastline in China may impose strong influences on the microbial communities and the biogeochemical cycles they support, and allow for the weakening or even loss of certain ecological functions. Such changes would threaten the critical role of coastal wetland ecosystems in global carbon cycling and climate change regulation.

4.2 | *S. alterniflora* invasion reduces the complexity and robustness of co-occurrence networks

Soils are home to hundreds of millions of microorganisms interacting with each other and driving the fundamental soil processes (Faust & Raes, 2012; Layeghifard et al., 2017). Dissecting the structure of soil microbial coexistence is important for a deeper understanding of the evolution of soil ecological functions in response to environmental changes. The impact of plant invasion on soil microbial networks has mostly been studied at the local scale, but works at large spatial scales (i.e., continental scale) is still lacking (Chen & Wen, 2021; Zhang et al., 2020). Here, we found that the continental-scale SA invasion causes a significant

reduction in connectivity between nodes within network and a consequent reduction in network stability (Figure 6; Figure S14). Our study was consistent with that of others who also found a decrease in network complexity of fungal communities after *S. alterniflora* invasions in a salt marsh ecosystem (Zhang et al., 2021). On the contrary, previous study reported that *S. alterniflora* invasion enhanced the bacterial interactions in the Yellow River Estuary of China (Zhang et al., 2020). There are several reasons that may be used to support our findings. First, the biotic homogenization after *S. alterniflora* invasion can be used to explain the reduction in network complexity and stability, because microorganisms within a community become more similar when biotic homogenization occurs and would outcompete for similar resources, resulting in increased competition within the community (Olden et al., 2004). Secondly. *S. alterniflora* invasion strongly altered soil physic-chemical properties, and this drastic environmental change exerts a strong selection pressure on the soil bacterial community, thus excluding more microorganisms from the network and leading to a simpler network (Ratzke et al., 2020). This seems to be supported elsewhere by the fact that as the environment changed, more microorganisms were replaced following the *S. alterniflora* invasion (Figure S9). Thirdly, high diversity was accompanied by low network complexity. Within a community, microorganisms can influence each other through competition, mutualism, predation, and other actions (Faust & Raes, 2012). A community with high diversity means that the habitat retains a greater diversity of microbial taxa that could compete for the same type of resource, thus making the network become less complex (Farrer et al., 2019). The reduced strength of the interactions between microorganisms leads to a more unstable network which, if it collapses, will make it difficult to recover and maintain normal ecological functioning (Olesen et al., 2007).

4.3 | Changes in the soil bacteriome are associated with those in carbon pools

Coastal wetlands are important blue carbon ecosystems on Earth, with stronger carbon sequestration than other biomes (Alongi, 2014). Given such a large soil carbon pool in coastal wetland soils, it's subtle changes may have important implications for global climate change. Therefore, it is ecologically important to explore the coupling relationship between soil microbiome and carbon pools, as microorganisms play key roles in mediating the carbon dynamic. In this study, we provided evidence that multiple environmental factors jointly regulate soil TC (Figure 7). Soil TC was increased after *S. alterniflora* invasion (Figure 2), and this positive effects of *S. alterniflora* on soil TC have been extensively verified in previous studies (Barry et al., 2021; Li et al., 2020; Zhang et al., 2021). Noteworthy, a positive association between network complexity and TC was found. A community with high network complexity implies that microorganisms within the community are more closely linked. The existence of complementarity effects between microorganisms, as when species use differentiated resources they are more likely to co-exist within a community, leading to complementarity effects and positively contributing to ecological functions (Godoy et al., 2020; Turnbull et al., 2016).

In addition, the relationship between microbes and soil TC was weakened after *S. alterniflora* invasion (Figure 7c). *S. alterniflora*, C4 perennial herb, has a strong carbon sequestration capacity and can allocate the fixed carbon to the soil through root exudations and litter input. Although *S. alterniflora* invasion significantly impacting the modularity of ecological networks influencing the relative abundance of specific microbial modules including multiple soil individual taxa (Figure 6c). The positive influence of *S. alterniflora* on organic carbon is likely to disconnect the multiple microbial metabolic connections needed to extract carbon resources in low carbon ecosystems, favoring the proportion of certain microbial individual taxa (microbial modules within the ecological network) over others. The disconnection between carbon and soil microbial communities associated with plant invasions still have unknown consequences for the environment, yet increase the uncertainty on the capacity of carbon blue ecosystems to regulate global carbon cycling compared with native environments. Currently, many countries are actively promoting efforts to achieve carbon neutrality goals. Given the excellent carbon sink capacity of coastal wetlands, the decoupling of soil microorganisms and carbon pools after *S. alterniflora* invasion may limit the potential of microorganisms in balancing carbon sink and source.

5 | CONCLUSIONS

Our work provides the first continental-scale evidence of the impacts of plant invasions on soil microbiomes. We show the biogeography of soil bacterial communities following continental-scale plant invasions, considering diversity, community structure, co-occurrence network and link with function. Plant invasion dramatically increased bacterial alpha diversity, while decreased the complexity and robustness of the co-occurrence networks. Plant invasion also caused significant biotic homogenization, and weakened the role of microbes in mediating soil TC accumulation. Overall, these findings have broadened our knowledge on the soil bacterial biogeography following plant invasion in Chinese coastal wetlands, which helps to understand their compositional and functional dynamic under global change.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in figshare at https://doi.org/10.6084/m9.figshare.19534285.v4.

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