

Mangrove re-establishment links deep-soil carbon enrichment with greater microbial phosphorus acquisition after *Spartina alterniflora* control

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Abstract

Aims

Mangrove re-establishment after invasive-plant control may alter soil carbon distribution and microbial nutrient acquisition, but these responses are rarely evaluated together across soil depths. We tested whether a *Kandelia obovata* patch re-established after *Spartina alterniflora* removal showed higher deep-soil organic carbon (SOC) and greater microbial allocation to phosphorus (P) acquisition than an extant *S. alterniflora* reference.

Methods

At one Minjiang Estuary site, we compared one extant *S. alterniflora* patch with *K. obovata* and *Phragmites australis* patches established approximately five years after *S. alterniflora* removal. SOC concentration, fixed-depth SOC stock, and soil and microbial C:N:P stoichiometry were measured at 0–100 cm in August 2023 and January 2024. Potential extracellular enzyme activities and ecoenzymatic vector metrics were assessed in surface soil.

Results

Relative to the reference, SOC concentrations in the *K. obovata* patch were 13.65% and 12.80% higher at 30–60 and 60–100 cm, respectively, although campaign-pooled fixed-depth SOC stock did not differ significantly. Mean potential acid phosphatase activity was 201.96% higher, and mean vector angle was greater (82.34° vs. 75.86°), indicating stronger relative allocation to P acquisition among the assayed enzymes. Total soil P did not differ significantly, whereas microbial biomass P at 60–100 cm was lower and surface microbial biomass N:P was higher in the *K. obovata* patch than in the reference.

Conclusions

These site-level findings show that post-control assessments should distinguish SOC concentration from fixed-depth stock and integrate depth-resolved carbon measurements with microbial nutrient-acquisition indicators.

Introduction

Estuarine and coastal wetlands store disproportionate amounts of organic carbon (C) because high primary productivity is coupled with sediment accretion and long-term burial (Wang et al. 2013; Macreadie et al. 2019). Biological invasion can reorganize these carbon pools and the processes that maintain them (Hensel et al. 2021). Introduced to the Chinese coast in 1979, *Spartina alterniflora* spread across extensive intertidal areas through high salinity tolerance and vigorous clonal growth (Zhou et al.

2025; Nie et al. 2023). China has consequently expanded control programs that combine eradication with the establishment of replacement vegetation (Min et al. 2025). Where hydrological and climatic conditions are suitable, mangrove re-establishment converts invasive stands to woody habitat and modifies belowground inputs, sediment trapping, and microbial resource demand (Qi et al. 2024; Gong et al. 2025; Wang and Sardans 2025). These responses may develop at different rates and depths, creating potential divergence among soil C concentration, area-based stock, and microbial nutrient acquisition (Fan et al. 2024; Ascenzi et al. 2025; Chen et al. 2026). Their joint evaluation is therefore necessary to characterize soil development after invasive-plant control.

S. alterniflora invasion alters SOC sources, stability, and microbially mediated biogeochemical processes (Xia et al. 2021; Nie et al. 2023; Rolando et al. 2024), while vegetation established after removal can reshape sediment properties and blue-carbon storage (Song et al. 2023; Beselly et al. 2025). Mangrove roots, litter, and rhizosphere inputs can extend carbon delivery into the soil profile, and microbial processing may generate products that associate with minerals and persist (Yin et al. 2025; Li et al. 2026). These mechanisms make a connection between deep-soil SOC enrichment and microbial resource allocation plausible, but evidence that the two signatures co-occur after mangrove re-establishment remains limited. The unresolved question is whether mangrove-associated deep-soil carbon enrichment coincides with a distinct microbial nutrient-acquisition response.

Phosphorus (P) provides a plausible nutrient dimension of this linkage because increased organic-matter inputs can raise microbial P demand even when bulk total P does not differ among patches (Hu et al. 2023; Nong et al. 2025; Rumeau et al. 2025). Microorganisms may respond by allocating more extracellular-enzyme activity to P acquisition, with consequences for substrate processing and estimated carbon use efficiency (Moorhead et al. 2016; Luo et al. 2022; Tao et al. 2023). Potential acid phosphatase activity and ecoenzymatic vector angle describe relative allocation among the assayed enzymes rather than realized P uptake or P limitation, while microbial biomass P and microbial biomass N:P provide complementary profile-level indicators (Mooshammer et al. 2014). Combining these measurements with depth-resolved SOC concentration and fixed-depth stock separates microbial allocation from the mass-based and area-based dimensions of the soil carbon pool.

This study compared one extant *S. alterniflora* patch with *P. australis* and *K. obovata* patches established approximately five years after *S. alterniflora* removal in the Minjiang Estuary. The extant patch served as a contemporaneous reference rather than a pre-control baseline, and one patch per state limited inference to cross-sectional site contrasts. The *K. obovata* patch represented mangrove re-establishment in a more tide-influenced middle- to lower-intertidal setting, whereas the *P. australis* patch provided environmental context from a freshwater-influenced upper-intertidal setting. Two site-level hypotheses were tested, with fixed-depth stock evaluated as a complementary area-based metric. The first predicted higher SOC concentrations at 30–100 cm in the *K. obovata* patch than in the reference. The second predicted greater relative allocation to P acquisition in surface soil of the *K. obovata* patch even without a corresponding difference in total soil P, expressed through potential acid phosphatase activity and ecoenzymatic vector angle and evaluated alongside profile microbial biomass P and N:P. This framework

distinguishes co-occurring carbon and microbial signatures from temporal recovery or causation and clarifies how complementary indicators can characterize post-control soil development.

Materials and methods

Study area and vegetation establishment

The study was conducted in the Shanyutan Wetland of the Minjiang Estuary, southeastern China (25°50'43"–26°09'42" N, 119°05'36"–119°41'05" E; Fig. 1). The region has a subtropical humid monsoon climate, a mean annual temperature of approximately 19.7°C, and precipitation concentrated mainly in spring (Ren et al. 2026). Regular semidiurnal tides interact with river discharge and seawater intrusion, and reported surface-water salinity fluctuates by approximately 4–8‰ over a tidal cycle (Yang et al. 2022a). *P. australis* occurs primarily in freshwater-influenced upper-intertidal habitats (Guan et al. 2017), whereas *K. obovata* was selected for assisted mangrove planting in a more tide-influenced setting at the study site. The species therefore represent two locally implemented vegetation-establishment settings that occupy distinct hydrogeomorphic niches. *S. alterniflora* was first recorded in the Minjiang Estuary in 2002, and systematic removal and vegetation establishment began around 2018.

Historical remote-sensing images indicate that the areas later planted with *P. australis* and *K. obovata* had been dominated by *S. alterniflora* before control (Yuan et al. 2026). Between May and August 2018, *S. alterniflora* was removed by mowing, rotary tillage to approximately 30–50 cm, belowground-rhizome removal, and surface leveling. Approximately 30 days after clearing, replacement vegetation was planted between July and September 2018. *P. australis* was established using rhizome-bearing transplants from designated donor populations, whereas *K. obovata* was planted as one-year-old seedlings at 0.5–1.0 m × 0.5–1.0 m spacing. Periodic post-planting maintenance supported establishment. Both planted patches had been established for approximately five years when sampling began in August 2023.

Because *S. alterniflora* occupied several intertidal zones, replacement vegetation was selected according to local inundation and salinity conditions. The *P. australis* patch occupied an upper-intertidal area with stronger freshwater influence and lower salinity, whereas the *K. obovata* patch occupied a more tide-influenced middle- to lower-intertidal area suitable for mangrove growth. One extant *S. alterniflora* patch and the two planted patches were sampled (Fig. 1). Each patch contained four nested 2 m × 2 m plots that served as spatial subsamples rather than independent replicates of each wetland state. Plots were placed in relatively level areas on the same continuous tidal flat. Plot-specific elevation, inundation frequency, water depth, and porewater salinity were unavailable, leaving patch identity confounded with hydrogeomorphic position. The comparisons therefore characterize differences among the three sampled patches rather than isolated plant-identity effects.

Sample collection

Soils were sampled in August 2023 and January 2024 in the extant *S. alterniflora*, planted *K. obovata*, and planted *P. australis* patches. After surface litter was removed, five subsamples were collected in an S-shaped pattern within each plot and composited into one plot-level sample. Samples from 0–30, 30–60, and 60–100 cm were sealed, cooled, and transported to the laboratory. Each composite was divided into portions that were air-dried for physicochemical and elemental analyses, stored at 4°C for microbial biomass measurements, or frozen at -20°C for enzyme assays. Potential extracellular enzyme activities and the derived vector and estimated CUE metrics were measured only in 0–30 cm samples.

Sample analysis

Soil physicochemical properties

Soil water content (WC) was determined by oven-drying at 105°C for 24 h. Bulk density (BD) was determined using undisturbed soil cores dried at 105°C (Lu 2000; Li et al. 2026). Soil pH was measured in a 2.5:1 soil–water suspension using a portable pH meter (Starter 300, USA). Electrical conductivity (EC) was measured in a 5:1 soil–water suspension using a conductivity meter (2265FS, USA).

Soil nutrients and C stock

Soil organic C (SOC) and total nitrogen (TN) were determined using an elemental analyzer (Elementar Vario MAX CN, Germany). Total phosphorus (TP) was determined by sulfuric acid–perchloric acid digestion (Carter and Gregorich, 1993), followed by quantification with a continuous flow analyzer (Skalar SAN++, Netherlands).

SOC stock was calculated as (Wiesmeier et al. 2012; Fowler et al. 2023):

$$\text{SOC}_{\text{stock}} = \text{SOC}_{\text{concentration}} \times \text{BD} \times \text{Thickness} \times 0.1$$

where SOC stock is in Mg C·ha⁻¹, SOC concentration in g·kg⁻¹, BD in g·cm⁻³, and thickness in cm.

Microbial biomass C (MBC) and N (MBN) were determined by chloroform fumigation-extraction (Murphy et al. 2000). Fumigated and unfumigated soil extracts were analyzed for dissolved organic C (TOC-VCPH, Shimadzu, Japan) and N (Skalar SAN++, Netherlands). Microbial biomass P (MBP) was determined by fumigation-extraction with spike-recovery quality control (Brookes et al. 1982): 5 g soil was spiked with 125 µL KH₂PO₄ standard (1000 µg·mL⁻¹), placed in a chloroform-free desiccator for 24 h, extracted with 0.5 mol·L⁻¹ NaHCO₃ at a 1:4 soil-to-solution ratio, and acidified with 300 µL concentrated sulfuric acid before P quantification (Skalar SAN++, Netherlands).

MBC, MBN, and MBP were calculated as:

$$\text{MBC} = \Delta E_C / K_C$$

$$MBN = \Delta E_N / k_N$$

$$MBP = \Delta E_P / k_P / K_b$$

where ΔE_C , ΔE_N , and ΔE_P are the differences between fumigated and non-fumigated extracts; $K_C = 0.45$, $k_N = 0.54$, $k_P = 0.40$ (Brookes et al. 1982; Jenkinson et al. 2004); and K_b is the P recovery rate.

Soil enzyme activities

Potential activities of four hydrolytic enzymes [β -glucosidase (BG), cellobiohydrolase (CBH), acid phosphatase (ACP), and N-acetyl- β -D-glucosaminidase (NAG)] and one oxidative enzyme [phenol oxidase (PHO)] were assayed following Saiya-Cork et al. (2002) and Bach et al. (2013). A 1.0 g fresh-soil sample was suspended in 125 mL of 0.05 mol L⁻¹ acetate buffer (pH 5.0) with stirring for 10 min, after which a 200 μ L aliquot of the suspension was transferred to a 96-well microplate with the corresponding substrate (Supplementary Material 1). Hydrolytic reactions were incubated at 20°C in darkness for 4 h, terminated with 10 μ L of 1.0 mol L⁻¹ NaOH, and measured fluorometrically (BioTek, USA). Oxidative reactions were incubated for 18 h. Enzyme activity was expressed as μ mol g⁻¹ h⁻¹.

Ecoenzymatic vector analysis described relative allocation among the assayed C-, N-, and P-acquiring enzymes (Moorhead et al. 2016). Vector length (VL) represents C acquisition relative to nutrient acquisition, whereas vector angle (VA) contrasts allocation to P acquisition with allocation to the assayed N-acquiring enzyme:

$$VL = (x^2 + y^2)^{0.5}$$

$$VA = (180/\pi) \times \text{atan2}(y, x)$$

where $x = (BG + CBH)/(BG + CBH + ACP)$ and $y = (BG + CBH)/(BG + CBH + NAG)$. Larger VL represents greater C acquisition relative to nutrient acquisition. $VA > 45^\circ$ denotes greater relative allocation to P acquisition than to N acquisition among the assayed enzymes, whereas $VA < 45^\circ$ denotes the converse. Vector angle was calculated using the mathematical argument order $\text{atan2}(y, x)$, with the same orientation retained in the software implementation.

Microbial CUE was calculated following Sinsabaugh et al. (2016):

$$CUE = CUE_{\max} \times \{(S_{C:N} \times S_{C:P}) / [(K_{C:N} + S_{C:N}) \times (K_{C:P} + S_{C:P})]\}^{0.5}$$

$$S = B / L \times (1/EEA)$$

$$S_{C:P} = B_{C:P} / L_{C:P} \times (1/EEA_{C:P})$$

where $CUE_{\max} = 0.6$; $K_{C:N} = K_{C:P} = 0.5$; $B_{C:N} = \text{MBC:MBN}$ and $B_{C:P} = \text{MBC:MBP}$; $L_{C:N} = \text{SOC:TN}$ and $L_{C:P} = \text{SOC:TP}$; $EEA_{C:N} = (\text{BG} + \text{CBH})/\text{NAG}$ and $EEA_{C:P} = (\text{BG} + \text{CBH})/\text{ACP}$.

Statistical analysis

All analyses were performed using SPSS 27.0, R 4.0.3, and Canoco 5.0. Normality and variance homogeneity were assessed using Shapiro-Wilk and Bartlett tests, respectively, and logarithmic transformation was applied when required. Results are reported as mean $\pm$ SE, with statistical significance set at $p < 0.05$. Profile variables were evaluated using linear mixed-effects models (LMMs; lme4 and lmerTest packages), with wetland state, sampling campaign, soil depth, and their interactions as fixed effects and plot identity as a random effect. Surface-soil enzyme activities, vector metrics, and estimated CUE were evaluated using LMMs with wetland state, sampling campaign, and their interaction as fixed effects and plot identity as a random effect. Significant fixed effects were followed by Tukey-adjusted pairwise contrasts. Plot identity accounted for repeated measurements, but the four plots were spatial subsamples within one patch per state. State contrasts therefore quantify differences among the sampled patches rather than replicated pathway or species effects.

Mantel tests (LinkET package) described associations between SOC variables and soil properties, while random forests (randomForest package) provided exploratory predictor-importance rankings. Associations of SOC concentration with C/N or C/P and of fixed-depth stock with bulk density were interpreted descriptively because the ratios contain SOC and bulk density is an explicit stock component. Redundancy analysis (RDA; Canoco 5.0) described covariance between surface-soil enzyme activities and environmental variables. Because estimated CUE was calculated from enzyme and SOC-related inputs included in the ordination, its position was also interpreted descriptively rather than as independent mechanistic evidence. Figures were produced using Origin 2021 and the ggplot2 and ggpubr packages in R.

Results

Depth profiles of SOC concentration and stock

SOC concentration differed most clearly below 30 cm between the mangrove-re-established *K. obovata* patch and the extant *S. alterniflora* reference (Fig. 2). Across campaigns, mean SOC concentrations at 30–60 and 60–100 cm were 15.15 ± 0.44 and $15.33 \pm 0.38 \text{ g kg}^{-1}$ in the *K. obovata* patch, compared with 13.33 ± 0.27 and $13.59 \pm 0.22 \text{ g kg}^{-1}$ in the reference. The *K. obovata* values were 13.65% and 12.80% higher, respectively ($p < 0.01$ and $p < 0.05$). Campaign-pooled fixed-depth SOC stock, however, did not differ significantly between these patches at any depth. At 30–60 cm, stock was lower in the *K. obovata* patch in August ($p < 0.05$) but not in January. In the *P. australis* patch, stock was lower than in the reference at 0–30 cm (41.26 ± 2.57 versus $58.51 \pm 5.64 \text{ Mg C ha}^{-1}$, $p < 0.05$) and 60–100 cm (52.88 ± 4.93 versus $73.02 \pm 4.52 \text{ Mg C ha}^{-1}$, $p < 0.01$).

Soil nutrients and microbial biomass stoichiometry

Total soil P did not differ significantly among patches or depths, whereas total N showed depth-specific patch contrasts (Fig. 3). Total N did not differ among patches at 0–30 cm but differed at 30–60 and 60–100 cm ($p < 0.05$). At 30–60 cm, total N was higher in the *K. obovata* patch than in the *S. alterniflora* reference, with the *P. australis* patch intermediate. At 60–100 cm, total N was higher in the *K. obovata* patch than in the *P. australis* patch, with the reference intermediate. Bulk-soil C/N, C/P, and N/P did not differ significantly among patches ($p > 0.05$).

Microbial P indicators provided the clearest biomass contrasts. Microbial biomass P (MBP) decreased with depth in all patches and was lower in the *K. obovata* patch at 60–100 cm ($17.22 \pm 1.83 \text{ mg kg}^{-1}$) than in the reference ($25.13 \pm 1.97 \text{ mg kg}^{-1}$, $p < 0.05$). At 0–30 cm, microbial biomass N:P was higher in both planted patches than in the reference ($p < 0.05$). Microbial biomass C (MBC) did not differ significantly at 0–30 or 60–100 cm, but at 30–60 cm it was lower in the *P. australis* and *K. obovata* patches (27.69 ± 7.22 and $30.28 \pm 4.54 \text{ mg kg}^{-1}$) than in the reference ($51.75 \pm 3.84 \text{ mg kg}^{-1}$, $p < 0.05$). Microbial biomass N decreased with depth in the *S. alterniflora* and *P. australis* patches but not in the *K. obovata* patch, although it did not differ significantly among patches within any depth. Microbial biomass C:N in the *P. australis* patch was lower than in both other patches at 30–60 cm and lower than in the reference at 60–100 cm ($p < 0.05$).

Enzyme activities, estimated CUE and vector metrics

Potential enzyme activities varied among patches and campaigns (Fig. 4). Acid phosphatase (ACP) activity was higher in the *K. obovata* patch than in both other patches during August and higher than in the *S. alterniflora* reference during January ($p < 0.05$). Cellobiohydrolase (CBH) remained higher in the *K. obovata* patch than in both other patches during both campaigns, whereas N-acetylglucosaminidase (NAG) exceeded both other patches only during August ($p < 0.05$). β -glucosidase (BG) was higher in both planted patches than in the reference during August and higher in the *K. obovata* patch than in the *P. australis* patch during January ($p < 0.05$). Phenol oxidase (PHO) was higher in both planted patches than in the reference during both campaigns ($p < 0.05$). Across campaigns, mean ACP, CBH, and NAG activities in the *K. obovata* patch were 1497.10 , 72.64 , and $86.61 \text{ } \mu\text{mol g}^{-1} \text{ h}^{-1}$, respectively. These means were 201.96%, 131.34%, and 144.45% higher than the corresponding *S. alterniflora* means ($p < 0.01$).

Ecoenzymatic vector metrics and estimated CUE showed campaign-dependent contrasts (Fig. 5). Although vector angle did not differ significantly among patches during August, it was higher in the *K. obovata* patch than in the reference during January and across both campaigns (82.11° versus 67.77° and 82.34° versus 75.86° , respectively; $p < 0.05$). The higher angle denotes greater relative allocation to P acquisition among the assayed enzymes. Vector length was higher in the *P. australis* patch than in the *K. obovata* patch during August ($p < 0.05$) but did not differ significantly among patches during January. Both planted patches had lower estimated CUE than the reference during August but higher values

during January ($p < 0.05$). Across campaigns, mean estimated CUE was 0.24, 0.13, and 0.16 in the *S. alterniflora*, *P. australis*, and *K. obovata* patches, respectively.

All observed vector angles exceeded 45° , showing greater relative allocation to P acquisition than to the assayed N-acquiring enzyme (Fig. 6). In pooled regressions, estimated CUE was negatively associated with vector length ($R^2 = 0.21$) and positively associated with vector angle ($R^2 = 0.31$), whereas vector angle was negatively associated with vector length ($R^2 = 0.56$). Because the three indices share enzyme inputs, these regressions describe covariance among derived metrics and do not represent independent mechanistic relationships.

Soil correlates of SOC concentration and stock

Across all samples, SOC concentration was associated with TN, C/P, and N/P in Mantel tests ($p < 0.01$), whereas fixed-depth stock was associated with bulk density ($p < 0.01$) and pH ($p < 0.05$; Fig. 7a). Exploratory random forests ranked TN (22.64%), C/P (21.32%), C/N (20.66%), and TP (14.31%) most highly for SOC concentration (Fig. 7b). For stock, bulk density ranked first (35.58%), followed by TP (10.72%), TN (8.21%), pH (8.09%), and C/N (7.57%; Fig. 7c). C/P and C/N include SOC, while bulk density enters the stock calculation directly, so these rankings partly reflect mathematical coupling. The remaining rankings represent exploratory associations and do not establish causation.

Surface enzyme–soil relationships

RDA1 and RDA2 explained 61.92% and 13.68% of the variation in surface-soil enzyme activities, respectively (75.60% cumulatively, $p = 0.004$; Fig. 8). Samples from the *S. alterniflora* and *K. obovata* patches were positioned mainly on the negative and positive sides of RDA1, respectively. BG, CBH, and NAG aligned with surface SOC, whereas ACP and PHO aligned with TP and microbial biomass C:P. The estimated CUE vector was oriented opposite to surface SOC and the hydrolytic enzymes. Because estimated CUE shares enzyme- and SOC-derived terms with variables in the ordination, its position is descriptive rather than independent mechanistic evidence.

Discussion

Deep-soil carbon enrichment and concentration–stock divergence

The clearest carbon contrast associated with mangrove re-establishment occurred below 30 cm, where SOC concentration in the sampled *K. obovata* patch exceeded that in the extant *S. alterniflora* reference by 13.65% at 30–60 cm and 12.80% at 60–100 cm. Mangrove roots, rhizodeposition, and chemically complex litter can increase belowground substrate supply, while woody-root development and tidal sediment trapping may redistribute carbon through the profile (Song et al. 2023; Beselly et al. 2025; Scheibe et al. 2023; Yin et al. 2025). Microbial processing and mineral association may further extend

the residence time of mangrove-derived and imported organic matter (Li et al. 2026). The measured profile may therefore integrate recent plant inputs with inherited and laterally supplied material (Zhou et al. 2023; Zhang et al. 2024; Fang et al. 2023), producing a deep-soil concentration signal without a corresponding surface contrast.

Control-related disturbance and hydrogeomorphic setting provide alternative explanations for the observed profiles. Mowing, rotary tillage, rhizome removal, and surface leveling may have redistributed inherited SOC and altered soil mass within the planted patches, while salinity, sedimentation, and pre-control conditions may also have differed among patches (Powlson et al. 2014). The *P. australis* patch further illustrates this hydrogeomorphic boundary because its upper-intertidal, freshwater-influenced setting differs from the more tide-influenced *K. obovata* setting, and its lower SOC concentration and stock cannot be attributed to reed identity alone. The observed profiles therefore represent realized post-control states shaped jointly by vegetation, control legacy, and site setting (Chen et al. 2026).

SOC concentration and fixed-depth stock describe different dimensions of the soil carbon pool (Smith et al. 2020). When campaigns were pooled, fixed-depth stock did not differ significantly between the *K. obovata* patch and the reference at any sampled depth, although stock was lower in the *K. obovata* patch at 30–60 cm during August. Because stock incorporates concentration, bulk density, and layer thickness, the high random-forest importance of bulk density largely reflects its direct role in the calculation rather than an independent mechanism (Haden et al. 2020; Smith et al. 2020). Deep-soil concentration enrichment was therefore the clearest mangrove-associated carbon signal, whereas area-based storage remained sensitive to soil mass, disturbance history, and hydrogeomorphic setting.

Microbial investment in P acquisition as the dominant functional contrast

Soil–microbial C:N:P stoichiometry can provide complementary information on microbial nutrient status and resource allocation that may not be evident from bulk-soil nutrient pools (Moorhead et al. 2013; Chen et al. 2024). Microbial allocation to P acquisition was the clearest functional contrast associated with the *K. obovata* patch. Relative to the *S. alterniflora* reference, mean potential ACP activity was 201.96% higher and mean vector angle was greater (82.34° versus 75.86°). Because all observed vector angles exceeded 45°, the larger angle in the *K. obovata* patch denotes stronger relative allocation to P acquisition among the assayed enzymes rather than the onset of P limitation (Moorhead et al. 2016; Sun et al. 2023). Lower microbial biomass P at 60–100 cm and higher surface microbial biomass N:P provide complementary evidence of a P-centered contrast between the *K. obovata* patch and the reference.

This allocation contrast occurred without a significant difference in total soil P. Estuarine P pools are strongly influenced by depositional parent material and mineral adsorption (Wang and Huang, 2021; Hu et al. 2023; Rumeau et al. 2025), and bulk total P includes forms that may not be readily accessible to plants or microorganisms. Microbial enzyme allocation can therefore differ between patches whose total-P pools do not differ significantly (Achat et al. 2010). Because available soil P, porewater P, and

plant P status were not measured, the evidence supports altered relative allocation to P acquisition rather than depletion of the total-P pool or realized P uptake.

ACP formed part of a broader increase in potential enzyme activity. BG, CBH, NAG, and PHO were also higher in the *K. obovata* patch in at least one campaign, so the P-centered inference rests most strongly on vector angle and the contrast between ACP and the assayed N-acquiring enzyme rather than on ACP alone. Mangrove litter, roots, and rhizosphere inputs may increase substrate supply and chemical complexity, while microbial biomass, pH, salinity, and assay conditions can also affect potential activity per unit soil (Sinsabaugh et al. 2008; Yang et al. 2022b; Yin et al. 2025). The broader enzyme response suggests that increased substrate processing accompanied the stronger allocation to P acquisition.

Estimated CUE changed in opposite directions between the August and January campaigns, and its shared enzyme inputs with the vector metrics prevent the regressions among these indices from serving as independent mechanistic evidence. Estimated CUE is therefore contextual rather than load-bearing evidence for the core interpretation. By contrast, the convergence of ACP, vector angle, microbial biomass P, and microbial biomass N:P identifies greater relative allocation to P acquisition as the dominant functional response in the mangrove-re-established patch.

A depth-separated carbon–phosphorus linkage

The carbon and P responses co-occurred across different soil depths: higher SOC concentration at 30–100 cm accompanied greater relative allocation to P acquisition at 0–30 cm in the mangrove-re-established *K. obovata* patch. Developing roots and litter could plausibly increase both organic-substrate supply and microbial P demand, while microbial products may subsequently enter mineral-associated pools (Cotrufo et al. 2013; Li et al. 2026). Because the responses were measured at one post-control stage and at different depths, they do not resolve a pathway from surface enzyme activity to deep SOC enrichment. Their co-occurrence nevertheless defines a site-level carbon–P signature across complementary soil dimensions.

Covariance analyses supported the separation among these dimensions rather than a single common driver. SOC concentration aligned with TN and bulk-soil stoichiometric ratios, whereas fixed-depth stock aligned most strongly with bulk density. Within surface soil, BG, CBH, and NAG aligned with SOC, while ACP and PHO aligned with TP and microbial biomass C:P. Some variables share mathematical terms, so these associations cannot identify independent drivers, and the surface RDA does not explain the deep SOC contrast. Instead, the analyses show that carbon and microbial indicators provide complementary information about the sampled soil states.

The central advance is therefore the identification of a depth-separated carbon–P signature that would be missed by any single metric. Deep-soil SOC concentration captured subsurface enrichment, fixed-depth stock constrained the area-based interpretation, and ACP, vector angle, microbial biomass P, and microbial biomass N:P captured microbial allocation. Integrating these measurements provides a clearer basis for evaluating mangrove-associated soil development after *S. alterniflora* control.

Implications for post-control assessment

Post-control assessment should distinguish SOC concentration from area-based stock and pair both measures with depth-matched microbial and plant nutrient indicators. SOC concentration describes carbon per unit soil mass, fixed-depth stock describes storage within a specified profile, and enzyme allocation describes potential microbial resource acquisition (Wiesmeier et al. 2019). Evaluating these metrics together can identify co-occurring carbon and nutrient responses without treating any one measure as a proxy for another.

Testing whether this site-level association generalizes requires replicated chronosequences or before-after designs matched for tidal elevation, hydrology, and control history. Such designs should align surface and deep measurements of SOC concentration, bulk density, equivalent-soil-mass stock, available and porewater P, roots, microbial necromass, and mineral-associated organic carbon (Qin et al. 2024; Ascenzi et al. 2025). These measurements would test whether the carbon–P signature persists, strengthens, or decouples as mangrove soils develop.

Management relevance also depends on whether these soil responses coincide with elevation gain, sediment accretion (Sidik et al. 2025), greenhouse-gas exchange (Liang et al. 2024), biodiversity, and restoration cost (Su et al. 2021). Combining these ecosystem outcomes with depth-resolved carbon and nutrient measurements would clarify when mangrove re-establishment supports durable post-control soil development beyond the Minjiang Estuary.

Conclusion

At this Minjiang Estuary site, the approximately five-year mangrove-re-established *K. obovata* patch combined 13.65% and 12.80% higher SOC concentration at 30–60 and 60–100 cm with greater surface microbial allocation to P acquisition than the contemporaneous extant *S. alterniflora* reference. Mean potential ACP activity was 201.96% higher and mean ecoenzymatic vector angle was greater (82.34° versus 75.86°), even though total soil P did not differ significantly and microbial biomass P at 60–100 cm was lower. When campaigns were pooled, fixed-depth SOC stock did not differ significantly between the patches, showing that concentration and area-based storage captured distinct dimensions of the carbon response. Within the cross-sectional design, these depth-separated signals identify a site-level carbon–P association and show why post-control assessment should integrate profile carbon metrics with depth-matched microbial nutrient indicators.

Declarations

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CRediT authorship contribution statement

Xiaoying Ren: Writing-original draft, Visualization, Conceptualization, Software, Data curation. **Jiahao Peng:** Visualization, Formal analysis, Conceptualization. **WeiQi Wang:** Writing-review and editing, Methodology, Resources, Project administration, Validation, Funding acquisition. **Yuan Li:** Writing-review and editing, Methodology. **Ning Hou:** Writing-review and editing. **Jordi Sardans:** Writing-review and editing, Methodology.

Declaration of competing interest

The authors declared that there is no conflict of interest.

Data availability

The data supporting this study are available in Mendeley Data (Version 1; DOI: 10.17632/wmvc944xkz.1).

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Figures

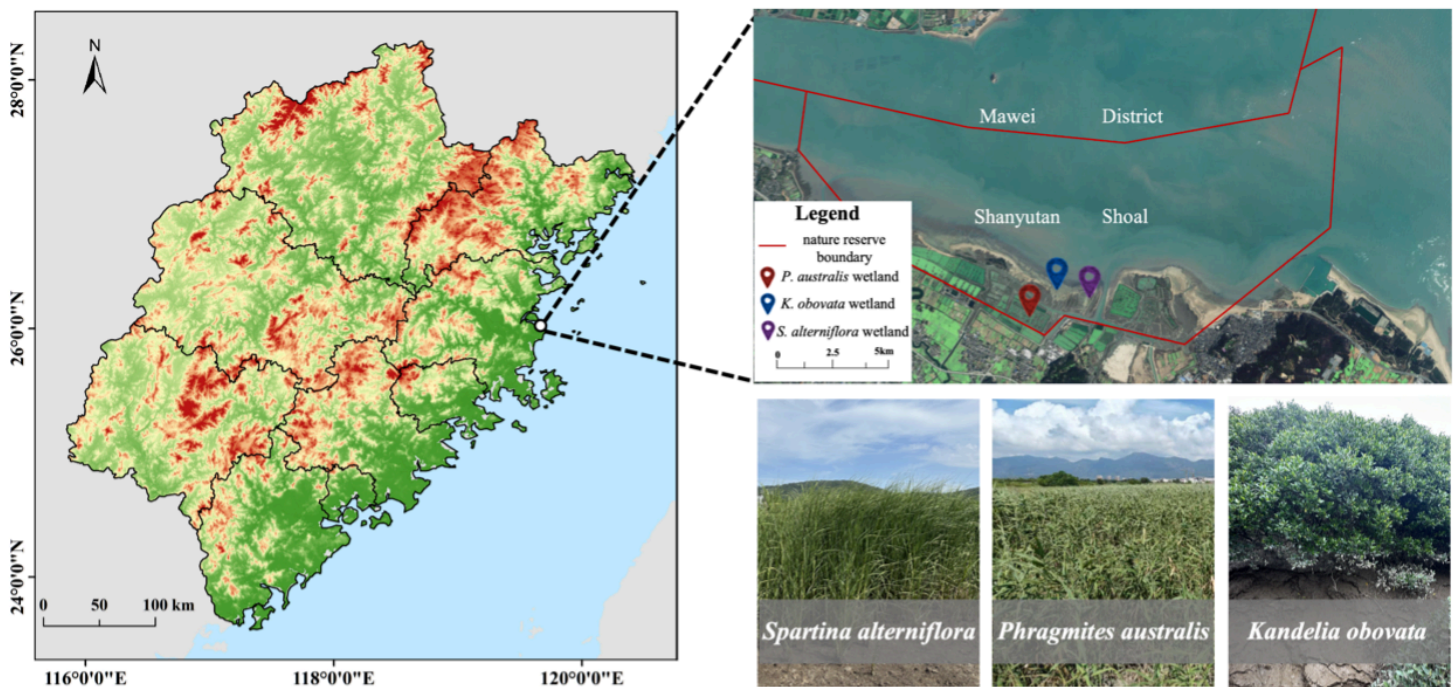


Figure 1

Study location and sampled wetland states in the Shanyutan Wetland, Minjiang Estuary, Fujian Province, southeastern China. The left map locates the study region within Fujian Province, and the satellite inset shows Shanyutan Shoal in Mawei District; the red outline marks the nature reserve boundary. Red, blue, and purple markers identify the sampled *Phragmites australis*, *Kandelia obovata*, and extant *Spartina alterniflora* patches, respectively. The *P. australis* and *K. obovata* patches were established after *S. alterniflora* removal in 2018, while extant *S. alterniflora* served as the contemporaneous reference. Four 2 m × 2 m plots were nested within each patch. Photographs show *S. alterniflora*, *P. australis*, and *K. obovata* from left to right.

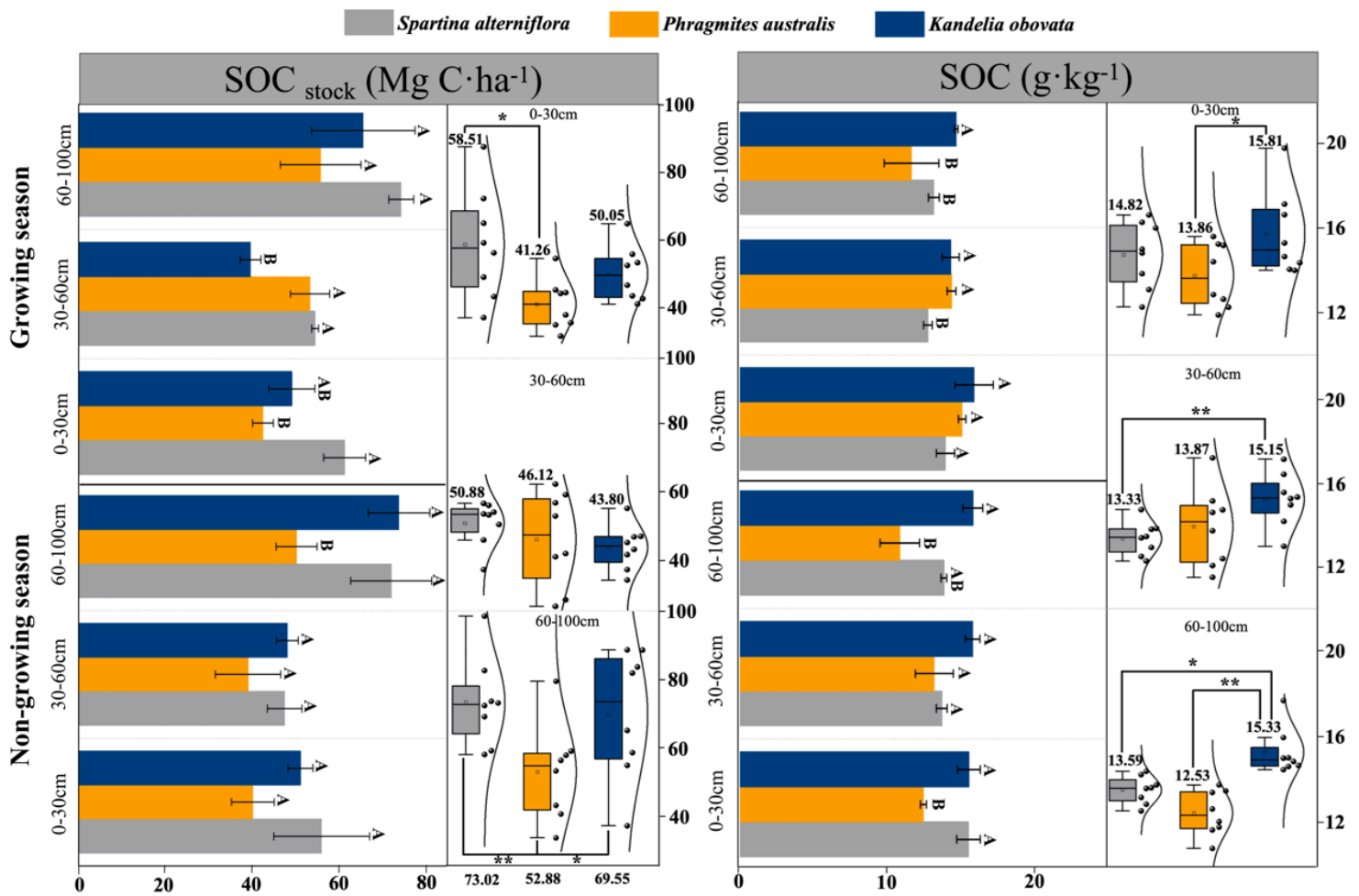


Figure 2

Soil organic carbon (SOC) concentration and layer-specific fixed-depth stock in the three sampled wetland states. SOC stock (left; Mg C ha⁻¹) and concentration (right; g kg⁻¹) are shown for the 0–30, 30–60, and 60–100 cm layers during the August 2023 and January 2024 campaigns. Horizontal bars show means $\pm$ SE for four plots per state, campaign, and depth. Raincloud summaries combine both campaigns and show plot-campaign observations, boxplots, half-violin distributions, and pooled means. Different uppercase letters indicate Tukey-adjusted differences among wetland states within the same depth and campaign ($p < 0.05$); shared letters indicate no significant difference. Brackets in the pooled summaries mark pairwise contrasts between connected states (* $p < 0.05$; ** $p < 0.01$).

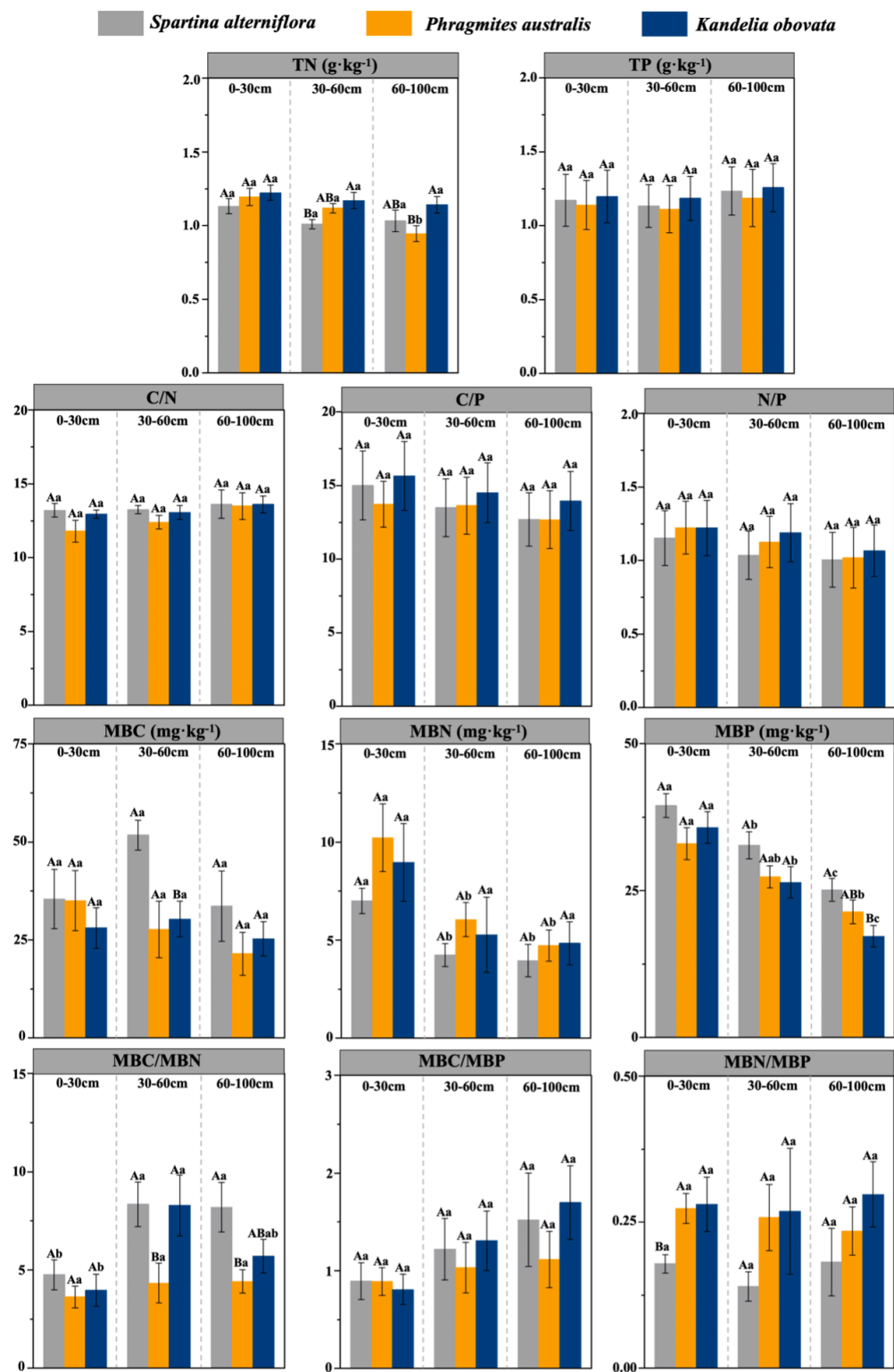


Figure 3

Bulk-soil nitrogen and phosphorus concentrations and stoichiometry, together with microbial biomass pools and stoichiometry, across wetland states and soil depths. Panels show total nitrogen (TN), total phosphorus (TP), bulk-soil C/N, C/P, and N/P, microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP), and their biomass ratios. Bulk-soil ratios represent SOC/TN, SOC/TP, and TN/TP. TN and TP are expressed as g kg^{-1} , microbial pools as mg kg^{-1} , and ratios are dimensionless. Values are

campaign means $\pm$ SE based on four plots per state. Different uppercase letters indicate Tukey-adjusted differences among wetland states within a soil depth, while different lowercase letters indicate differences among depths within a wetland state ($p < 0.05$); shared letters indicate no significant difference.

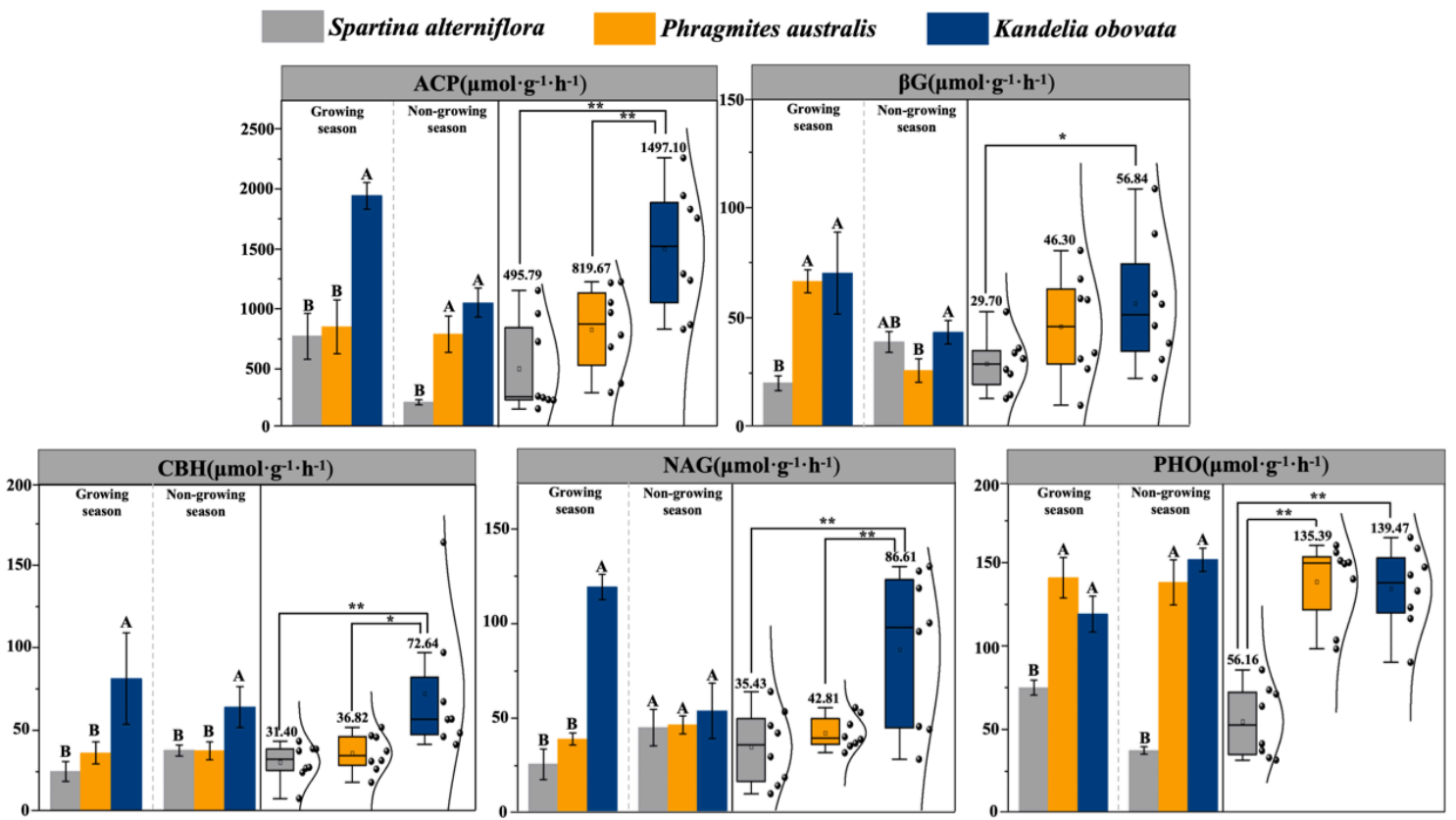


Figure 4

Potential extracellular enzyme activities in surface soils (0–30 cm) among the three sampled wetland states during the two sampling campaigns. Enzymes are acid phosphatase (ACP), β -glucosidase (BG; labeled β G in the panel), cellobiohydrolase (CBH), N-acetyl- β -D-glucosaminidase (NAG), and phenol oxidase (PHO); activities are expressed as $\mu\text{mol g}^{-1} \text{h}^{-1}$. Campaign-specific bars show means $\pm$ SE for four plots per state in August 2023 and January 2024. Raincloud summaries combine both campaigns and show individual observations, boxplots, half-violin distributions, and pooled means. Different uppercase letters indicate Tukey-adjusted differences among wetland states within a campaign ($p < 0.05$). Brackets mark the displayed pairwise contrasts ($*p < 0.05$; $**p < 0.01$).

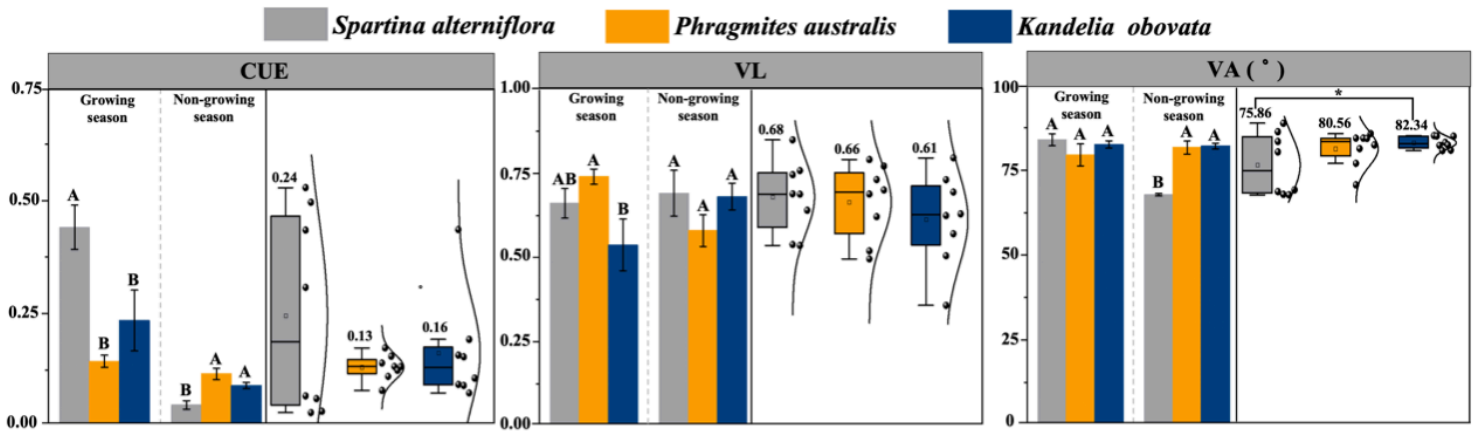


Figure 5

Estimated microbial carbon use efficiency and ecoenzymatic vector metrics in surface soils (0–30 cm) among the sampled wetland states. CUE is estimated microbial carbon use efficiency, VL is vector length, and VA is vector angle in degrees; CUE and VL are dimensionless. Larger VL indicates greater allocation to C acquisition relative to nutrient acquisition, whereas $VA > 45^\circ$ indicates greater relative allocation to P acquisition than to the assayed N-acquiring enzyme. Campaign-specific bars show means $\pm$ SE for four plots per state in August 2023 and January 2024. Raincloud summaries combine both campaigns and show individual observations, boxplots, half-violin distributions, and pooled means. Different uppercase letters indicate Tukey-adjusted differences among wetland states within a campaign ($p < 0.05$). Single and double asterisks denote $p < 0.05$ and $p < 0.01$, respectively.

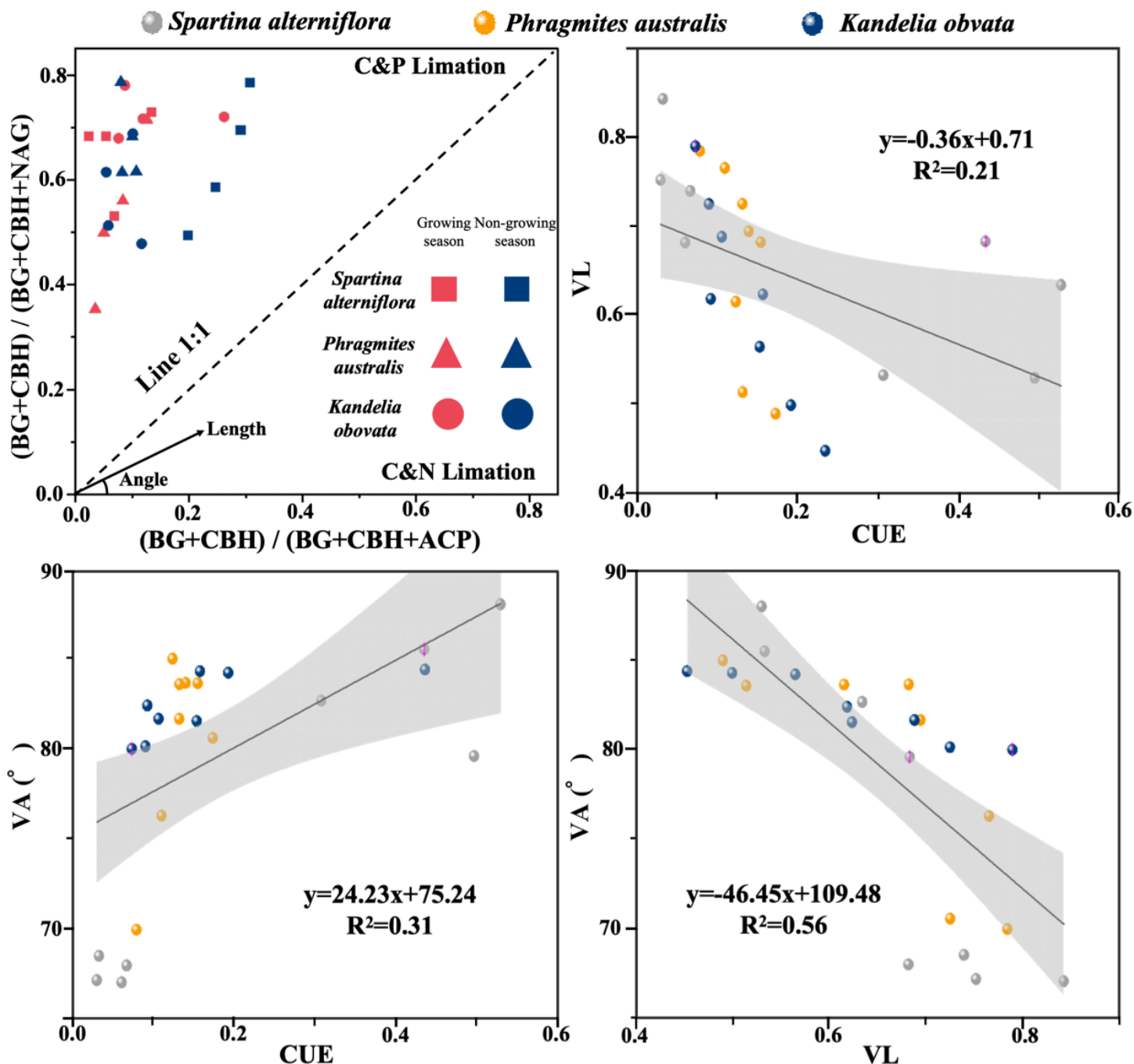


Figure 6

Ecoenzymatic allocation coordinates and descriptive relationships among estimated microbial carbon use efficiency, vector length, and vector angle in surface soils (0–30 cm). The upper-left panel plots $x = (BG + CBH) / (BG + CBH + ACP)$ against $y = (BG + CBH) / (BG + CBH + NAG)$. The origin ($x = 0, y = 0$) is a mathematical reference rather than an observed baseline. Distance from the origin represents VL, and the angle from the x-axis represents VA. The dashed 1:1 line corresponds to $VA = 45^\circ$. Values above the line indicate greater relative allocation to P acquisition than to the assayed N-acquiring enzyme. Red and blue symbols denote the August 2023 and January 2024 campaigns, while symbol shapes denote the sampled patches. The other panels regress VL against CUE, VA against CUE, and VA against VL. Gray

lines and bands show pooled fitted regressions and 95% confidence intervals; point colors denote patch identity. Equations and R^2 values are displayed.

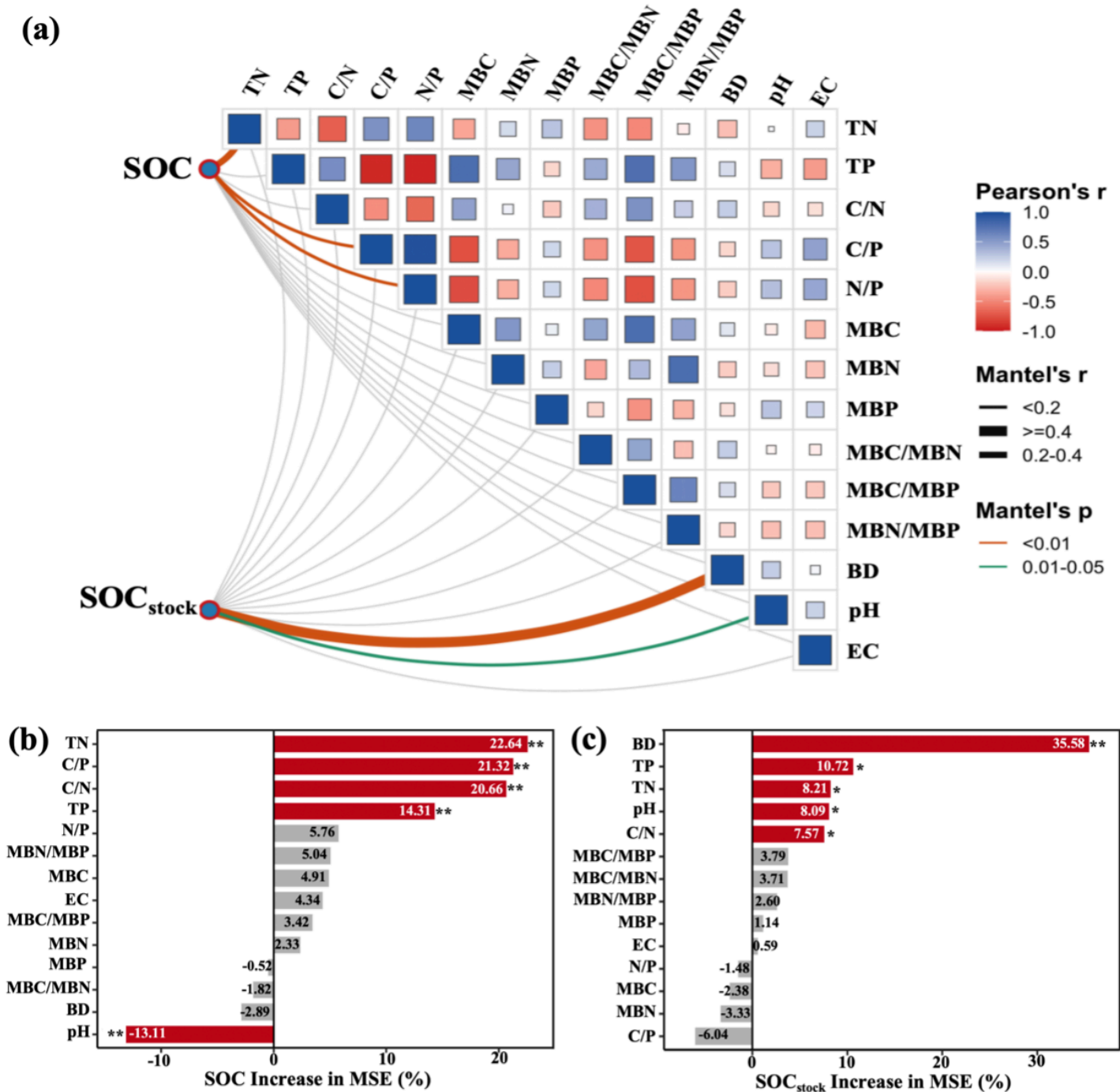


Figure 7

Associations and exploratory predictor rankings for SOC concentration and fixed-depth stock across wetland states, soil depths, and sampling campaigns. Panel (a) overlays Mantel-test links between each SOC response and the soil and microbial variables on a Pearson correlation matrix. Matrix color shows the sign and magnitude of Pearson's r , while square size shows $|r|$. Mantel-link width denotes $r < 0.2$, 0.2 –

0.4, or ≥ 0.4 ; orange, green, and gray links denote $p < 0.01$, $0.01 \leq p < 0.05$, and $p \geq 0.05$. Panels (b) and (c) rank random-forest predictor importance for concentration and stock as the percentage increase in mean squared prediction error after permutation. Red bars identify predictors with asterisk-marked permutation significance, whereas gray bars indicate predictors without a significance mark. Asterisks denote $*p < 0.05$ and $**p < 0.01$. TN, total nitrogen; TP, total phosphorus; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; MBP, microbial biomass phosphorus; BD, bulk density; EC, electrical conductivity.

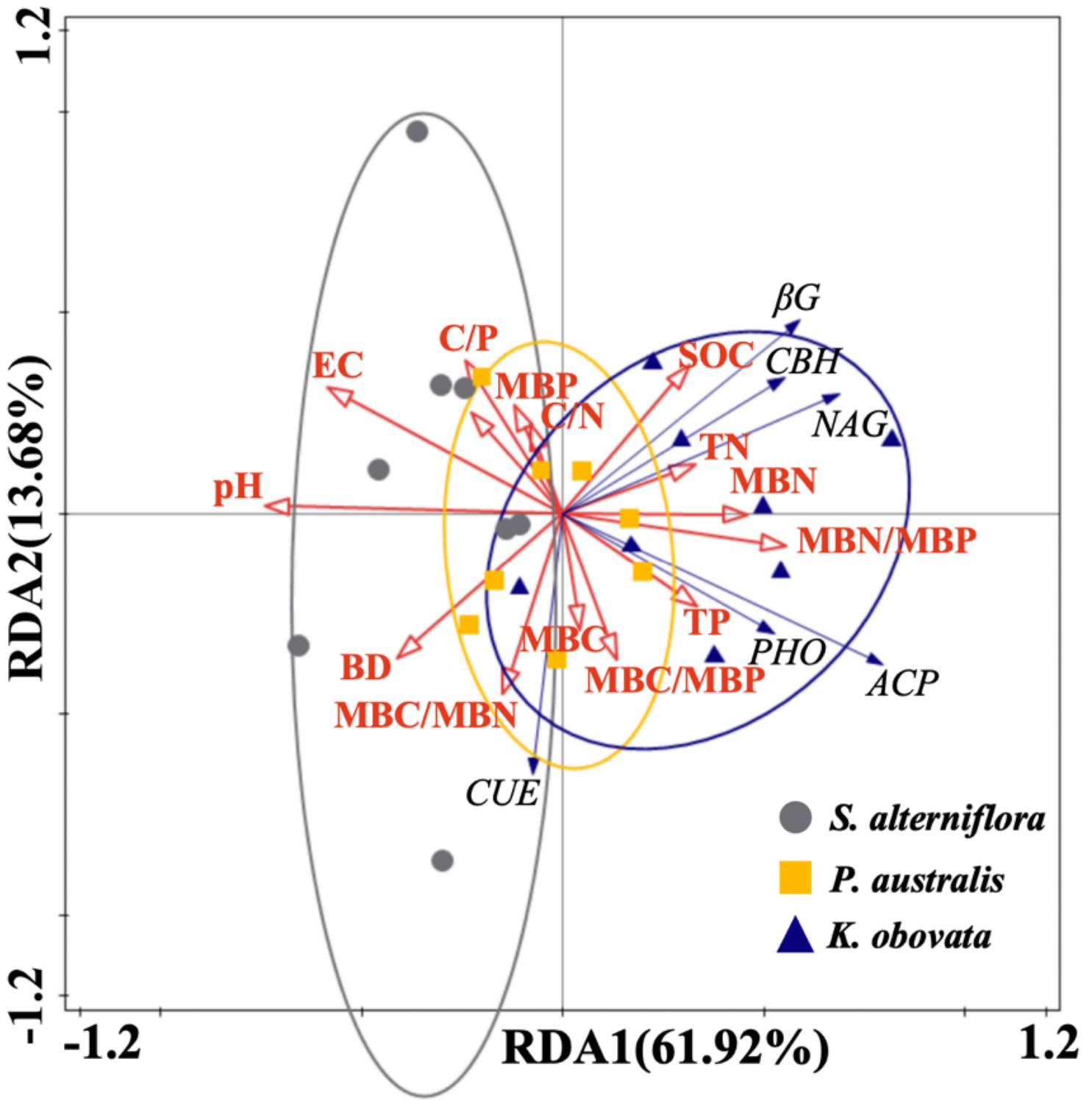


Figure 8

Redundancy-analysis (RDA) biplot of potential extracellular enzyme activities, estimated microbial carbon use efficiency (CUE), and soil physicochemical and microbial variables in surface soil (0–30 cm) across the two sampling campaigns. RDA1 and RDA2 explain 61.92% and 13.68% of the variation, respectively, for a cumulative 75.60% (overall $p = 0.004$). Blue vectors represent potential enzyme activities and estimated CUE, while red vectors represent soil properties and microbial pools or ratios; vector direction and length describe covariance with the ordination axes. BG, β -glucosidase; CBH, cellobiohydrolase; NAG, N-acetyl- β -D-glucosaminidase; PHO, phenol oxidase; ACP, acid phosphatase; MBC, MBN, and MBP, microbial biomass C, N, and P.

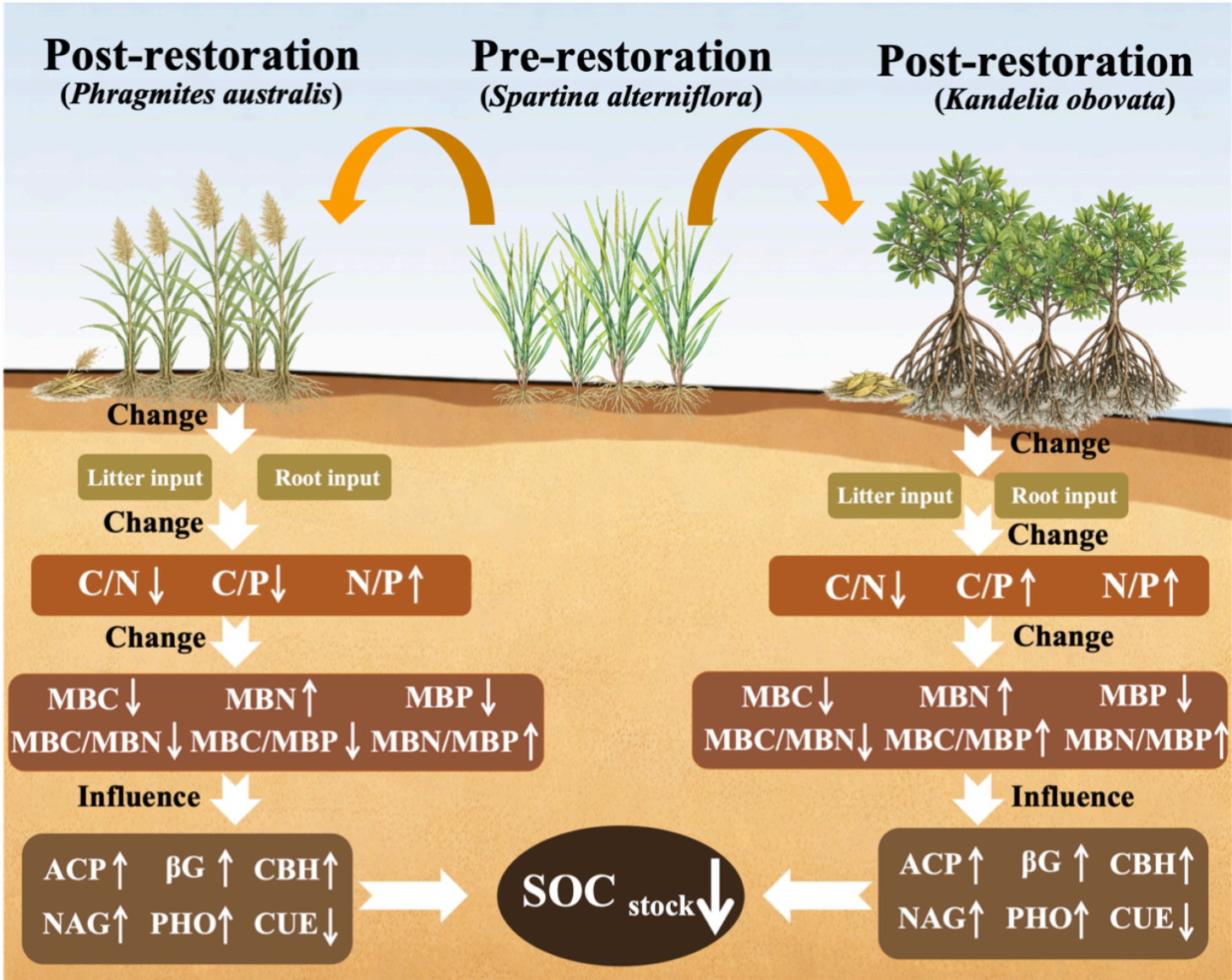


Figure 9

Legend not included with this version

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