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Research article

Divergent biotic-abiotic mechanisms of soil organic carbon storage between bulk and rhizosphere soils of rice paddies in the Yangtze River Delta

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ABSTRACT

Rice paddies serve as important reservoirs of soil organic carbon (SOC) and are hotspots for microbial-mediated carbon conversion. Understanding the regulatory mechanisms of SOC in rice paddies is important for carbon sequestration management under global warming. Most previous studies on the influencing factors of paddy SOC have focused on a single habitat, such as bulk soil (BS) or rhizosphere soil (RS). However, the divergence in SOC regulatory mechanisms between BS and RS, the mediating role of rhizoplane soil (RP) microbial communities on SOC in RS, and the interactive effects of multiple influencing factors on habitat-specific SOC remain poorly quantified, particularly at regional scales. Herein we used piecewise structural equation modeling and random forest model to explore the effects of biotic-abiotic factors on SOC between BS and RS of rice paddies in the Yangtze River Delta. Significant differences in SOC, soil physicochemical, and microbial community properties between BS and RS. Soil physical and chemical properties had the greatest effect on SOC, with a standardized total effect of 0.76 vs. 0.53 for BS, and 0.72 vs. 0.94 for RS, respectively. The direct effect of microbial communities on SOC in RS (standardized direct effect, 0.33) was significant and positive, while it was marginal in BS (standardized direct effect, 0.14). Methane microbial communities in RP on SOC in RS were also markedly important. Notably, soil metallic elements had a significant positive effect on SOC in BS with a standardized path coefficient of 0.30 ($P < 0.05$), but a negative effect on SOC in RS with a path coefficient of -0.18 ($P < 0.05$). Soil physical properties had a positive indirect effect on SOC in BS with an indirect effect of 0.39 through its effect on soil chemical properties, metallic elements, and microbial community properties, while a positive indirect effect on SOC in RS with an indirect effect of 0.66 mainly through its effect on soil chemical properties. The indirect effects of climatic factors and agricultural management on SOC between BS and RS were also nonnegligible. Our study reveals the complex interactive influence of various categories of biotic-abiotic factors with different functions on paddy SOC between BS and RS at a regional scale, as well as the contribution of microbial communities in RP to SOC in RS. This study improves our understanding of the regulatory mechanisms for SOC storage and offers valuable guidance for paddy soil carbon sequestration management.

1. Introduction

Soil organic carbon (SOC) is the largest carbon pool in terrestrial ecosystems, playing a critical role in global carbon cycle (Batjes, 1996). SOC influences soil structure, fertility, agricultural productivity, and ecosystem functioning (Davidson and Janssens, 2006). Rice paddies, rich in SOC, serve as an important agricultural carbon reservoir (Liu et al., 2021). Due to the gradual accumulation of organic carbon in rice paddies under the unique water tillage and maturation process (Liu et al., 2019), the carbon storage of rice paddies is more than 30 % higher than that of the corresponding upland soils per unit area (Qin et al., 2013). The carbon sequestration efficiency of rice paddies is more than 39 %–127 % higher than their adjacent upland counterparts (Chen et al., 2021). Within the context of climate change and intensive human activities, it is important to explore the underlying mechanisms driving SOC spatial distribution in rice paddies for sustainable management and

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carbon sequestration.

Rice paddies exhibit distinct SOC formation mechanisms compared to other soil types due to their unique hydrothermal conditions (Kögel-Knabner et al., 2010). Anaerobic environments under flooded conditions generally slow organic matter decomposition, promoting SOC accumulation (Wu, 2011). In contrast, non-flooded periods increase soil microbial activity and SOC turnover. SOC formed under the alternating two periods is influenced by various factors, including climatic factors, soil physicochemical properties, microbial community properties, and agricultural management (Luo et al., 2017; Philippot et al., 2024; Wu et al., 2024b; Chen et al., 2025). Numerous studies have been conducted on the above influencing factors (Davidson and Janssens, 2006; Crystal-Ornelas et al., 2021), and more attention has been recently paid to soil microorganisms (e.g., bacteria, fungi, and archaea). This is probably because they can be an important source of SOC (Kallenbach et al., 2016), and play a unique role in SOC accumulation and decomposition process (Lehmann and Kleber, 2015; Jansson and Hofmockel, 2020). Particularly, microbial community properties, such as community composition, diversity, and network complexity, influence the stability and turnover of SOC in rice paddies (Wang et al., 2023; Wu et al., 2024b).

In the special redox environment of rice paddies, methane microorganisms emerge as keystone taxa that not only mediate methane flux but also orchestrate SOC transformation through substrate competition and metabolic cross-feeding (Cai et al., 2016; Jansson and Hofmockel, 2020). This dual role positions methane microorganisms at the nexus of SOC stabilization and greenhouse gas emission dynamics in rice paddies. The centrality of methane microorganisms to rice paddy carbon budgets is underscored by their capacity to couple SOC turnover with atmospheric feedbacks. Methanogens (e.g., *Methanosarcina*) drive hydrogenotrophic and acetoclastic pathways that compete with iron reducing bacteria for organic substrates, thereby regulating SOC mineralization rates. Concurrently, methanotrophs (e.g., *Methylocystis*, *Methylomonas*) oxidize CH_4 to CO_2 while synthesizing refractory biomarkers, creating a microbial carbon pump that redirects labile SOC toward stabilized pools (Cai et al., 2016; Qian et al., 2023; Zheng et al., 2024a). Such redox-dependent functional partitioning ultimately dictates whether rice paddies act as net carbon sinks or sources.

Bulk soil (BS), rhizosphere soil (RS), and rhizoplane soil (RP) show gradient differences in physicochemical properties and microbial activities, and all three were coupled dynamically through carbon flow, oxygen gradient, and biomigration, which jointly shaped the spatial heterogeneity of soil carbon pools in rice paddies (Kuzyakov, 2010; Edwards et al., 2015; Fan et al., 2017). Previous studies on SOC have focused on a single habitat, such as BS (Luo et al., 2017; Wang et al., 2023) or RS (Luo et al., 2021; Villarino et al., 2021). In recent years, increasing attention has been paid to the differences in microbial communities among BS, RS, and RP (Fan et al., 2017). Differences between BS and RS led to differences in the stability and carbon uptake of soil microbial communities (Lange et al., 2024). A previous study showed that bacterial community richness was significantly higher in BS when compared with RS in rice paddies, but community distances for RS bacteria were significantly higher than in BS (Tian et al., 2022). Another study showed that Actinobacteria and Bacteroidetes were significantly more abundant in RP, compared to RS and BS; while Chloroflexi was significantly less abundant in RP compared to the other habitats (Fan et al., 2017). Soil microorganisms in RS and RP receive carbon metabolites from the plant through root exudates, thereby promoting the accumulation of SOC in RS. Furthermore, plant root exudates promote the enrichment of methanogens in RP, while RS oxidizing layer promotes the activity of methanotrophs, forming a “methane-producing-consuming” microregion that regulates the spatial distribution of SOC in rice paddies (Zheng et al., 2024a). Thus, methane microbial-mediated methane fluxes from rice paddies are closely related to long-term carbon storage. However, existing studies have not comprehensively analyzed the differences in microbial community properties in different habitats of rice paddies. The divergence in SOC regulatory mechanisms between BS and RS, and the mediating role of microbial communities in RP on SOC content in RS are still unknown.

SOC is usually affected by several biotic and abiotic factors together. An important issue is to quantify the interactive influence of multiple factors. Previous studies have mainly quantified the impact pathways of climate, soil physicochemical properties or microbial communities, and quantified their direct and indirect effects (Deng et al., 2023; Wang et al., 2023; Fan et al., 2025). A study indicated that microbial metabolic limitation regulated by carbon nitrogen phosphorus stoichiometric ratio stimulated SOC accumulation in rice paddies (Wang et al., 2024). Recently, some scholars have also begun to pay attention to agricultural management (Wu et al., 2024a; Xin et al., 2024; Abrar et al., 2025) and soil metallic elements (Moore et al., 2023; Pei et al., 2024; Zeng et al., 2024). However, the interactive effects of multiple influencing factors on SOC in different habitats of rice paddies are still unclear.

The Yangtze River Delta (YRD) is one of the major agricultural and rice-producing regions in China, and multiple factors and management have regulated SOC in the YRD region. Thus, it can be an optimal area to identify the regulatory mechanisms for SOC between BS and RS of rice paddies. Particularly, we focus on combining the comprehensive microbial communities (bacteria and archaea) and the functional microbial communities (methanotrophs and methanogens) in the three habitats (BS, RS, and RP) to analyze their contributions to SOC, incorporating climatic factors, soil physicochemical properties, metallic elements, and agricultural management. We addressed three research hypotheses in the current study. First, SOC drivers differ between BS and RS due to habitat-specific microbial-plant-environment interactions. Second, microbial communities in RP may mediate SOC storage in RS due to proximity distance and cascading effects among microorganisms. Third, soil metallic elements drive SOC differently due to soil physicochemical and microbial community differences in BS and RS. Based on the above hypotheses, the objectives of the study are: (1) to compare the differences in SOC, soil physicochemical, and microbial community properties between BS and RS in Yangtze River Delta paddies; (2) to quantify and compare how climatic factors, soil physicochemical properties, metallic elements, microbial community properties, and agricultural management interactively regulate SOC storage between BS and RS; (3) to examine whether the microbial communities in RP mediate SOC storage in RS. This study aims to provide a scientific basis for understanding soil carbon cycling mechanisms in different habitats and improving soil carbon management in rice paddies.

2. Materials and methods

2.1. Study sites and soil sampling

The study area is in the YRD (including Jiangsu Province, Zhejiang Province, and Chongming Island of Shanghai), located in eastern China (Fig. 1a). The area belongs to the warm-temperate and subtropical summer monsoon climate, spanning warm-temperate semi-humid and subtropical humid climate zones. Most of the area lies in the subtropical humid climate zone. The northern area comprises flatlands, while the southern area is characterised by hilly terrain (Fig. 1b). Rice is the main crop in the region, and most of the rice grown in this region is double-cropping rice.

Sampling was carried out within rice paddies in late September to early October. We chose to sample during this period, which is the mature stage of rice growth (Bhattacharyya et al., 2013). The determination of sampling sites was mainly based on the Latin Hypercube sampling method (Minasny and McBratney, 2006). We sampled 32 sites scattered across typical rice paddies (Fig. 1b) in the area (117°30′ E–121°37′ E, 28°28′ N–34°35′ N) and these sampling sites were at least 5 km apart. The mean annual temperature (MAT) of the sample sites ranges from 14.2 °C to 18.5 °C, and the mean annual precipitation (MAP) ranges from 717.5 mm to 1661.3 mm. The pH of the sample sites

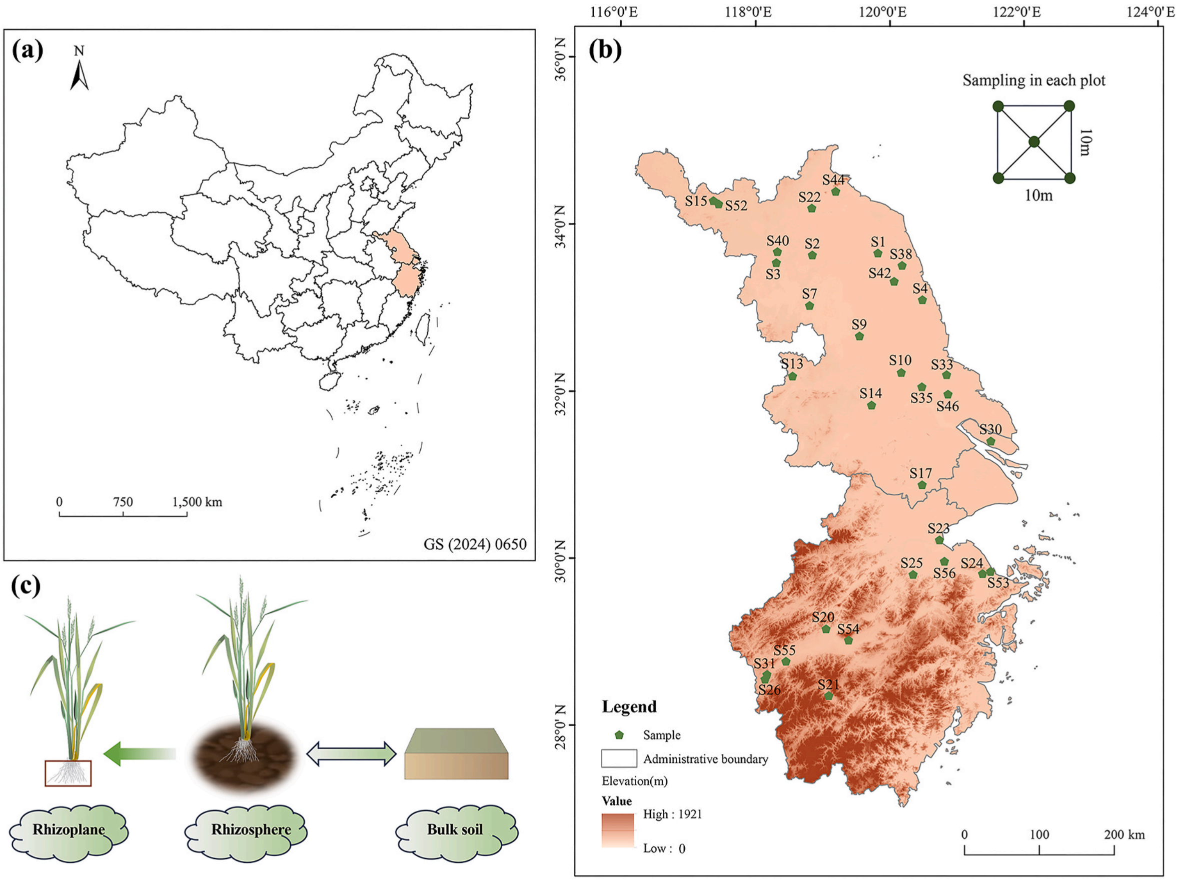


Fig. 1. Location of the study area (a), the geographic distribution of sampling sites in rice paddies (b), and schematic representation of different habitats (c).

ranges from 5.2 to 8.4 (Fig. S1), and the sum of the proportions of silt and clay ranges from 58.4 % to 90.0 %.

A 10 m × 10 m square area at each sampling site was established to sampled five points within the square (Mao et al., 2020). At each sampling site, we sampled three different habitats i.e., BS, RS, and RP (Fig. 1c; the sampling method referred to Mendes et al., 2014; Edwards et al., 2015; Fan et al., 2018), and collected a total of 96 samples. In this square area, we collected five rice plants using a shovel from each of the four corners and the center. Excess loosely bound soils around the rhizosphere were shaken off. The soil around the roots was carefully collected with disposable sterile rubber gloves, and then mixed to form a composite sample, and used as RS. Using a soil drill, inter-montane soil was collected at each of the five sampling points at a depth of 0–20 cm adjacent to the collected rice plants and then mixed to form a composite sample, and used as BS. We brought back the rice plants in a sterile ziplock bag along with other soil samples to the laboratory after collecting RS samples, where the root system of the rice plants was cut and shredded, and soil solids were collected by ultrasound and centrifugation by adding sterile ultrapure water to a Falcon tube, which was then preserved in a 50 mL sterile centrifuge tube, and used as RP.

The RP and a part of BS and RS samples in 50 mL sterile centrifuge tubes were immediately stored at −80 °C for DNA extraction and high-throughput sequencing. We placed the remaining BS and RS in a 4 °C refrigerator for subsequent determination of soil physicochemical properties. SOC was determined by the $K_2Cr_2O_7$-H_2SO_4 oxidation method (Mebius, 1960) with air-dried soil. Although RP did not have enough samples for soil physicochemical properties, soil microbial communities in RP were measured because they may influence soil microbial communities or SOC in RS.

2.2. *Soil physicochemical properties and metallic elements measurements, and other data collection on abiotic factors*

pH was measured by a pH electrode (PHS-3G, Shanghai, China). Soil water content (SWC) and soil bulk density (SBD) were determined by oven-drying fresh soil. Total nitrogen (TN) concentration was determined by the Kjeldahl method (Bremner, 1960). Ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) concentrations were determined by Ultraviolet–Visible spectrophotometry (Hood-Nowotny et al., 2010). Total phosphorus (TP) concentration was determined by sodium hydroxide melting and molybdenum antimony colorimetric method. Available phosphorus (AP) concentration was determined by $NaHCO_3$ extraction and molybdenum antimony colorimetric method (Olsen, 1954). Total potassium (TK), available potassium (AK), exchangeable Ca (ExCa), and exchangeable Mg (ExMg) concentrations were determined by atomic absorption spectrometry (Page, 1982). Ca, Mg, Mn, Cu, Cd, and Cr concentrations were determined using a microwave (Kubrakova and Toropchenova, 2013). Available Mn (AMn), available Cu (ACu), available Cd (ACd), and available Cr (ACr) concentrations were measured by inductively coupled plasma optical emission spectrometry (ICP-OES; iCCP 6300, Thermo Scientific, USA) (de Santiago-Martín et al., 2015).

Climatic factors included MAT, MAP, soil transpiration evapotranspiration ratio (T/ET) (Niu et al., 2020), transpiration (T) (Niu et al., 2020), evapotranspiration (ET) (Niu et al., 2020), snow water equivalent (SWE) (https://ldas.gsfc.nasa.gov/gldas/), and shortwave radiation (Srad) (Abatzoglou et al., 2018). MAT and MAP from 1990 to 2020 for each sampling site were obtained from the WorldClim database (https://www.worldclim.org/). Agricultural management was used from Jiangsu (https://tj.jiangsu.gov.cn/2021/index.htm), Shanghai (https://tjj.sh.gov.cn/tjnj/tjnj2021.htm), and Zhejiang (https://tjj.zj.gov.cn/art/20

21/10/28/art_1525563_58951576.html) Statistical Yearbook 2021, including fertilization, total crops sown area (TCSA), gross agricultural product (GAP), total power of agricultural machinery (TPAM), and pesticide usage. Vegetation factors included fractional vegetation cover (FVC) and net primary production (NPP), which were used from the MODIS satellite imagery MOD13A1 and MOD17A3H products, respectively. Terrain factors were used from the global 30 m resolution digital elevation model (DEM) data of the National Aeronautics and Space Administration (NASA) in 2020 (https://www.earthdata.nasa.gov/esds/competitive-programs/measures/nasadem) including elevation, topographic wetness index (TWI), stream power index (SPI), and slope. Atmospheric deposition was used from ChinaHighAirPollutants (CHAP) dataset (Wei et al., 2023) including SO_2 and NO_2.

2.3. DNA extraction, illumina sequencing, and sequence processing

Total soil genomic DNA was extracted from the bulk, rhizosphere, and rhizoplane soil samples using Fast DNA® SPIN kit for soil (MP Biomedicals, Santa Ana, CA, USA) according to manufacturer's instructions. After soil genomic DNA extraction, the integrity of the extracted genomic DNA was detected by 1 % (w/v) agarose gel electrophoresis. Using NanoDrop 2000 Ultraviolet–Visible spectrophotometer to detect DNA purity and concentration (Thermo NanoDrop 2000 Technologies, Wilmington, DE, USA). The soil genomic DNA was stored at −20 °C to maintain its stability until further analyses were performed after the extraction process.

An aliquot of 45 ng of purified DNA was taken from each sample to be used as the template for polymerase chain reaction (PCR). The bacteria and archaea specific V4-V5 hypervariable region of the 16S rRNA gene were performed using the primer pairs 515F/907R (5′-GTGCCAGCMGCCGCGG-3′ and 5′-CCGTCAATTCMTTTRAGTTT-3′) and Arch519F/Arch915R (5′-CAGCCGCCGCGGTAA-3′ and 5′-GTGCTCCCCCGCCAATTCCT-3′), respectively (Caporaso et al., 2011; Cai et al., 2016; Jiao et al., 2018). The methanotrophs (pmoA) and methanogens (mcrA) specific hypervariable region of the pmoA-11 and mcrA-2 gene were performed using the primer pairs A189F/mb661R (5′-GGNGACTGGGACTTCTGG-3′ and 5′-CCGGMGCAACGTCYTTACC-3′) and Mlas-mod-F/mcrA-rev-R (5′-GGYGGTGTMGGDTTCACMCARTA-3′ and 5′-CGTTCATBGCGTAGTTVGGRTAGT-3′), respectively (Kolb et al., 2003; Steinberg and Regan, 2009). Illumina adapters and unique barcodes were incorporated into the end of the reverse primer (Berg et al., 2012).

The reactions were performed in a final volume of 25 μL containing 2.5 μL 10 × PCR buffer II, 0.5 unit of AccuPrime Taq DNA Polymerase High Fidelity (Invitrogen, Carlsbad, CA, USA), 2 μL of template DNA, and 3 μL of forward and reverse primers. The PCR products were checked by agarose gel electrophoresis, and the target bands were recovered by gel tapping and purified using the AxyPrep Gel Purification Kit (Axygen Scientific Inc., USA). PCR products were sequenced using an Illumina MiSeq platform (300 bp paired-end) (Guangdong Magigene Biotechnology Co., Ltd., Guangzhou, China).

Raw sequencing data were qualified through screening and the removal of sequences that were shorter than 200 bp, with a quality score below 20 ($Q < 20$), contained ambiguous bases or did not exactly match the primer sequences and barcode tags. Passed sequences were dereplicated and subjected to the DADA2 algorithm to identify indel-mutations and substitutions (Callahan et al., 2016). The trimming and filtering were performed on paired reads with a maximum of two expected errors per read. We removed the singlet amplicon sequence variants (ASVs) and those classified as mitochondria and chloroplasts to improve data reliability. The phylogenetic affiliation of bacteria and archaea ASVs was analyzed by uclust algorithm (http://www.drive5.com/usearch/manual/uclust_algo.html) against the SILVA (version SSU138.1) database (http://www.arb-silva.de) using a confidence threshold of 80 %. The pmoA and mcrA ASVs were analyzed by uclust algorithm against the NCBI-NT database (https://ftp.ncbi.nlm.nih.gov/blast/db/). The raw sequence data were deposited in the public NCBI database (http://www.ncbi.nlm.nih.gov/) under the BioProject ID: PRJNA1185113 (Bacteria), PRJNA1185154 (Archaea), PRJNA1185190 (pmoA), and PRJNA1185433 (mcrA).

2.4. Community structure, co-occurrence network, and community assembly

Non-metric multi-dimensional scaling (NMDS) ordination, the Wilcoxon test, and analysis of similarities (ANOSIM) were used to investigate differences in soil microbial communities (bacteria, archaea, pmoA, and mcrA) between BS, RS, and RP. The community structure between samples were analyzed using the Bray-Curtis similarity of soil microbial ASVs abundance. These analyses were conducted using "vegan" (Oksanen et al., 2024) and "ggsignif" (Ahlmann-Eltze and Patil, 2021) R packages.

Network analysis has proven helpful in deciphering complex microbial interaction patterns. Our study focused on two composite networks that included comprehensive microbial communities (bacteria&archaea) and functional microbial communities (pmoA&mcrA), and rather than a single taxonomic network. Thus, bacteria&archaea and pmoA&mcrA internetworks were constructed for each habitat, and the sub-network topology properties were extracted to describe soil microbial network complexity. To determine the co-occurrence patterns of microorganisms in BS, RS, and RP, six networks (three communities of bacteria&archaea and three communities of pmoA&mcrA) were constructed. Microbial phylotypes with relative abundances less than 0.01 % were excluded from the analysis. Then, the bacteria&archaea and pmoA&mcrA ASVs were merged into an abundance table, respectively. Pairwise Spearman correlations between ASVs were calculated, and *P* values were adjusted by the Benjamini and Hochberg false discovery rate (FDR) test (Benjamini and Hochberg, 1995). The cutoff of the FDR-adjusted *P*-values was 0.05, and Spearman correlations with a coefficient of less than 0.60 were also removed. Only robust ($|r| > 0.60$) and statistically significant ($P < 0.05$) correlations were incorporated into the network analyses. The analysis was conducted using "WGCNA" R package to calculate the Spearman correlation matrix between ASVs for different habitats (Langfelder and Horvath, 2008). The network topological properties used in this study included the Nodes number, Average weight degree, Mean distance, Betweenness centralization, Network density, and Clustering coefficient. Because these topological properties were tightly correlated, we used the first and second components (Network.PC1 and Network.PC2) of the six selected topological properties to denote the network complexity (Wang et al., 2023).

Stochasticity is a fundamental driver of community structure, especially in surface soils (Mo et al., 2024). To determine the potential importance of stochastic processes on community assembly, we used a neutral community model to predict the relationship between ASV detection frequency and their relative abundance across the wider metacommunity (Sloan et al., 2006), applying nonlinear least-squares (NLS) to generate the best fit between the frequency of ASVs occurrence and their relative abundance.

2.5. Piecewise structural equation modeling

The piecewise structural equation modeling (piecewiseSEM) was employed to explore the underlying influences of biotic and abiotic factors on SOC between BS and RS. The piecewiseSEM in our study is to split the complex model into several independent linear mixed-effect models for local estimation (Table S3). The use of piecewiseSEM is particularly useful in large-scale correlative studies due to its ability to partition the causal influences among multiple variables and separate the direct and indirect effects of each variable (Lefcheck, 2016; Tian et al., 2021; Liu et al., 2022).

We first constructed an a priori conceptual model following previous research (Delgado-Baquerizo et al., 2017; Tian et al., 2021; Liu et al., 2022). Several composite variables were set, including climatic factors (composed of MAT, MAP, SWE, Srad, T/ET, T, and ET), soil physical properties (composed of SWC, SBD, and soil texture), soil chemical properties (composed of TN, TP, TN/TP, AP, TK, AK, NH_4^+-N, NO_3^--N, and pH), soil metallic elements (composed of Ca, Mg, Mn, Cu, Cd, Cr, AMn, ACu, ACd, ACr, ExCa, and ExMg), agricultural management (composed of fertilization, TCSA, GAP, TPAM, and pesticide usage), and soil microbial community properties (composed of diversity, NMDS1, NMDS2, Network.PC1, and Network.PC2 of bacteria, archaea, bacteria&archaea, pmoA, mcrA, and pmoA&mcrA). Particularly, we extracted the principal components for the six sub-network topology properties (Nodes number, Average weight degree, Mean distance, Betweenness centralization, Network density, and Clustering coefficient) to obtain Network.PC1 and Network.PC2 as network complexity of soil microbial communities (Wang et al., 2023). We calculated the first and second axis scores of NMDS (NMDS1 and NMDS2) as soil microbial community structure, respectively. Then, based on the a priori model, we gradually modified and constructed the final model (Fig. 9 and Table S3).

Fisher's C test (when $0.05 < P < 1.00$) was used to judge the goodness of the modeling results and then stepwise modified our models according to the pathway significance ($P < 0.05$) and the goodness of the model (Tian et al., 2021; Liu et al., 2022). The variance inflation factor (VIF) for biotic and abiotic factors included in the piecewiseSEM were all less than 10 (O'brien, 2007). We used conditional R^2 (R_C^2) and marginal R^2 (R_M^2) to evaluate how the built models explain the variance of composite variables to SOC. The R_C^2 and R_M^2 represent the proportion of variance explained by all composite variables with and without accounting for random effects of the "sampling site", respectively (Nakagawa and Schielzeth, 2013). These analyses were conducted using "piecewiseSEM" (Lefcheck, 2016), "nlme" (Pinheiro et al., 2024), and "lme4" (Bates et al., 2015) R packages.

2.6. Statistical analyses

All statistical analyses and figures were done using the R software (version 4.4.1) (R Core Team, 2024). Shannon, simpson, pielou, and richness were performed using the "picante" (Kembel et al., 2010) and "vegan" (Oksanen et al., 2024) R packages. The differences in SOC, main abiotic factors, Shannon diversity of soil microbial communities and sub-network topological properties in different habitats were tested using Wilcoxon test, and the differences in soil microbial dominant taxa in different habitats were tested using Student's t-test. Homogeneity of variances was tested by Levene's test, and the normal distribution of residues was tested by the Shapiro test.

Random forest was used to quantify the relative importance of each factor on SOC between BS and RS. In the random forest model, the number of decision trees (ntree) is set as 1000, and the number of cross-validation folds is 15. Random forest analysis was performed using the "randomForest" (Liaw and Wiener, 2002), "rfPermute" (Archer, 2023), and "caret" (Kuhn, 2008) R packages. Spearman's rank correlation analysis was used to evaluate their relationships between SOC and top25 influencing factors identified by random forest importance analysis. Spearman's rank correlation analysis was performed using the "Hmisc" R package (Harrell, 2025). The data were Z-score normalised before analysis to remove the effect of the dimension using the "vegan" package (Oksanen et al., 2024). We used the ggplot2 (Wickham, 2016), ggdist (Kay, 2024), maptools (Bivand and Lewin-Koh, 2023), and corrplot (Wei and Simko, 2021) R packages for graphing.

3. Results

3.1. Differences of SOC and soil properties between BS and RS

Significant spatial heterogeneity was observed in SOC and soil physicochemical properties across the study area (Fig. S1), with distinct distribution patterns between BS and RS. Specifically, TP and pH demonstrated north-to-south decreasing gradients in both soil compartments (Fig. S1). Comparative analysis revealed pronounced differences between BS and RS: SOC (BS: 11.18 ± 4.53 g/kg; RS: 17.84 ± 7.32 g/kg), TN, TN/TP, AP, NH_4^+-N, SWC, ACu, ACd, and AMn were elevated in RS, whereas TP, AK, and ExMg exhibited inverse trends (Fig. 2; Wilcoxon test, $P < 0.05$).

3.2. Divergence of SOC content across main soil property gradients between BS and RS

The SOC in BS and RS increased significantly with the increase of TN, TN/TP, SWC, and ACd (Fig. 3; $P < 0.01$). Specifically in BS, SOC accumulation was further mediated by NH_4^+-N and ACu levels. Conversely, the SOC in BS decreased significantly with the increase of pH and SBD (Fig. 3a). The SOC in RS also decreased significantly with the increase of SBD (Fig. 3b; $P < 0.01$), though without significant pH-mediated regulation.

3.3. Soil microbial community composition, diversity, structure, and sub-network topological properties between BS, RS, and RP

We analyzed the relative abundances of the top12 dominant taxa and differences in top8 dominant taxa of bacteria, archaea, pmoA, and mcrA at the phylum, order, genus, and genus level in BS, RS, and RP, respectively (Fig. 4). The results showed that the top8 dominant taxa had significant differences between BS, RS, and RP (Fig. 4; Student's t-test, $P < 0.05$). The relative abundances of biomarkers also showed significant differences in BS, RS, and RP (Figs. S2 and S3; Linear discriminant analysis, $P < 0.05$).

The Shannon diversity of bacteria and pmoA showed significant differences between BS, RS, and RP (Fig. 5a; Wilcoxon test, $P < 0.05$). The Shannon diversity of archaea showed a significant difference between BS and RP (Fig. 5a; Wilcoxon test, $P < 0.05$). Especially, the Shannon diversity of all microbial communities in RS was generally higher than that in BS (Fig. 5a).

Habitat-specific divergence emerged in the significant associations between microbial dominant taxa and SOC (Fig. 5b). Interestingly, *Methylocystis* in RS and RP showed a significant synergistic positive effect on SOC in RS (Fig. 5b). The Nitrospirota ($R = 0.49$, $P < 0.01$) and Thermococcales ($R = 0.39$, $P < 0.05$) in BS were significantly positively correlated with SOC in BS. The Firmicutes ($R = -0.36$, $P < 0.05$) in BS was significantly negatively correlated with SOC in BS. The Chloroflexi ($R = 0.45$, $P < 0.05$), *Methylocystis* ($R = 0.40$, $P < 0.05$), and *Candidatus Methanoperedens* ($R = 0.58$, $P < 0.001$) in RS were significantly positively correlated with SOC in RS. The Proteobacteria ($R = -0.50$, $P < 0.01$) in RS was significantly negatively correlated with SOC in RS. The Nitrosopumilales ($R = 0.41$, $P < 0.05$) and *Methylocystis* ($R = 0.48$, $P < 0.01$) in RP were significantly positively correlated with SOC in RS. The Halobacteriales ($R = -0.40$, $P < 0.05$), *Methylomonas* ($R = -0.45$, $P < 0.01$), and *Methylomicrobium* ($R = -0.54$, $P < 0.01$) in RP were significantly negatively correlated with SOC in RS (Fig. 5b).

NMDS ordination and Wilcoxon tests showed that community structure of bacteria differed significantly between RP and RS, as well as between RP and BS (Fig. 6a; Wilcoxon test, $P < 0.05$). The community structure of archaea and pmoA differed significantly between BS, RS, and RP (Fig. 6b and c; Wilcoxon test, $P < 0.05$). While the community structure of mcrA did not differ significantly between BS, RS, and RP (Fig. 6d; Wilcoxon test, $P > 0.05$). Analysis of similarities (ANOSIM) demonstrated distinct differences in bacteria ($R = 0.44$, $P = 0.001$), archaea ($R = 0.43$, $P = 0.001$), pmoA ($R = 0.25$, $P = 0.001$), and mcrA ($R = 0.07$, $P = 0.003$) between BS, RS, and RP (Fig. 6).

Network topology analysis revealed enhanced co-occurrence complexity in RS (Fig. 7 and S4). For bacteria&archaea and pmoA&mcrA, the Network density, Average weight degree, and Clustering coefficient in RS were higher than those in BS and RP (Fig. 7). For

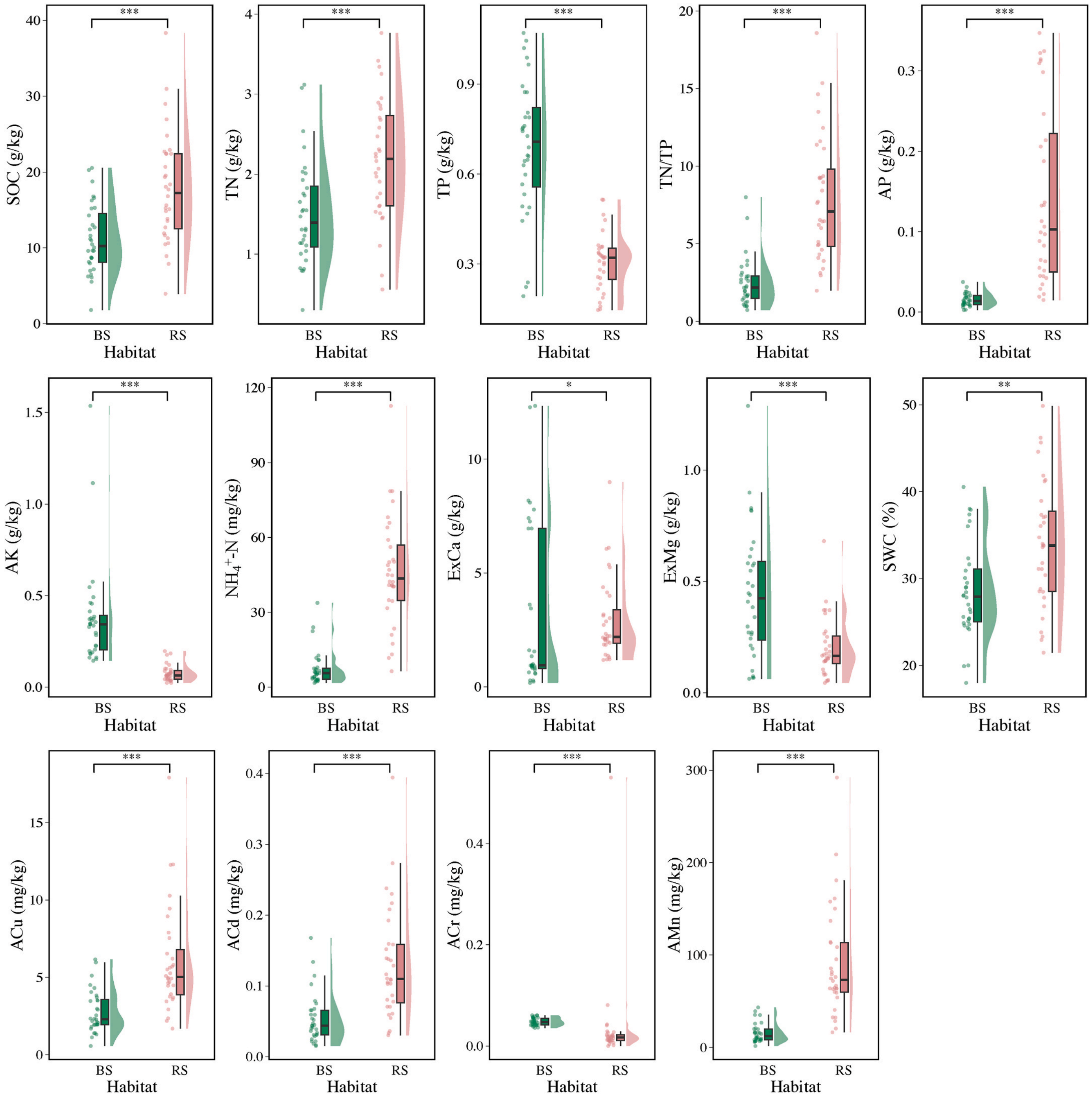


Fig. 2. Differences in SOC, soil physicochemical properties, and metallic elements between bulk (BS) and rhizosphere soils (RS). The differences were tested using Wilcoxon test (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$).

bacteria&archaea, the Nodes number, Network density, and Clustering coefficient were markedly different between BS, RS, and RP (Fig. 7a; Wilcoxon test, $P < 0.05$). For pmoA&mcrA, the microbial sub-network topological properties except Nodes number were markedly different between BS, RS, and RP (Fig. 7b; Wilcoxon test, $P < 0.05$).

The neutral community model results indicated that relative contribution of stochastic processes increased gradually of pmoA&mcrA from BS to RS, then to RP (Fig. S5b). The construction of bacteria&archaea and pmoA&mcrA was more influenced by stochastic processes and less by deterministic processes in RP (Fig. S5). Although the diffusion of microorganisms in RP was more restricted compared to RS, the distance between RS and RP was closer, and the microorganisms in RP were sufficient to diffuse into RS, which had a certain impact on the microorganisms and SOC in RS. Therefore, the microorganisms in RP were included in the RS analysis to analyze the impact of microorganisms on SOC in RS comprehensively.

3.4. Quantifying main influencing factors for SOC between BS and RS via random forest importance analysis

It showed that soil physical (SBD, SWC) and chemical properties (TN, SOC/TP, TN/TP) were the most important variables of SOC between BS and RS according to the random forest importance (Fig. 8). The first ranking factor of SOC in BS was SBD (Fig. 8a), while in RS was TN

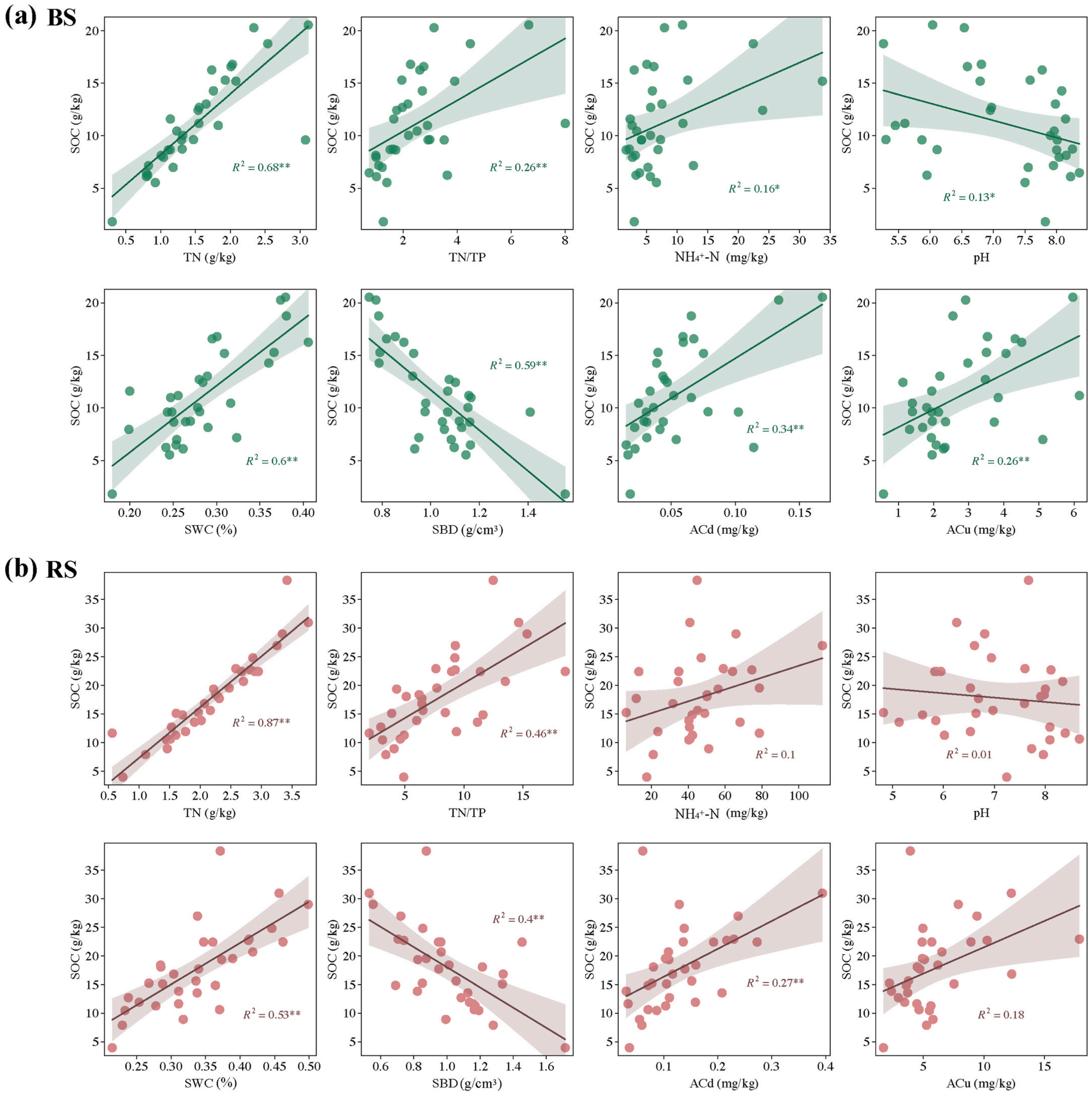


Fig. 3. SOC content diverges along physicochemical properties and metallic element gradients between (a) bulk (BS) and (b) rhizosphere soils (RS). The solid line represents the trends, and the shaded area represents the standard error of the estimate. TN/TP, TN to TP ratio. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

(Fig. 8b). The overall impact of soil microbial community properties on SOC was important in BS (Fig. 8a), accounting for 25.65 % within the top25 influencing factors, second only to soil physical (27.46 %) and chemical (28.14 %) properties (Table S1). The mcrA NMDS2, Bacteria NMDS1, and Bacteria *Nitrospirota* in BS contributed significantly more to SOC in BS ($P < 0.05$). The overall impact of soil metallic elements on SOC was also important in BS, accounting for 11.52 % within the top25 influencing factors (Fig. 8a and Table S1).

Soil microorganisms in RP had an important influence on SOC in RS (Fig. 8b). The overall impact of soil microbial community properties on SOC was more important in RS (Fig. 8b and Table S2) compared to BS (Fig. 8a), accounting for 39.56 % within the top25 influencing factors, second only to soil chemical properties (43.33 %). The RP pmoA Richness, RP pmoA&mcrA Richness, RS Archaea Network.PC2, RS Archaea *Halobacteriales*, RS mcrA *Candidatus Methanoplasma*, RS pmoA *Candidatus Methylumidiphilus*, RS pmoA&mcrA Network.PC2, and RP pmoA NMDS1 contributed significantly more to SOC in RS compared to BS. But soil physical properties (10.06 %) and soil metallic elements (1.97 %) contributed less in RS (Fig. 8b and Table S2). In particular, the community of pmoA&mcrA in RS and RP attributed dominant influence on SOC in RS compared to bacteria&archaea (Fig. 8b), while the community of bacteria&archaea in BS attributed dominant influence on SOC in BS compared to pmoA&mcrA (Fig. 8a).

Correlation analysis showed the TN, SWC, SOC/TP, TN/TP, ACu,

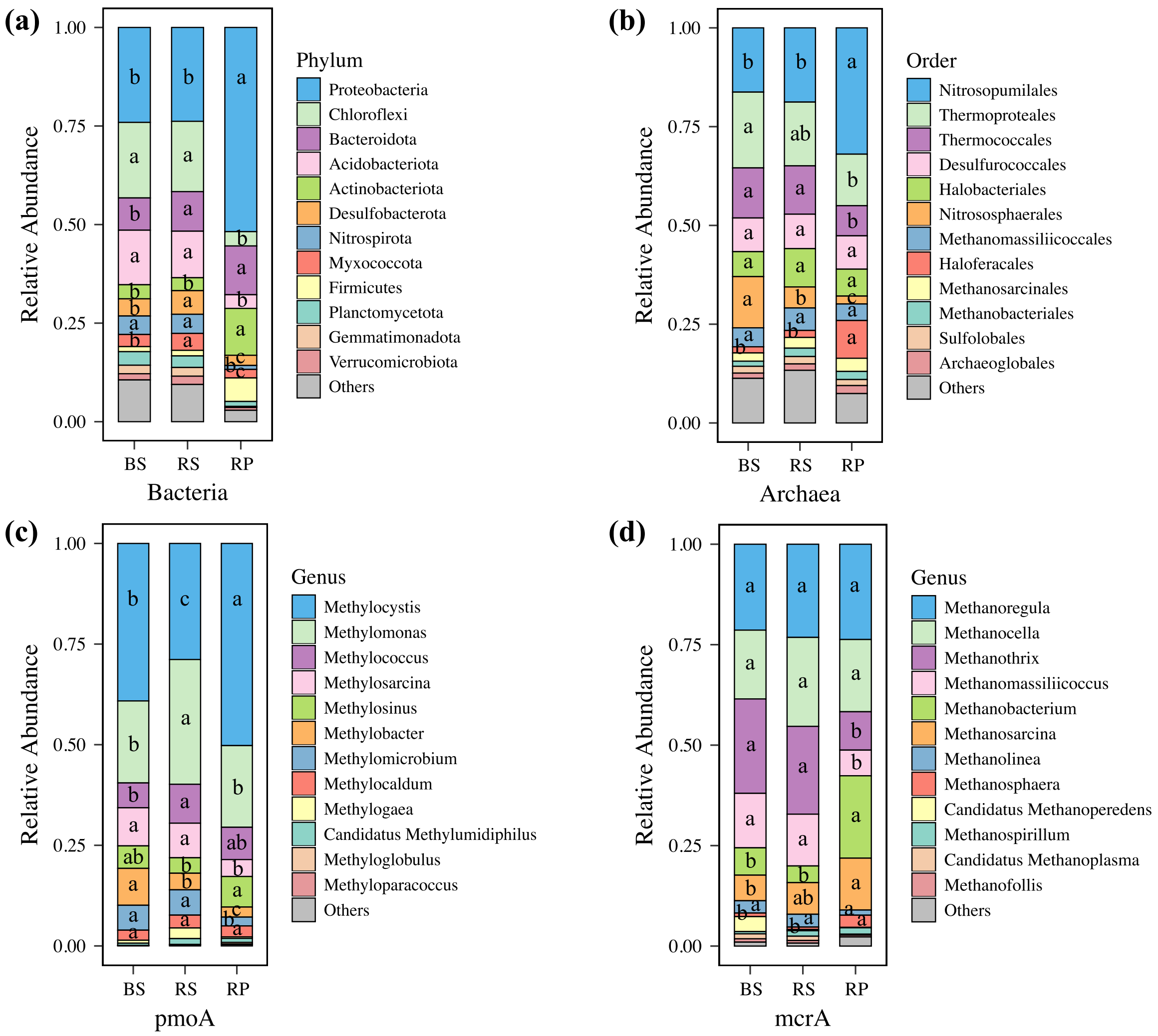


Fig. 4. Differences in soil microbial community composition in bulk (BS), rhizosphere (RS), and rhizoplane soils (RP). (a) Relative abundance of dominant phyla of bacteria in top12 and differences in top8 dominant phyla; (b) Relative abundance of dominant order of archaea in top12 and differences in top8 dominant order; (c) Relative abundance of dominant genus of methanotrophs (pmoA) in top12 and differences in top8 dominant genus; (d) Relative abundance of dominant genus of methanogens (mcrA) in top12 and differences in top8 dominant genus. Different lowercases letters within each column in the same sub-figure represent significant differences between BS, RS, and RP (Student's t-test, $P < 0.05$).

Bacteria Nitrospirota, ACd, pmoA Nodes number, and mcrA Nodes number in BS were significantly positively correlated with SOC in BS, and SBD was significantly negatively correlated with SOC (Fig. 8a). The TN, SOC/TP, SWC, SOC/TN, TN/TP, TWI, RS mcrA *Candidatus Methanoperedens*, and RS mcrA *Methanofollis* were significantly positively correlated with SOC in RS, and SBD was also significantly negatively correlated with SOC (Fig. 8b). The RP pmoA *Methylocystis* was significantly positively correlated with SOC in RS, and RP pmoA Richness and RP pmoA&mcrA Richness were significantly negatively correlated with SOC in RS (Fig. 8b).

3.5. *Decoupling direct and indirect biotic-abiotic drivers of SOC through piecewise structural equation modeling between BS and RS*

The piecewiseSEM showed that 88 % and 92 % of SOC variations could be explained by the selected variables in BS and RS, respectively (Fig. 9). SOC was directly significantly influenced by soil physical properties (i.e., SWC and SBD; $R^2_{\mathrm{M}} = 0.10$, $P < 0.001$), chemical properties (i.e., TN and TN/TP; $R^2_{\mathrm{M}} = 0.37$, $P < 0.01$), and metallic elements (i.e., Mg, Mn, Cd, ACu, and ExCa; $R^2_{\mathrm{M}} = 0.59$, $P < 0.05$) in BS (Fig. 9a and Table S4). The direct effects of soil physical (standardized direct effect was 0.36) and chemical (standardized direct effect was 0.36) properties were the largest in BS. In terms of the indirect effect in BS, soil physical properties had a great positive indirect effect (standardized indirect effect was 0.39) on SOC through its positive effect on soil chemical properties, metallic elements, and microbial community properties. Soil chemical properties had a positive indirect effect (standardized indirect effect was 0.17) on SOC mainly through its positive effect on soil metallic elements. The indirect effects of climatic factors (standardized indirect effect was 0.28) and agricultural management (standardized indirect effect was 0.26) on SOC were also great (Fig. 9a).

SOC was directly significantly influenced by soil chemical properties (i.e., TN, AK, NH_4^+-N, and pH; $R^2_{\mathrm{M}} = 0.70$, $P < 0.001$), metallic elements (i.e., ACu, ACd, ACr, and ExMg; $R^2_{\mathrm{M}} = 0.43$, $P < 0.05$) and microbial community properties (i.e., RS Bacteria Simpson, RS pmoA&mcrA Richness, RS Bacteria NMDS2, RS pmoA&mcrA Network.PC2, RP pmoA&mcrA Richness, RP pmoA&mcrA NMDS2, and RP pmoA&mcrA Network.PC2; $R^2_{\mathrm{M}} = 0.58$, $P < 0.001$) in RS (Fig. 9b and Table S5). The direct effect of soil chemical properties (standardized direct effect was 0.74) was the largest in RS, followed by soil microbial community properties (standardized direct effect was 0.33). Soil microbial communities, especially functional microbial communities (pmoA&mcrA) in

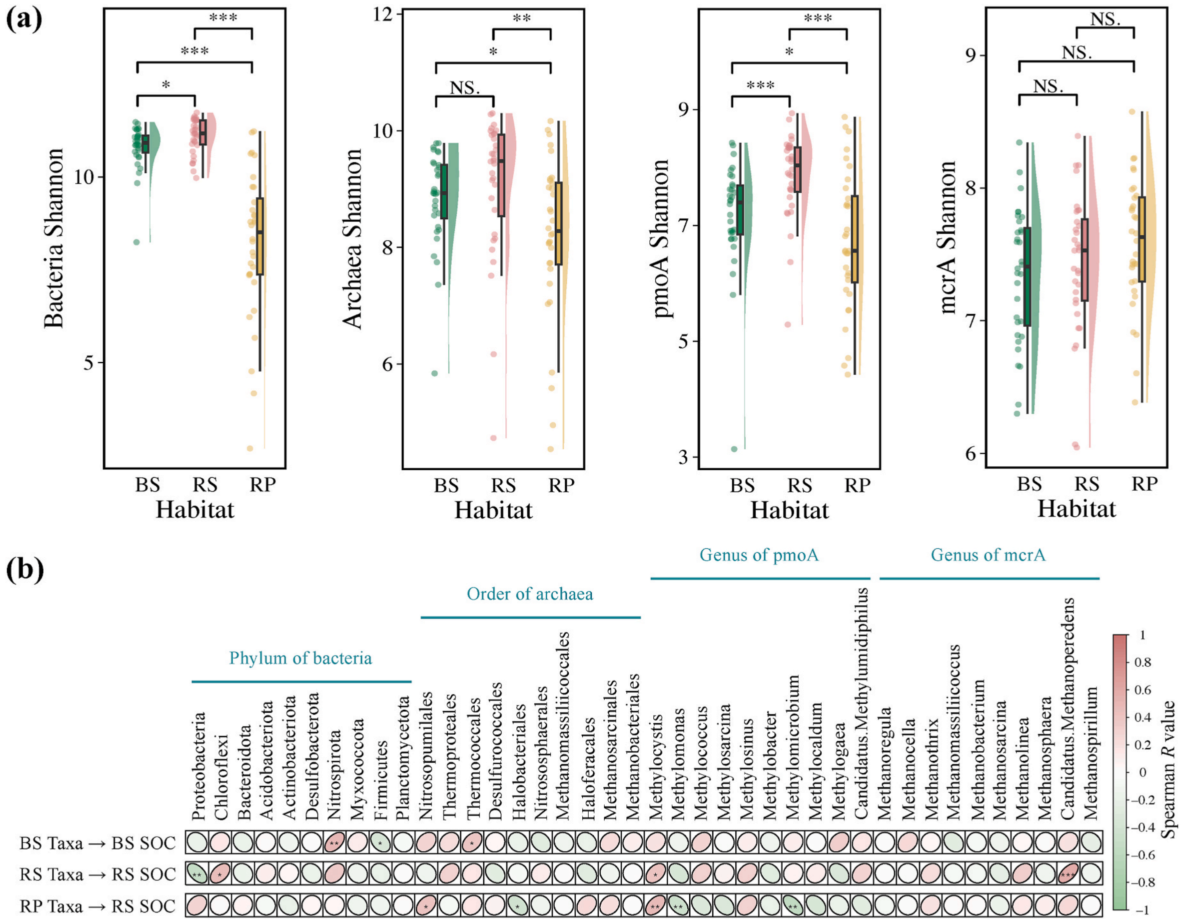


Fig. 5. Differences in diversity of soil microbial communities and correlation analysis between microbial dominant taxa and SOC in bulk (BS), rhizosphere (RS), and rhizoplane soils (RP). (a) Differences in Shannon diversity of soil microbial communities between BS, RS, and RP was tested using Wilcoxon test (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, NS.: not significant). (b) The correlation analysis between the top10 microbial dominant taxa and SOC (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$). Among them, BS Taxa → BS SOC represents the correlation analysis between the top10 microbial dominant taxa in BS and SOC in BS; RS Taxa → RS SOC represents the correlation analysis between the top10 microbial dominant taxa in RS and SOC in RS; RP Taxa → RS SOC represents the correlation analysis between the top10 microbial dominant taxa in RP and SOC in RS.

RS had a significant positive direct effect on SOC in RS (Fig. 9b and Table S5). Conversely, the direct effect of soil microbial communities on SOC in BS was insignificant (Fig. 9a and Table S4). In terms of the indirect effect in RS, soil physical properties had a great positive indirect effect (standardized indirect effect was 0.66) on SOC mainly through its positive effect on soil chemical properties. Soil chemical properties had a positive indirect effect (standardized indirect effect was 0.21) on SOC mainly through its positive effect on soil microbial communities (Fig. 9b).

Soil methane microbial community properties (RP pmoA&mcrA Richness, NMDS2, and Network.PC2) in RP were also important influencing factors on SOC in RS (Fig. 9b). To conclude, climatic factors and agricultural management had a marginal direct effect on SOC, however, they markedly indirectly influenced SOC by influencing other factors in BS and RS (Fig. 9). Soil chemical properties in BS and RS positively influenced SOC. Soil metallic elements positively influenced SOC in BS (Fig. 9a), but negatively influenced SOC in RS (Fig. 9b). The effect of abiotic factors on soil microbial community properties further significantly influenced SOC in RS (Fig. 9b). In addition, there were marked differences in the observed variables retained in piecewiseSEM in soil chemical properties, metallic elements, and microbial community properties between BS and RS (Fig. 9).

4. Discussion

4.1. Differences in SOC, biotic, and abiotic factors between BS and RS of rice paddies

In this study, we observed significant differences in SOC, main abiotic factors (soil physical properties, chemical properties, and metal elements), and soil microbial community properties between BS and RS of rice paddies at a regional scale. In terms of abiotic factors, the TN, TN/TP, AP, NH_4^+-N, ACu, ACd, and AMn in RS were significantly higher than those in BS. RS is a biological hotspot, characterized by physical and chemical properties that differ substantially from the surrounding BS (Kuzyakov, 2010). One reason is that root exudation of low-molecular-weight organic acids in RS can fundamentally alter the biogeochemistry of this environment (Fan et al., 2017; Zhao et al., 2022). We found the SOC content in RS was significantly higher than that in BS. This difference is related to rhizosphere secretions (Fan et al., 2021; Wei et al., 2022), key microbial taxa (e.g., Chloroflexi, *Methylocystis*, *Candidatus Methanoperedens*; Figs. S2 and S3) (Shih et al., 2017) and the increased amorphous iron oxide pool in RS that could bind organic carbon in plant roots (Arredondo et al., 2019). Furthermore, RS sustained a greater capacity for microbial necromass contribution to the SOC pool than BS (Wang et al., 2025).

Our study found that the diversity of microbial communities in RS was higher than that in BS. A previous study also showed that the mean

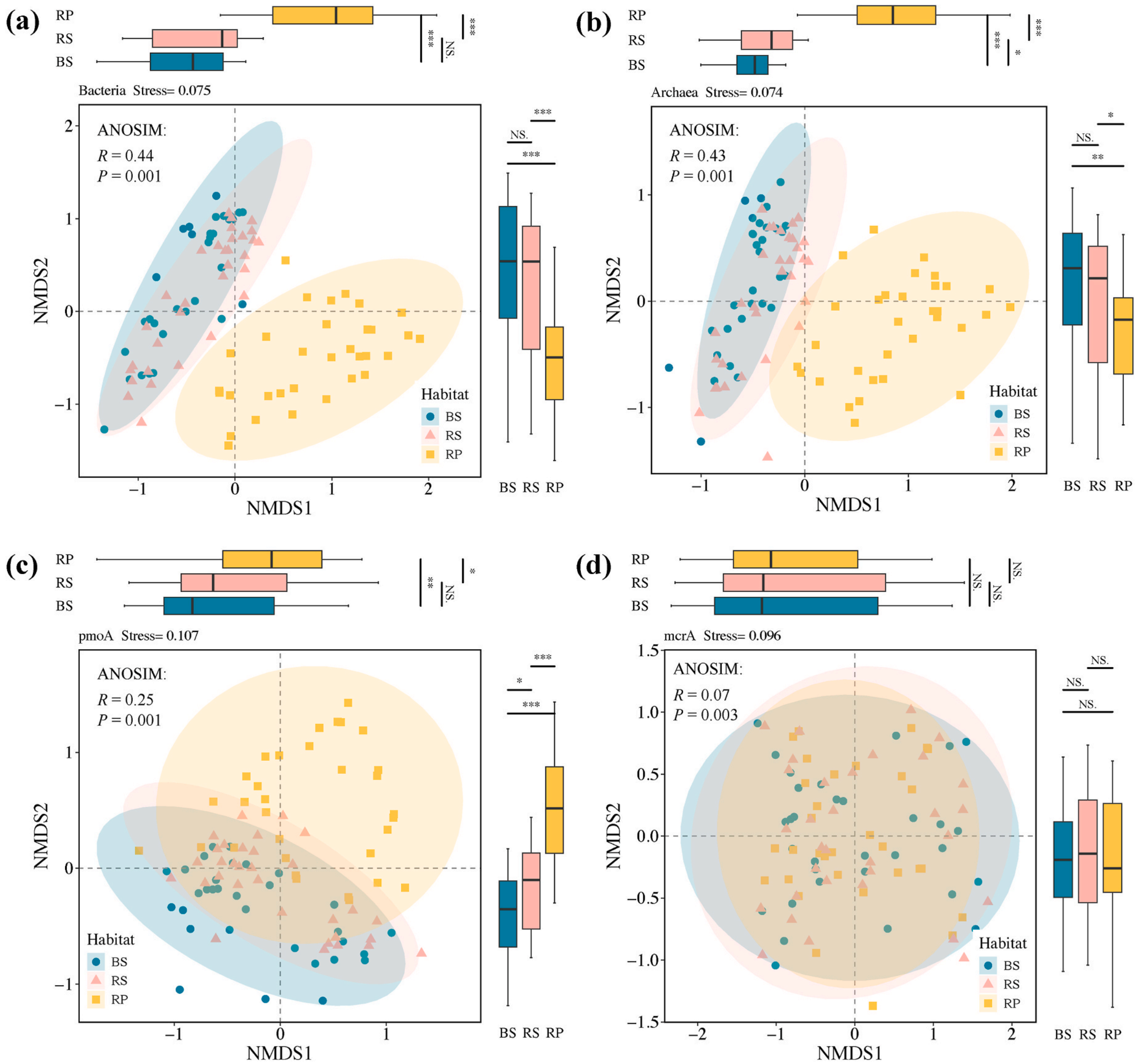


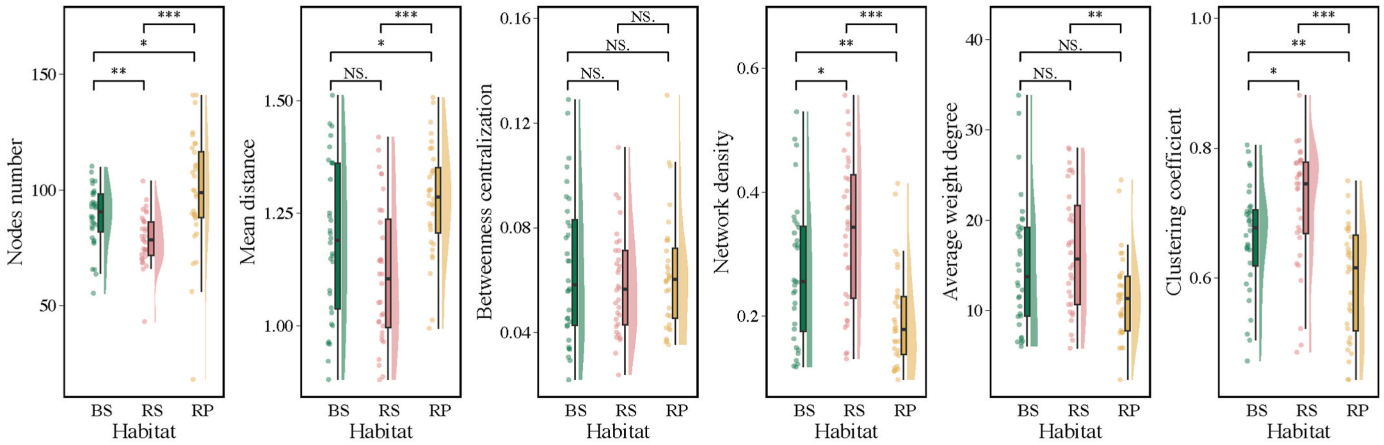
Fig. 6. Differences in soil microbial community structure in bulk (BS), rhizosphere (RS), and rhizoplane soils (RP). (a) Bacteria; (b) Archaea; (c) methanotrophs (pmoA); (d) methanogens (mcrA). Differences in NMDS1 or NMDS2 of soil microbial communities between BS, RS, and RP were tested using Wilcoxon test (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, NS.: not significant).

diversity of microbial communities in RS was higher than that in BS of rice paddies (Edwards et al., 2015). We found that the community structure of archaea and pmoA differed significantly between BS and RS and the network complexity (including Clustering coefficient, Network density, and Average weight degree) of microbial communities in RS was higher than that in BS (Fig. 7 and S4). A previous study on bacterial communities in rice paddies showed that the microbial co-network and community structure differed markedly between BS and RS (Tian et al., 2022). The main reason is that growing plant roots changes the chemical identity, quantity and diversity of resources available to soil microbial communities (Zolla et al., 2013; Zhao et al., 2022), through processes of both addition and removal of diverse chemical compounds from soil (Jones et al., 2004). In turn, this altered resource availability changes the selective pressures experienced by soil microbes. The outcome of these complex processes is the development of microbial communities in RS that differ markedly from the source communities in BS (Minz et al., 2013). Microbial community structural differences between BS and RS might also be attributable to microbial interactions involving both competition and cooperation (Edwards et al., 2015). Moreover, microorganisms in RS played a part in shaping their selective environment by modulating plant root exudation (Bakker et al., 2015).

4.2. Regulatory mechanisms of biotic and abiotic factors on SOC between BS and RS of rice paddies

Our study based on 32 samples found significant differences in regulatory mechanisms of biotic and abiotic factors on SOC between BS and RS of rice paddies at a regional scale. In the ranking of influencing factors in BS, soil physical and chemical properties had the largest direct effect on SOC, followed by metallic elements. Soil chemical properties had the largest direct effect on SOC in RS, followed by microbial community properties and metallic elements. The positive relationships between SOC and soil physicochemical properties (N, C/N, N/P, AP, and SWC) found in our study are consistent with the previous studies (Sahrawat, 2004; Wang et al., 2023). Some scholars found that those properties were positively correlated with SOC due to nutrient sufficiency could increase crop biomass, allowing more plant-derived carbon to enter the soil (Ni et al., 2021; Hu et al., 2022), thereby facilitating SOC increases. Furthermore, a study found that precipitation, SWC, AP, and microbial network complexity contributed significantly to SOC in BS (Wang et al., 2023). In RS, a study elucidated that rhizodeposits promoted SOC formation and accumulation via microbial biomass and necromass (Luo et al., 2021). Another study also found rhizodeposition

(a) Bacteria&Archaea



(b) pmoA&mcrA

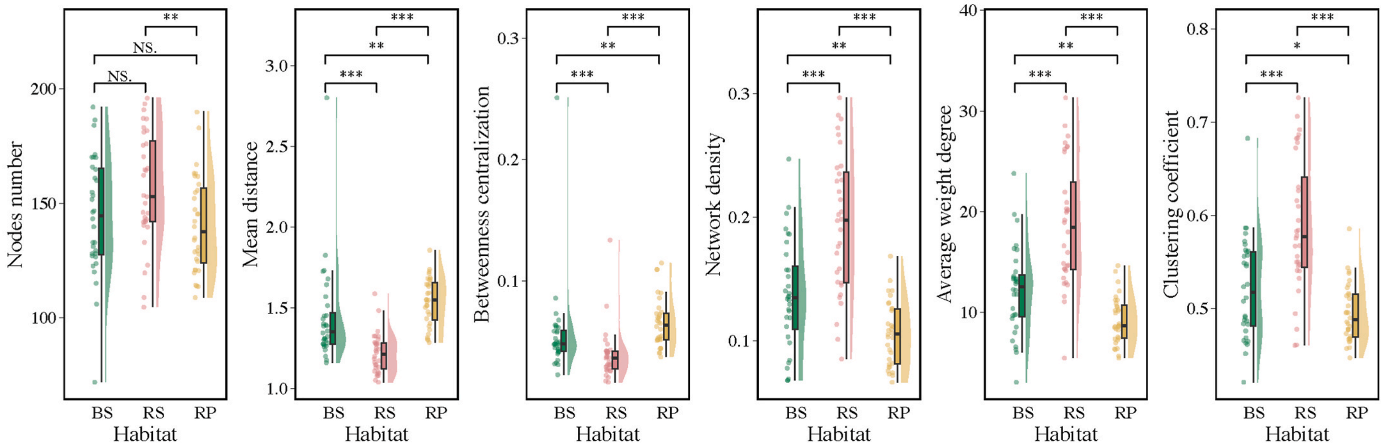


Fig. 7. Differences of sub-network topology properties for bacteria&archaea and pmoA&mcrA between bulk (BS), rhizosphere (RS), and rhizoplane soils (RP). pmoA, methanotrophs; mcrA, methanogens. The P value is from a Wilcoxon test (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, NS.: not significant).

was highly efficient carbon sources for mineral-associated organic carbon formation and then promote the accumulation of SOC in RS (Villarino et al., 2021).

Interestingly, we found that soil metallic elements had a significant positive effect on SOC in BS, but a negative effect on SOC in RS. The mineralization of organic carbon can be inhibited in BS due to the chemical protective effect of ExCa, Mg, Mn, etc. on organic carbon, thereby promoting SOC accumulation in BS (Moore et al., 2023; Xiao et al., 2025). Furthermore, fertilization may increase mineral availability and improve soil aggregation, and affect the bioavailability of soil metallic elements in BS of rice paddies (Huang et al., 2018). This also confirmed our study that agricultural management positively affected soil metallic elements by affecting soil physicochemical properties, indirectly promoting SOC accumulation in BS. Although soil microorganisms are highly active in RS, the protective effects of Ca, Mg, and other substances on SOC may reduce the microbial carbon use efficiency, thereby inhibiting SOC accumulation (Dai et al., 2023; Pei et al., 2024). Rhizodeposition and root chemistry also may influence SOC interaction with minerals and metals (Poirier et al., 2018). In addition, iron manganese oxides may promote electron transfer in methanotrophs and enhance the stability of carbon mineral composites by adsorbing extracellular polymeric substances, thereby promoting SOC accumulation in RS (Ouboter et al., 2024). The above research confirmed that soil metallic elements indirectly promoted SOC content in RS by regulating microbial community properties in our study. During the formation and decomposition of SOC, microbial redox transformations of Mn and Cr determine their solubility and bioavailability (Philippot et al., 2024). Therefore, the negative effects of metallic elements on SOC in RS can be offset by adjusting the abundance of relevant microorganisms. Moreover, the toxicity or availability of Mn and Cr as electron acceptors or donors thus influences the ecological responses of soil microorganisms (Philippot et al., 2024). Meanwhile, changes in Cr concentration can also pose potential risks to the soil environment, affecting soil quality and health. For example, the interaction between Cd and Cu may exacerbate the problem of antibiotic resistance genes entering the food chain, which can have severe consequences on the environment and the wellness of humankind (Pan et al., 2024).

We found that microbial community properties in RS and RP contributed markedly to SOC and had a significant positive effect on SOC in RS. This may indicate that soil microbial communities in RS and RP promoted SOC accumulation in RS. In RS, where oxygen and root exudates are abundant, methanotrophic activity is generally higher, leading to enhanced carbon cycling and potentially promoting SOC (Fan et al., 2021; Wei et al., 2022). However, methanogenic activity is usually suppressed by the availability of oxygen and the competitive presence of methanotrophs in RS (Rajendran et al., 2024). In contrast, the activity of methanotrophs in BS, away from plant roots, is often lower due to limited oxygen availability and nutrients (Edwards et al., 2015), which may cause the insignificant influence of microbial communities on SOC in BS. Type I/II methanotrophs convert methane to CO_2 via particulate methane monooxygenase while assimilating carbon via the ribulose monophosphate or serine cycle under aerobic conditions (Zheng et al.,

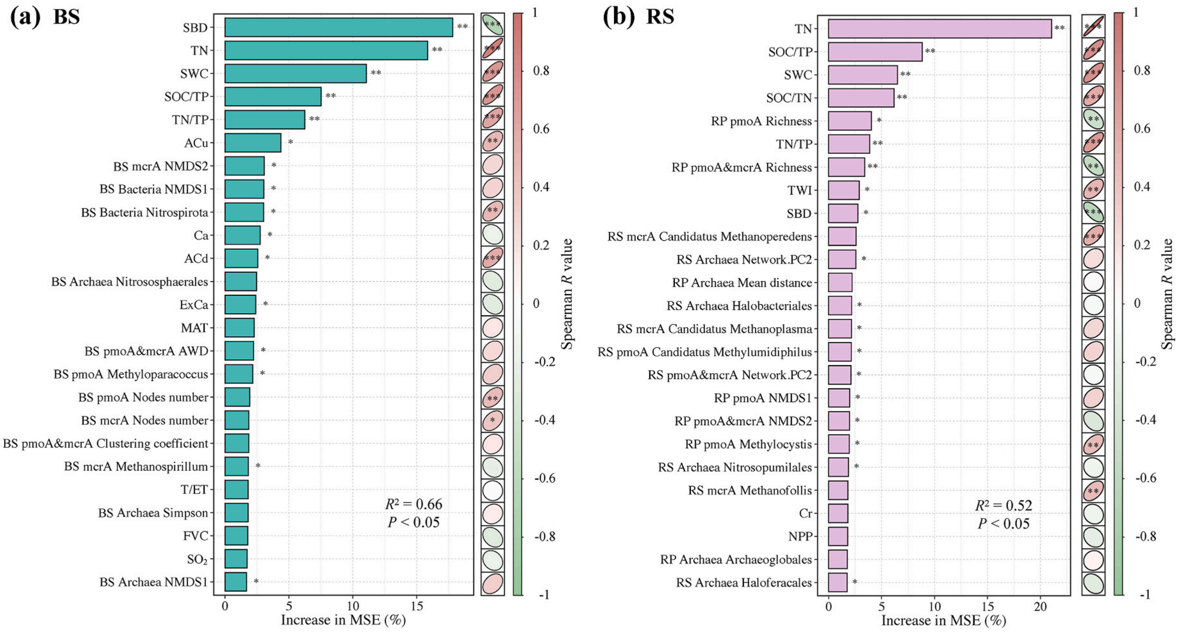


Fig. 8. Relative importance of biotic and abiotic factors on SOC between (a) bulk (BS) and (b) rhizosphere soils (RS). MSE, percentage of increase of mean square error (%). Bars marked with "*", "**", and "***" represent variables that are significant at the <0.05, <0.01 and < 0.001 level, respectively. R^2 and P values are the variance explained (i.e., goodness of fit) and significance of the random forest model. Spearman R value is the correlation between SOC and the top25 influencing factors. SOC/TP, SOC to TP ratio; SOC/TN, SOC to TN ratio; TN/TP, TN to TP ratio; NMDS1, first axis scores of NMDS; NMDS2, second axis scores of NMDS; Network. PC1, first principal component extracted using sub-network topological properties; Network.PC2, second principal component extracted using sub-network topological properties; pmoA, methanotrophs; mcrA, methanogens; AWD, average weight degree.

2024a). This validated the results of this study that type II methanotrophs (e.g., *Methylocystis*) in RP had a pronounced positive contribution to SOC in RS. Microorganisms consumed oxygen when degrading root exudates, resulting in brief or sustained hypoxic conditions in RP, leading to an increase in the abundance of methanogens and promoting SOC consumption (Qian et al., 2023). In our study, the higher abundance and diversity of methanotrophs in RS, and the higher rate of methane oxidation and carbon assimilation could offset the SOC loss caused by methane produced by methanogens in RP. Higher network complexity of methane microbial communities in RS and community structure in RP might have a positive effect on SOC in RS. Similarly, a previous study found that microbial network complexity had a significant positive effect on SOC in rice paddies (Wang et al., 2023). Although the diffusion of microorganisms in RP was more restricted compared to RS, the distance between RS and RP was closer. The microorganisms in RP were sufficient to diffuse into RS, which had a certain impact on the microorganisms and SOC in RS. Furthermore, the root exudation was impacted by soil properties such as nutrient availability or deficiency (Lu et al., 1999), and was sensitive to the microorganisms that colonize the RP, thereby affecting SOC in RS (De-la-Pena et al., 2008).

Our results also demonstrated that the indirect effects of climatic factors, soil physical properties, and agricultural management were larger than their direct effects on SOC between BS and RS, reflecting the importance of the interactions between biotic and abiotic factors. Furthermore, soil physical properties had a great positive indirect effect on SOC in BS through its positive effect on soil chemical properties, metallic elements, and microbial community properties, but a great positive indirect effect on SOC in RS mainly through its positive effect on soil chemical properties. Similarly, a study on methane emissions from a paddy field showed that soil abiotic factors could regulate methanotrophic community composition and atmospheric methane uptake in paddy soils, thereby promoting SOC accumulation (Zheng et al., 2024b). This implies that the sensitivity of SOC to climate change may be buffered by changes in soil properties and soil microbial communities (Ni et al., 2021; Li et al., 2024).

We found that agricultural management, such as fertilization, TPAM and GAP, had a positive indirect effect on SOC through its positive effect on physical and chemical properties between BS and RS. Lin et al. (2025) reported that fertilization could increase SOC in BS by augmenting the proportion of macroaggregate and carbon concentration in soil aggregate sizes. In addition, agricultural management had a significant positive effect on soil microbial communities in BS, which further had an important influence on SOC. However, agricultural management did not have a significant effect on soil microbial communities in RS. This may be related to the RS microenvironment (Minz et al., 2013; Wei et al., 2022), paddy root exudates (Zolla et al., 2013), and the main functional microbial communities (Edwards et al., 2015; Rajendran et al., 2024) and carbon-acquisition enzyme activities (Chen et al., 2023) in RS and RP. A global meta-analysis on enzyme activities supported this point, as they found that the nitrogen sensitivity of carbon-acquisition enzyme (β-1,4-glucosidase, phenol oxidase, and peroxidase) activities in RS was significantly lower than that of BS (Chen et al., 2023). The response of soil microbial communities to fertilization and other agricultural management is relatively weak due to the high nutrient content in RS.

4.3. Implications, limitations, and future directions

Our study fills a gap in the regulatory mechanisms of SOC in different habitats of rice paddies at a regional scale. The divergent regulatory mechanisms of SOC storage between BS and RS imply that different habitats should be considered when studying soil carbon cycling processes in rice paddies. Furthermore, when optimizing agricultural management (e.g., fertilization) aiming at carbon accumulation, synergistic consideration of soil properties, climate, and soil microbial communities is imperative.

There are some limitations of our study. First, county-level agricultural management data (e.g., fertilization, TCSA, GAP, TPAM, and pesticide usage) derived from the Jiangsu, Shanghai, and Zhejiang Statistical Yearbook 2021 may lack site-specific resolution for precisely delineating management effects on SOC at the plot scale. Future studies would benefit from higher-resolution spatial datasets to elucidate linkages between agricultural interventions and SOC accumulation

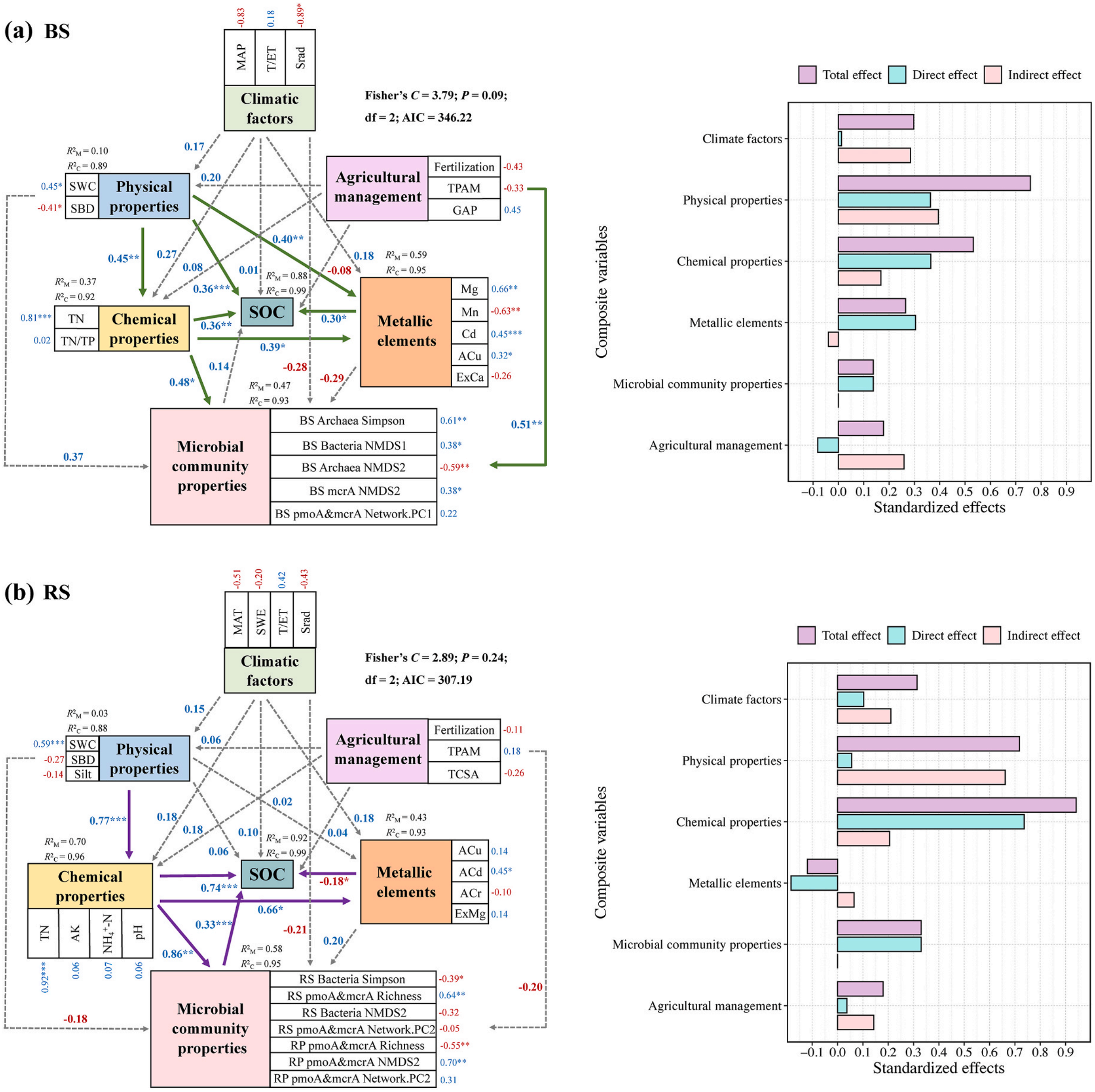


Fig. 9. Piecewise structural equation modeling (piecewiseSEM) accounting for the direct and indirect effects of biotic and abiotic factors on SOC between (a) bulk (BS) and (b) rhizosphere soils (RS). The biotic and abiotic factors including climatic factors, physical properties, chemical properties, metallic elements, agricultural management, and microbial community properties. Numbers adjacent to arrows are path coefficients (partial regression) which represent the directly standardized effect size of the relationship. The standardized path coefficients in the paths are shown in blue if positive or in red if negative. Significant paths ($P < 0.05$) are represented by solid lines, and non-significant paths ($P > 0.05$) are represented by dashed lines in the figure. Numbers adjacent to measured variables are their coefficients with composite variables. Relationships between residual variables of measured predictors are not shown. Significance levels of each predictor are $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Standardized effects (total, direct, and indirect effects) based on piecewiseSEM are shown in the figure on the right. df, degree of freedom; AIC, Akaike information criterion. NMDS1, first axis scores of NMDS; NMDS2, second axis scores of NMDS; Network.PC1, first principal component extracted using sub-network topological properties; Network.PC2, second principal component extracted using sub-network topological properties.

mechanisms. Second, mechanism experiments should be synergistically integrated with large-scale analysis, as exemplified in our study, to advance understanding of soil carbon transformation processes.

5. Conclusions

We employed the effects of biotic and abiotic factors on driving SOC storage at a regional scale using random forest model and piecewiseSEM, and revealed the regulatory mechanisms on SOC storage between BS and RS of rice paddies differed markedly. Soil physical and chemical properties had the largest direct effect on SOC in BS, while soil chemical properties had the largest direct effect on SOC in RS. Microbial community properties in RS exhibited a more significant positive direct effect (the second largest direct effect) on SOC compared to those in BS. Notably, microbial communities in RP had an important influence on the SOC content in RS. Soil available metal elements and methane microbial community properties exhibited a more pronounced effect on SOC in RS than in BS. Interestingly, soil metallic elements positively influenced SOC in BS, but negatively influenced SOC in RS. Furthermore, the indirect effects of climatic factors, soil physical properties, and agricultural management on SOC exceeded their direct effects in BS or RS. Soil physical properties had a positive indirect effect on SOC in BS through its effect on soil chemical properties, metallic elements, and microbial community properties, but a positive indirect effect on SOC in RS mainly through its effect on soil chemical properties. Moreover, agricultural management influenced SOC indirectly through soil physical and chemical properties. In terms of the total effect, physical properties, chemical properties, and climate were the most influencing factors for SOC in BS, while chemical properties, physical properties, and microbial community properties were the most influencing factors for SOC in RS. Accordingly, deciphering the complex and divergent regulatory mechanisms of SOC storage in different habitats is vital for understanding microbe-driven ecological processes and functions in rice paddies. This study offers novel insights into the regulatory mechanisms of SOC storage in different habitats of rice paddies at a regional scale and provides valuable references for future soil carbon sequestration management in rice paddies.

CRediT authorship contribution statement

Jie Liu: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lin Yang:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Jonathan Miles Adams:** Writing – review & editing. **Lei Zhang:** Writing – review & editing, Supervision. **Jie Wang:** Writing – review & editing. **Ren Wei:** Writing – review & editing. **Chenghu Zhou:** Writing – review & editing, Supervision.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2025.126179.

Data availability

Data will be made available on request.

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