1 **Title:** Continental-scale plant invasions reshuffle the soil microbiome of blue carbon

2 ecosystems

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4 **Running Title:** Soil microbiome after plant invasions

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54 **Abstract**

55 Theory and experiments support that plant invasions largely impact aboveground

56 biodiversity and function. Yet, much less is known on the influence of plant invasions

57 on the structure and function of the soil microbiome of coastal wetlands, one of the

58 largest major reservoirs of biodiversity and carbon on Earth. We studied the

59 continental-scale invasion of *Spartina alterniflora* (SA) across 2,451 km of Chinese

60 coastlines as our model-system, and found that SA invasion can largely influence the

61 soil microbiome (across six depths from 0-100 cm), compared with the most common

62 microhabitat found before invasion (mudflats, Mud). In detail, SA invasion was

63 positively associated with bacterial richness, but also resulted in important biotic

64 homogenization of bacterial communities, suggesting plant invasion can lead to

65 important continental scale trade-offs in the soil microbiome. We found that plant

66 invasion changed the community composition of soil bacterial communities across the

67 soil profile. Moreover, the bacterial communities associated with SA invasions where

68 less responsive to climatic changes than those in native Mud microhabitats,

69 suggesting that these new microbial communities might become more dominant under

70 climate change. Plant invasion also resulted in important reductions in the complexity

71 and stability of microbial networks, decoupling the associations between microbes

72 and carbon pools. Taken together, our results indicated that plant invasions can largely

73 influence the microbiome of coastal wetlands at the scale of China, representing the

74 first continental-scale example on how plant invasions can reshuffle the soil

75 microbiome, with consequences for the myriad of functions that they support.

76

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* (SA), 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.

SA 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). SA 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 SA invasion is important for an in-depth understanding of the ecological consequences of SA invasion. Currently, the distribution area of SA in mainland China has reached 545.80 km^2 by 2015 (Liu et al., 2018), ranging from Liaoning province to Guangdong province across more than 20 latitudes (Liu et al., 2018; Zuo et al., 2012). We compared the influence of SA to the most common microhabitat found before invasion (i.e., Mud; Fig. 1). China has approximately 7,474.6 km^2 of coastal wetlands, dominated by 5,379.8 km^2 of Mud microhabitats (Wang et al., 2020). By doing so, we

4

109 conducted the first continental-scale example on how soil microbiome responds to
110 plant invasions. The extensive occupation of SA in coastal wetlands provides a
111 suitable experimental platform for this study to assess the ecological impact of plant
112 invasion on soil microbial communities within its invasive range.

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

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

136

2. Material and methods

2.1 Study sites

Based on the extent area of SA 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) site (Fig. 1a). These sites belong to temperate monsoon climate or tropical-subtropical monsoon climate, with a mean annual temperature ranging from 4.17 °C to 23.98 °C (Fig. 1a).

At each site, we sampled soils under paired individuals of Mud and SA microsites (Fig. 1b). Within each site, the selected Mud and SA 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 SA habitats were compared to reflect the ecological consequences of SA invasion on the most common native habitats before invasion for two reasons: 1) Emerging evidence suggest that the expansion of SA 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

164   processing scheme. Specifically, a quadrat with 50 × 50 meters was established at

165   each site for Mud and SA habitats, respectively (Fig. 1b). Within this quadrat, three

166   replicates for Mud habitat and four replicates for SA habitat were randomly sampled,

167   with at least 15 meters away in geographical distance from each other. Then, around

168   each replicate, five intact soil cores (~within 1 m to the centroid point) were randomly

169   collected by using PVC pipes (5 cm diameter, 100 cm length). Here, considering the

170   highly developed root system of SA that might alters the microbial distributions

171   between soil depths, soil in different depths were collected up to 100 cm maximum. It

172   is important to note that due to the differences in soil textures, individual plots could

173   only be collected to a maximum depth of 40-60 cm. After achiving the soil cores, they

174   were then divided into 6 sections (0-10 cm, 10-20 cm, 20-40 cm, 40-60 cm, 60-80 cm,

175   and 80-100 cm) using a stainless steel knife, referring to different soil depths (Fig. 1c).

176   The corresponding soils from all the 5 soil cores were completely homogenized as a

177   replicate. Soil samples were sealed immediately after removing gravel stones and

178   other debris. Finally, a total of 407 soil samples were obtained, of which 177 and 230

179   samples for Mud and SA habitat, respectively (Table S1). All the soil samples were

180   stored on ice during the transportation.

181   A suite of 18 environmental variables were obtained, including 11 soil properties

182   and 7 climatic factors (Gao et al., 2022). For soil variables, soil temperature of

183   corresponding sample was determined *in-situ* using a mercury thermometer. Soil

184   redox potential (Eh, mV) and salinity/pH were measured by inserting portable probes

185   into the corresponding depth, by using the ExStikTM RE300 (USA) and ExStikTM

186   EC500 (USA), respectively. In the laboratory, soil water content was determined by

187   30 °C oven-drying of 50 g fresh soil to a constant weight. The air-dried soils were

188   ground into powder and sieved through a 2 mm mesh sieve. Soil electrical

189   conductivity (EC) was determined at 25 °C by using a conductivity meter (Leici

190   DDS-307, China). Soil organic matter (SOM) was measured based on the loss on

191   ignition at 550 °C for 6 h after 105 °C oven-dried (Heiri et al., 2001). Soil total carbon

192 (TC), total nitrogen (TN) and total sulfur (TS) were determined using a Vario EL III

193 Elemental Analyzer (Elementar, Hanau, Germany), and then the carbon-to-nitrogen

194 ratio (C/N) was calculated. For the climatic factors, the mean annual temperature

195 (MAT), mean annual precipitation (MAP), isothermality, precipitation seasonality, and

196 temperature seasonality were compiled from the WorldClim version 2

197 (https://www.worldclim.org/) at 30 arc-second resolutions. Aridity index and potential

198 evapotranspiration (PET) were obtained from Global Aridity Index and Potential

199 Evapotranspiration (ET0) Climate Database v2 (https://cgiarcsi.community),

200 respectively.

201

202 **2.3 Soil DNA extraction and high-throughput sequencing**

203 Soil DNA was extracted by using FastDNA SPIN Kit (MP Biomedicals, Santa Ana,

204 CA, USA), according to the manufacturer's instructions. Then, the quality and

205 quantity of the extracted DNA were checked by using NanoDrop2000

206 spectrophotometer (NanoDrop Tech, Wilmington, USA) and 1% agarose gel

207 electrophoresis, respectively. The V4-V5 region of the bacterial *16S rRNA* gene was

208 amplified using the primers 515F (5'-GTGCCAGCMGCCGCGG-3') and 907R (5'-

209 CCGTCAATTCMTTTRAGTTT-3'). PCR reactions were performed on ABI

210 GeneAmp® 9700 (ABI, Waltham, MA, USA). High-throughput sequencing was

211 performed on an Illumina MiSeq PE300 platform (Illumina, Inc., San Diego, CA,

212 USA). All the generated raw sequences have been deposited in the Genome Sequence

213 Archive in National Genomics Data Center, China National Center for

214 Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences, under

215 the accession number of CRA005208 (https://bigd.big.ac.cn/gsa/browse/CRA005208).

216

217 **2.4 Bioinformatic analyses**

218 Raw reads were processed using the amplicon sequence variants (ASV) method by

219 Quantitative Insight into Microbial Ecology 2 (QIIME2) (Bolyen et al., 2019).

220   Sequences with poor-quality (read length < 200 bp or average quality score < 25)

221   were discarded (Ladau et al., 2018). Finally, a total of 23,508,265 sequences were

222   obtained. The clean sequences were denoised using the DADA2 pipeline (Callahan et

223   al., 2016), and generated a total of 49,497 ASVs. Representative sequences were used

224   to construct the phylogenetic tree and for species annotations. SILVA database

225   (https://www.arb-silva.de/) was applied to assign the taxonomy. All the soil samples

226   were rarefied to 28,759 sequences (minimum) per sample for downstream analyses.

227

228   **2.5 Co-occurrence network constructions**

229   All the 407 soil samples were used to construct the co-occurrence networks by using

230   the *WGCNA* package (Langfelder & Horvath, 2012). ASVs that occur in less than 5

231   soil samples were excluded (Gao et al., 2021), leaving a total of 5, 134 ASVs. The

232   pairwise Spearman correlations matrix among ASVs were calculated, and p values

233   were corrected using Benjamini Hochberg's correction (Benjamini et al., 2006).

234   Nodes with correlations greater than 0.5 and $p < 0.001$ were retained (Delgado-

235   Baquerizo et al., 2018). The networks were visualized using Gephi (version 0.9.2).

236   Then, the ecological modules within the network were identified in Gephi, and the

237   relative abundance of each ecological module was calculated by averaging the

238   standardized relative abundance (Z-score). Sub-networks were generated from the

239   original network by preserving the presented nodes and edges of the target samples.

240   The topological parameters of all the generated networks were calculated, including

241   betweenness centrality (the number of times a node acts as a bridge along the shortest

242   path between two other nodes), closeness centrality (the number of steps required to

243   access all other nodes from a given node), clustering coefficient (a ratio of the number

244   of links between the neighbors of a node, and the maximum number of links that

245   could possibly exist between its neighbors) and degree (number of edges connecting a

246   node to other nodes). The average degree (avgK), represents the average number of

247   edges per node, was used to describe the network complexity (Yao et al., 2014).

248 Natural connectivity provides a sensitive discrimination of network structural

249 robustness. Thus, network robustness was estimated by removing nodes in the static

250 network to assess how quickly the natural connectivity degraded (Peng & Wu, 2016;

251 Wu et al., 2010).

252

253 **2.6 Statistical analyses**

254 To explore the environmental variables affecting bacterial alpha diversity, the

255 importance of variables was analyzed using the partial correlations method (Kim,

256 2015). We identified the major environmental factors that influencing the taxa's

257 relative abundance (i.e., the top ten phyla and core species). Multiple linear

258 regressions were fitted separately between taxa's relative abundance and

259 environmental variables using the lm function. Backward stepwise regression was

260 then performed to filter variables using stepAIC in *MASS* package. For the optimal

261 model obtained, the contribution of the main environmental variables to the total

262 variance in taxa's relative abundance was assessed using the calc.relimp function in

263 *relaimpo* package. A classification of random forest model was used to identify

264 biomarkers between Mud and SA habitats on class level using the *randomForest*

265 package (Liaw & Wiener, 2002). Then, the accuracy of the classification model was

266 assessed by receiver operating characteristic (ROC) and the prediction of confusion

267 matrix. Those ASVs that occurring in more than 50% samples were defined as core

268 species for Mud and SA habitat, respectively. Covariate adjusted principal coordinates

269 analysis (aPCoA) was used to describe pairwise dissimilarity between samples based

270 on the Weighted Unifrac distance (Shi et al., 2020). Pairwise permutational

271 multivariate analysis of variance (PERMANOVA) was used to test the significance of

272 differences in bacterial communities between soil samples. To better understand the

273 biodiversity patterns and to explore their underlying mechanisms, compositional

274 dissimilarities (BDtotal) were divided into Repl (species replacement) and RichDiff

275 (difference in abundance) components using the *adespatial* package. Dissimilarity-

276   overlap curve (DOC) was used to test whether the underlying ecological dynamics of

277   microbiomes are universal across all communities or unique to individual

278   communities (Kalyuzhny & Shnerb, 2017). We used a variety of methods to explore

279   the role of environmental factors in influencing bacterial communities. Firstly, the

280   variation partitioning analysis (VPA) was performed to estimate the effects of space,

281   depth, climate and soil factors on bacterial community using the *vegan* package. Then,

282   Mantel and partial Mantel tests were applied to examine the relationships between

283   environmental variables and bacterial communities. Finally, these relationships were

284   tested by the multiple regression on distance matrices (MRM) using the *ecodist*

285   packages, after filtering the environmental variables with high multicollinearity

286   (Spearman $\rho^2 > 0.7$) (Wang et al., 2017). Fast expectation-maximization microbial

287   source tracking (FEAST) analysis was used to estimate the source proportion of

288   bacteria in SA soils that derived from Mud soils, and vice versa (Shenhav et al.,

289   2019). All analyses involving R software were performed under R version 4.0.5 (R

290   Core Team, 2018), unless otherwise stated.

291   Structural equation model (SEM) was used to evaluate the direct and indirect

292   effects of biotic (i.e., module abundance, bacterial richness, network complexity) and

293   abiotic factors (i.e., climatic factors, soil properties) on soil TC. The predicted causal

294   relationships of the SEM were constructed based on prior knowledge (Delgado-

295   Baquerizo et al., 2020). All the environmental variables were treated as independently

296   observed variables in the SEM, rather than latent variable. Prior to SEM analysis, the

297   multicollinearity of environmental variables was examined by using the *Hmisc*

298   package, and those variables with Spearman ρ^2 greater than 0.7 were removed. Then,

299   Random Forest analyses were performed to identify the key predictors that

300   influencing soil TC by using the *rfPermute* package. A 1,000 permutation was then

301   applied to compute the null distribution and calculate the *p* values. Since most of the

302   variables were not normally distributed, the bootstrapping method were used to

303   evaluate the probability that the path coefficients differed from zero (Delgado-

304   Baquerizo et al., 2017). Then, three indices were used to assess the SEM performance

305   (Schermelleh-Engel & Moosbrugger, 2003): (1) Chi-square (x^2) test, a good fit is

306   defined as $0 \leq x^2/\text{df}$ (degree of freedom) ≤ 2, and $0.05 < p \leq 1.00$; (2) Root mean

307   square error of approximation (RMSEA), in which a good fit is defined as $0 \leq$

308   RMSEA ≤ 0.05; (3) Bollen-Stine bootstrap test, a good fit is defined as $0.10 <$ Bollen-

309   Stine bootstrap $p \leq 1.00$. The standardized total effect of each predictor on soil TC

310   was calculated. SEM analyses were performed in AMOS 21 (IBM SPSS Inc., Chicago,

311   IL, USA).

312

313   **3. Results**

314   **3.1 Plant invasions causes drastic changes in soil environments and on the**

315   **biodiversity and community composition of soil bacteriomes**

316   A total of 18 environmental variables were obtained, including 11 soil properties (i.e.,

317   pH, salinity) and 7 climatic factors (i.e., MAT, MAP) (Table S2). Results show that

318   environmental variables varied considerably among habitats, sites, and soil depths. SA

319   soils have higher EC, salinity, total nitrogen (TN), SOM and water content than that

320   of Mud soils, but lower pH values. We further showed that plant invasions cause

321   important homogenization in soil environments. In particular, we found that important

322   soil properties such as Eh, soil temperature, TC, TS, C/N, and water content had

323   smaller changes in environmental variability than those in Mud soils. Yet, Mud habitat

324   and SA habitat had similar levels of total carbon ($p = 0.08$) (Fig. 2a). Both in the Mud

325   and SA habitats, the soil carbon content had a distinct latitudinal distribution pattern,

326   showing the highest at approximate 34°N regions. Not only that, compared to SA

327   habitat, the soil carbon in Mud habitat exhibited a larger increase with increasing soil

328   depth and then a subsequent decrease (Fig. 2b). Compared to Mud habitat, carbon

329   accumulation in SA habitats was mainly between the soil surface and 60 cm below

330   ground.

331   We then investigated patterns in soil microbial diversity. We used richness

332   (observed ASVs) to present the taxonomic alpha diversity of bacteria. Richness ($p <$

333   0.05) were significantly higher in SA soils compared with Mud soils, but not for all

334   the sites (Fig. 3a, Table S3). In general, richness increased with latitude but decreased

335   with soil depths (Fig. 3b, Fig. S1). We analyzed soil properties against richness for

336   revealing the main predictors. Three-ways ANOVAs showed that richness was mainly

337   influenced by the sample site heterogeneity ($F = 42.37$, $p < 0.001$) (Table S4). Partial

338   correlations analyses suggested that soil properties, rather than climatic factors, were

339   the primary factors in influencing richness (Fig. 3c). Among all the environmental

340   variables, soil temperature was found to be the best predictor (Fig. S2, Table S5).

341   Binomial regressions showed that richness decreasing when soil temperature beyond

342   the optimum ranges (Fig. 3d).

343   Venn diagram showing the share and unique ASVs, with 15,259 ASVs and

344   24,591 ASVs unique to Mud and SA soils respectively (Fig. S3a). A total of 60 phyla

345   were identified from all the soil samples, and the most abundant phyla was

346   *Proteobacteria*, *Chloroflexi*, and *Epsilonbacteraeota*, and their relative abundances

347   varied considerably across different sites and depths (Fig S3b). Among the top ten

348   phyla, SA soils have significantly ($p < 0.05$) higher relative abundances of

349   *Bacteroidetes*, *Acidobacteria*, *Planctomycetes*, *Gemmatimonadetes*, *Nitrospirae*, and

350   *Latescibacteria* than Mud soils, while have lower *Epsilonbacteraeota* (Fig. 4a).

351   Distinct latitudinal distributions were observed for these phyla, and the measured

352   environmental variables could explain 21.43%-74.01% of the variations in their

353   relative abundances (Fig. 4b, Fig. S4).

354   We identified 72 classes as the biomarkers between Mud and SA soils (Fig. S5a).

355   Both the results of area under curve (AUC = 0.77) of ROC and prediction of

356   confusion matrix (0.21 < error < 0.22) suggested a high accuracy of the classification

357   model (Fig. S5b, Table S6). These biomarkers included *Spirochaetia*, *Phycisphaerae*,

358   *Clostridia*, and others (Fig. S5c). In addition, at the 50% occurrence threshold, we

359   identified 8 and 5 core ASVs for Mud and SA soils respectively (Fig. 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 (Fig. S6b, c, Fig. 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* < 0.001) differences in the soil bacterial communities between Mud and SA habitats (Fig. 5a). PERMANOVA suggested that soil bacterial communities were mainly influenced by the factor of sample sites (*pseudo-F* = 23.41, *p* < 0.001), followed by depths (*pseudo-F* = 8.84, *p* < 0.001) and habitats (*pseudo-F* = 8.54, *p* < 0.001) (Table S8). We then showed that, in general, soil microbiomes in SA 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 SA soils compare to Mud soils (Fig. 5b, Fig. S8). A significant pattern of distance decay relationship (DDR) was observed, with the slopes of DDR of the SA soils higher than that of Mud soils, suggesting higher spatial turnover of bacterial community after plant invasion (Fig. 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 SA soils respectively (Fig. S9). DOC analysis further revealed that these ecological dynamics of bacterial communities were universal across metacommunities (Fig. S10a). Microorganisms could spread from one habitat to another. We used FEAST (source tracking) method to quantify this dispersal event, and found a higher proportion of Mud was derived from SA than vice versa (Fig. S10b).

388   We then used multiple approaches to explore the environmental variables

389   influencing the bacterial community structure. Firstly, partial Mantel tests

390   demonstrated the critical role of soil properties and climatic factors in affecting

391   bacterial communities (Table S9), which were further confirmed by the VPA analyses

392   (Fig. S11a). Then, the relationships between bacterial communities and environmental

393   variables were tested by Mantel tests (Fig. S11b, Table S10). MRM method, an

394   extension of partial Mantel analysis which offers several advantages over partial

395   Mantel tests, were applied to explore the key predictors (Table S11, Fig. S12). MRM

396   revealed that TS and TN were the decisive factors in influencing bacterial

397   communities for Mud and SA soils, respectively.

398

399   **3.3 *S. alterniflora* invasion reduces the complexity and robustness of co-**

400   **occurrence networks**

401   Co-occurrence networks were used to identify soil bacterial taxa organized within

402   closely-linked ecological clusters. The network consists of 4,230 nodes and 41,506

403   edges, with a high number of major nodes belong to phylum of *Proteobacteria*

404   (51.49%), *Bacteroidetes* (17.02%), *Chloroflexi* (7.85%), and *Epsilonbacteraeota*

405   (6.93%) (Fig. S13a). There are many nodes clustered together (modules) in the

406   network, of which, there are 7 modules containing more than 300 nodes, namely

407   Modules #36 (14.96%), Modules #45 (10.47%), Modules #27 (9.57%), Modules #25

408   (9.27%), Modules #42 (7.75%), Modules #22 (7.71%), and Modules #37 (7.66%) (Fig.

409   6a, Fig. S13b). Each module contains a wide variety of bacterial taxa, among which

410   the more abundant class groups include *Campylobacteria*, *Deltaproteobacteria*, and

411   *Bacilli* (Fig. 6b). The relative abundance of these modules was significantly ($p < 0.05$)

412   higher in SA habitats than in Mud habitats, including Modules #22, Modules #42, and

413   Modules #45 (Fig. 6c). Network properties characterize the physical structure of the

414   network, and the Mud network has significantly ($p < 0.05$) higher closeness centrality

415   (Mud: 0.20; SA: 0.19) and degree (Mud: 19.27; SA: 17.50) than the SA network,

416   suggesting a more complex network of Mud habitat (Fig. S14a, b). In addition, the

417   natural connectivity of the network decreased sharply as the proportion of removed

418   nodes increases, but the slope of the decline of Mud network was lower than that of

419   the SA network, means a more stable network of the Mud network (Fig. 6d). In order

420   to understand the spatial distributions of the network, sub-networks were constructed,

421   and results showed that the network complexity increases with increasing latitude for

422   both Mud and SA soils (Fig. 6e, Fig. S14c).

423

424   **3.4 Associations between bacterial communities and soil carbon pools**

425   SEM analysis helps us to understand the relationships between soil bacterial

426   communities and soil TC, while considering a variety of biotic and abiotic factors

427   simultaneously. Based on the initial model (Fig. S15), a Random Forest analysis was

428   performed to identify the key environmental factors (Fig. S16). SEM results showed

429   that soil TC was regulated by a combination of environmental factors, of which, the

430   most important were soil properties and climatic factors (Fig. 7). Notably, bacterial

431   community also played an important role in influencing TC, in detail, TC was

432   significantly and positively correlated with avgK (path coefficient = 0.15 ***) and

433   module #45 (path coefficient = 0.15 ***), while significantly and negatively

434   correlated with module #36 (path coefficient = -0.35 ***). Not only that, the presence

435   of aboveground plants also has a positive effect on soil TC (Fig. 7a). By analyzing the

436   total effect of each predictor, we found that ST was the most important factor in

437   regulating soil TC (Fig. 7b). From the results of the linear regression analysis, the

438   slope of the correlation between module #45 and avgK and TC was reduced in SA

439   habitats compared to Mud habitats, implying that the role of these bacterial taxa in TC

440   accumulation was diminished (Fig. 7c). Overall, these results have revealed the strong

441   associations between bacterial communities and soil TC, and which were reduced

442   after plant invasion.

443

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 SA 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 SA invasion significantly ($p < 0.05$) increased bacterial alpha diversity in Chinese coastal wetlands (Fig. 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 SA 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,

17

472   Venn diagrams showed the share and unique ASVs, with 15,259 ASVs and 24,591

473   ASVs unique to Mud and SA soils respectively (Fig. S3a). These results suggest that

474   plant invasions can have a previously unreported impact on soil biodiversity, as

475   previously found for plant and animal diversity.

476   We further provided unprecedented evidences that continental-scale SA invasion

477   in China can cause important biotic homogenization of soil bacterial community (Fig.

478   5a, b, Fig. S8). Biotic homogenization following biological invasions is commonly

479   found for aboveground biodiversity (Stotz et al., 2019). For example, it has been

480   shown in many studies that biological invasions could lead to biotic homogenization

481   across different ecosystems, for animals and plants (Delgado-Baquerizo et al., 2021;

482   Gossner et al., 2016; Leprieur et al., 2007; Zhang et al., 2019). However, much less

483   studied are the impacts of plant invasions on soil microbiomes. The occurrence of

484   biotic homogenization does not conflict with our earlier observation that SA invasions

485   increase alpha diversity, as increases in local alpha diversity are usually accomplished

486   at the expense of beta diversity (Whittaker, 1972). Biotic homogenization is a process

487   whereby some species in a community are replaced by other species (Olden et al.,

488   2004; Olden & Poff, 2003). The invasion of SA could lead to biotic homogenization

489   of soil microbial community by altering soil physic-chemical properties, making them

490   more homogenous. In addition, compared to SA habitats, the dominant original native

491   ecosystems (Mud soils) are not protected by any vegetation, and are more susceptible

492   to environmental disturbance, which may cause greater microbial fluctuations. Not

493   only that, but bacteria in SA soils appeared to have a greater dispersal capacity than

494   that in Mud soils, as indicated by the results of source tracking (Fig. S10b), which

495   may favor biotic homogenization within the habitats. As a result, biotic

496   homogenization causes a narrowing of ecological niches within the community,

497   making microbes more responsive to environmental change and thus accelerating the

498   spatial turnover of the bacterial community (Fig. 5c). However, it is currently unclear

499   whether biotic homogenization also occurs at the genetic and functional levels

500   following SA invasion, beyond the taxonomic level. It has been shown that biotic

501   homogenization appears to reduce the multifunctionality in forest ecosystems (Van

502   Der Plas et al., 2016). Therefore, we speculate that SA invasion along the coastline in

503   China may impose strong influences on the microbial communities and the

504   biogeochemical cycles they support, and allow for the weakening or even loss of

505   certain ecological functions. Such changes would threaten the critical role of coastal

506   wetland ecosystems in global carbon cycling and climate change regulation.

507

508   **4.2 *S. alterniflora* invasion reduces the complexity and robustness of co-**

509   **occurrence networks**

510   Soils are home to hundreds of millions of microorganisms interacting with each other

511   and driving the fundamental soil processes (Faust & Raes, 2012; Layeghifard et al.,

512   2017). Dissecting the structure of soil microbial coexistence is important for a deeper

513   understanding of the evolution of soil ecological functions in response to

514   environmental changes. The impact of plant invasion on soil microbial networks has

515   mostly been studied at the local scale, but works at large spatial scales (i.e.,

516   continental scale) is still lacking (Chen & Wen, 2021; Zhang et al., 2020). Here, we

517   found that the continental-scale SA invasion causes a significant reduction in

518   connectivity between nodes within network and a consequent reduction in network

519   stability (Fig. 6, Fig. S14). Our study was consistent with that of others who also

520   found a decrease in network complexity of fungal communities after SA invasions in

521   a salt marsh ecosystem (Zhang et al., 2021). On the contrary, previous study reported

522   that SA invasion enhanced the bacterial interactions in the Yellow River Estuary of

523   China (Zhang et al., 2020). There are several reasons that may be used to support our

524   findings. Firstly, the biotic homogenization after SA invasion can be used to explain

525   the reduction in network complexity and stability, because microorganisms within a

526   community become more similar when biotic homogenization occurs and would

527   outcompete for similar resources, resulting in increased competition within the

528   community (Olden et al., 2004). Secondly. SA invasion strongly altered soil physic-

529   chemical properties, and this drastic environmental change exerts a strong selection

530   pressure on the soil bacterial community, thus excluding more microorganisms from

531   the network and leading to a simpler network (Ratzke et al., 2020). This seems to be

532   supported elsewhere by the fact that as the environment changed, more

533   microorganisms were replaced following the SA invasion (Fig. S9). Thirdly, high

534   diversity was accompanied by low network complexity. Within a community,

535   microorganisms can influence each other through competition, mutualism, predation,

536   and other actions (Faust & Raes, 2012). A community with high diversity means that

537   the habitat retains a greater diversity of microbial taxa that could compete for the

538   same type of resource, thus making the network become less complex (Farrer et al.,

539   2019). The reduced strength of the interactions between microorganisms leads to a

540   more unstable network which, if it collapses, will make it difficult to recover and

541   maintain normal ecological functioning (Olesen et al., 2007).

542

543   **4.3 Changes in the soil bacteriome are associated with those in carbon pools**

544   Coastal wetlands are important blue carbon ecosystems on Earth, with stronger carbon

545   sequestration than other biomes (Alongi, 2014). Given such a large soil carbon pool in

546   coastal wetland soils, it's subtle changes may have important implications for global

547   climate change. Therefore, it is ecologically important to explore the coupling

548   relationship between soil microbiome and carbon pools, as microorganisms play key

549   roles in mediating the carbon dynamic. In this study, we provided evidence that

550   multiple environmental factors jointly regulate soil total carbon (Fig. 7). Soil TC was

551   increased after SA invasion (Fig. 2), and this positive effects of SA on soil TC have

552   been extensively verified in previous studies (Barry et al., 2021; Li et al., 2020; Zhang

553   et al., 2021). Noteworthy, a positive association between network complexity and TC

554   was found. A community with high network complexity implies that microorganisms

555   within the community are more closely linked. The existence of complementarity

556   effects between microorganisms, as when species use differentiated resources they are

557   more likely to co-exist within a community, leading to complementarity effects and

558   positively contributing to ecological functions (Godoy et al., 2020; Turnbull et al.,

559   2016).

560   In addition, the relationship between microbes and soil TC was weakened after

561   SA invasion (Fig. 7c). SA, C4 perennial herb, has a strong carbon sequestration

562   capacity and can allocate the fixed carbon to the soil through root exudations and litter

563   input. Although SA invasion significantly impacting the modularity of ecological

564   networks influencing the relative abundance of specific microbial modules including

565   multiple soil individual taxa (Fig. 6c). The positive influence of SA on organic carbon

566   is likely to disconnect the multiple microbial metabolic connections needed to extract

567   carbon resources in low carbon ecosystems, favoring the proportion of certain

568   microbial individual taxa (microbial modules within the ecological network) over

569   others. The disconnection between carbon and soil microbial communities associated

570   with plant invasions still have unknown consequences for the environment, yet

571   increase the uncertainty on the capacity of carbon blue ecosystems to regulate global

572   carbon cycling compared with native environments. Currently, many countries are

573   actively promoting efforts to achieve carbon neutrality goals. Given the excellent

574   carbon sink capacity of coastal wetlands, the decoupling of soil microorganisms and

575   carbon pools after SA invasion may limit the potential of microorganisms in balancing

576   carbon sink and source.

577

578   **5. Conclusions**

579   Our work provides the first continental-scale evidence of the impacts of plant

580   invasions on soil microbiomes. We show the biogeography of soil bacterial

581   communities following continental-scale plant invasions, considering diversity,

582   community structure, co-occurrence network and link with function. Plant invasion

583   dramatically increased bacterial alpha diversity, while decreased the complexity and

584   robustness of the co-occurrence networks. Plant invasion also caused significant

585   biotic homogenization, and weakened the role of microbes in mediating soil TC

586   accumulation. Overall, these findings have broadened our knowledge on the soil

587   bacterial biogeography following plant invasion in Chinese coastal wetlands, which

588   helps to understand their compositional and functional dynamic under global change.

589

602

603   **Conflict of interest**

604   The authors declare that they have no conflict of interest.

605

606   **Data availability statement**

607   The data that support the findings of this study are openly available in figshare at

608   https://doi.org/10.6084/m9.figshare.19534285.v4

609

610   **Figure legends**

611   **Fig. 1|** The sample sites and their corresponding MAT along the coastal wetlands in

612 China (a). The locations of each replicate in a 50*50 m quadrat for Mud and SA

613 habitats. Five intact soil cores were randomly collected and completely mixed as a

614 replicate (b). Schematic diagram of the sampling depth for each sample site (c). Mud:

615 bare mudflat; SA: *Spartina alterniflora*; HLD: Huludao; TG: Tanggu; DY: Dongying;

616 LYG: Lianyungang; YC: Yancheng; CM: Chongming; YQ: Yueqing; XP: Xiapu; YX:

617 Yunxiao; ZH: Zhuhai; BH: Beihai; ZJ: Zhanjiang; MAT: mean annual temperature.

618

619 **Fig. 2|** Soil total carbon (TC) content in coastal wetlands. The latitudinal pattern of

620 soil TC for Mud and SA habitats respectively (a). Variation of soil TC with depth (b).

621 For abbreviations, see the legends of Fig. 1.

622

623 **Fig. 3|** The influence of plant invasions on the response of bacterial richness to

624 environmental variability. Difference in bacterial richness between Mud and SA

625 habitats, and the significance was examined by Wilcoxon rank sum test (a). Variations

626 of bacterial richness in various depths (b). Partial Spearman correlations between

627 bacterial richness and the groups of environmental variables (i.e., space, climate,

628 habitat, depth and soil) (c). All observed variables were grouped into corresponding

629 groups and constructed as latent variables. Latent variables were constructed using the

630 Partial Least Squares Path Modeling (PLS-PM) method. The relationships between

631 richness and soil temperature (d). Both linear and quadratic models were used to fit

632 these relationships, and best models were selected based on a low Akaike's

633 information criterion (AIC) value. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. For

634 abbreviations, see the legends of Fig. 1.

635

636 **Fig. 4|** The relative abundance of the bacterial top ten phyla. Differences in the

637 relative abundance of the top ten bacterial phyla between Mud and SA habitats (a).

638 Trends in the relative abundance of the top ten bacterial phyla with latitude (b). * $p <$

639 0.05; ** $p < 0.01$; *** $p < 0.001$. For abbreviations, see the legends of Fig. 1.

640

Fig. 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 SA 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. $* p < 0.05$; $** p < 0.01$; $*** p < 0.001$. For abbreviations, see the legends of Fig. 1.

651

Fig. 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 SA 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). $* p < 0.05$; $** p < 0.01$; $*** p < 0.001$. For abbreviations, see the legends of Fig. 1.

662

Fig. 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.

668   Solid and dashed arrows indicate positive and negative effects, respectively. R^2 value

669   means the proportion of variance explained. The standardized total effects of each

670   predictor attributes on soil TC (b). The relationships between soil TC and bacterial

671   communities (Mod #36, Mod #45, and avgK) (c). Solid and dashed lines indicate

672   significant and insignificant correlations, respectively. Long: longitude; Lat: latitude;

673   Mod: module; Iso: isothermality; MAP: mean annual precipitation; PET: potential

674   evapotranspiration; avgK: average degree; ST: soil temperature; TN: total nitrogen;

675   TC: total carbon. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. For abbreviations, see the

676   legends of Fig. 1.

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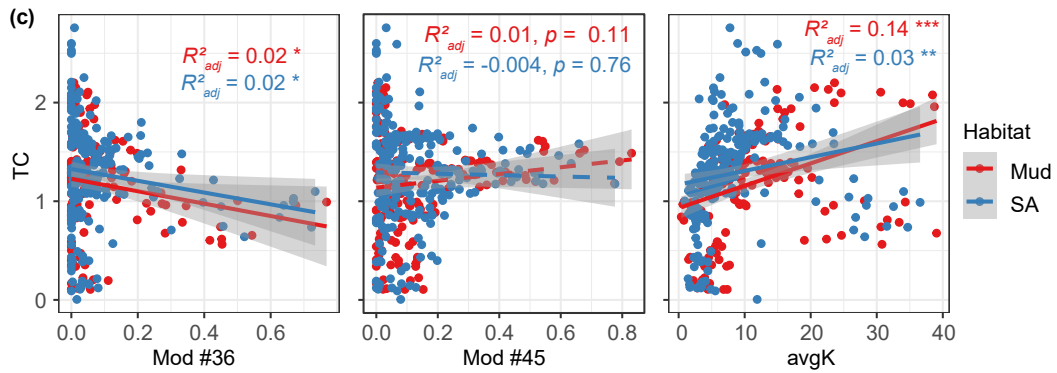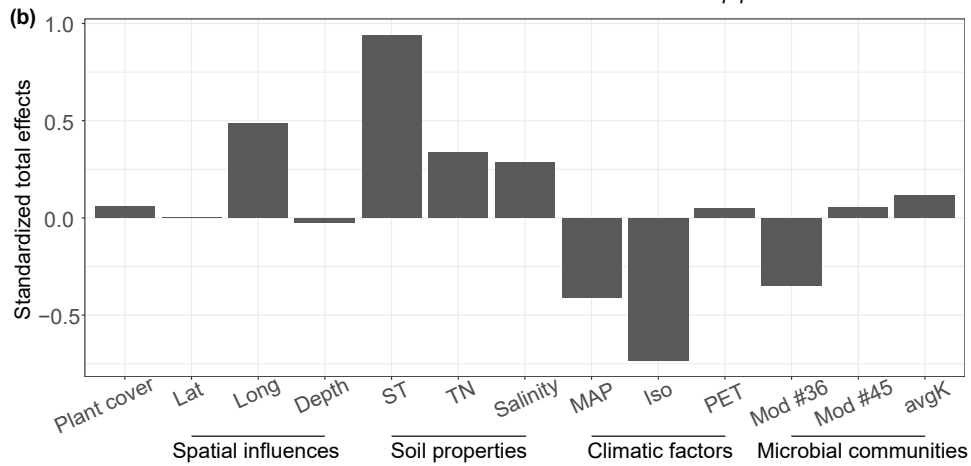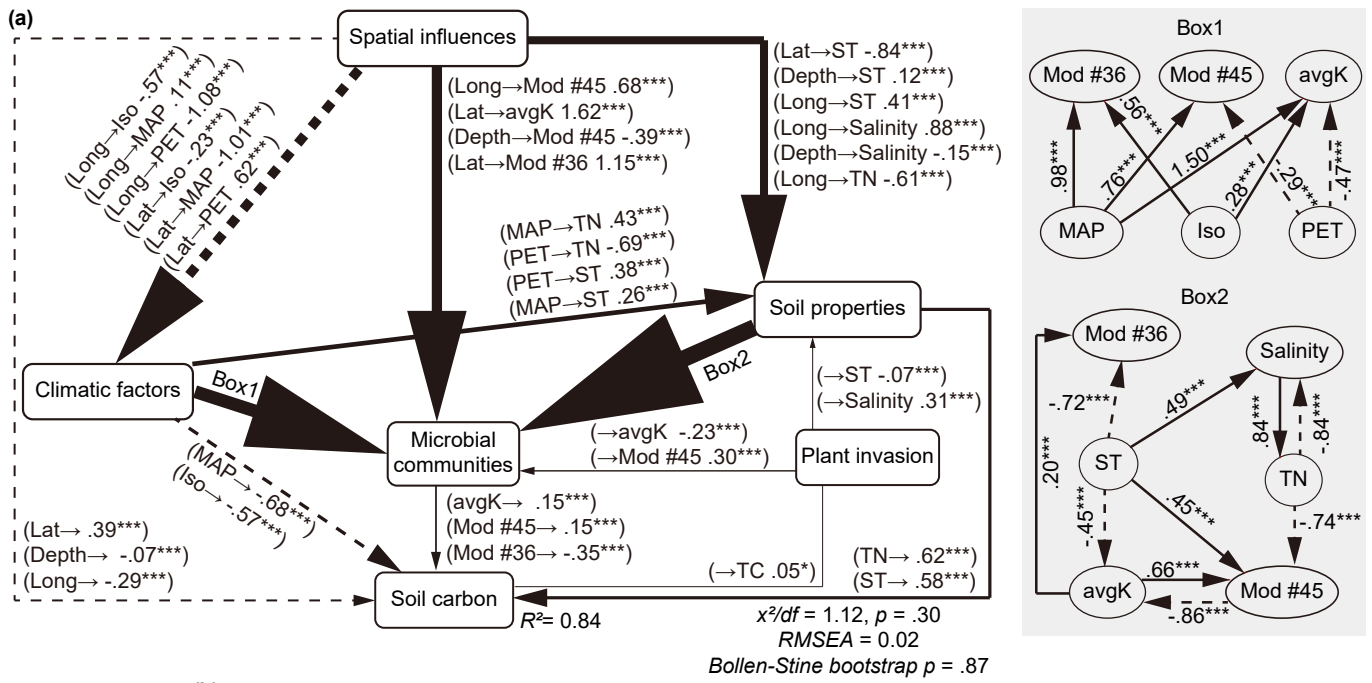900 Zhao, Q. (2021). *Spartina alterniflora* invasions reduce soil fungal diversity and

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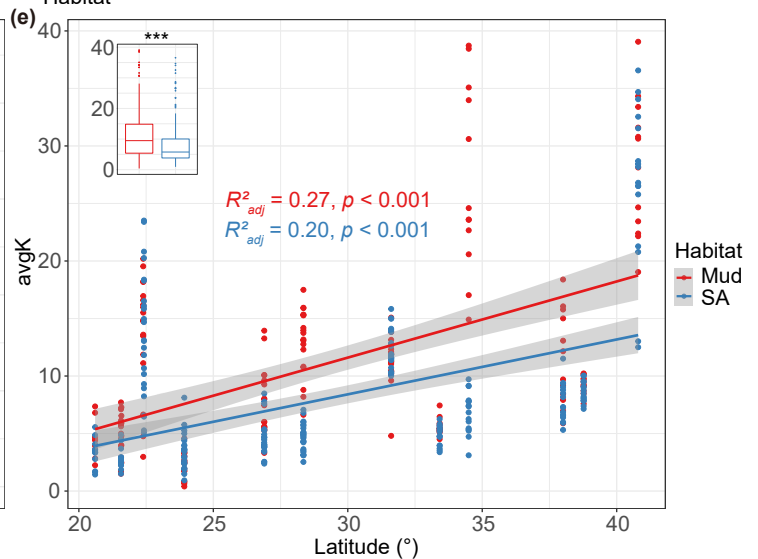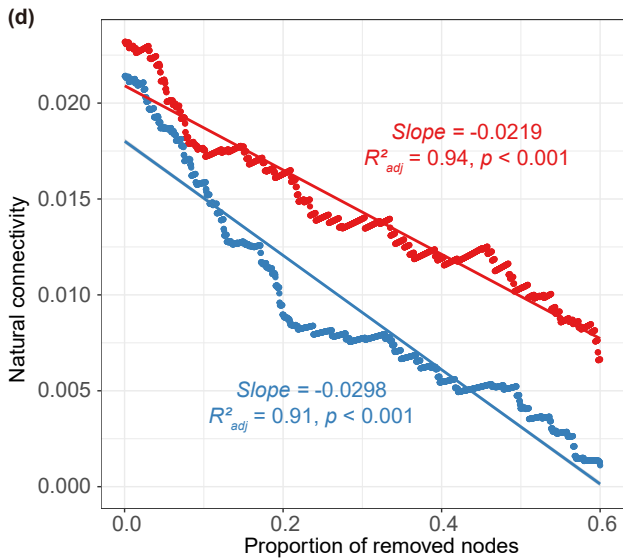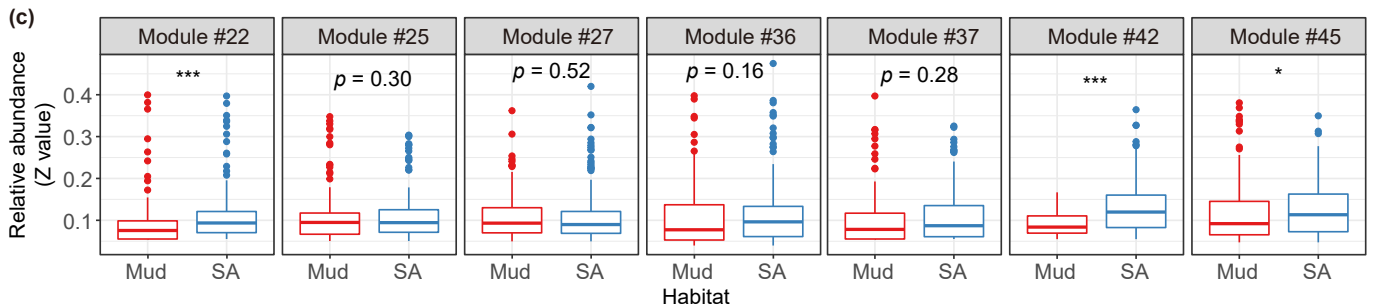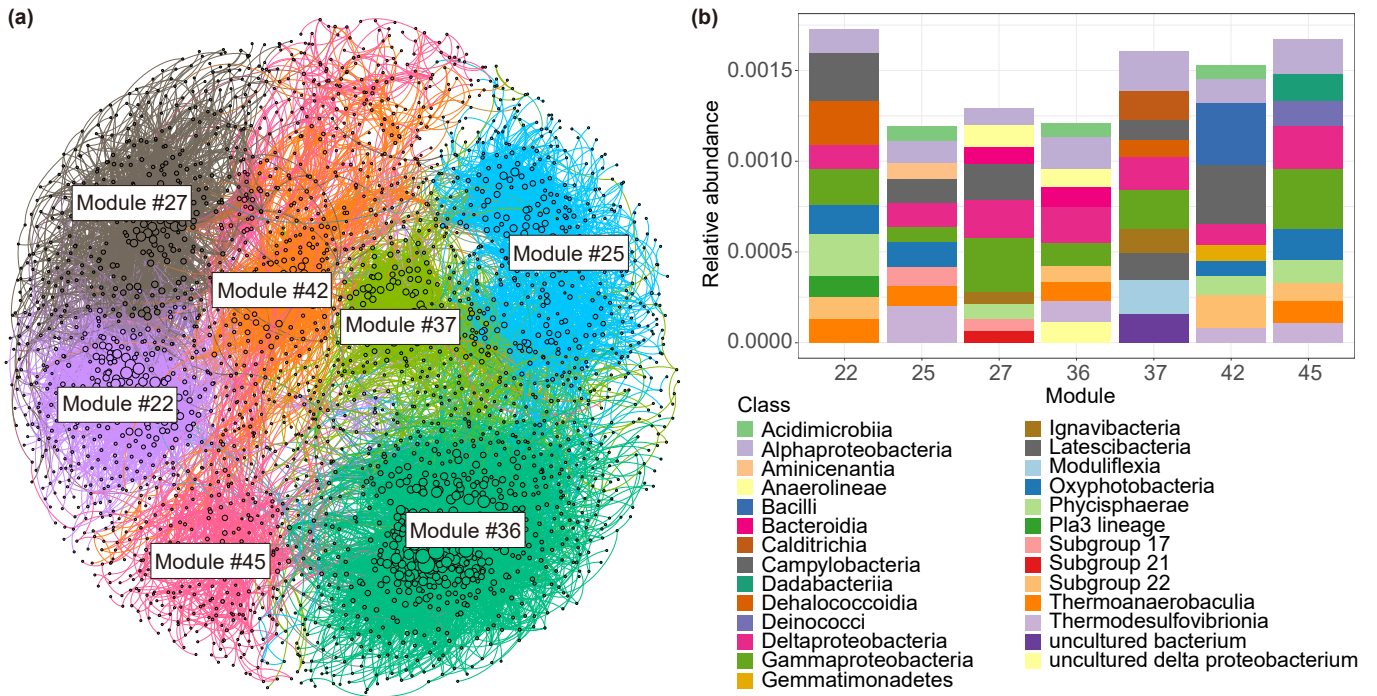
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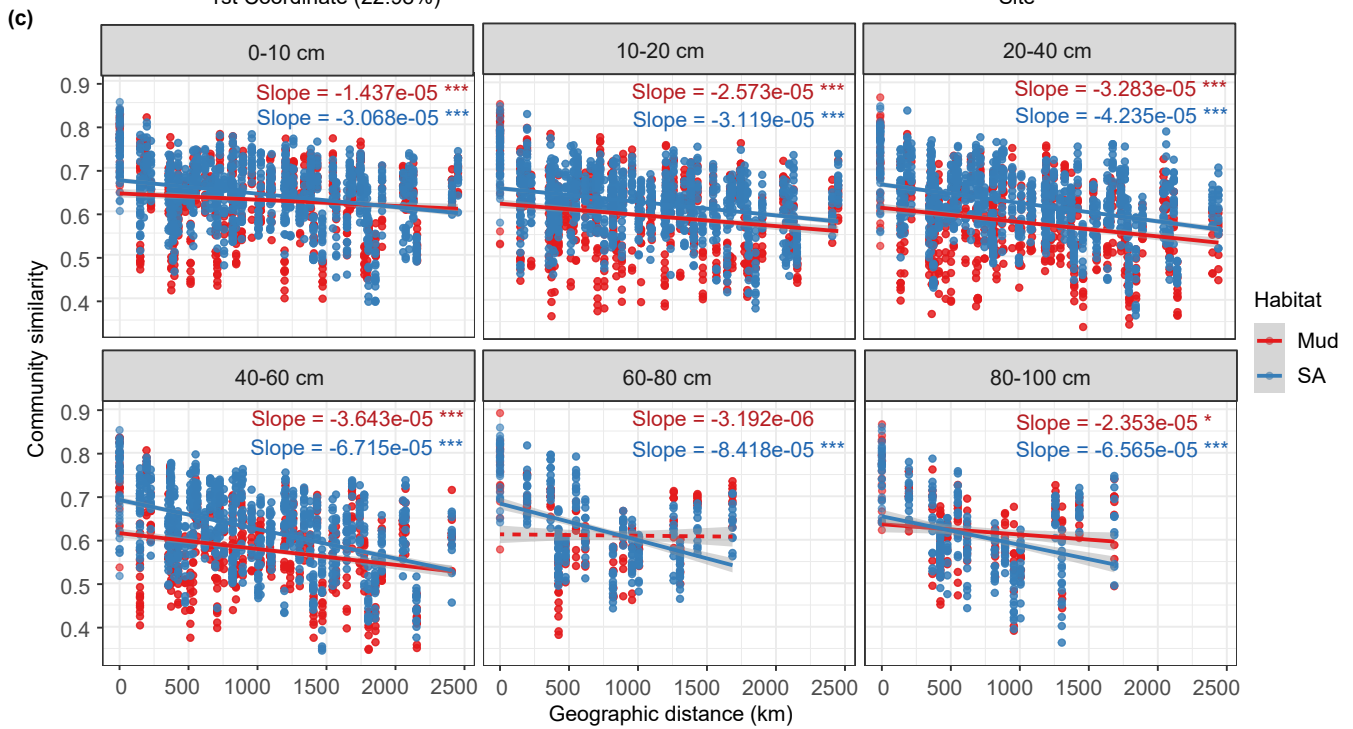
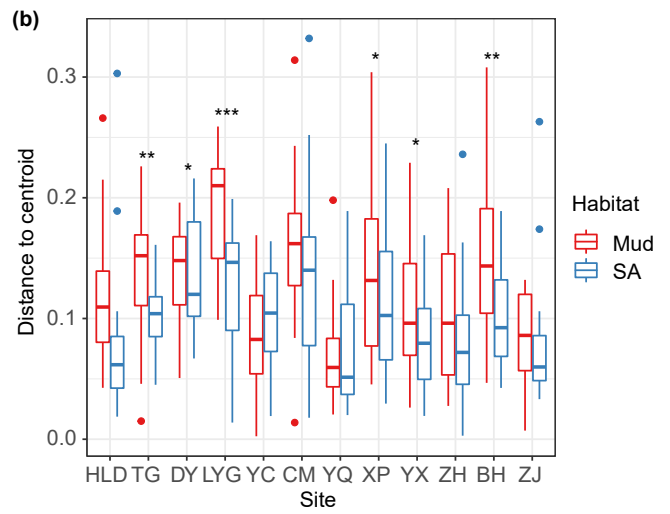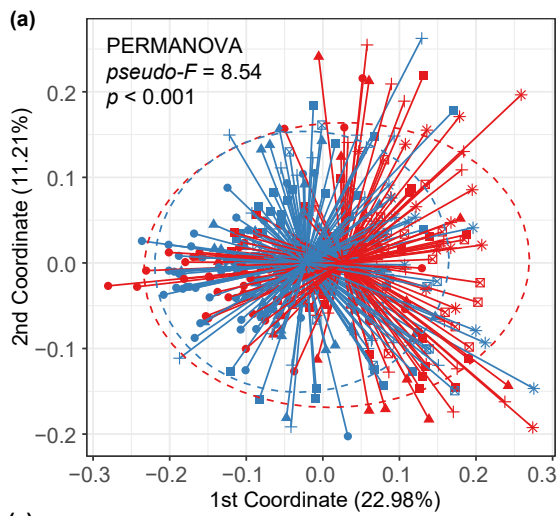Zhang, Y., Pennings, S. C., Li, B., & Wu, J. (2019). Biotic homogenization of wetland nematode communities by exotic *Spartina alterniflora* in China. *Ecology*, *100*(4), e02596. https://doi.org/10.1002/ecy.2596
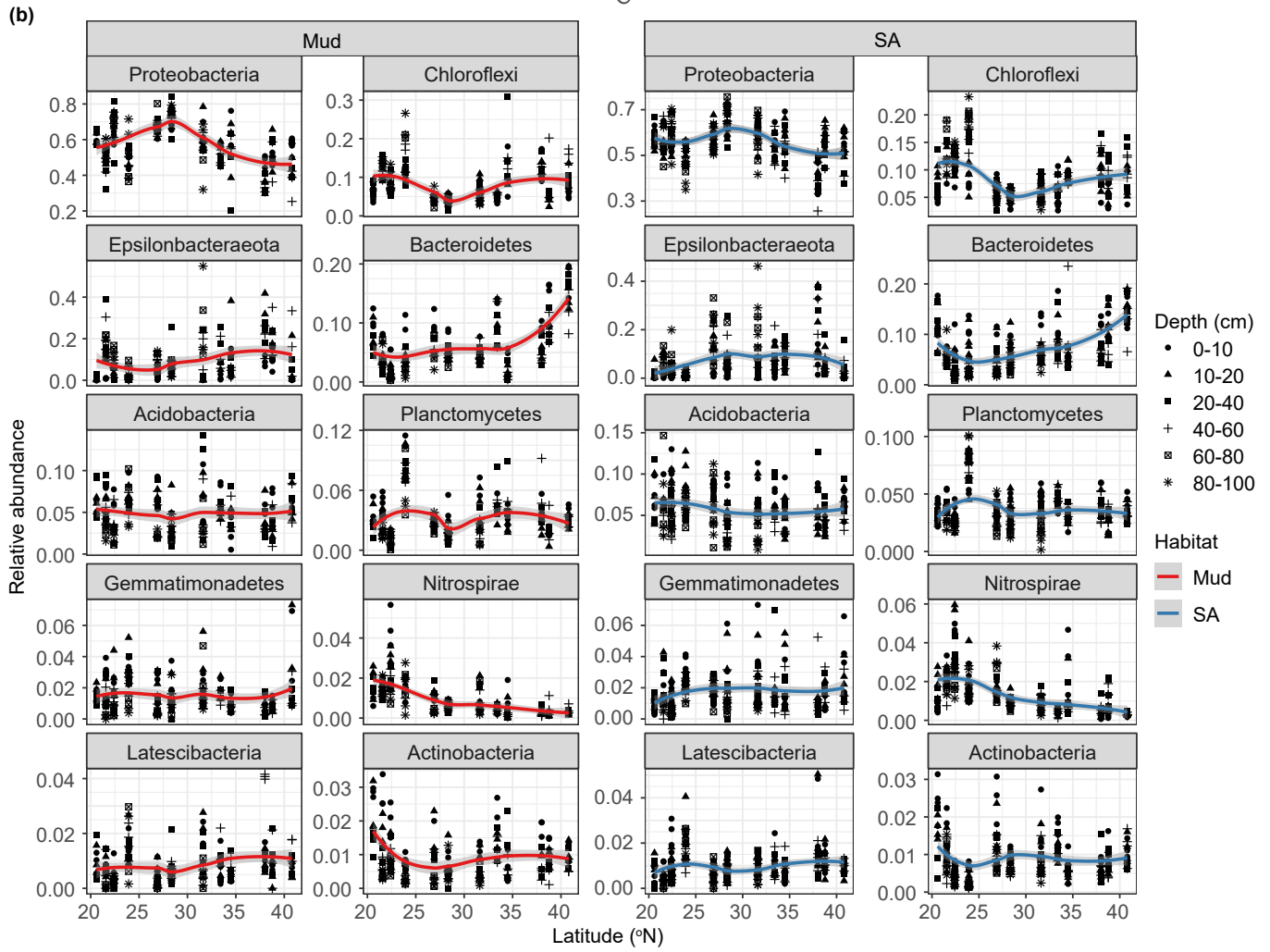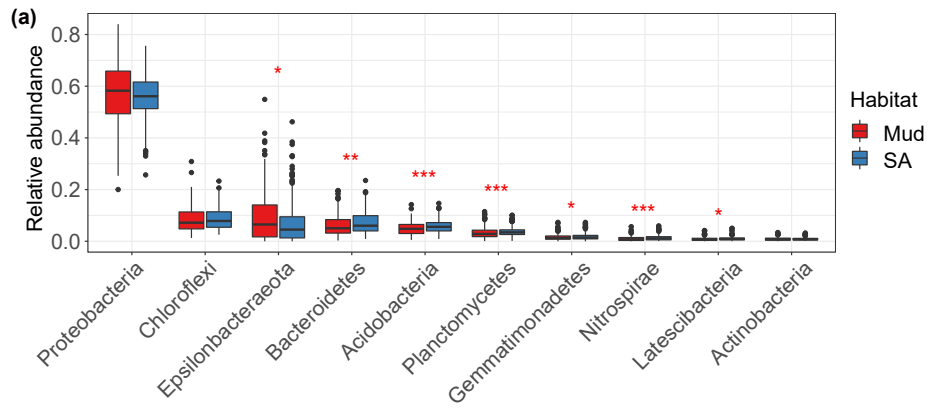
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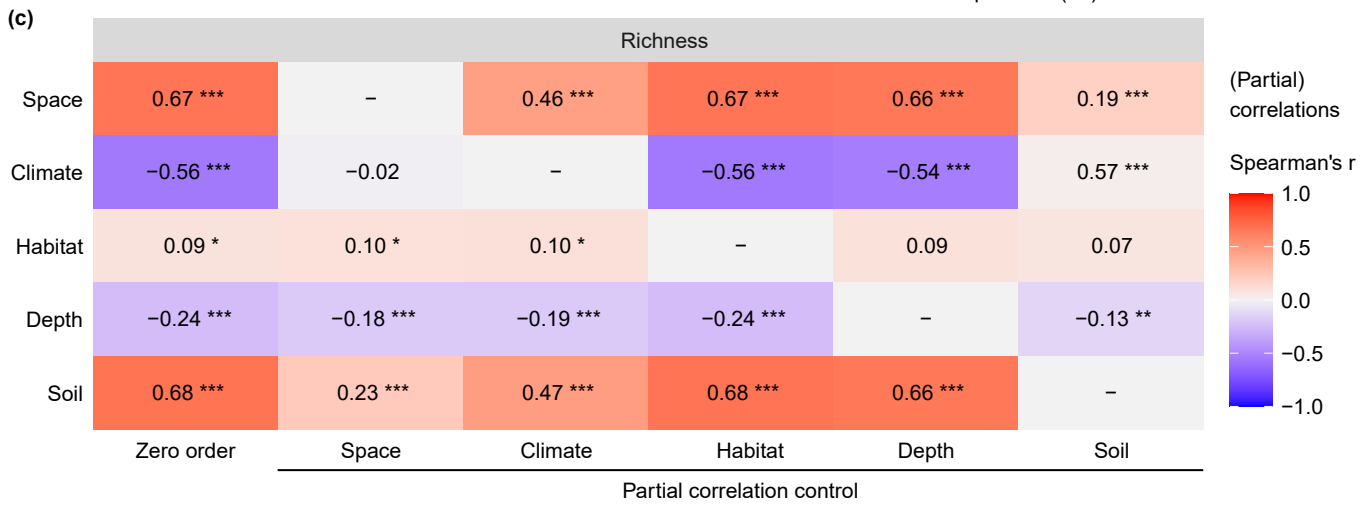
(a)

Spatial influences

(Long→Iso -.57***)
(Long→MAP .11***)
(Long→PET -1.08***)
(Lat→Iso -.23***)
(Lat→MAP 1.01***)
(Lat→PET .62***)

(Long→Mod #45 .68***)
(Lat→avgK 1.62***)
(Depth→Mod #45 -.39***)
(Lat→Mod #36 1.15***)

(Lat→ST -.84***)
(Depth→ST .12***)
(Long→ST .41***)
(Long→Salinity .88***)
(Depth→Salinity -.15***)
(Long→TN -.61***)

(MAP→TN .43***)
(PET→TN -.69***)
(PET→ST .38***)
(MAP→ST .26***)

Climatic factors   Box1   Soil properties

(→ST -.07***)
(→Salinity .31***)

(MAP→ -.68***)
(Iso→ -.57***)

Microbial communities

(→avgK -.23***)
(→Mod #45 .30***)

Plant invasion

(Lat→ .39***)
(Depth→ -.07***)
(Long→ -.29***)

(avgK→ .15***)
(Mod #45→ .15***)
(Mod #36→ -.35***)

(→TC .05*)

(TN→ .62***)
(ST→ .58***)

Soil carbon R^2= 0.84

x^2/df = 1.12, p = .30
$RMSEA$ = 0.02
Bollen-Stine bootstrap p = .87

Box1

Mod #36   Mod #45   avgK

.98***   .76***   1.50***   .28***   -.29***   -.47***   .56***

MAP   Iso   PET

Box2

Mod #36   Salinity

-.72***   .49***   .84***   -.84***

.20***   ST   .45***

-.45***   -.74***

avgK   .66***   Mod #45

-.86***

(b)

(c)

R^2_{adj} = 0.02 *
R^2_{adj} = 0.02 *

R^2_{adj} = 0.01, p = 0.11
R^2_{adj} = -0.004, p = 0.76

R^2_{adj} = 0.14 ***
R^2_{adj} = 0.03 **

TC

Mod #36   Mod #45   avgK

Habitat
Mud
SA

(a) Network diagram showing Module #27, Module #42, Module #25, Module #37, Module #22, Module #45, Module #36

(b) Relative abundance by Module (22, 25, 27, 36, 37, 42, 45)

Class
- Acidimicrobiia
- Alphaproteobacteria
- Aminicenantia
- Anaerolineae
- Bacilli
- Bacteroidia
- Calditrichia
- Campylobacteria
- Dadabacteriia
- Dehalococcoidia
- Deinococci
- Deltaproteobacteria
- Gammaproteobacteria
- Gemmatimonadetes
- Ignavibacteria
- Latescibacteria
- Moduliflexia
- Oxyphotobacteria
- Phycisphaerae
- Pla3 lineage
- Subgroup 17
- Subgroup 21
- Subgroup 22
- Thermoanaerobaculia
- Thermodesulfovibrionia
- uncultured bacterium
- uncultured delta proteobacterium

(c) Relative abundance (Z value) across Module #22 (***), Module #25 (p = 0.30), Module #27 (p = 0.52), Module #36 (p = 0.16), Module #37 (p = 0.28), Module #42 (***), Module #45 (*) by Habitat (Mud, SA)

(d) Natural connectivity vs Proportion of removed nodes

$Slope$ = -0.0219
R^2_{adj} = 0.94, p < 0.001

$Slope$ = -0.0298
R^2_{adj} = 0.91, p < 0.001

(e) avgK vs Latitude (°)

***

R^2_{adj} = 0.27, p < 0.001
R^2_{adj} = 0.20, p < 0.001

Habitat
- Mud
- SA

(a)

PERMANOVA
pseudo-F = 8.54
p < 0.001

Depth (cm)
● 0-10
▲ 10-20
■ 20-40
+ 40-60
⊠ 60-80
✳ 80-100

Habitat
● Mud
● SA

(b)

Habitat
Mud
SA

(c)

0-10 cm	10-20 cm	20-40 cm
Slope = -1.437e-05 ***	Slope = -2.573e-05 ***	Slope = -3.283e-05 ***
Slope = -3.068e-05 ***	Slope = -3.119e-05 ***	Slope = -4.235e-05 ***

40-60 cm	60-80 cm	80-100 cm
Slope = -3.643e-05 ***	Slope = -3.192e-06	Slope = -2.353e-05 *
Slope = -6.715e-05 ***	Slope = -8.418e-05 ***	Slope = -6.565e-05 ***

Habitat
Mud
SA

(a)

(b)

(d)

$Y = -1.0876X^2 + 20.7427X + 364.9738$
$R^2_{adj} = 0.50$, $p < 2.2e\text{-}16$

$Y = -1.7037X^2 + 39.8749X + 241.9680$
$R^2_{adj} = 0.45$, $p < 2.2e\text{-}16$

Depth (cm)
● 0-10
▲ 10-20
■ 20-40
+ 40-60
⊠ 60-80
✳ 80-100

Habitat
— Mud
— SA

(c)

	Zero order	Space	Climate	Habitat	Depth	Soil
Space	0.67 ***	–	0.46 ***	0.67 ***	0.66 ***	0.19 ***
Climate	−0.56 ***	−0.02	–	−0.56 ***	−0.54 ***	0.57 ***
Habitat	0.09 *	0.10 *	0.10 *	–	0.09	0.07
Depth	−0.24 ***	−0.18 ***	−0.19 ***	−0.24 ***	–	−0.13 **
Soil	0.68 ***	0.23 ***	0.47 ***	0.68 ***	0.66 ***	–

Richness

(Partial) correlations

Spearman's r
1.0
0.5
0.0
−0.5
−1.0

Partial correlation control

(a)

MAT (°C)
23.98
4.17

2000
before invasion

2017
after invasion

Comparison before and after plant invasion,
taking the YX site as an example.

(b)

50 m

50 m

Mud

SA

● *S. alterniflora* patches
★ Replicates
● Soil cores

(c)

Depth (cm)	HLD	TG	DY	LYG	YC	CM	YQ	XP	YX	ZH	BH	ZJ
0-10	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●
10-20	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●
20-40	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●
40-60	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●
60-80						●●	●●	●●	●●	●●	●●	
80-100						●●	●●	●●	●●	●●	●●	

● Mud
● SA

Types of contribution: Research articles

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

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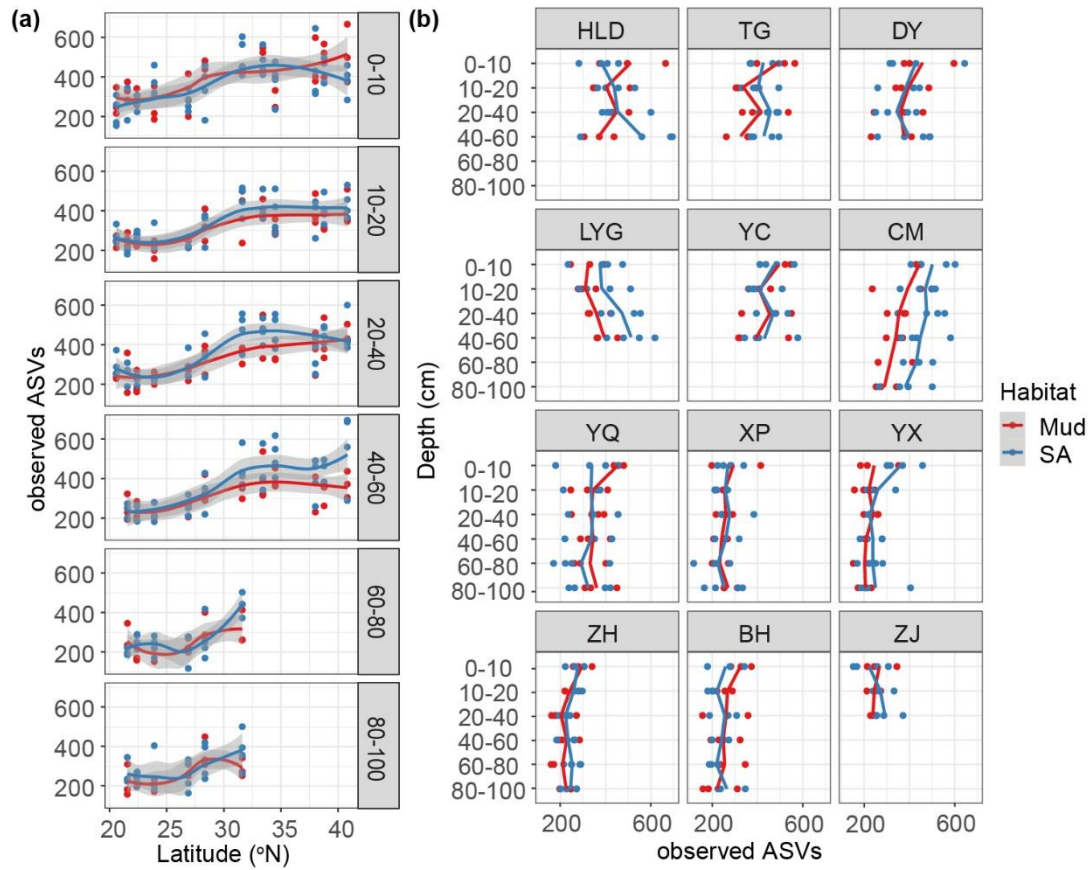
Fig. S1| Changes of soil bacterial alpha diversity. Variations of observed ASVs in various depths with latitude (a). Variations of observed ASVs with soil depth in each site (b). Mud: bare mudflat; SA: *Spartina alterniflora*; HLD: Huludao; TG: Tanggu; DY: Dongying; LYG: Lianyungang; YC: Yancheng; CM: Chongming; YQ: Yueqing; XP: Xiapu; YX: Yunxiao; ZH: Zhuhai; BH: Beihai; ZJ: Zhanjiang.
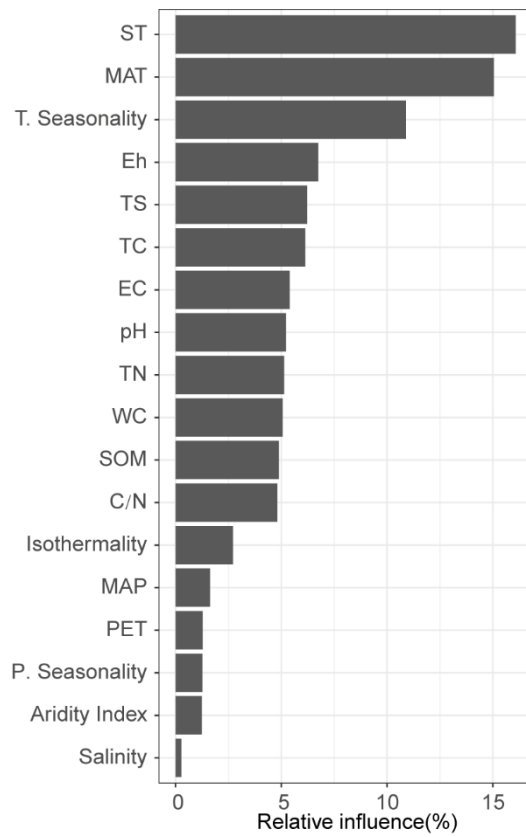
Fig. S2| Random forest analysis was used to identify the key environmental factors in affecting observed ASVs. The relative importance of the environmental factors was ranked in a descending order. ASVs: amplicon sequence variants; Eh: redox potential; ST: soil temperature; EC: electrical conductivity; TN: total nitrogen; TC: total carbon; TS: total sulfur; SOM: soil organic matter; WC: water content; MAP: mean annual precipitation; MAT: mean annual temperature; PET: potential evapotranspiration.
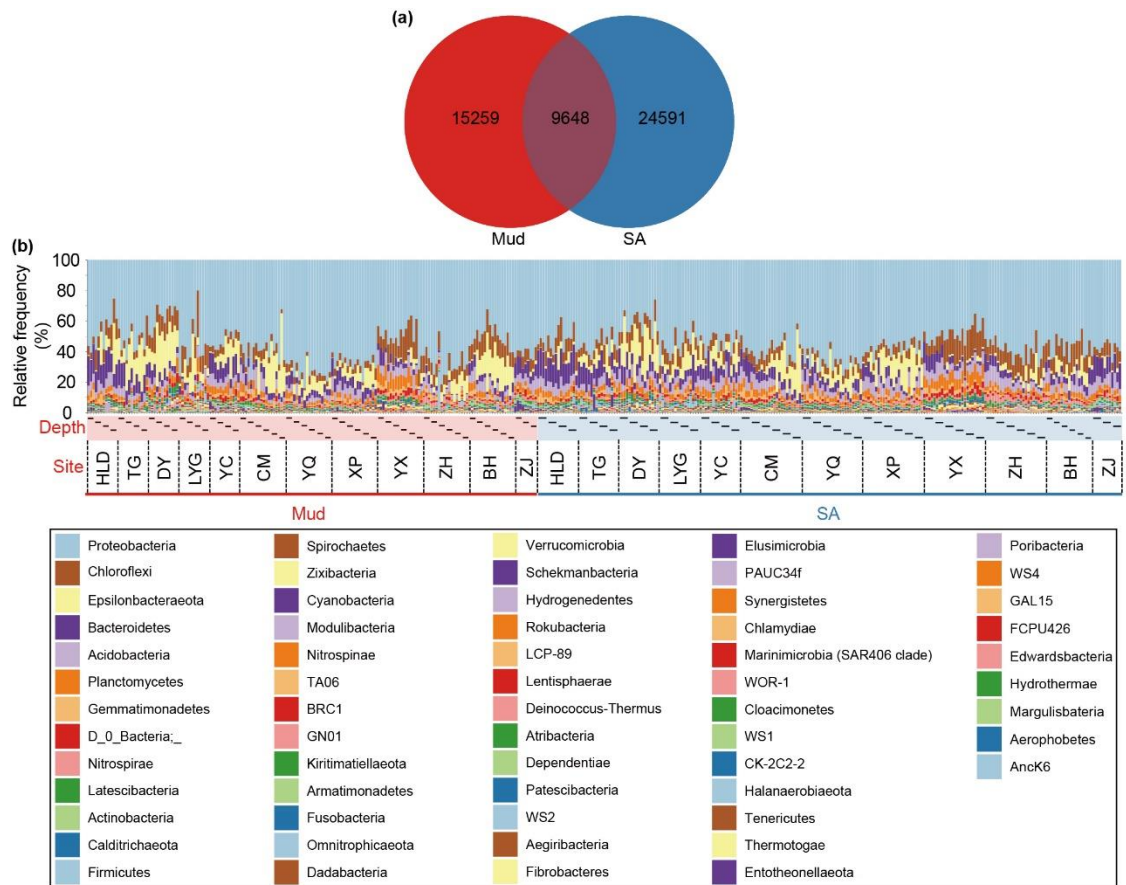
Fig. S3| The number of unique and shared ASVs of Mud and SA habitats (a). The relative abundance of identified bacterial phyla (b). Different colors of the columns represent different bacterial phyla. For abbreviations, see the legends of Fig. S1.
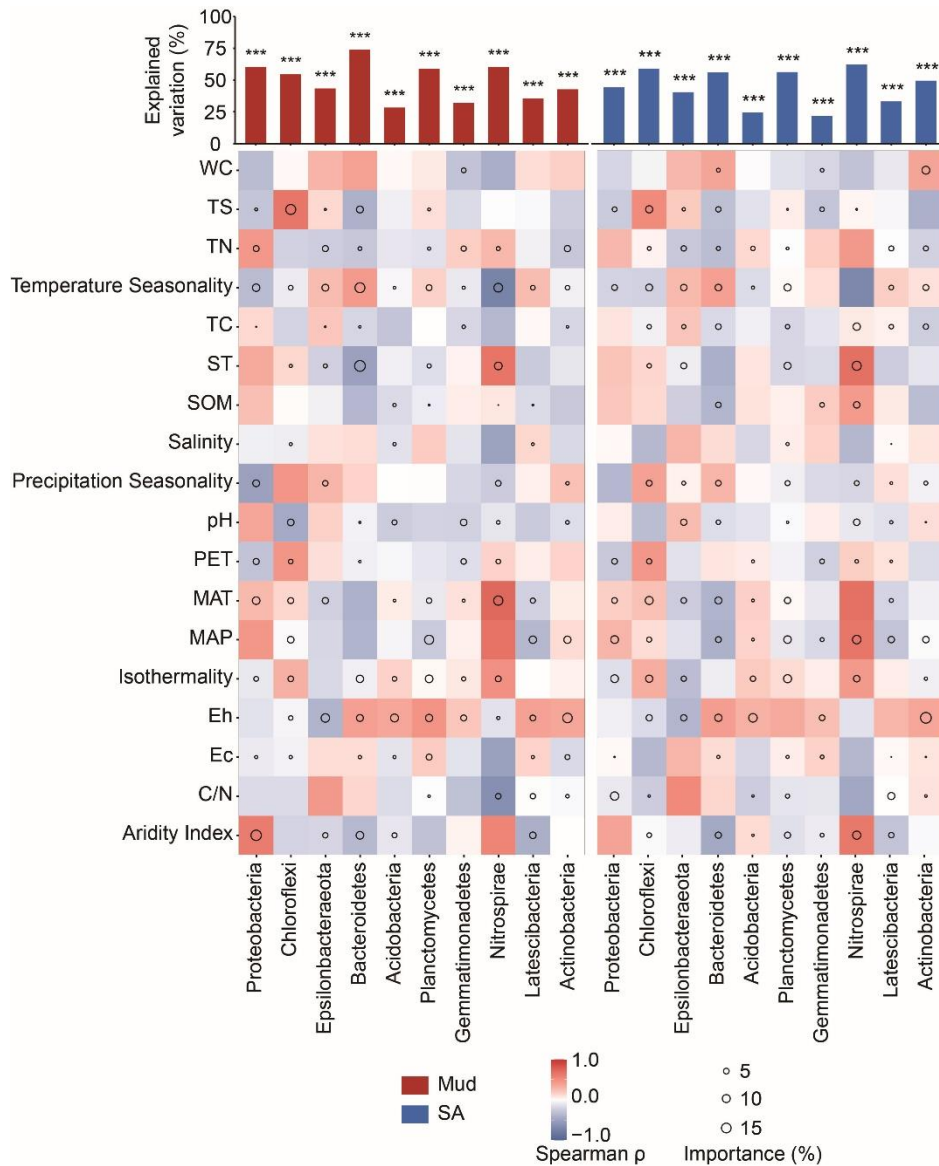
Fig. S4| Contributions of soil properties to the differences in relative abundances of microbial phyla based on correlation and best multiple regression model. The heat map represents the Spearman correlation coefficients between soil properties and phyla. The bars represent the total contribution of soil properties in explaining microbial variation, and the circle size indicates the importance of soil properties, which is obtained by multiple linear regression and variance decomposition analysis. For abbreviations, see the legends of Fig. S1 and Fig. S2. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
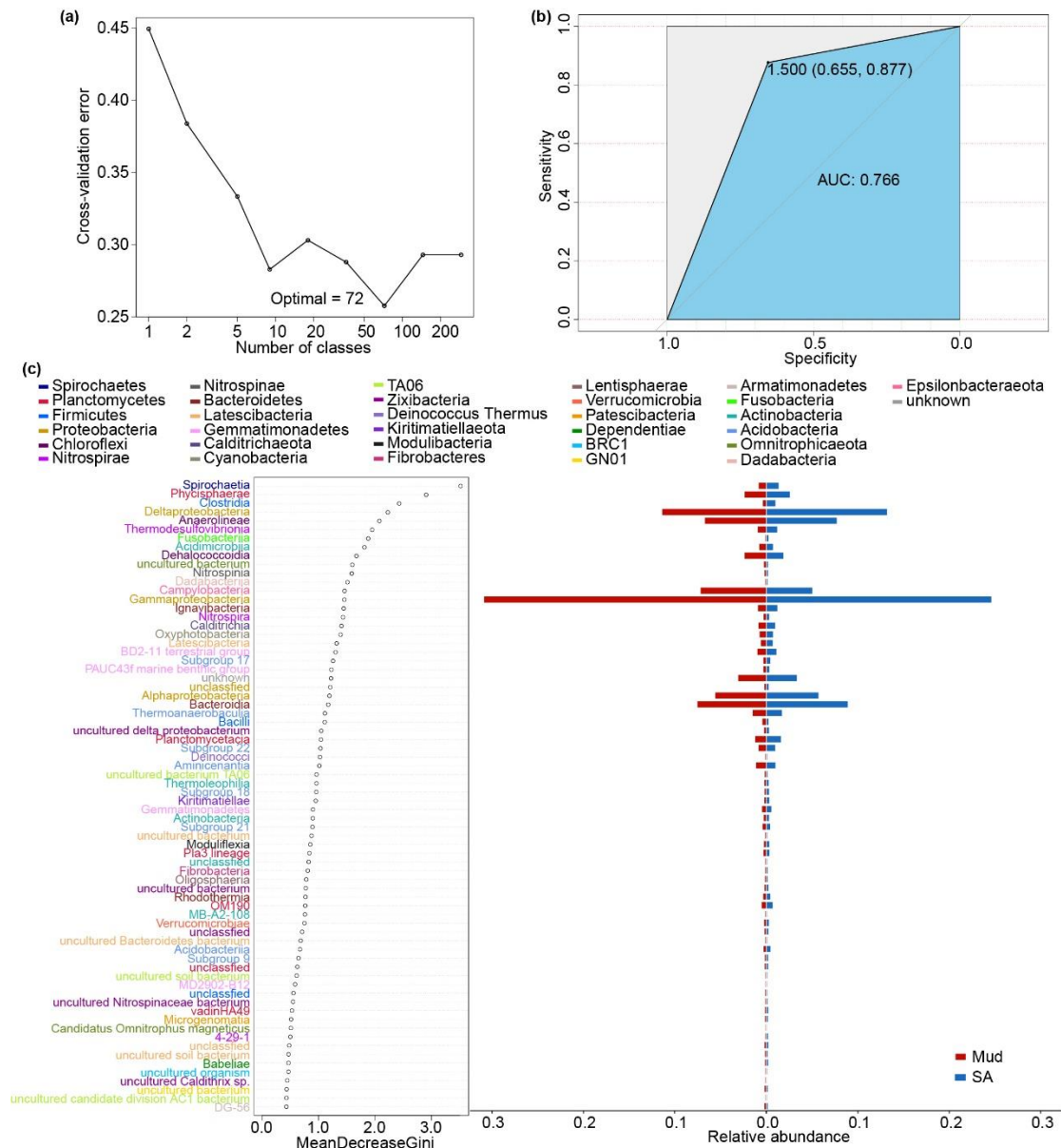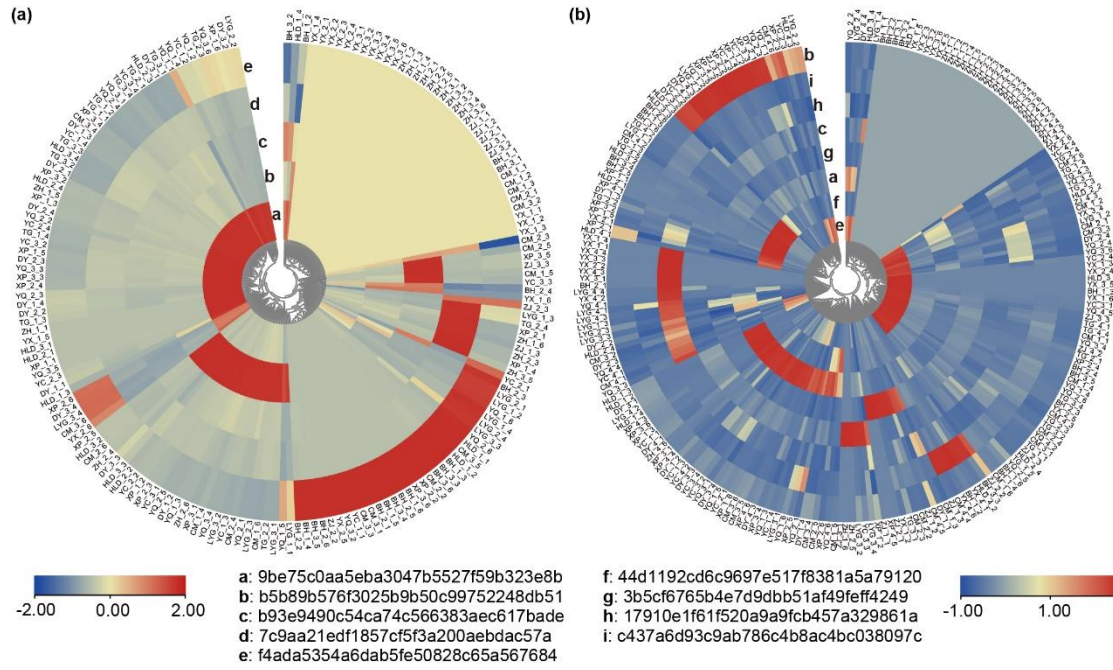
Fig. S5| Random Forest analysis demonstrating biomarkers in Mud and SA soils. The cross-validation error as a function of the number of input classes used to classify against group (a). AUC score for evaluation of random forest classification models (b). Biomarkers ranking in a descending order of their importance to the accuracy of the model (c). The colors of the classes represent the phyla to which they belong to. For abbreviations, see the legends of Fig. S1.
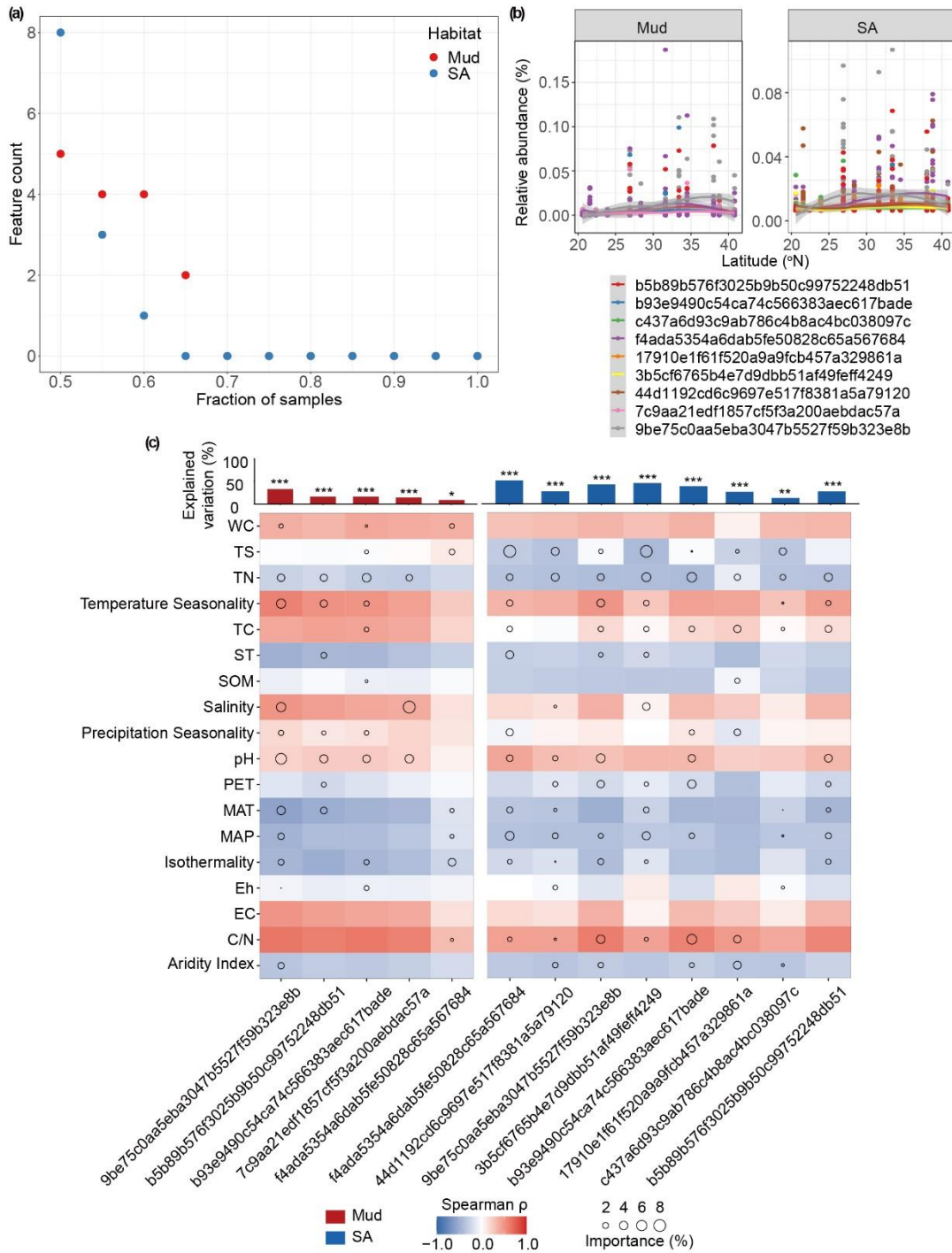
Fig. S6| Identification of core species. Relationship between changes in the number of core species and their proportional occurrence in the samples (a). Variation in relative abundance of core species with latitude (b). Contributions of soil properties to the differences in relative abundances of core species based on correlation and best multiple regression model. The heat map represents the Spearman correlation coefficients between soil properties and core species. The bars represent the total contribution of soil properties in explaining microbial variation, and the circle size indicates the importance of soil properties, which is obtained by multiple linear regression and variance decomposition analysis (c). For abbreviations, see the legends of Fig. S1 and Fig. S2.

(a)

(b)

a: 9be75c0aa5eba3047b5527f59b323e8b	**f**: 44d1192cd6c9697e517f8381a5a79120
b: b5b89b576f3025b9b50c99752248db51	**g**: 3b5cf6765b4e7d9dbb51af49feff4249
c: b93e9490c54ca74c566383aec617bade	**h**: 17910e1f61f520a9a9fcb457a329861a
d: 7c9aa21edf1857cf5f3a200aebdac57a	**i**: c437a6d93c9ab786c4b8ac4bc038097c
e: f4ada5354a6dab5fe50828c65a567684	

Fig. S7| Heat map showing the relative abundance of core species in all samples for Mud (a) and SA (b) soils. The lower-case letters in the diagram represent their corresponding ASVs. For abbreviations, see the legends of Fig. S1.
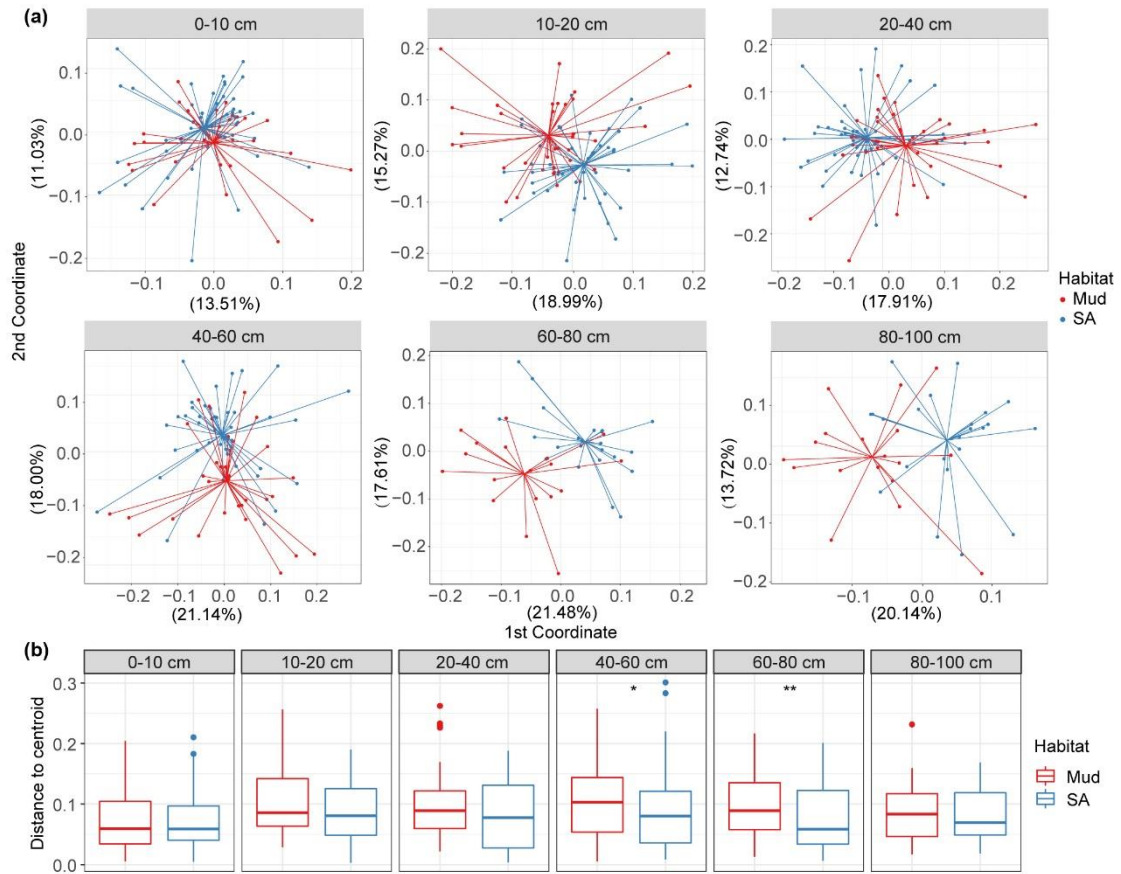
Fig. S8| Results of the covariate adjusted principal coordinates analysis (aPCoA) based on the Weighted UniFrac distance revealing the pairwise dissimilarities between Mud and SA habitats for each soil depth (a). Distance of soil bacterial community to centroid point for each soil depth (b). For abbreviations, see the legends of Fig. S1. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
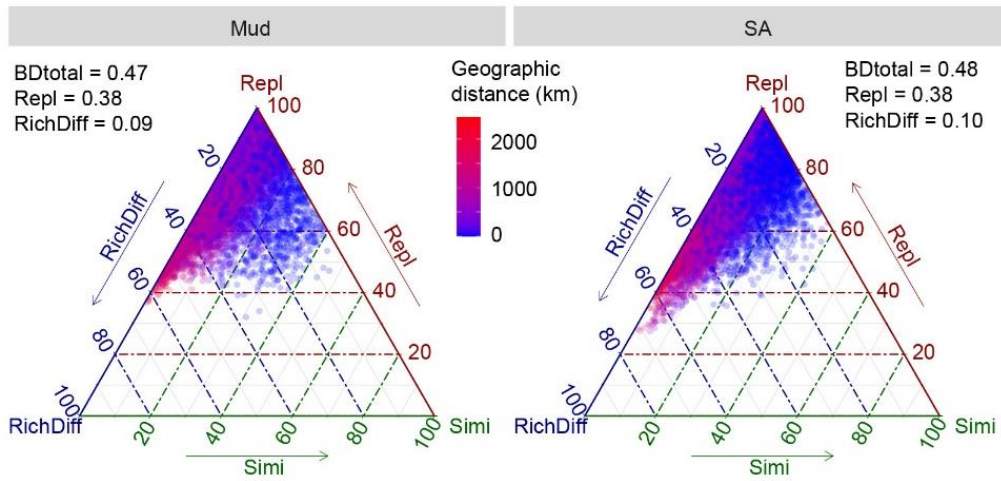
Fig. S9| Variation in the relative contribution of beta diversity components with geographic distance. For abbreviations, see the legends of Fig. S1.
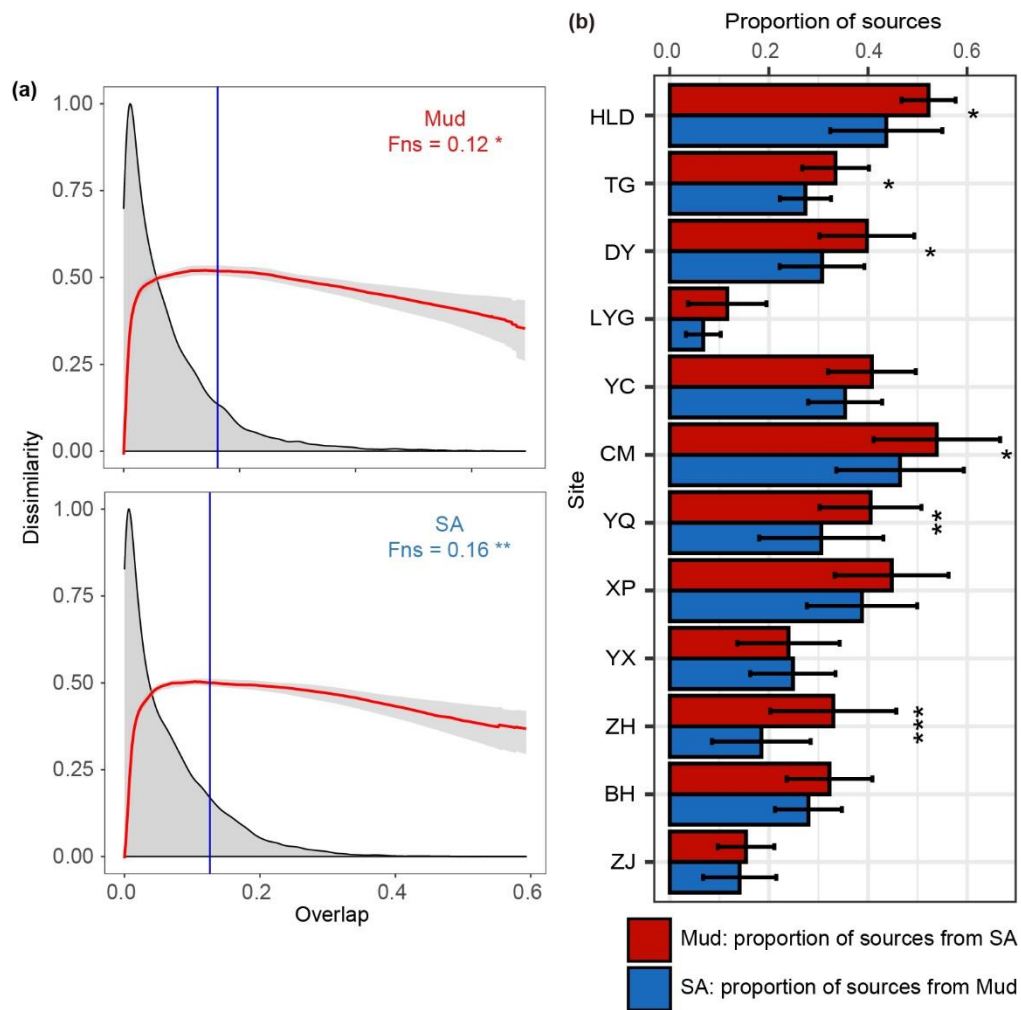
Fig. S10| Dissimilarity Overlap curve was used to examine whether the ecological dynamics of the bacterial communities are universal across all communities or unique to individual communities (a). Fast expectation-maximization microbial source tracking (FEAST) analysis reveals the proportion of sources in Mud or SA habitats, respectively (b). For abbreviations, see the legends of Fig. S1. $* p < 0.05$; $** p < 0.01$; $*** p < 0.001$.
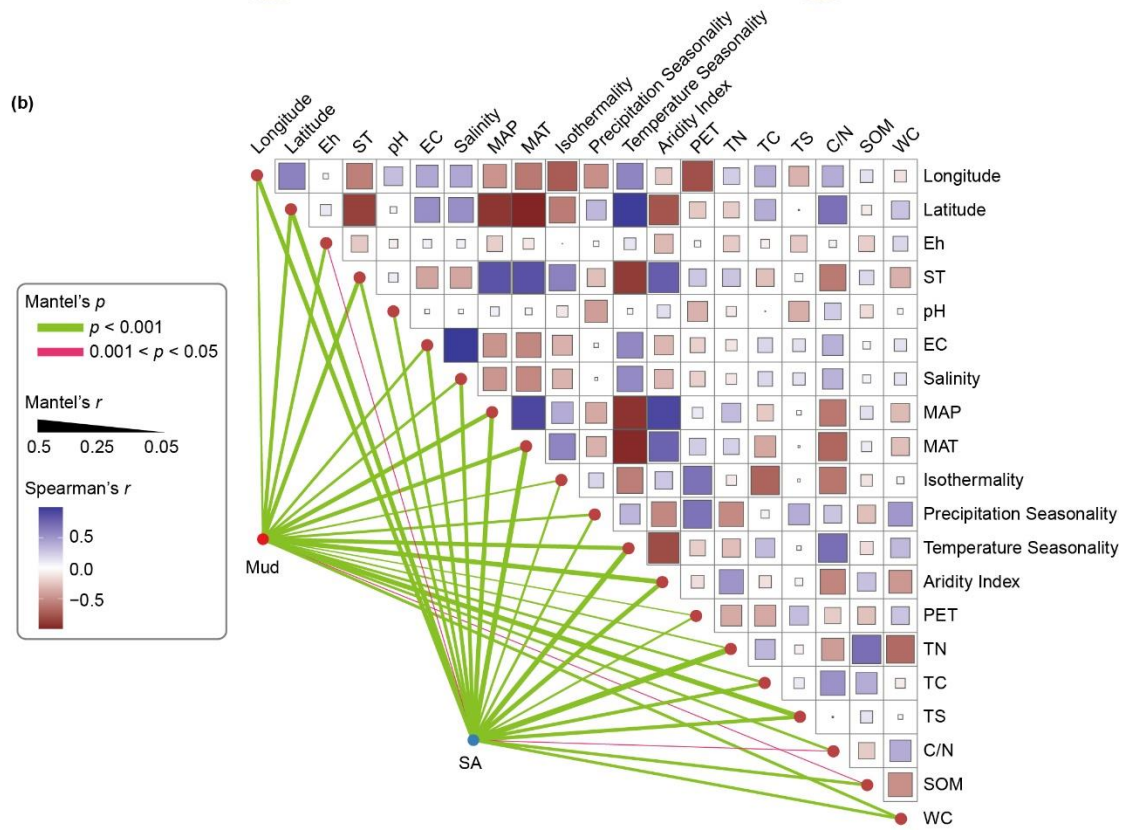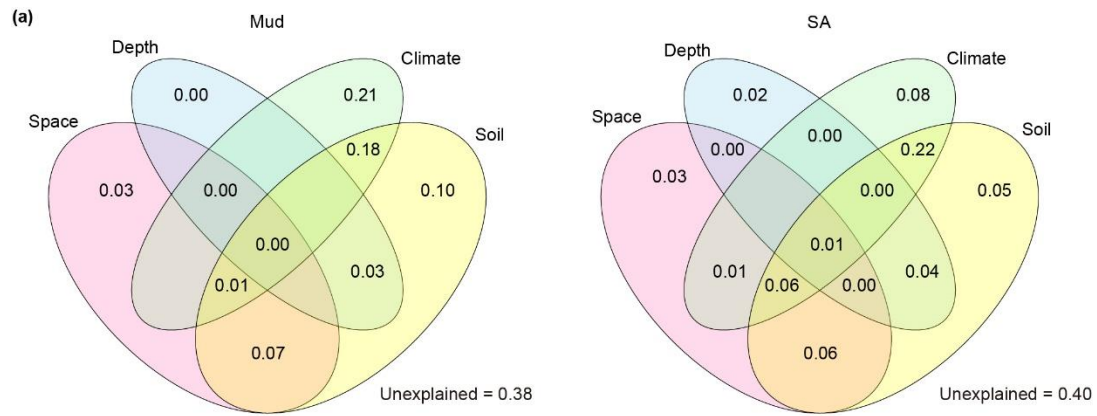
Fig. S11| The results of variation partitioning analysis (VPA) showed the dependent and independent effects of space, depth, climate and soil factors in influencing bacterial communities (a). The Spearman correlations between environmental factors, and the Mantel correlations between bacterial communities and soil properties, for Mud and SA habitats respectively. The thickness and the color of the line represents the magnitude of the Mantel's *r* and *p* value (b). For abbreviations, see the legends of Fig. S1 and Fig. S2.
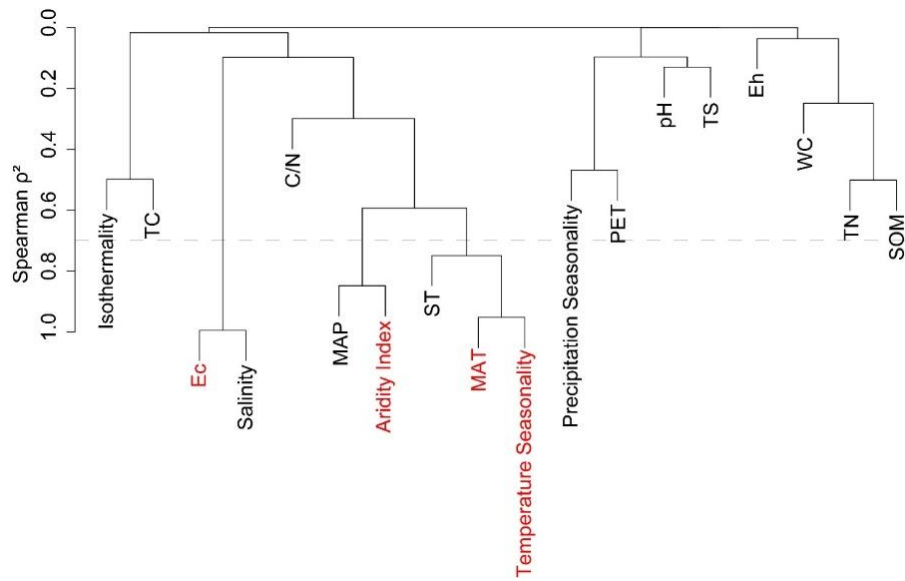
Fig. S12| To avoid the effect of multicollinearity, the redundancy of environmental variables was evaluated. The dashed line is Spearman ρ^2 equal to 0.7, and the environment variables marked in red font are those deleted in the subsequent analysis. For abbreviations, see the legends of Fig. S2.
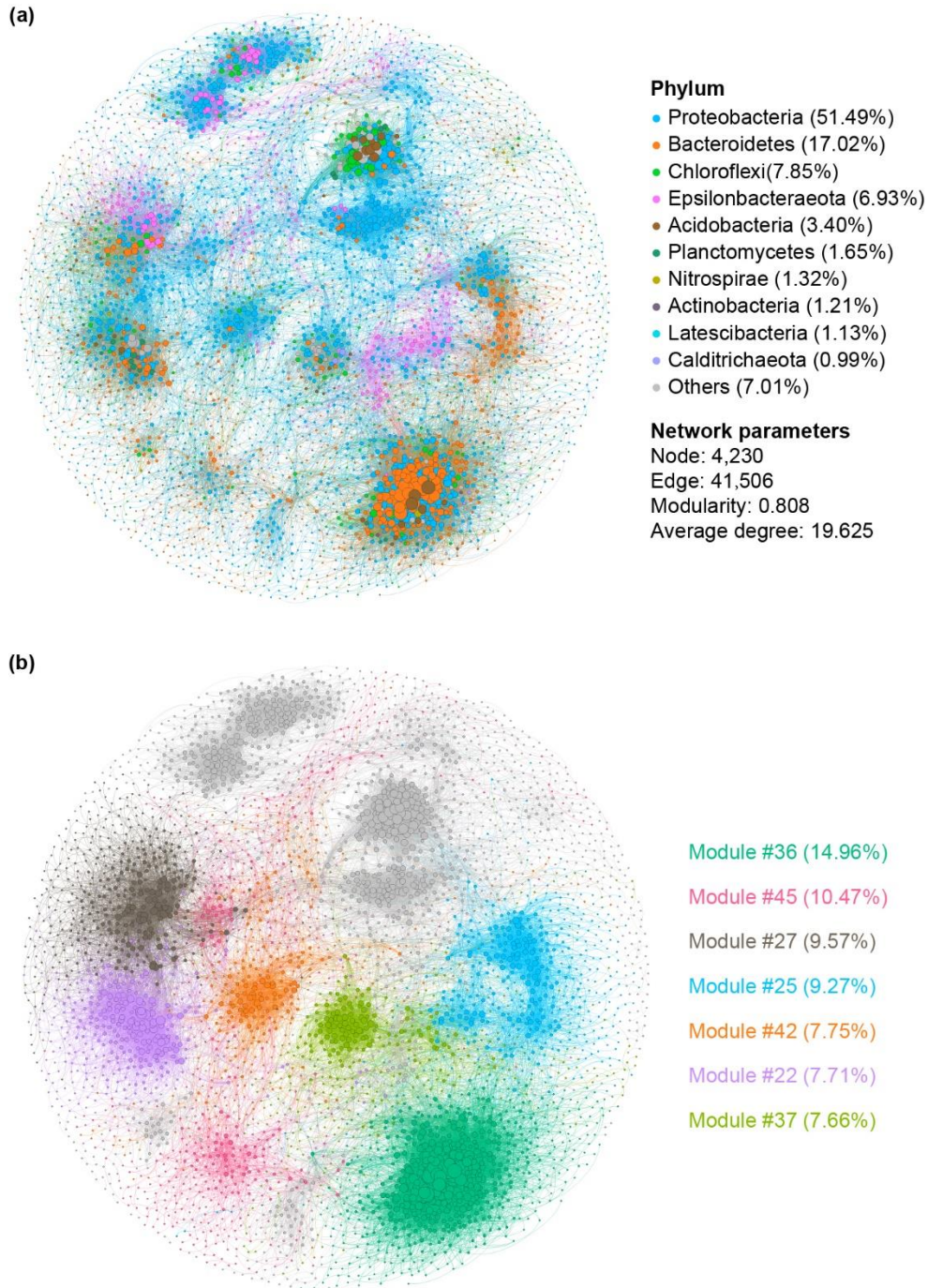
(a)

Phylum
- Proteobacteria (51.49%)
- Bacteroidetes (17.02%)
- Chloroflexi(7.85%)
- Epsilonbacteraeota (6.93%)
- Acidobacteria (3.40%)
- Planctomycetes (1.65%)
- Nitrospirae (1.32%)
- Actinobacteria (1.21%)
- Latescibacteria (1.13%)
- Calditrichaeota (0.99%)
- Others (7.01%)

Network parameters
Node: 4,230
Edge: 41,506
Modularity: 0.808
Average degree: 19.625

(b)

Module #36 (14.96%)

Module #45 (10.47%)

Module #27 (9.57%)

Module #25 (9.27%)

Module #42 (7.75%)

Module #22 (7.71%)

Module #37 (7.66%)

Fig. S13| Co-occurrence network of soil bacterial communities. The colors of the nodes represent the different dominant bacterial phyla (a). The percentage in parentheses represents the percentage of bacteria of that phylum within the network. The color of the nodes represents the different ecological modules (b). Modules with more than 300 nodes are marked with different colors. The percentage in parentheses represents the percentage of that module within the network.
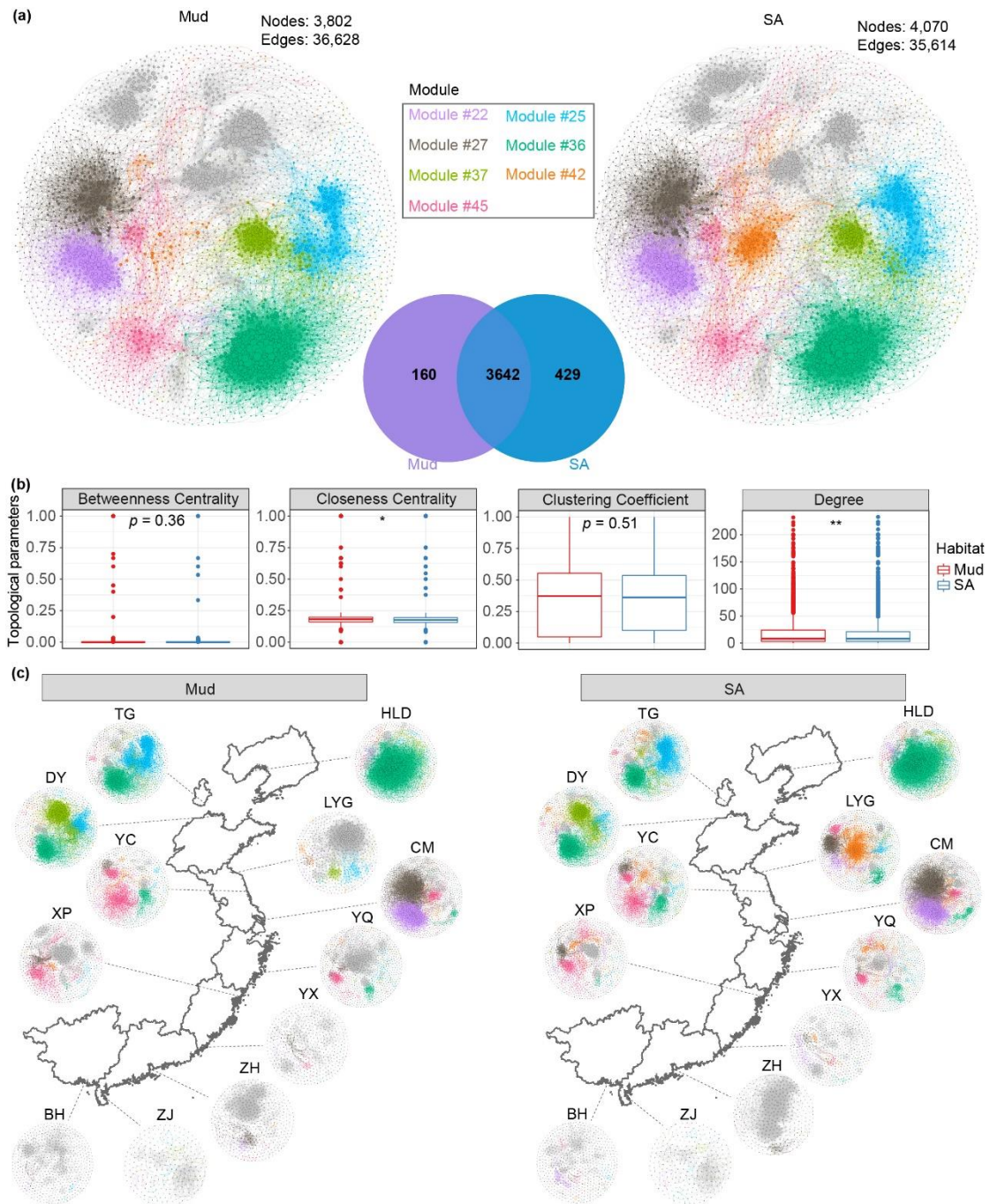
Fig. S14| Bacterial co-occurrence network for Mud and SA soils respectively. Venn diagram showing the number of unique and shared ASVs for Mud and SA networks (a). The color of the nodes represents the different ecological modules. The differences of topological parameters between Mud and SA networks (b). The latitudinal distribution of co-occurrence network for Mud and SA soils respectively (c). $p < 0.05$ *; $p < 0.01$ **. For abbreviations, see the legends of Fig. S1.
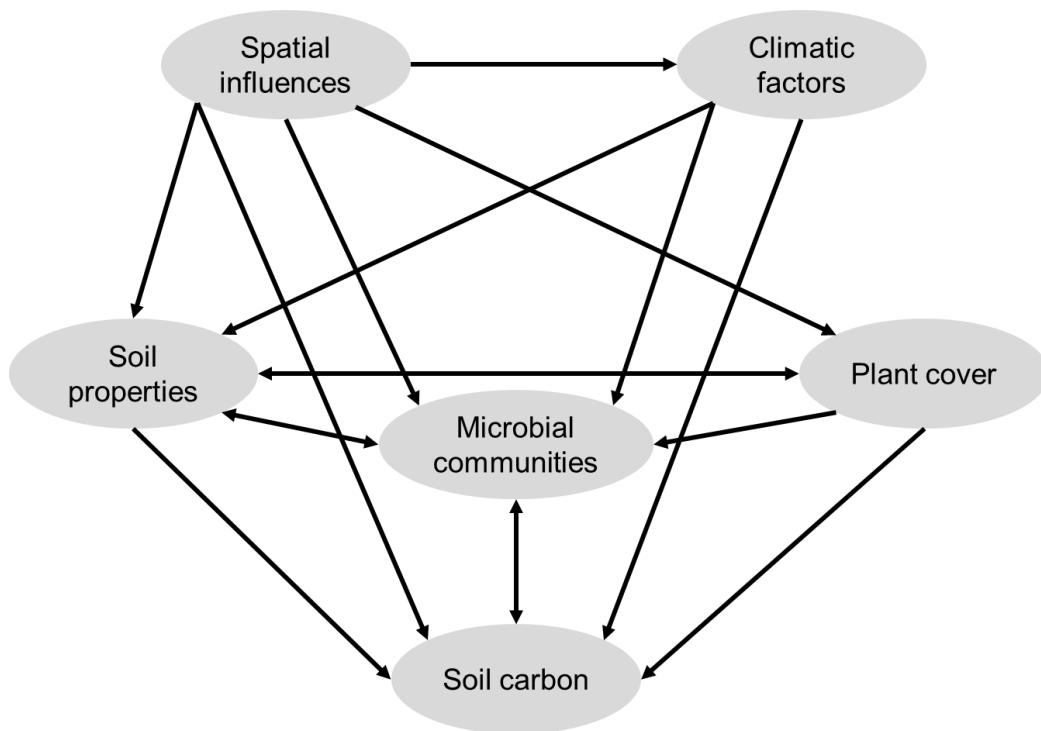
Fig. S15| The initial model of structure equation modeling analysis. The arrows indicate the predicted causal relationships.
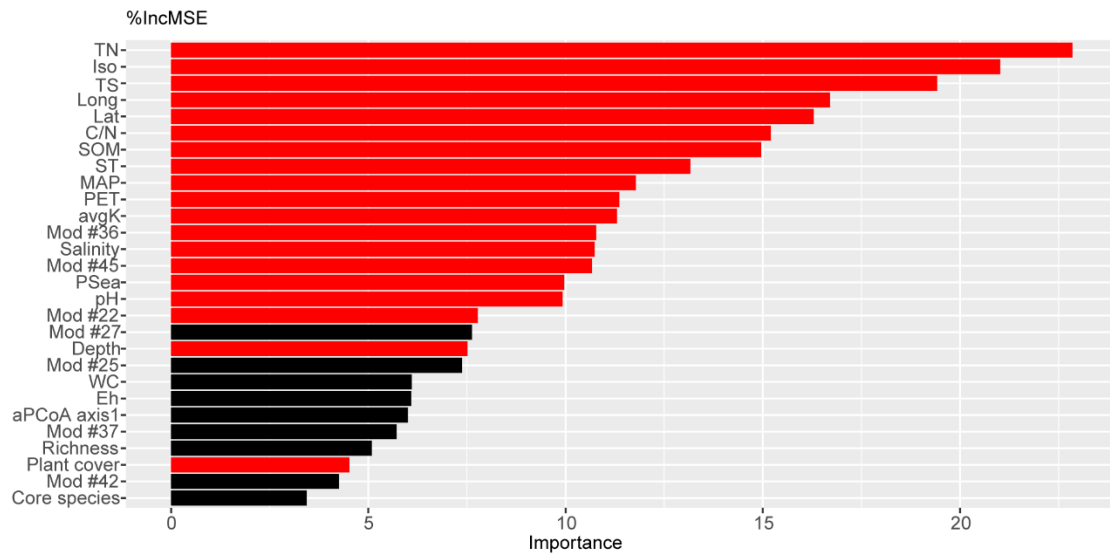
Fig. S16| Random Forest analyses were conducted to identify the most important predictors in influencing soil TC. The graphic shows importance scores scaled by ranked importance. For abbreviations, see the legends of Fig. S2.