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Spartina alterniflora invasion exacerbates soil microbial carbon and phosphorus co-limitations and alters microbial carbon and nitrogen use efficiency in the coastal wetlands of eastern China

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Abstract

Background Enzymatic stoichiometry reflects microbial relative resource limitations by linking microbial nutritional demands with soil nutrient availability, yet how plant invasion-induced changes in vegetation, soil properties, and microbial communities modulate these limitations and metabolic efficiency remains undetermined. Here, we employed enzymatic stoichiometry and vector modeling to assess microbial relative resource limitations in invasive *Spartina alterniflora* salt marsh in comparison to those in bare flat and in native *Suaeda salsa* and *Phragmites australis* salt marshes, and systematically linked these limitations to microbial carbon (C) and nitrogen (N) use efficiencies (CUE and NUE, respectively) across coastal wetland ecosystems of eastern China.

Results Our analyses showed predominant phosphorus (P) limitation of soil microbial metabolism in bare flat and native *S. salsa* and *P. australis* salt marshes, contrasting with dual C-P co-limitation observed in invasive *S. alterniflora* salt marsh. *S. alterniflora* invasion intensified microbial P limitation compared with bare flat, while simultaneously inducing the most pronounced C limitation among all plant communities. The microbial C limitation induced by *S. alterniflora* invasion drove reductions in microbial CUE, whereas microbial NUE increased, establishing an antagonistic relationship between these metabolic efficiencies. Microbial resource constraints and nutrient use efficiencies (CUE/NUE) in soils were coordinately controlled by plant traits, soil properties, and microbial attributes. Partial least squares path modeling analysis identified soil organic C (SOC) chemical fractions (e.g., aromatic C, alkyl C, dissolved organic C) as predominant positive drivers of microbial C limitation and NUE, while simultaneously suppressing microbial CUE. Simultaneously, plant traits were identified as the foremost contributor to microbial P limitation, followed by microbial attributes as the second-most influential positive factor.

Conclusions This study revealed that *S. alterniflora* invasion fundamentally shifted microbial nutrient limitation from predominant P limitation in bare flat and native salt marshes to dual C-P co-limitation, while simultaneously inducing the strongest microbial C limitation among all communities. This invasion-induced microbial C limitation

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drove a reduction in microbial CUE but an enhancement of NUE. SOC accumulation increased with decreasing microbial CUE following *S. alterniflora* invasion, a tradeoff potentially linked to divergent nutrient limitations across ecosystems. This study provided empirical evidence for microbially-mediated soil C sequestration mechanisms underlying plant invasion-induced ecosystem transformations.

Keywords Enzymatic stoichiometry, Microbial nutrient use efficiency, Microbial relative resource limitation, Plant invasion, Salt marsh

Introduction

Biological invasions by alien species have emerged as a critical driver of ongoing global change (Dickie et al. 2017), generating serious ecological, economic, and social impacts (Brazeiro et al. 2024). Alien plant species frequently establish dense monocultures that outcompete native plant communities (Gallé et al. 2023), reduce species diversity (Cano-Barbacid et al. 2023; Xu et al. 2023), and impair ecosystem functions and services (Hanberry 2023; Hunter and DeBerry 2023). Plant invasion dramatically transforms vegetation community composition and biomass distribution patterns (Gallé et al. 2023). This subsequently alters the quantity and quality of plant-derived organic matter entering the soil (Yang et al. 2019; Beyene et al. 2024). These changes modify soil physicochemical properties (Gallé et al. 2023). Ultimately, invasion restructures soil microbial communities (Yang et al. 2016b), with particularly strong effects on bacterial and fungal diversity and composition (Yang et al. 2016b, 2019, 2020). Consequently, such transformations can profoundly impact ecosystem processes and functions, notably by affecting biogeochemical cycles of carbon (C) and nutrients (Yang et al. 2016a; Lee et al. 2018; Beyene et al. 2024). Nevertheless, the effects of plant invasion on microbial resource limitations and nutrient use efficiencies, and critically, how the resulting metabolic alterations further influence soil C dynamics and nutrient cycling, are still unclear.

Soil microorganisms play a central role in maintaining C and nutrient cycles via their critical involvement in organic matter decomposition and soil organic matter regulation (Neurauter et al. 2023; Guo et al. 2024). However, the majority of free-living microbial communities are constrained by energy (i.e. C) or essential nutrients, such as nitrogen (N) or phosphorus (P) (Moorhead et al. 2016; Deng et al. 2019). Soil extracellular enzymes secreted by microbes can facilitate the breakdown of plant and microbial cell walls, catalyze the depolymerization of macromolecules, and generate bioavailable substrates for microbial utilization (Deng et al. 2019), thereby alleviating microbial metabolic limitations (Hu et al. 2024). Microbial communities optimize resource distribution by synthesizing extracellular C-, N-, and P-acquiring enzymes to address resource limitations (Sinsabaugh

and Moorhead 1994; Zhai et al. 2025). Soil extracellular enzyme activities (EEA) can effectively reflect the biogeochemical equilibrium linking microbial metabolic processes, nutrient demands, and soil nutrient availability (Deng et al. 2019). Meanwhile, soil enzymatic stoichiometry (EES) serves as a critical indicator of microbial energy and nutrient requirements, revealing microbial resource allocation strategies during energy and nutrient acquisition (Abay et al. 2024; Lian et al. 2024). Recently, within EES theory framework, a vector-based analytical model was developed to assess microbial metabolic constraints at local, regional, and global scales, which calculates the “vector length” (indicative C acquisition) and “vector angle” (representing N or P acquisition) for plotted vectors to symbolize the relative enzymatic activities of C:N vs. C:P acquisition (Moorhead et al. 2013, 2016). For instance, Guo et al. (2024) employed the method to show that soil microbial P limitation is prevalent in global terrestrial ecosystems, accounting for 83.78% of 1245 observations. Cui et al. (2023) also utilized this method to demonstrate that microbial C limitation is present in only about 22% of global terrestrial surface soil sites, based on 847 out of 2476 observations. Thus, quantifying invasion-induced changes in EEA, EES, and enzyme vector metrics (length/angle) provides key insights into plant invasion-driven shifts in microbial resource limitations.

Substrate stoichiometry and availability, microbial nutrient demand and metabolic limitations can affect microbial nutrient use efficiencies (Fuchslueger et al. 2019). Microorganisms can adapt their nutrient use efficiencies in response to varying nutrient availabilities and stoichiometric imbalances between resources and microbial requirements (Manzoni et al. 2012; Sinsabaugh et al. 2016). The efficiency with which microorganisms convert assimilated C and N into biomass is quantified as microbial C use efficiency (CUE) and N use efficiency (NUE), which quantitatively reflect the allocation of C and N resources between microbial biomass production and mineralization losses (Mooshammer et al. 2014; Sinsabaugh et al. 2016; Iven et al. 2023). Increased microbial CUE and NUE correspond to enhanced C and N retention in microbial biomass, resulting in reduced respiratory C losses (CO₂ emissions) and decreased N release as inorganic forms, respectively (Mooshammer et al. 2014;

Sinsabaugh et al. 2016; Fuchslueger et al. 2019). Microbial CUE has been extensively recognized as a crucial parameter governing short-term C cycling dynamics (Hagerty et al. 2018). Higher CUE values generally enhance soil organic carbon (SOC) accumulation (Kallenbach et al. 2016; Tao et al. 2023). Additionally, the integration of microbial CUE and NUE better bridges enzymatic processes, microbial biomass, and soil nutrient availability (Lian et al. 2024). Thus, elucidating the linkages between microbial resource limitations and nutrient use efficiencies (i.e., microbial CUE and NUE) could enhance our understanding of how microbial nutrient metabolism response to plant invasion, while simultaneously elucidating the effects of microbial nutrient cycling dynamics on soil organic C and N sequestration.

Microbial resource limitations and nutrient use efficiencies are mediated by multiple driving factors (Cruz-Paredes and Rousk 2024; Pan et al. 2024), including climate factors (e.g., precipitation, temperature, and humidity) (Sinsabaugh et al. 2016; Li et al. 2022), plant traits (e.g., litter and fine root) (Pan et al. 2024; Shi et al. 2024), soil nutrient availability and their stoichiometry (Soares and Rousk 2019), soil physicochemical properties (e.g., temperature, moisture, and pH) (Stark et al. 2014; Jones et al. 2019; Kang et al. 2022), microbial community composition and biomass stoichiometry (Pan et al. 2024), and environmental stresses (Manzoni et al. 2012; Hu et al. 2022). For instance, soil microbial N limitation was predominantly observed in temperate regions, while microbial P limitation primarily occurred in tropical and cold zones (Guo et al. 2024). Elevated plant productivity could exacerbate plant–microbe competition for soil N and P, thereby diminishing nutrient availability and restricting microbial nutrient acquisition (Pan et al. 2024). Soil physicochemical properties (e.g., pH and moisture) directly and indirectly regulate microbial metabolic processes through changes in substrate availability and soil C:N:P stoichiometric ratios (Deng et al. 2019), and further affect microbial CUE and NUE (Jones et al. 2019). Plant invasion has been reported to significantly impact plant productivity (Carboni et al. 2021), modify soil physicochemical characteristics (Yang et al. 2020; Gallé et al. 2023), alter soil C, N, and P cycling processes (Yang et al. 2016a; Lee et al. 2018; Beyene et al. 2024; Zhang et al. 2024), and restructure microbial communities (Stanek et al. 2023). Accordingly, clarifying primary determinants of microbial resource limitations and nutrient use efficiencies during plant invasion may advance mechanistic comprehension of how invasion-induced changes alter microbial metabolism in soils.

Coastal wetlands function as critical ecotones between terrestrial and marine ecosystems and rank among Earth's most fragile, responsive, and biologically

productive ecosystems (Zhang et al. 2024). Coastal wetlands play critical roles in regulating biogeochemical (e.g., C, N, and P) cycling and mitigating climate change (Macreadie et al. 2021; Zhang et al. 2024). However, extensive coastal wetland ecosystems worldwide are increasingly jeopardized by invasive plant species proliferation (Hessini et al. 2020). *Spartina alterniflora* Loisel., a rhizomatous C_4 perennial grass originating from Atlantic coastal ecosystems spanning the American continents, has been intentionally introduced intertidal zones of coastal wetlands across multiple continents for coastal stabilization purposes. *S. alterniflora* is classified among the most pernicious invasive species in coastal wetlands globally, demonstrating particularly aggressive colonization strategies in coastal wetlands. Following its introduction to China in 1979, *S. alterniflora* has rapidly expanded along eastern coastal margins through colonization of unvegetated bare flat and competitive substitution of native halophytes including such as *Suaeda salsa* (Linn.) Pall. and *Phragmites australis* (Cav.) Trin. ex Steud. (Yang et al. 2019, 2020). *S. alterniflora* invasion has been demonstrated to enhance net primary productivity, while modifying soil physicochemical properties (Yang et al. 2016b), altering soil C-N-P biogeochemical cycling (Yang et al. 2016a; Zhang et al. 2024), and reshaping soil microbial communities (Yang et al. 2016b, 2019, 2020) and C-cycling functional genes (Zhang et al. 2025). Previous extensive research has characterized microbial resource limitations in terrestrial ecosystems (Cui et al. 2023; Guo et al. 2024), yet comparable understanding of coastal wetland remains critically lacking. Furthermore, how *S. alterniflora* invasion impacts microbial resource limitations and nutrient use efficiencies in coastal wetland ecosystems, and how these changes subsequently regulate soil C dynamics and nutrient cycling remain poorly understood. To address this knowledge gap, we investigated the effects of *S. alterniflora* invasion on soil EEA, EES, microbial resource limitations (vector length and angle), and nutrient use efficiencies (CUE and NUE) at three soil depths (0–10, 10–20, and 20–30 cm) in 20-year-old invasive *S. alterniflora* salt marsh, comparatively analyzed with unvegetated bare flat, indigenous *S. salsa* and *P. australis* communities in the coastal wetlands of eastern China. Accordingly, we established the following hypotheses for verification: (1) Soil microbial metabolism in coastal wetland ecosystems is primarily constrained by P, as mineral-bound P release through parent material weathering remains rate-limited, creating persistent P deficiency conditions; (2) *S. alterniflora* invasion exacerbates microbial C limitation through the inputs of massive refractory plant residues; (3) the exacerbation of microbial C limitation following

S. alterniflora invasion further constrained microbial CUE, thereby reducing SOC accumulation through triggered more soil C release.

Materials and methods

Site descriptions

The study site was situated within the core zone of Yancheng Wetland National Nature Reserve for Rare Birds (32° 36′–34° 28′ N, 119° 51′–121° 5′ E). This reserve is located within the ecotone between the warm-temperate and northern subtropical zones, experiences a continental monsoon climate characterized by mean annual temperature of 13.6 °C coupled with mean annual precipitation of 1024 mm (Yang et al. 2019). This region was designated as China's largest coastal wetland nature reserve, functioning as a critical stopover point for migratory birds traversing the Northeast Asian-Australasian flyway and contributing significantly to global biodiversity conservation efforts. In 1983, *S. alterniflora* was introduced to this reserve with the aim of accelerating siltation and stabilizing tidal flat lands. *S. alterniflora* has proliferated exponentially, developing dominant monocultural salt marsh systems through four decades of ecological colonization. The bare flat and *S. alterniflora* salt marsh occupy distinct zones within the intertidal environment, with the former predominantly established in lower intertidal regions, and the latter characterizing mid-intertidal areas, which are subject to semidiurnal tides. Before the invasion of *S. alterniflora*, the seaward intertidal area existed as unvegetated bare flat, lacking any plant cover (Yang et al. 2020). As native C_3 halophytes, *S. salsa* and *P. australis* occupy distinct niches, with *S. salsa* inhabiting the upper intertidal zone and *P. australis* located above the high tide level of the spring tide (Fig. S1). The coastal vegetation in the reserve exhibits a distinct spatial zonation pattern from seaward to upland areas: bare flat, followed by *S. alterniflora*, then *S. salsa*, and finally *P. australis* communities (Fig. S1). There was rarely overlap between them.

Field sample collection

Four parallel 5 km×50 m transects were established in July 2021, spanning sea-land gradients that successively crossed the bare flat (control), *S. alterniflora*, *S. salsa*, and *P. australis* vegetation zones within the reserve (Fig. S1). A single sampling site was designated for each vegetation type along every transect, yielding four sites per transect (Fig. S1). *S. alterniflora* community at the sampling sites represent a 20-year invasion, which occupied the bare flat before their colonization (Yang et al. 2019). Within each transect's sampling site, three 2 m×2 m plots were systematically deployed using random sampling. From each plot, three 5 cm×10 cm (diameter×depth) soil

cores were sampled randomly at three depth intervals: 0–10 cm, 10–20 cm, and 20–30 cm. For each depth layer, 3 soil cores per plot (totaling 9 cores per depth) within each transect's sampling site were pooled and homogenized to produce final composite samples. In total, 48 soil samples (4 vegetation types×4 transect replicates×3 soil depth intervals) were obtained. Using a standardized cutting ring method, soil bulk density (BD) measurements were conducted at three soil layers (0–10 cm, 10–20 cm, and 20–30 cm) within each sampling site. For each transect's sampling site, aboveground vegetation was harvested from three 1 m×1 m quadrats, with parallel root sampling employing three 10 cm×10 cm (diameter×depth) soil cores retrieved randomly at three depth tiers (0–10 cm, 10–20 cm, and 20–30 cm) using a root auger. Each soil sample was sieved (2.0 mm mesh), homogenized, and subdivided into five representative aliquots. The first soil subsample was transferred to a pre-weighed aluminum container for soil moisture determination. The second and third soil subsamples were cryopreserved at –20 °C and –80 °C, respectively, designated for extracellular enzyme activity assays and phospholipid fatty acid (PLFA) biomarker profiling. The fourth soil subsample was refrigerated at 4 °C for subsequent microbial biomass C (MBC) and dissolved organic C (DOC) analyses. The fifth soil subsample was prepared through sequential air-drying and fine sieving (0.15 mm) for determination of soil properties and SOC fractions.

Analyses of basic soil and plant properties

Aboveground and root materials underwent standardized rinsing followed by 65 °C drying until mass stabilization, enabling sequential measurement of aboveground biomass (AB) and root biomass (RB). Soil moisture content was quantified through standardized oven-drying (105 °C, 48 h) of fresh samples. Soil pH was analyzed with a calibrated pH meter (1:2 soil-to-water mixture), whereas salinity was evaluated via conductivity meter measurements (1:5 soil-to-water suspension). Soil DOC was isolated via 0.5 M K_2SO_4 extraction under 60-min shaking, followed by 0.45 µm membrane filtration and subsequent quantification with a Shimadzu TOC-L CPH analyzer (TOC/TN mode). Plant (i.e., foliar, stem, root) organ C and SOC levels were determined by a Vario PYRO Cubic Element Analyzer (Elementar, Germany). Soil samples underwent 24-h 1 M HCl pretreatment to eliminate carbonates preceding analytical procedures. The dried fine powders obtained from leaf, stem, root, and soil samples were subjected to acid digestion with H_2SO_4 followed by subsequent extraction using 1 M KCl solution. N content in foliar, stem, and root tissues, along with soil total nitrogen (TN) content, were quantified in the resultant extracts employing an AA3 continuous flow

analyzer (Auto Analyzer 3; SEAL Analytical, Germany). The P content in foliar, stem, root tissues, and soil TP were analyzed through acid digestion using 8 mL concentrated H_2SO_4 (98%) and 10 mL HClO_4 (72%) at 300 °C for 30 min, with 0.5–1.0 g dry samples processed (Sparks et al. 1996). The extraction of soil available phosphorus (AP) was performed using 0.5 M NaHCO_3 (Olsen and Sommers 1982), while both AP and total phosphorus (TP) were quantified with an AA3 continuous flow analyzer. Soil ammonium nitrogen ($\text{NH}_4^+\text{-N}$) and nitrate nitrogen ($\text{NO}_3^-\text{-N}$) concentrations were analyzed on an AA3 continuous flow analyzer subsequent to 1 M KCl extraction. The soil net nitrogen mineralization rate was determined based on pre- and post-incubation extractable inorganic N measurements, following the method of Chen et al. (2023). A two-step H_2SO_4 hydrolysis method (Rovira and Vallejo 2002) was employed to quantify the concentrations of labile organic carbon (LC) and recalcitrant organic carbon (RC) in soil samples. The soil recalcitrance carbon index (RIC) was determined by applying the following equation (Rovira and Vallejo 2002):

$$\text{RIC (\%)} = \text{RC/SOC} \times 100\% \quad (1)$$

Solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy was performed to determine SOC chemical fractions. The soil samples (0.1 g) treated with a 10% hydrofluoric acid solution were investigated via solid-state ^{13}C NMR spectra with a Bruker BioSpin AVANCE 400 MHz WB spectrometer, which was fitted with a standard (4 mm) bore CPMAS probe, having a scan number of 5000 and spin rate of 10 kHz. The ^{13}C NMR spectra were categorized into four characteristic regions based on chemical shift ranges: alkyl C (0–45 ppm), O-alkyl C (45–110 ppm), aromatic C (110–160 ppm), and carbonyl C (160–220 ppm) (Finn et al. 2015). The experimental procedures were carried out as outlined in Yang et al. (2022).

Soil extracellular enzyme activities and microbial attribute assays

Extracellular enzyme activities related to C acquisition (β -1,4-glucosidase, BG; cellobiohydrolase, CB), N acquisition (β -1,4-*N*-acetylglucosaminidase, NAG; leucine amino peptidase, LAP), and P acquisition (alkaline phosphatase, ALP) were measured through standard fluorometric techniques (German et al. 2011). Fluorometric determination of soil extracellular enzyme activities was performed using 200 μM substrate solutions labeled with either 4-methylumbelliferone or 7-amino-4-methylcoumarin fluorescent markers. Briefly, soil suspensions were prepared by homogenizing 1 g fresh soil in 90 mL Tris buffer via magnetic stirring. Hydrolytic enzymes were analyzed fluorometrically using black

96-well polystyrene plates (Whatman Inc., NJ, USA). Tris buffer, soil suspension, standard solution (10 μM 4-MUB), and corresponding enzyme-specific fluorescent substrate solutions (200 μM) were dispensed onto the well plate in a rigorously defined order. After 5-h dark incubation at 25 °C, enzymatic reactions were stopped with 10 μL NaOH (1 mol/L). Fluorescence quantification was performed immediately using a DTX880 microplate reader (Beckman-Coulter) with excitation at 360 nm and emission detection at 450 nm (German et al. 2011). The activities of soil extracellular enzymes were quantified in $\text{nmol g}^{-1} \text{h}^{-1}$ (dry weight). Soil enzymatic C:N, C:P, and N:P ratios were calculated through logarithmic transformations: C:N ratio as $\ln(\text{BG} + \text{CB}) / \ln(\text{NAG} + \text{LAP})$, C:P ratio as $\ln(\text{BG} + \text{CB}) / \ln(\text{ALP})$, and N:P ratio as $\ln(\text{NAG} + \text{LAP}) / \ln(\text{ALP})$, consistent with Sinsabaugh et al. (2008). The soil microbial biomass C (MBC), N (MBN), and P (MBP) were determined via the chloroform fumigation-extraction technique established by Vance et al. (1987). Further, both the soil bacterial and fungal biomass were quantified via PLFA analysis as described by Bossio and Scow (1998), with experimental protocols implemented according to Yang et al. (2016b).

Microbial resource/nutrient limitations and nutrient use efficiencies

Microbial resource/nutrient limitations in soil microbes were assessed via enzymatic activity vector characteristics, vector length and vector angle parameters were derived from the computational framework established by Moorhead et al. (2016):

$$\text{Vector length} = \text{SQRT}(x^2 + y^2) \quad (2)$$

$$\text{Vector angle} (^{\circ}) = \text{DEGREES}[\text{ATAN2}(x, y)] \quad (3)$$

where, x represents to the relative C- vs. P-acquiring enzymatic activities (e.g., $(\text{BG} + \text{CB}) / (\text{BG} + \text{CB} + \text{ALP})$), and y denotes the relative C- vs. N-acquiring activities (e.g., $(\text{BG} + \text{CB}) / (\text{BG} + \text{CB} + \text{NAG} + \text{LAP})$). Vector length indicates microbial C limitation intensity, and longer vector lengths correspond to heightened microbial C limitation. Angles $> 45^{\circ}$ denote P limitation, while angles $< 45^{\circ}$ reflect N limitation. P limitation strengthens with increasing angle, whereas N limitation intensifies as the angle decreases.

The microbial CUE and NUE values were calculated through metabolic equilibrium modeling approaches derived from ecological stoichiometry (Sinsabaugh et al. 2016):

$$\text{CUE}_{\text{C:N}} = \text{CUE}_{\text{max}}[\text{S}_{\text{C:N}} / (\text{S}_{\text{C:N}} + \text{K}_{\text{N}})] \quad (4)$$

$$S_{C:N} = (1/EEA_{C:N})(B_{C:N}/L_{C:N}) \quad (5)$$

$$NUE_{N:C} = NUE_{max}[S_{N:C}/(S_{N:C} + K_C)] \quad (6)$$

$$S_{N:C} = (1/EEA_{N:C})(B_{N:C}/L_{N:C}) \quad (7)$$

where, CUE_{max} is the maximal microbial growth efficacy that retains a constant value of 0.60 based on thermodynamic constraints; and NUE_{max} is standardized to a constant value of 1. $S_{C:N}$ and $S_{N:C}$ are scalar that refer to the extent to which the distribution of enzymatic activities compensates for disparities between the composition of available nutrient resources and that of the microbial biomass; $EEA_{C:N}$ and $EEA_{N:C}$ are the enzymatic activity ratios, i.e., $EEA_{C:N} = (BG + CB)/(NAG + LAP)$, $EEA_{N:C} = (NAG + LAP)/(BG + CB)$. $B_{C:N}$ and $B_{N:C}$ are the microbial biomass ratios, i.e., $B_{C:N} = MBC/MBN$, $B_{N:C} = MBN/MBC$. $L_{C:N}$ and $L_{N:C}$ are the elemental ratios of the consumed substrates, i.e., $L_{C:N} = DOC/(NH_4^+ - N + NO_3^- - N)$, $L_{N:C} = (NH_4^+ - N + NO_3^- - N)/DOC$. The half-saturation constants K_C and K_N were assigned a constant value of 0.50 (Sinsabaugh et al. 2016).

Statistical analyses

The effects of plant communities and soil depth, respectively, on ecosystem parameters (plant-soil-microbial properties, enzyme activities and their stoichiometry, vector length, vector angle, microbial CUE and NUE) were statistically analyzed using one-way ANOVA in SPSS v25.0. Significant inter-group mean differences were analyzed using Duncan's test with a significance threshold of $P < 0.05$. A two-way ANOVA (SPSS Statistics 25.0) was implemented to test the interactive effects of plant communities and soil depth on integrated variables: plant-soil-microbial properties, enzymatic activities/stoichiometry, vector geometry (length/angle), and microbial nutrient use efficiencies (CUE, NUE). Linear regression analysis was performed to assess (1) enzymatic stoichiometric linkages [$\ln(BG + CB)$ vs. $\ln(NAG + LAP)$; $\ln(BG + CB)$ vs. $\ln(ALP)$; $\ln(NAG + LAP)$ vs. $\ln(ALP)$]; (2) microbial nutrient use efficiencies vs. vector-defined nutrient limitations; and (3) intrinsic vector parameter covariation (vector length-angle relationships) using the 'lm' function in the 'stats' package in R software (v. 4.2.2,

<https://www.r-project.org>). Pearson's correlation analysis was employed to examine the relationships between soil enzyme activities and their stoichiometry, vector length, vector angle, CUE, NUE, and environmental factors using the 'corr.test' function in the 'corrplot' package in R software (v. 4.2.2, <https://www.r-project.org>). The relative contribution of plant-soil-microbial predictors to variability in vector length, vector angle, microbial CUE and NUE was assessed via random forest analysis using the 'randomForest' package (v. 4.2.2) in R. Partial least squares path modeling (PLS-PM) was used to analyze both the direct and indirect links between the plant, soil, and microbial attributes with the vector length, vector angle, microbial CUE and NUE, respectively, using 'innerplot' function in the 'plsmp' package in R software (v. 4.2.2, <https://www.r-project.org>). Principal component analysis (PCA) was conducted to identify variable subsets maximizing explained variance in vector length, vector angle, microbial CUE and NUE. In the principal component analysis, variables with absolute factor loadings exceeding 0.5 were retained for subsequent PLS-PM, while the outer model employed a more stringent threshold of factor loadings > 0.7 to exclude less contributory independent variables (Zhang et al. 2024). We further selected the significant factors for random forest analysis from the factors that meet the above screening criteria for PLS-PM analysis to improve the PLS-PM results.

Results

Plant traits, soil properties, and microbial attributes

The *S. alterniflora* community displayed significantly greater aboveground biomass and foliar C:N ratios compared to other plant communities (Figs. S2a and S3a). Root biomass in the upper 20 cm soil profile was considerably higher in *S. alterniflora* community than in *P. australis* community (Fig. S2b). The *S. alterniflora* community exhibited markedly elevated root C:N values in the upper 20 cm soil profile compared to *S. salsa* and *P. australis* communities (Fig. S4a). Within the 0–30 cm soil profile, *S. alterniflora* community consistently exhibited the highest values across multiple soil parameters, including soil moisture, salinity, SOC, DOC, $NH_4^+ - N$, RC, SOC:TN, SOC:TP, $DOC:(NH_4^+ - N + NO_3^- - N)$ relative to other plant communities (Figs. 1 and S5). The

(See figure on next page.)

Fig. 1 Soil properties (mean $\pm$ SE, $n=4$) of bare flat, *S. alterniflora*, *S. salsa*, and *P. australis* communities. Different uppercase letters over the bars imply statistically significant differences at the $\alpha=0.05$ level between plant communities at the same soil depth. Different lowercase letters over the bars imply statistically significant differences at the $\alpha=0.05$ level between soil depths in the same plant community. BD: bulk density; SOC: soil organic carbon; TN: soil total nitrogen; TP: soil total phosphorus; DOC: soil dissolved organic carbon; $NH_4^+ - N$: ammonium nitrogen; $NO_3^- - N$: nitrate nitrogen; AP: available phosphorus; LC: labile organic carbon; RC: recalcitrant organic carbon; RIC: recalcitrant index for carbon. BF: bare flat; SA: *Spartina alterniflora*; SS: *Suaeda salsa*; PA: *Phragmites australis*

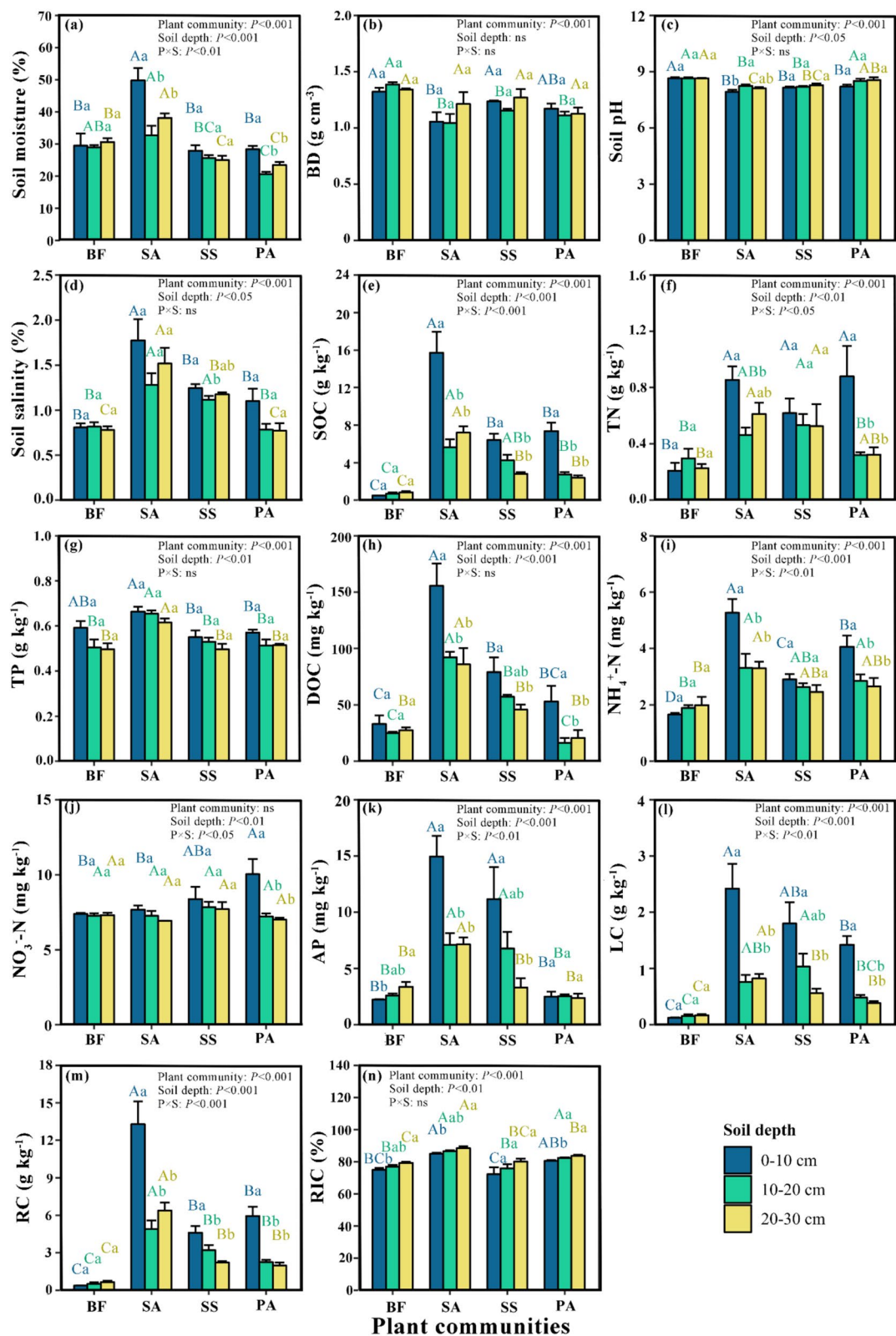


Fig. 1 (See legend on previous page.)

highest soil (0–10 cm and 20–30 cm) LC, RIC, and SOC:TN levels were observed in *S. alterniflora* community (Figs. 1 and S5a). The proportions of soil (0–30 cm) alkyl C and aromatic C in *S. alterniflora* community were substantially higher than those in *S. salsa* and *P. australis* communities (Fig. S6a and c). The proportion of soil (0–30 cm) O-alkyl C peaked in *P. australis* community, but reached a minimum in *S. alterniflora* community (Fig. S6b). Surface soils (0–10 cm) in *S. alterniflora* and *P. australis* communities exhibited higher MBC and MBN concentrations than bare flat and *S. salsa* community (Fig. S7a and b). The *S. alterniflora* community exhibited maximum MBP concentration in both surface (0–10 cm) and subsurface (20–30 cm) soil layers compared with other plant communities (Fig. S7c). The *S. alterniflora* community exhibited the highest bacterial and fungal PLFA (0–30 cm soil depth), indicating dominant microbial biomass accumulation compared to other plant communities (Fig. S7g, h).

Soil extracellular enzyme activities and their stoichiometry

Soil C-, N-, and P-acquiring enzymes, enzyme C:N and C:P ratios were strongly affected by plant communities (Fig. 2). Within the 0–30 cm soil profile, *S. alterniflora* community exhibited the highest C-acquisition enzyme activities (BG+CB) and enzymatic C:N ratio among all plant communities (Fig. 2a, d). The highest activities of N-acquiring enzymes (NAG+LAP) in soil profiles were detected in *S. alterniflora*, *S. salsa*, and *P. australis* communities at 0–10 cm depth, in *S. alterniflora* and *S. salsa* communities at 10–20 cm depth, and exclusively in *S. alterniflora* community at 20–30 cm soil depth (Fig. 2b). Soil (0–10 cm and 20–30 cm) P-acquiring enzyme (ALP) activities were greatest in *S. alterniflora* community (Fig. 2c). Surface soil layer (0–10 cm) enzyme C:P ratio was notably higher in *S. alterniflora* and *S. salsa* communities than in bare flat and *P. australis* community, while reaching peak level in *S. alterniflora* community at the 10–30 cm soil layers (Fig. 2e). Linear regression analysis of C-N-P enzymatic activities showed distinct deviations from the 1:1:1 stoichiometric ratio in $\ln(\text{BG} + \text{CB}) : \ln(\text{NAG} + \text{LAP}) : \ln(\text{ALP})$ across plant communities (Fig. S8).

Soil microbial nutrient limitations and nutrient use efficiencies

The soil enzymatic stoichiometric characteristics differed between plant communities (Fig. 3), demonstrating that *S. alterniflora* invasion substantially altered microbial nutrient supply. The vast majority of datapoints were above the 1:1 line, which indicated strong microbial P limitation in the coastal wetlands of eastern China (Fig. 3a). *S. alterniflora* community exhibited maximum vector length across the 0–30 cm soil profile (Fig. 3b).

Vector angles ranged from 47.4 to 76.1° at 0–30 cm soil layers among plant communities of the coastal wetlands (Fig. 3c). The bare flat, *S. alterniflora*, *S. salsa*, and *P. australis* communities showed soil microbial P limitation (rather than N), as evidenced by vector angles consistently >45° (Fig. 3c). Vector angles reached peak levels at 0–20 cm soil layers in *S. alterniflora*, *S. salsa*, and *P. australis* communities, revealing more pronounced microbial P limitation compared to bare flat (Fig. 3c). Linear regression analysis demonstrated a significant ($P < 0.05$) inverse correlation between vector length and vector angle (Fig. 3d).

Microbial CUE at 0–30 cm soil layers in *S. alterniflora* community was considerably lower than that in the other plant communities (Fig. 4a). Conversely, *S. alterniflora* community exhibited the highest microbial NUE within the 0–30 cm soil profile, contrasting with other plant communities (Fig. 4b). Linear regression analysis demonstrated that microbial CUE had significantly negative correlation with vector length and microbial NUE, respectively (Fig. 5a, e).

Important environmental variables shaping soil enzyme activities and their stoichiometric ratios

Pearson's correlation analysis revealed that soil C-, N-, P-acquiring enzymes, and enzyme C:N ratio exhibited significantly positive correlations with AB, RB, root C:N, stem C:N, stem N:P, and foliar C:N, soil moisture, salinity, SOC, TN, TP, DOC, $\text{NH}_4^+\text{-N}$, AP, SOC:TN, SOC:TP, and $\text{DOC}:(\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N})$ ratios, LC, RC, alkyl C, aromatic C, carbonyl C, MBP, fungal PLFA, and bacterial PLFA, which were negatively correlated with soil pH, BD, and MBC:MBP ratio (Fig. 6). Soil enzyme C:P ratio was positively correlated with AB, RB, stem N:P, foliar C:N, soil moisture, salinity, SOC, TP, DOC, AP, SOC:TN, SOC:TP, $\text{DOC}:(\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N})$, LC, RC, aromatic C, MBP, fungal PLFA, and bacterial PLFA, which was negatively correlated with soil pH, $(\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N})\text{:AP}$, MBC:MBP, and MBN:MBP (Fig. 6). Soil enzyme N:P ratio was negatively related to root C:N, stem C:N, stem N:P, foliar C:N, foliar C:P, alkyl C, O-alkyl C, and aromatic C (Fig. 6).

Key factors regulating microbial nutrient metabolic limitations and use efficiencies

Pearson's correlation analysis showed that the vector length was significantly positively associated with AB, stem N:P, foliar C:N, moisture, salinity, SOC, TP, DOC, AP, SOC:TN, SOC:TP, and $\text{DOC}:(\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N})$ ratios, RC, RIC, fungal PLFA, and bacterial PLFA (Fig. 6). The vector angle revealed significantly positive correlations with AB, RB, C:N, C:P, and N:P ratios of roots, stems, and leaves, SOC, TN, $\text{NH}_4^+\text{-N}$, SOC:TN,

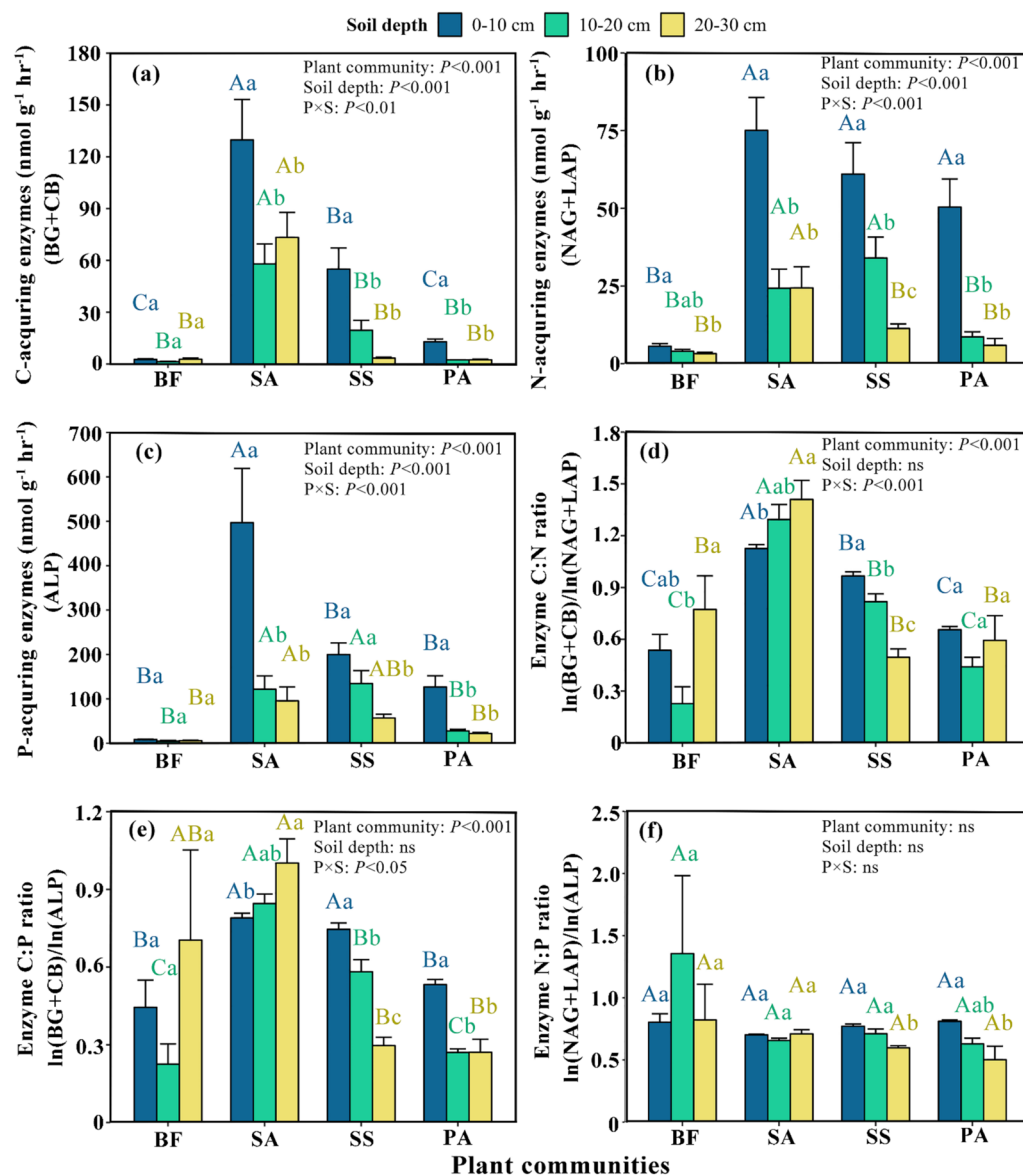


Fig. 2 The **a** C-acquiring enzymes (BG+CB), **b** N-acquiring enzymes (NAG+LAP), **c** P-acquiring enzymes (ALP), **d** enzyme C:N ratio, **e** enzyme C:P ratio, and **f** enzyme N:P ratio (mean $\pm$ SE, $n=4$) in bare flat, *S. alterniflora*, *S. salsa*, and *P. australis* communities. Different uppercase letters over the bars imply statistically significant differences at the $\alpha=0.05$ level between plant communities at the same soil depth. Different lowercase letters over the bars imply statistically significant differences at the $\alpha=0.05$ level between soil depths in the same plant community. BF: bare flat; SA: *Spartina alterniflora*; SS: *Suaeda salsa*; PA: *Phragmites australis*

SOC:TP, and TN:TP ratios, LC, alkyl C, O-alkyl C, aromatic C, carbonyl C, MBN, and F:B ratio (Fig. 6). Soil microbial CUE exhibited significant negative correlation with AB, RB, C:N ratio of roots, stems, roots, stem N:P ratio, soil moisture, salinity, SOC, TN, TP, DOC, $\text{NH}_4^+\text{-N}$, AP, SOC:TN, SOC:TP, and $\text{DOC}:(\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N})$ ratios, LC, RC, RIC, alkyl C, aromatic C, carbonyl C, MBN, MBP, fungal PLFA, and bacterial

PLFA (Fig. 6). Soil microbial NUE revealed a completely opposite trend compared with microbial CUE (Fig. 6).

Random forest analysis revealed that soil aromatic C, foliar C:N, AB, RIC, F:B ratio, soil moisture, alkyl C, $(\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N})\text{:AP}$ ratio, O-alkyl C, bacterial PLFA, DOC, root C:P, $\text{DOC}:(\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N})$, foliar C:P, and foliar N:P, and AP had the major and significant influences on vector length (Fig. 7a). Soil

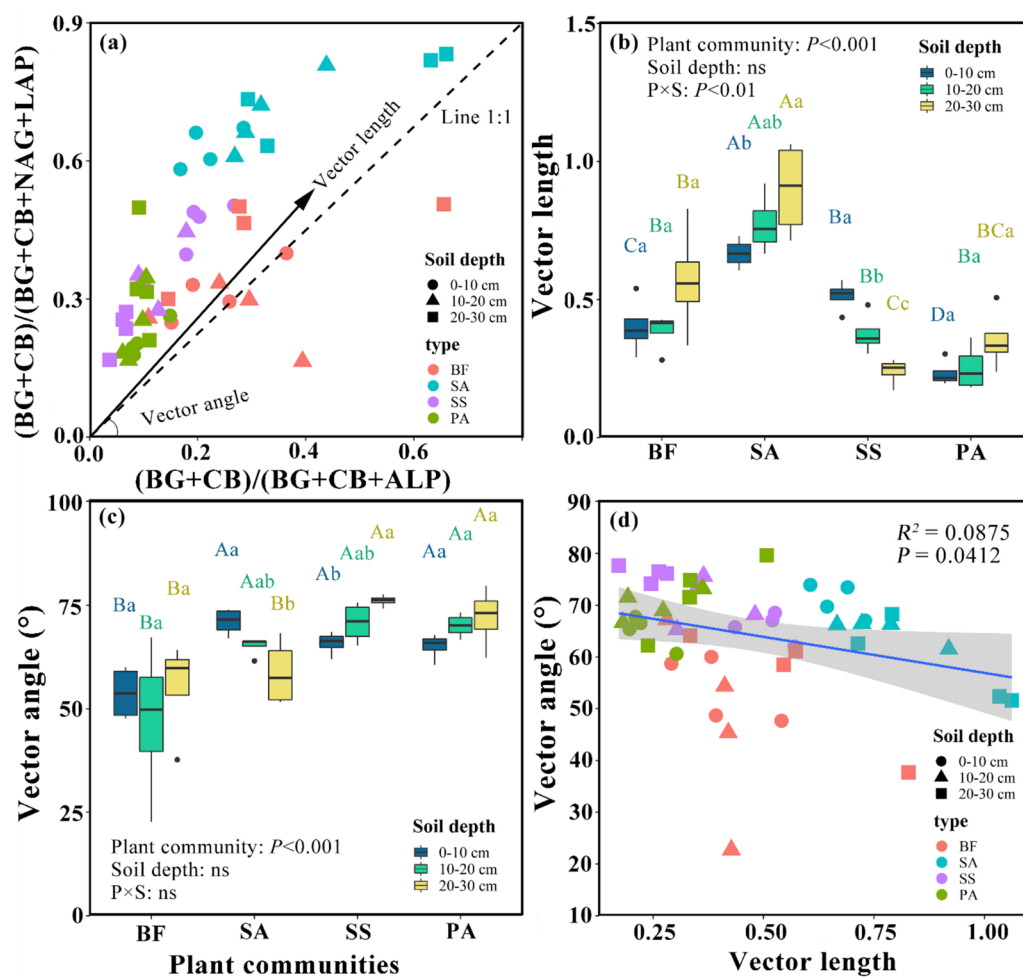


Fig. 3 Extracellular enzyme stoichiometry of the relative proportions of C to N acquisition versus C to P acquisition (a), the variation of vector length and angle (b and c) (mean $\pm$ SE, $n=4$) and their relationships (d). BG: β -1,4-glucosidase; CB: β -D-cellobiosidase; NAG: β -1,4-*N*-acetylglucosaminidase; LAP: L-leucine aminopeptidase; ALP: alkaline phosphatase; vector length and angle represent soil microbial C and N/P limitation, respectively. Different uppercase letters over the bars imply statistically significant differences at the $\alpha=0.05$ level between plant communities at the same soil depth. Different lowercase letters over the bars imply statistically significant differences at the $\alpha=0.05$ level between soil depths at the same plant community. Linear regression analysis to identify the relationships of microbial C limitation with microbial N/P limitation. BF: bare flat; SA: *Spartina alterniflora*; SS: *Suaeda salsa*; PA: *Phragmites australis*

O-alkyl C, RC, SOC:TP, SOC, LC, alkyl C, foliar N:P, SOC:TN, root C:N, bacterial PLFA, RIC, and foliar C:P were the most important driving factors for the variations in vector angle (Fig. 7b). Soil aromatic C, DOC, DOC:(NH_4^+ -N + NO_3^- -N), foliar C:N, F:B ratio, MBC:MBN, salinity, AB, AP, (NH_4^+ -N + NO_3^- -N):AP, alkyl C, and bacterial PLFA were the vital driving factors for the variations in microbial CUE (Fig. 7c). Soil DOC, DOC:(NH_4^+ -N + NO_3^- -N), aromatic C, F:B ratio, AP, (NH_4^+ -N + NO_3^- -N):AP, foliar C:N, MBC:MBN, salinity, AB, bacterial PLFA, MBN, alkyl C, DOC:AP, and RB were the most crucial drivers for the variations in microbial NUE (Fig. 7d).

PLS-PM analysis identified soil properties as the dominant positive driver of vector length (total effect: 0.591; Fig. 8b), exerting both a direct positive effect (direct effect: 0.565) and indirect effects mediated through microbial attributes (Fig. 8a and b). Plant traits were the second strongest positive contributor to vector length (total effect: 0.447; Fig. 8b), with their impacts primarily mediated through the positive direct effects of plant traits on soil properties (indirect effect: 0.451; Fig. 8a). Plant traits exerted the strongest positive influence on vector angle (total effect: 0.937; Fig. 8d), primarily through indirect pathways via their significant direct effects on microbial attributes (indirect effect: 0.749; Fig. 8c). Microbial

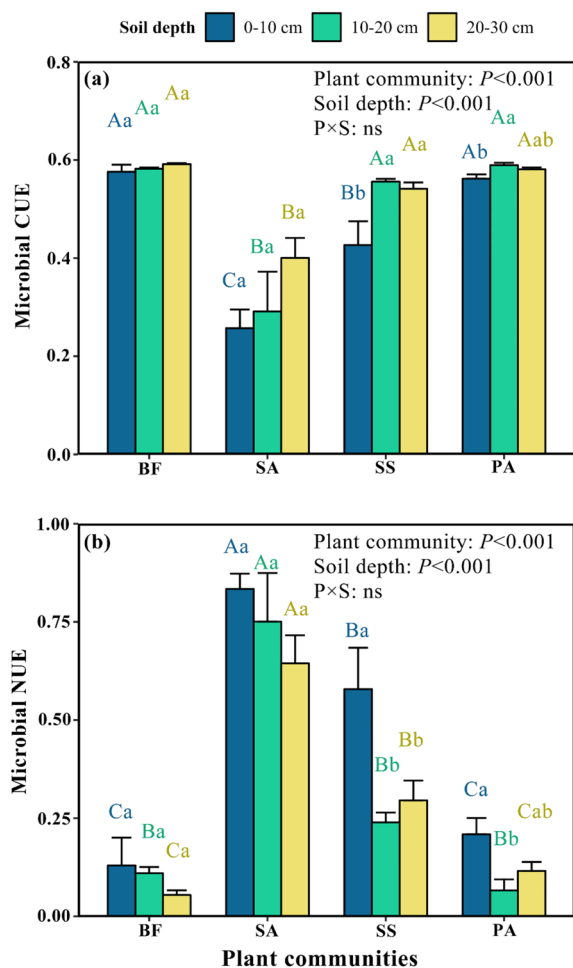


Fig. 4 **a** Microbial carbon use efficiency (CUE), **b** Microbial nitrogen use efficiency (NUE) (mean $\pm$ SE, $n = 4$) in the bare flat, *S. alterniflora*, *S. salsa*, and *P. australis* communities. Different uppercase letters over the bars imply statistically significant differences at the $\alpha = 0.05$ level between plant communities at the same soil depth. Different lowercase letters over the bars imply statistically significant differences at the $\alpha = 0.05$ level between soil depths in the same plant community. BF: bare flat; SA: *Spartina alterniflora*; SS: *Suaeda salsa*; PA: *Phragmites australis*

attributes ranked as the second most significant positive contributor to vector angle (total effect: 0.775; Fig. 8c). Soil properties exerted the strongest negative influence on microbial CUE (total effects: -0.718 ; Fig. 8f), predominantly through their direct effects (direct effect: -0.573 ; Fig. 8e). Plant traits exerted the second-largest negative total effects on microbial CUE (total effects: -0.658 ; Fig. 8f), primarily mediated by their indirect pathway through soil properties. This indirect effect (indirect effects: -0.434) resulted from the significant positive direct influence of plant traits on soil properties (Fig. 8e). Microbial attributes had direct and significant

negative effects on microbial CUE (direct effects: -0.284 ; Fig. 8e). Soil properties exhibited the greatest and most positive effects on microbial NUE (total effects: 0.849; Fig. 8h), which was primarily derived from the direct effects of soil properties on microbial NUE (direct effects: 0.817; Fig. 8g).

Discussion

Microbial P limitation in coastal wetlands and the effect of *S. alterniflora* invasion on microbial P limitation

As a vital parameter reflecting microbial energetic and nutritional requirements, soil EES reveals organisms' resource partitioning strategies in substrate acquisition (Abay et al. 2024; Lian et al. 2024). In this study, soil enzymatic ratios (C:N: 0.23–0.97; C:P: 0.23–0.75; N:P: 0.50–1.36) across the 0–30 cm soil profile in the bare flat, native *S. salsa*, and *P. australis* communities showed reduced C:N and C:P values relative to global averages (1.02 and 0.95) in terrestrial ecosystems (Fig. 2; Sinsabaugh et al. 2008, 2009; Feyissa et al. 2022). This reflects microbial metabolic demands where greater microbial investment prioritizes P acquisition over N and C. Consequently, the microbial resource partitioning hierarchy in these coastal wetland vegetation communities follows $P > N > C$ (Fig. 2). Meanwhile, the soil enzyme C:N, C:P, and N:P ratios in invasive *S. alterniflora* community across the 0–30 cm soil profile were 1.28, 0.88, and 0.69, respectively (Fig. 2). This indicated that microbial metabolic demands and investment strategies prioritize P acquisition over C and N, resulting in a resource acquisition hierarchy of $P > C > N$ in invasive *S. alterniflora* community. The greater microbial demands and investment for P relative to N and C (Fig. 2) confirmed P-limitation as the principal metabolic constraint in the bare flat, invasive *S. alterniflora*, and native *S. salsa* and *P. australis* communities.

Furthermore, the vector angle ($> 45^\circ$) derived from stoichiometric vector analysis (Fig. 3c) further indicated that P-limitation dominance in microbial metabolic processes within bare flat, invaded (*S. alterniflora*) and native (*S. salsa*, and *P. australis*) coastal wetland communities. Our observed microbial P limitation conflicts with Zhao et al. (2024)'s reported C-N co-limitation of microbial metabolism across salinity gradients in coastal wetland ecosystems. Our findings align with Sundareswar et al. (2003) and Luo and Gu (2018), and are further reinforced by Guo et al. (2024)'s recent evidence of predominant P limitation over N and C in terrestrial microbial metabolism. The P-limited microbial metabolism observed across coastal wetland vegetation aligns with the growth rate hypothesis, where fast-growing microorganisms exhibit inherent P demand for synthesizing rRNA and ATP to sustain population dynamics (Elser et al. 2000; Sterner and Elser

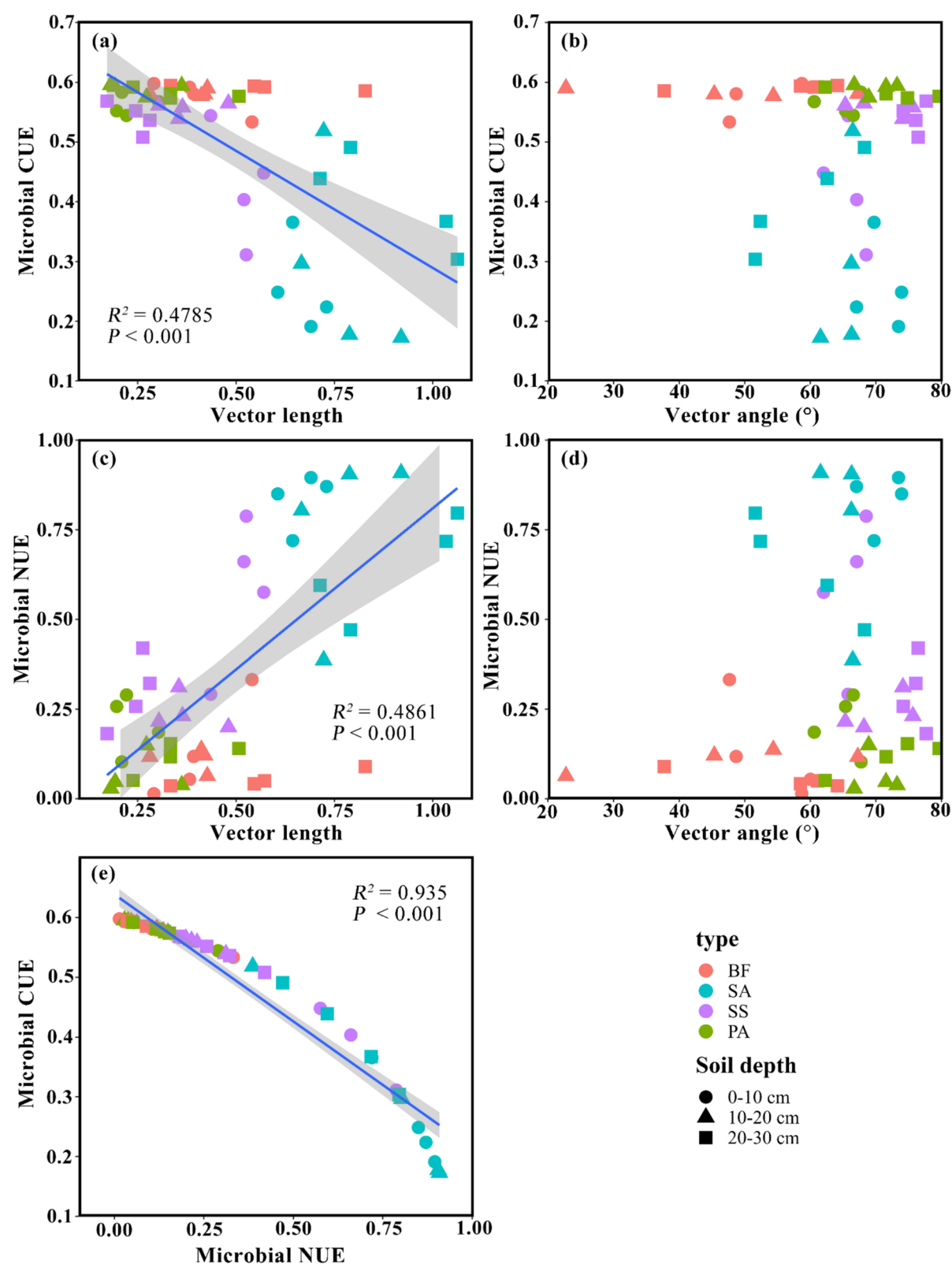


Fig. 5 Microbial CUE and NUE in the soil and their linear relationship with the vector length and vector angle ($n=4$), respectively. BF: bare flat; SA: *Spartina alterniflora*; SS: *Suaeda salsa*; PA: *Phragmites australis*

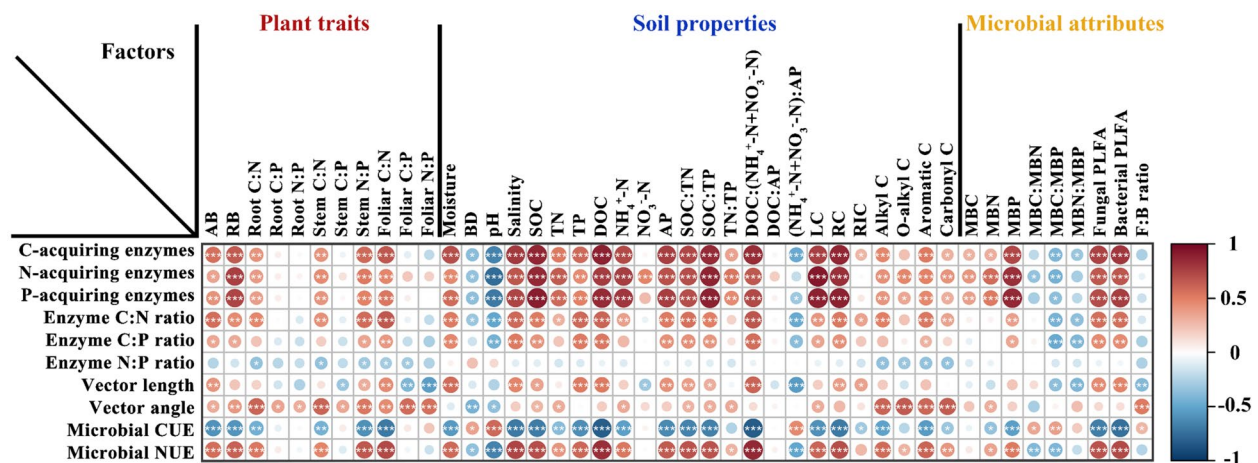


Fig. 6 Heatmap showing Pearson's correlations between environmental factors and soil (0–30 cm depths) extracellular enzymatic activities and enzyme stoichiometry, soil microbial metabolic limitation, and nutrient use efficiency ($n=4$). The colors of circles indicate the strength of the correlation. * $P<0.05$; ** $P<0.01$; *** $P<0.001$. AB: aboveground biomass; RB: root biomass; C: carbon; N: nitrogen; P: phosphorus; BD: bulk density; SOC: soil organic carbon; TN: total nitrogen; TP: total phosphorus; DOC: dissolved organic carbon; $\text{NH}_4^+\text{-N}$: ammonium nitrogen; $\text{NO}_3^-\text{-N}$: nitrate nitrogen; AP: available phosphorus; LC: labile organic carbon; RC: recalcitrant organic carbon; RIC: recalcitrant index for carbon; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; MBP: microbial biomass phosphorus; PLFA: phospholipid fatty acids; F:B ratio, fungal:bacterial PLFA ratio

2002; Guo et al. 2024). Additionally, the decomposition continuum exhibits sharp stoichiometric shifts: litter N:P (45:1) > soil N:P (17:1) > microbial N:P (6:1) (Sinsabaugh et al. 2008; Guo et al. 2024). This pattern demonstrates preferential N retention over P during decomposition. It further reinforces microbial P-limitation in detrital biogeochemical processes (Guo et al. 2024). Furthermore, Zechmeister-Boltenstern et al. (2015) reported that specific microbial taxa demonstrate preferential utilization of C substrates with higher organic N (e.g., organic polymers and humic substances). This substrate selection strategy may promote N availability to soil microbes (Guo et al. 2024). However, soil P bioavailability remains geochemically constrained by slow lithogenic weathering rates from parent material (Augusto et al. 2017), creating inherent P scarcity that reinforces microbial P-limitation in coastal wetland ecosystems.

As a pivotal soil P-acquiring enzyme, ALP mediates organic P mineralization into phosphate (Sinsabaugh et al. 2008; Guan et al. 2022), thereby serving as a key indicator for quantifying soil P bioavailability (Li et al. 2024). Our data revealed pronounced elevation of P-acquiring enzymes in *S. alterniflora* community (Fig. 2c), indicative of invasion-induced enhancement in soil P metabolic processes and consequent modulation of P biogeochemical cycling in coastal wetlands (Li et al. 2024). The vector analytical approach demonstrated *S. alterniflora* invasion induced pronounced enhancement of microbial P limitation in surface soils (0–20 cm) relative to bare flat (Fig. 3c). However, no differentiation in

microbial P limitation was observed among *S. alterniflora*, *S. salsa*, and *P. australis* communities at equivalent depths (Fig. 3c). Previous studies reveal soil microorganisms' intrinsic P dependency for ribosomal RNA and ATP synthesis to sustain population dynamics (Elser et al. 2000; Sterner and Elser 2002; Guo et al. 2024). *S. alterniflora* invasion triggered 8.58- and tenfold increases in bacterial and fungal PLFA biomass compared to bare flat (Fig. S7g, h), proportionally amplifying microbial P requirements for sustaining these expanded communities. Meanwhile, high productive traits of *S. alterniflora* (e.g., greatly increased aboveground and root biomass) drive rapid P bioaccumulation and intensify plant–microbe P competition (Fig. S2), that exacerbates microbial P limitation (Fig. 3c; Cheng et al. 2022; Pan et al. 2024). Additionally, soil nutrient stoichiometry has been recognized as one of the important factors to critically mediate microbial resource limitations (Zhang et al. 2023). *S. alterniflora* community exhibited increased SOC:TP and TN:TP ratios compared to bare flat (Fig. S5b, c), showing stoichiometric disequilibrium and soil P deficiency, that further exacerbated microbial P limitation in *S. alterniflora* soil relative to bare flat (Fig. 3c). Our PLS-PM analysis substantiated these mechanisms: microbial attributes (e.g., bacterial PLFA) exerted direct positive regulation on microbial P limitation, while plant traits demonstrated dominant indirect control through modulating microbial attributes (Fig. 8c). Random forest analysis revealed that the SOC:TP ratio ranked as the third-most influential predictor of microbial P limitation

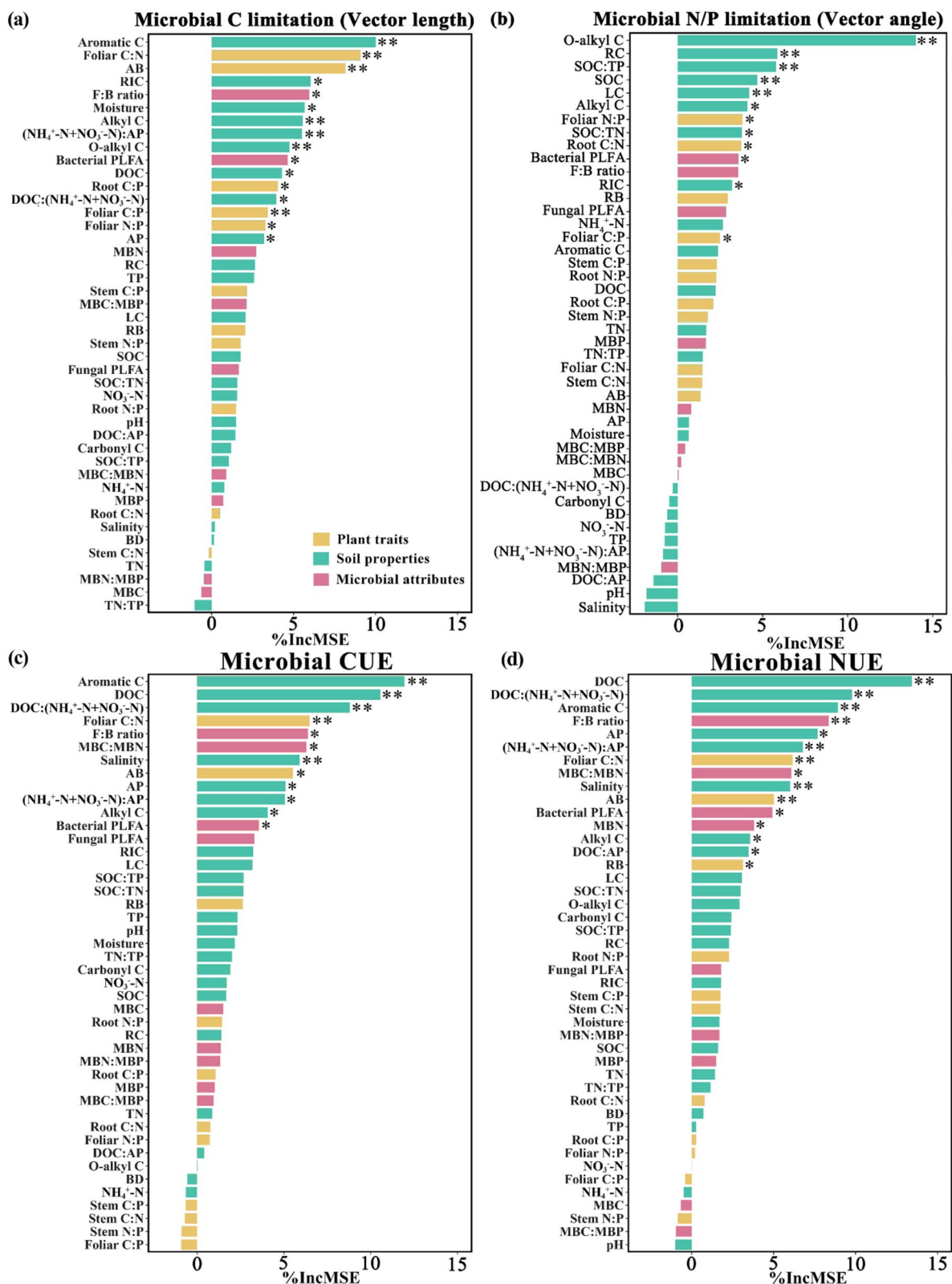


Fig. 7 Order of relative importance of properties selected for use in the model generated by the Random Forest algorithm to explain variations in the soil (0–30 cm depths) microbial C limitation (represented by vector length), microbial P limitation (represented by vector angle), CUE, and NUE ($n=4$), respectively. See Fig. 6 for abbreviations

(Fig. 7b). Additionally, soil O-alkyl C, RC, alkyl C, SOC, LC, and aromatic C were identified as principal drivers of microbial P limitation (Fig. 7b), findings corroborated by Cui et al. (2021) and Cheng et al. (2022) demonstrating higher SOC fractions exacerbated microbial P limitation.

Effect of *S. alterniflora* invasion on microbial C limitation

Contrary to terrestrial ecosystems, coastal wetlands' inherent C richness (Mitsch and Gosselink 2015) precludes microbial C limitation, as evidenced by our EES data showing non-limiting C conditions in the bare flat, native *S. salsa*, and *P. australis* salt marshes of coastal wetlands (Fig. 2). However, the results of soil enzyme C:N, C:P, and N:P ratios indicated that invasive *S. alterniflora* community was co-limited by P and C (Fig. 2). Furthermore, the greatest enzyme C:N and C:P ratios, and vector length across the 0–30 cm soil profile were observed in *S. alterniflora* community (Figs. 2d and e, 3b), which indicated that *S. alterniflora* soil (0–30 cm) had the greatest microbial C limitation among all plant communities; consistent with our second hypothesis. The extent of microbial C limitation showed strong dependence on substrate C availability relative to that of other nutrients (Chakrawal et al. 2022; Cui et al. 2023). The PLS-PM analysis revealed that soil properties, i.e., aromatic C, RIC, moisture, alkyl C, DOC, AP, and $\text{DOC}:(\text{NH}_4^+ - \text{N} + \text{NO}_3^- - \text{N})$ exerted the strongest direct positive influences on microbial C limitation (Fig. 8a). Furthermore, random forest analysis highlighted aromatic C, RIC, alkyl C, and O-alkyl C as principal determinants governing variations in microbial C limitation (Fig. 7a). Aromatic C, predominantly composed of lignin and tannin (Yang et al. 2022), contrasts with alkyl C derived from plant-derived biopolymers and microbial metabolites, both representing the most persistent organic matter components in soils (Cheng et al. 2017). O-alkyl C primarily originates from carbohydrate structures that constitute the principal fraction of labile organic C (Cheng et al. 2017). Our ^{13}C NMR spectra results showed that *S. alterniflora* soil possessed the greatest relative abundance of aromatic C and alkyl C, along with the lowest O-alkyl C proportion (Fig. S6). This implied that *S. alterniflora* invasion increased SOC chemical stability, resulting in

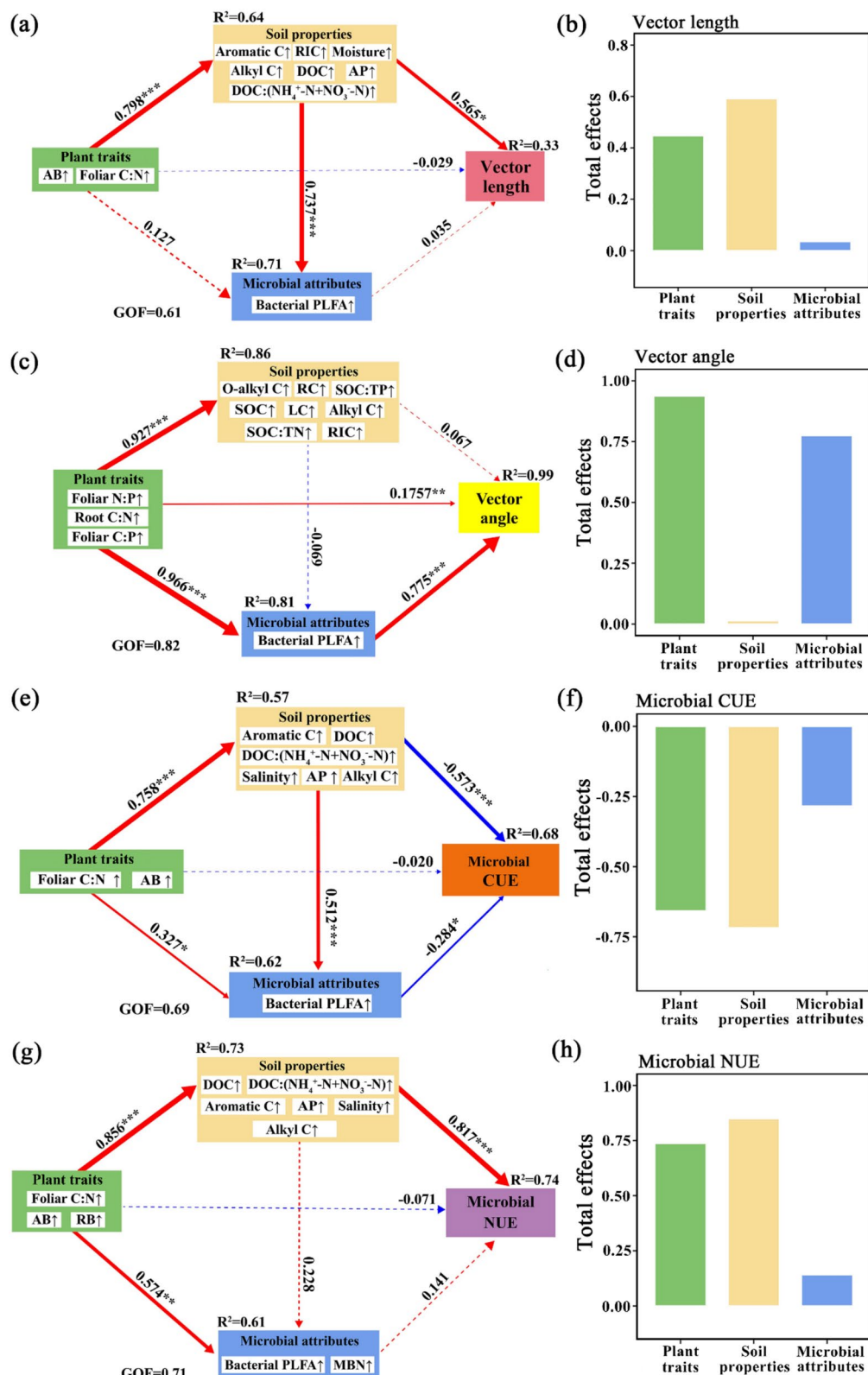
SOC within the *S. alterniflora* community being more recalcitrant to microbial decomposition. This finding was corroborated by observed significant increases in soil RC and RIC ratios following *S. alterniflora* invasion (Fig. 1), concurrent with enhanced biochemical stability of SOC. The greatly increased distribution of recalcitrant C (e.g., aromatic C and alkyl C), and reduced distribution of labile C (e.g., O-alkyl C) in *S. alterniflora* soil reduced substrate (soil-derived) C availability (Figs. 1, S6), thereby intensifying microbial C limitation (Fig. 3b). Moreover, *S. alterniflora* invasion significantly stimulated soil microbial proliferation (particularly bacterial and fungal communities) (Fig. S7, Yang et al. 2016b, 2019, 2020), thereby elevating microbial metabolic C demand and consequently exacerbating C limitation within the coastal ecosystem (Fig. 3b). Furthermore, aboveground biomass and foliar stoichiometric ratios (C:N, C:P, N:P) emerged as primary drivers of microbial C limitation (Fig. 7a). This finding aligns with Qiao et al. (2021), who demonstrated that microbial C limitation was partially determined by the quality of plant materials. As reported by Yang et al. (2009), *S. alterniflora* plant materials contain more recalcitrant substances (e.g., lignin), and its leaves and roots (0–20 cm soil layers) have lower quality, characterized by a higher C:N ratio, compared to *S. salsa* and *P. australis* communities (Figs. S3a, S4a). The substantial input of low-quality, recalcitrant *S. alterniflora* residues into soils caused significant biogeochemical alterations. These alterations particularly enhanced recalcitrant C components (aromatic C, alkyl C) while reducing labile C pools (Figs. 1, 8a, S6), culminating in intensified microbial C limitation within invaded ecosystems (Fig. 3a).

Impact of *S. alterniflora* invasion on soil microbial CUE and NUE

The microbial CUE in surface soils (0–30 cm) of bare flat, *S. salsa*, and *P. australis* communities ranged from 0.43 to 0.59 (Fig. 4a), which was markedly higher than the global mean value of 0.26 reported for various ecosystems (Sinsabaugh et al. 2016). *S. alterniflora* invasion significantly decreased microbial CUE (i.e., the mean microbial CUE across 0–30 soil profile was 0.32) compared to other plant communities (Fig. 4a). Microbial CUE typically declines

(See figure on next page.)

Fig. 8 Partial least squares path modeling (PLS-PM) was used to identify the possible pathways by which attributes controlled soil (0–30 cm depths) microbial C limitation (represented by vector length), microbial P limitation (represented by vector angle), CUE, and NUE ($n = 4$). Red and blue arrows indicate positive and negative relationships, respectively. Solid and dashed arrows indicate significant and insignificant relationships, respectively. The arrow width is proportional to the strength of the relationship. Numbers adjacent to arrows are the standardized path coefficients ($^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$). The symbols “↑” and “↓” indicate positive and negative correlations between the variables and the first component from the principal component analysis (PCA), respectively. R^2 associated with the response variables represents the proportion of variation explained by relationships with other variables. See Fig. 6 for abbreviations

**Fig. 8** (See legend on previous page.)

under nutrient-limited conditions, driven by microbial metabolic adaptations that prioritize enzyme production for nutrient acquisition over biomass synthesis (Mehnaz et al. 2019; Mganga et al. 2022). This was validated by our observation that microbial CUE presented significant negative correlation with microbial C limitation (Fig. 4a). Microbial CUE has been reported to be partially determined by the quality of organic matter (i.e., substrates) (Hu et al. 2022; Islam et al. 2023). For instance, simple substrates (e.g., sugars, amino acids) can be easily assimilated and require less activation energy, resulting in a higher CUE (Hu et al. 2022). In contrast, the enzymatic hydrolysis of complex macromolecules necessitates multi-step depolymerization processes and increased C expenditure, creating substantial metabolic costs that proportionally reduce microbial CUE (Soares and Rousk 2019; Gunina and Kuzyakov 2022; Islam et al. 2023). Our PLS-PM analysis highlighted soil properties as the most influential negative predictors of microbial CUE (Fig. 8e, f), with aromatic C, alkyl C, foliar C:N ratio, and aboveground biomass being the principal contributors to microbial CUE variation (Fig. 7c). Accordingly, it was deduced that greatly reduced microbial CUE in *S. alterniflora* soil was mainly attributed to the increased distribution of recalcitrant C (e.g., aromatic C and alkyl C), which required multiple enzymatic steps and thus consumed additional C for microbial decomposition (Figs. 1, 4a, and S6; Islam et al. 2023). The greatest microbial C limitation in *S. alterniflora* soil induced microbes to consume more energy to produce C-acquiring enzymes (Figs. 2a, 3b; Mganga et al. 2022). This process caused massive C loss; consequently, less C was allocated to microbial biomass, leading to the lowest microbial CUE (Fig. 4a). Additionally, Manzoni et al. (2012) reported that microbial CUE is reduced under environmental stress (e.g., salinity, pollution), as additional C is expended for respiration to deal with stress; thus, less C remains to allocate for microbial growth (Hu et al. 2022). The marked increase in soil salinity in *S. alterniflora* community likely reduced microbial CUE through enhanced C allocation for salt stress adaptation (Figs. 1d, 4a), a mechanism corroborated by significant negative correlations between microbial CUE and soil salinity in our experimental data (Figs. 6, 8e).

Interestingly, *S. alterniflora* invasion significantly increased microbial NUE relative to other plant communities (Fig. 4b). This finding corroborates earlier reports that higher soil C:N ratios (indicating N-deficiency) trigger microbial metabolic shifts (Mooshammer et al. 2014). These shifts manifest as decreased microbial CUE coupled with enhanced NUE, establishing an antagonistic relationship between microbial CUE and NUE (Fig. 5e; Mooshammer et al. 2014). Studies demonstrate distinct microbial N utilization strategies under contrasting

nutrient regimes (Mooshammer et al. 2014). Under N-deficient conditions, microorganisms preferentially allocate acquired organic N to biomass synthesis and growth processes, while simultaneously suppressing N excretion (e.g., ammonium release) to enhance microbial NUE and reduce N waste (Mooshammer et al. 2014; Sun et al. 2025). Consequently, increased N availability may reduce NUE as microbes shift their metabolic allocation patterns—decreasing N investment in anabolic growth processes while increasing proportional distribution toward catabolic pathways and subsequent net N mineralization (Mooshammer et al. 2014; Schleuss et al. 2021; Sun et al. 2025). The scientific rationale for these viewpoints was further corroborated by Pearson's correlation analysis, which demonstrated statistically significant negative correlations between microbial NUE and soil C:N ratios (i.e., SOC:TN) (Fig. 6). Additionally, our analyses identified DOC and MBN were the predominantly positive drivers for variations in microbial NUE (Figs. 7 and 8d). The marked increase in DOC (readily available nutrients for soil microbes) following *S. alterniflora* invasion promoted microbial growth (Fig. 1h; Yang et al. 2016b), with concomitant biomass accumulation evidenced by increased MBN (Fig. S7b). Thus, greatly enhanced microbial NUE occurred in invasive *S. alterniflora* soil with the highest soil C:N ratios (i.e., SOC:TN, N-deficiency; Figs. 4b and S5a). This likely resulted from dual mechanisms: preferential N retention in microbial biomass (increased MBN; Fig. S7b; Sun et al. 2025) and minimized N loss through constrained mineralization (lowest net N mineralization rate; Fig. S9; Schleuss et al. 2021) post-invasion. Together, these findings reflect microbial nutrient acquisition strategies during plant invasion (Fuchslueger et al. 2019).

Tradeoff between microbial CUE and SOC sequestration following *S. alterniflora* invasion

Contrary to our third hypothesis, SOC content increased with decreasing microbial CUE following *S. alterniflora* invasion (Figs. 1e, 4a), a tradeoff potentially linked to divergent nutrient limitations across ecosystems (Shi et al. 2024). Existing researches have demonstrated that the relationship between CUE and SOC can exhibit positive, negative, or decoupled patterns, governed by interplay of multiple ecological processes (Tao et al. 2023; He et al. 2024; Shi et al. 2024). Our findings align with Shi et al. (2024), who showed increased SOC content alongside declining microbial CUE during vegetation restoration. Coastal wetlands constitute essential blue C reservoirs with high sequestration capacity, exerting a crucial role in global C cycling (LaFond-Hudson and Sulman 2023). As an ecological engineer, *S. alterniflora* invasion exerts crucial C sequestration

capacities, predominantly mediated through SOC accumulation enhancement in intertidal biogeochemical cycles (Yang et al. 2016a; Qi and Chmura 2023). Although *S. alterniflora* invasion substantially boosted soil C pool accumulation (e.g., SOC, DOC, LC, RC; Fig. 1), concurrent microbial C limitation exacerbation and CUE decline emerged in invaded soils, primarily attributable to reduced substrate C availability (Figs. 1, 3b, 4a, 9, S6). Microbial CUE is a key regulator of global SOC storage (Tao et al. 2023). Enhanced microbial CUE typically promotes SOC accumulation (Kallenbach et al. 2016; Tao et al. 2023), while diminished CUE triggers soil C release (Iven et al. 2023; Tao et al. 2023).

Interestingly, although *S. alterniflora* invasion greatly decreased microbial CUE (Fig. 4a), its communities exhibited maximal SOC accumulation (Fig. 1a). This enhanced SOC sequestration following *S. alterniflora* invasion was principally attributed to substantial inputs of refractory plant-derived materials with low biodegradability (Figs. 9, S3a, S4a). Furthermore, greatly increased distribution of biochemically recalcitrant C (e.g., aromatic C and alkyl C) enhanced SOC chemical stability (Figs. 1, 9, S6; Yang et al. 2015; Zhang et al. 2022), prolonged soil C residence time (Basak et al. 2021), and suppressed C efflux (Fig. 9).

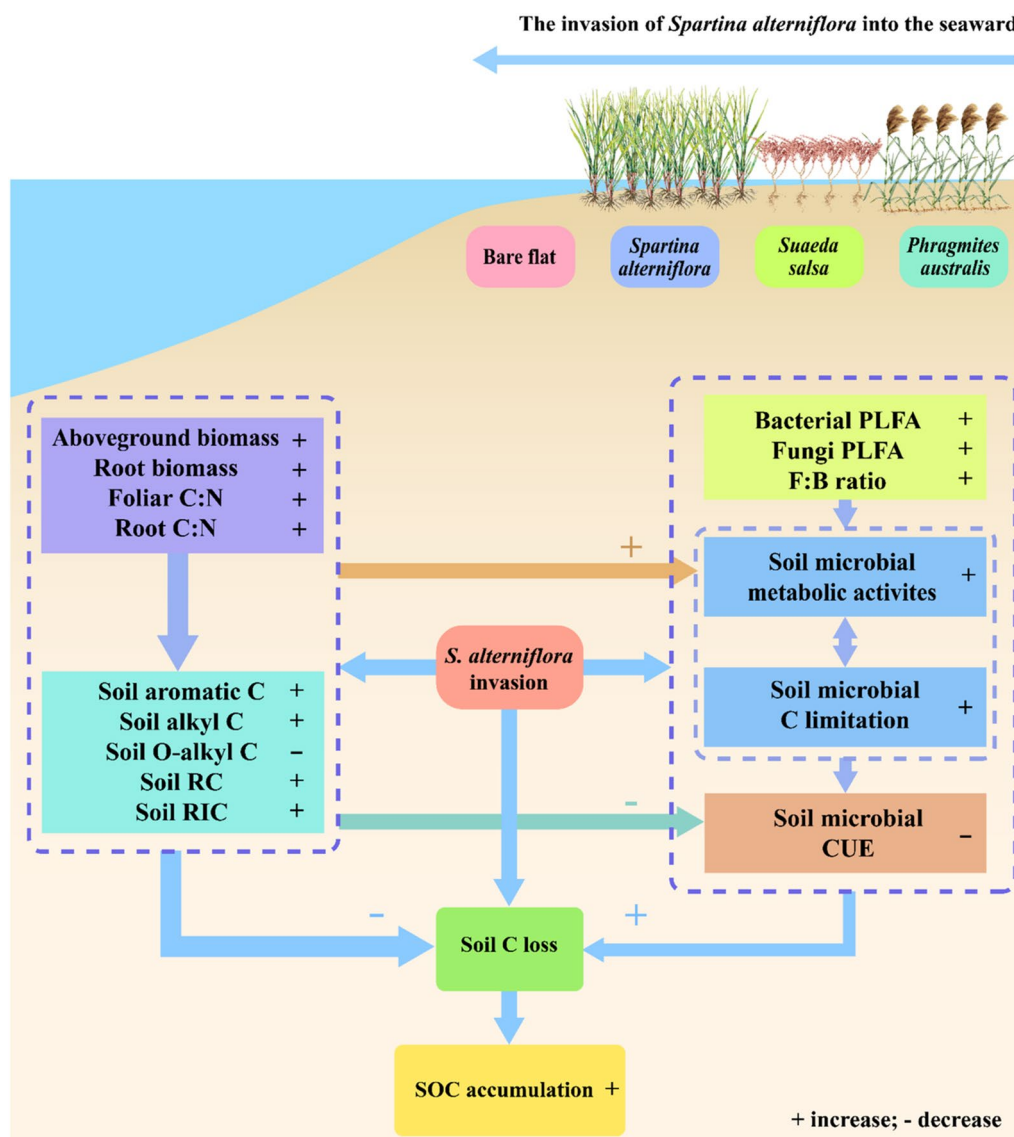


Fig. 9 Conceptual diagram for the influences of variations in microbial nutrient metabolisms on SOC accumulation along with *S. alterniflora* invasion. See Figs. 4 and 6 for abbreviations

Conclusions

Our findings demonstrate that P limitation constitutes the primary microbial metabolic constraint governing coastal wetlands in eastern China. Microbial metabolic processes in unvegetated bare flat and native halophyte communities (*S. salsa* and *P. australis*) remained unconstrained by either C or N, whereas invasive *S. alterniflora* salt marsh exhibited dual C-P limitations on microbial metabolism. *S. alterniflora* invasion aggravated microbial P limitation compared with bare flat, while simultaneously inducing the most pronounced microbial C limitation among all plant communities. While enhancing SOC sequestration, *S. alterniflora* invasion exhibited maximal C limitation on microbial metabolism, concurrent with the lowest CUE and highest NUE observed across plant communities. Random forest analysis identified aromatic C as the overwhelming driver of variations in microbial C limitation and CUE, which provided further evidence that SOC chemical fractions played a crucial role in regulating soil microbial C metabolism. We deduced that increased recalcitrant C fractions (e.g., aromatic C and alkyl C) under *S. alterniflora* invasion diminished soil bioavailable C pools, driving a compensatory microbial investment in C-acquiring enzymes that ultimately reduced microbial CUE through resource diversion. Despite low microbial CUE typically constraining SOC accumulation, *S. alterniflora* invasion greatly enhanced SOC sequestration through massive refractory plant-derived inputs. This enhancement occurred alongside concurrent increases in SOC chemical stability and prolongation of soil C residence time, which overrode microbial efficiency limitations. Our study provides a new process-based paradigm for understanding how microbial resource limitation and metabolic efficiencies respond to plant invasion and provides empirical evidence of microbial regulation of C and nutrient cycles, informing the microbial nutrient limitation status in coastal wetland ecosystems.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13717-025-00637-6>.

Additional file 1 (DOCX 2845 KB)

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Author contributions

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agreed and approved the manuscript for publication in *Ecological Processes*.

Competing interests

The authors declare that they have no competing interests.

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