

*Original Research*

# Regulation of the Soil Microbial Metabolism Through Alterations in the Vegetative Community in Wetlands

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## Abstract

Changes in vegetation communities are projected to have a greater impact on the turnover and storage of carbon in coastal wetlands by affecting soil organic matter decomposition. Microbial metabolism regulates the process of organic matter decomposition in the soil. However, there is still a need for a mechanistic framework to predict the effects of vegetation changes on soil microbial metabolism. Hence, this study aimed to evaluate the changing trends of microbial metabolic limitation and carbon use efficiency under natural succession and vegetation community degradation in a wetland using soil extracellular enzyme ecological stoichiometric ratios. The results showed that microorganisms at the degradation sites experienced higher carbon limitations compared to others. Microbial carbon use efficiency at the degradation sites was significantly lower ( $p < 0.05$ ). A trade-off between microbial carbon limitation and carbon use efficiency was observed, as these two factors were negatively associated. Furthermore, microbial carbon use efficiency showed a strong correlation with changes in soil pH. These findings suggest that, to balance microbial carbon limitation and mitigate the adverse effects of soil pH changes, microorganisms allocate more carbon from microbial carbon use efficiency toward the production of relevant extracellular enzymes.

**Keywords:** microbial metabolism, organic matter decomposition, soil properties, invasive plants, wetland ecosystem, carbon cycling

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## Introduction

Wetlands serve as vital ecotones bridging aquatic and terrestrial ecosystems and are a significant component of the global carbon pool [1, 2]. Despite wetlands covering just 5-8% of the earth's surface, they store approximately 15% of global terrestrial carbon [3, 4]. The substantial carbon storage in wetlands, particularly coastal wetlands, can be attributed to the high primary productivity of plants and the slow decomposition rate of belowground organic matter [5]. Thus, the coastal wetland soil carbon pool is sensitive to the vegetation community because plants are the most critical contributors to the soil carbon pool. In recent decades, global coastal wetland vegetation communities have experienced considerable changes, including degradation and/or succession, due to human interference and the invasion of alien species [7-9]. These alterations in wetland vegetation communities inevitably affect coastal wetlands' carbon sequestration function, ultimately exacerbating climate change.

The decomposition of soil organic matter is crucial to ecological processes because it regulates carbon, nutrients, and the conversion processes of other biogeochemical elements [10]. Soil extracellular enzymes are essential facilitators of decomposition [6]. Soil microorganisms typically generate a range of enzymes to enhance the decomposition of organic matter and acquire resources that are bound within it, particularly in situations where they encounter limitations in energy and/or nutrients [1, 11-13]. Therefore, organic matter's decomposition potential and quality could be reflected by the variety and activities of extracellular enzymes and the activities and metabolism of microorganisms in the soil [14, 15]. It has been reported that the metabolic limitation of soil microorganisms is an indicator of the accessibility and availability of soil resources [14, 15]. Microbial metabolic limitation can be evaluated by examining the stoichiometry of extracellular eco-enzymes, which is based on the theories of ecological stoichiometry and ecological metabolism developed by [16, 17]. The stoichiometry of extracellular eco-enzymes can link the availability of environmental resources with the resource acquisition strategies of microorganisms. The linkage is an intuitive response to the microbial metabolic requirements and is regulated by resource acquisition and competition with plants during soil organic matter decomposition [16-20]. Another crucial indicator for assessing the microbial metabolic effect on soil organic matter decomposition is microbial carbon use efficiency, which reflects the proportion of carbon used for physiological growth to total carbon uptake by microorganisms [18, 20]. Microorganisms invest more energy in enzymatic production and resource acquisition during organic matter decomposition to meet their metabolic requirements, and the energy allocated to new biomass biosynthesis is bound to decrease, resulting in low microbial carbon use efficiency [21]. Meanwhile, microbial carbon allocation generally

adheres to an economic principle, aiming to minimize resource expenditure while fulfilling requirements and coping with stresses [5, 22]. Thus, to alleviate environmental stresses and/or resource limitations, microorganisms will regulate the investment between resource acquisition and growth maintenance, inducing a trade-off between metabolic limitation and carbon use efficiency [5, 19].

Plant substrate input (i.e., litter, residues, and root exudates) is the soil's primary source of elemental resources and organic matter. Changes in vegetation communities could impact the microorganisms' metabolic limitations and carbon use efficiency in many ways [23]. For example, it could change the composition, quantity, and quality of the plant substrate input, the vegetation community's nutrient demands, and the microbial community's composition and function. This results in changes in the resource competition between plants and microorganisms and the resource allocation of microorganisms [24-26]. Additionally, alterations in the soil microenvironment, such as physicochemical properties and resource availability, caused by vegetation community degradation or succession are also related to the metabolic limitation and carbon use efficiency of microorganisms in wetland soils [21, 27]. However, there is currently a lack of mechanistic frameworks that fully elucidates the effects of alterations in vegetation communities on soil microbial metabolic limitation and carbon use efficiency. This knowledge gap hinders our understanding of the underlying mechanisms governing soil organic matter decomposition in response to vegetation community degradation or succession in coastal wetlands.

Coastal vegetation communities have been seriously damaged by human interference (e.g., fishing, land use development, environmental, and ecological pollution) and alien invasion through alteration, replacement, and degradation of native vegetation communities. These changes inevitably affect the decomposition of soil organic matter and the cycles of biogeochemical elements by affecting microorganisms' metabolic limitations and carbon use efficiency. Therefore, it is necessary to identify how metabolic limitation and carbon use efficiency of soil microorganisms vary during changes in vegetation community to understand soil microbial responses to vegetation communities alteration and their effects on soil carbon cycles.

Consequently, this study aimed to evaluate the changing trends of soil microbial metabolic limitation and carbon use efficiency under the degradation and succession of vegetation communities induced by the alien plant invasion in coastal wetlands using the ecological stoichiometric ratios of extracellular soil enzymes and to determine the driving factors of such changing trends. Our hypotheses were as follows: (1) both succession and degradation of the vegetation community will inevitably alter soil extracellular enzymatic activities and stoichiometry by changing the vegetative community; (2) the induced changes in soil

extracellular enzymatic activities lead to conversion and intensification of the microbial metabolic limitation via regulation of the soil microbial metabolism; and (3) the enhancement of soil microbial metabolic limitation reduces the efficiency of microbial carbon use.

## Materials and Methods

### Study Region Description and Design

The study region is located on the coastal wetland (32°45' N, 120°50' E) near Dongtai Town, Yancheng City, China. The native plant of this region is *Phragmites australis*. Since the 1990s, with the development of land use and the invasion of the alien plant *Spartina alterniflora*, the ecosystem of this region has suffered damage and vegetation community simplification. In some areas, the invasion of *Spartina alterniflora* has led to the occurrence of vegetation community succession, and some vegetation communities have even been replaced by *Spartina alterniflora*. The vegetation community has degraded considerably in other areas, including exposed tidal flats.

In the study region, nine areas of 5 × 5 m that included three different vegetation community sites (i.e., three replicates for each vegetation community type) were established in October 2022. There were three types of vegetation communities at the sites: the original vegetation community with dominant *Phragmites australis*; the succession site with dominant *Spartina alterniflora* and the vegetation community degradation site with a low plant cover (<10%). Sites with different vegetation types alternated, and the interval distance between two neighboring sites was at least 10 m.

### Soil Samples Collection and Preparation

Composite topsoil samples (0-10 cm) were collected by mixing the soil at 10 random points at each site. The collected composite soil samples were passed through a sieve (2 mm) to remove visible stones and plant debris before further analysis. Each composite sample was divided into two parts. One part was stored at 4°C in the refrigerator until the analyses for soil inorganic nitrogen, microbial biomass, and extracellular enzymatic activity. The other one was air-dried for the analysis of other soil properties.

### Soil Properties Measurement

Soil pH was measured using the potentiometry method. Soil inorganic nitrogen was measured using spectrophotometric methods [28, 29]. The water extraction method measured the soil's dissolved organic carbon and nitrogen [30]. The available phosphorus content in the soil was measured following the molybdate colorimetric method [31]. Microbial biomass carbon, nitrogen, and phosphorus contents in the

soil were measured using the chloroform fumigation extraction method [32-35]. Extracellular enzymatic activities of soil microorganisms were measured as described by [36]. The microbial extracellular enzymes measured were  $\beta$ -1,4-glucosidase (carbon-acquisition enzyme),  $\beta$ -1,4-xylosidase (carbon-acquisition enzyme),  $\beta$ -1,4-cellobiohydrolase (carbon-acquisition enzyme), L-leucine aminopeptidase (nitrogen-acquisition enzyme),  $\beta$ -1,4-N-acetylglucosaminidase (nitrogen-acquisition enzyme), and alkaline phosphatase (phosphorus-acquisition enzyme). The buffer solution was selected according to the pH range of the soil at the study sites [34]. All extracellular enzymatic reactions of soil microorganisms were incubated at 25°C for 2 h in the dark, except for  $\beta$ -1,4-xylosidase and L-leucine aminopeptidase, which were incubated for 6 h. Measurement at excitation and emission wavelengths of 355 and 460 nm was then performed using a multimode microplate reader.

### Statistical Analysis

Microbial metabolic limitations and carbon use efficiency in the soil were inferred using the stoichiometry of extracellular eco-enzymes. Soil microbial metabolic limitations were assessed by calculating vector length and angle, representing microbial carbon and nutrient (nitrogen or phosphorus) limitations. The calculation methods of soil microbial metabolic limitations and carbon use efficiency followed those described by [19, 21].

A one-way analysis of variance was carried out to determine statistical differences in soil properties, microbial metabolic limitations, and carbon use efficiency. Significant differences between means were established using Fisher's least significant difference test. Pearson correlation analyses were conducted to test the relationships between soil parameters, microbial metabolic limitations, and carbon use efficiency. Based on the results of Pearson correlation analyses, partial least squares path modeling was carried out to evaluate the possible pathway by which vegetation community alteration affects the metabolic limitations and carbon use efficiency of soil microorganisms. All analyses were conducted with R software 4.0.2 (R Core Team) [37]. Differences at  $p < 0.05$  were considered significant.

## Results and Discussion

### Effects of Vegetation Community Alteration on the Activities and Stoichiometry Soil Microbial Extracellular Enzymes in the Soil of Coastal Wetland

The mean activities of microbial extracellular carbon (EEA<sub>C</sub>), nitrogen (EEA<sub>N</sub>), and phosphorus (EEA<sub>P</sub>) acquisition enzymes were 1.85-12.09, 0.63-1.20, and 3.22-4.85, respectively. Only significant differences

among the study sites were observed in the EEAC and EEAP (all  $p < 0.01$ ). The  $EEA_C$  and  $EEA_N$  showed a similar variation trend among the study sites. The  $EEA_C$  and  $EEA_N$  in the vegetation community degradation site were 5.54 and 0.90 times and 4.93 and 0.89 times higher than those in the original and succession vegetation community sites, respectively. The  $EEA_p$  in the original vegetation community site was 0.38 and 0.29 times higher than those in the succession and degradation sites, respectively (Fig. 1(a,b,c)).

Researchers initially hypothesized that vegetation community alteration would reduce the extracellular enzyme activity of soil microorganisms because this alteration could lead to resource fluctuation, thereby inducing competition between plants and soil microorganisms and reducing the extracellular enzyme activity of soil microorganisms [38-39]. However, vegetation community succession considerably decreased  $EEA_p$ , compared to the original vegetation community sites. However, vegetation community degradation substantially increased and decreased  $EEA_C$  and  $EEA_p$ , respectively (Fig. 1). This might be because of higher changes in extracellular enzymes, which considerably fluctuate the physiochemical and biogeochemical properties of the soil and are mainly related to changes in the soil's available nutrients [17, 40-41]. Additionally, soil nutrient contents fluctuate due to the production of exhaustive crops and invasive alien plants, which draw higher nutrient levels from the soil according to their demands [41, 42]. This suggests that nutrient stoichiometry and imbalance may be one of the reasons for the production of soil enzymes and changes in their activities. This is supported by significant correlations between inorganic nitrogen and dissolved organic carbon and extracellular enzyme activities (Figure 4; Table S1). During vegetation community alterations (such as succession or degradation), the substrate (i.e., litter, residues, and root exudates) input into the soil from the plant may be an essential mechanism to regulate soil available nutrients. Therefore, adding mulch to the soil increases the water and nutrient retention capacity, which deliberately enhances soil carbon capacity, composition, and fertility to increase the production of crops [43-46]. Previous studies have demonstrated that the activity of microbial extracellular enzymes could be inhibited through interactions with substrates released by *Spartina alterniflora* [44]. This suggests that the difference in enzyme activity during vegetation community alteration may be caused by the interaction between the chemical compounds released by plant allelopathy or the secondary compounds and microbial functional groups in the soil [47-48].

Among all study sites, the mean ratios of  $EEA_C$  to  $EEA_N$  ( $EEA_{C:N}$ ),  $EEA_C$  to  $EEA_p$  ( $EEA_{C:P}$ ), and  $EEA_N$  to  $EEA_p$  ( $EEA_{N:P}$ ) ranged from 2.90 to 10.82, 0.38 to 3.22, and 0.13 to 0.32, respectively. The highest and lowest ratios were observed in the degradation and original vegetation sites, but differences were only significant ( $p < 0.01$ ) in  $EEA_{C:N}$  and  $EEA_{C:P}$ . The  $EEA_{C:N}$  and  $EEA_{C:P}$

in the degradation sites were significant, at 2.73 and 7.47 times and 2.21 and 3.35 times higher than those in the original and succession sites, respectively (Fig. 1(d,e,f)).

In this study, the extracellular enzyme stoichiometric ratio of soil microorganisms at succession and original sites decreased significantly compared to that at degradation sites (Fig. 1). This indicates that the presence of vegetation can reduce the extracellular enzyme stoichiometric ratio. In addition, the effects of succession and original sites on microbial extracellular enzymes are similar. One of the reasons for this may be that vegetation increases nitrogen deposition in the atmosphere and leads to a higher soil nitrogen content than at the degradation site. Such a nitrogen-rich environment would enhance the interaction between bacteria and fungi in the soil, thus reducing the extracellular enzyme activity of soil microorganisms [49].

It was predicted that changes in the vegetation community would lead to changes in the metabolic limitations of soil microorganisms, which could be reflected in the ecological enzymatic stoichiometry in the soil. However, contrary to this hypothesis, significant changes were only observed in microbial carbon limitations ( $p < 0.01$ ) between the degradation and succession sites (Fig. 2). Combined with the results of the considerable variations in  $EEA_C$ ,  $EEA_p$ , and  $EEA_{C:P}$ , microorganisms in the degradation sites experienced a higher carbon limitation than those in other sites, where the highest value of VL was observed. The relatively low soil pH may cause higher microbial carbon limitations in the altered vegetation community sites. This is especially true for degradation sites, where VL showed a close relationship with soil pH when compared to the original sites (Figs 3 and 4). Soil pH can alter the microbial community's abundance, composition, and structure [50]. The carbon requirements of microorganisms change as the microbial community converts.

#### Effects of Vegetation Community Alteration Soil Microbial Metabolic Limitations and Carbon Use Efficiency in the Soil of Coastal Wetland

The results revealed that VL is significantly positively correlated with pH ( $R^2 = 0.81$ ,  $p < 0.01$ ), inorganic nitrogen ( $R^2 = 0.57$ ,  $p < 0.05$ ),  $EEA_C$  ( $R^2 = 0.91$ ,  $p < 0.01$ ),  $EEA_{C:N}$  ( $R^2 = 0.99$ ,  $p < 0.01$ ), and  $EEA_{C:P}$  ( $R^2 = 0.91$ ,  $p < 0.01$ ). The VA is significantly correlated with  $EEA_C$  ( $R^2 = -0.81$ ,  $p < 0.01$ ),  $EEA_N$  ( $R^2 = -0.97$ ,  $p < 0.01$ ),  $EEA_{C:P}$  ( $R^2 = -0.83$ ,  $p < 0.01$ ), and  $EEA_{N:P}$  ( $R^2 = -0.99$ ,  $p < 0.01$ ). The microbial carbon use efficiency showed a significant correlation with  $EEA_C$  ( $R^2 = -0.87$ ,  $p < 0.01$ ),  $EEA_{C:N}$  ( $R^2 = -0.92$ ,  $p < 0.01$ ), and  $EEA_{C:P}$  ( $R^2 = -0.87$ ,  $p < 0.01$ ) (Fig. 3). The partial least squares path modeling analysis showed that soil property changes caused by vegetation community alteration affected extracellular enzyme

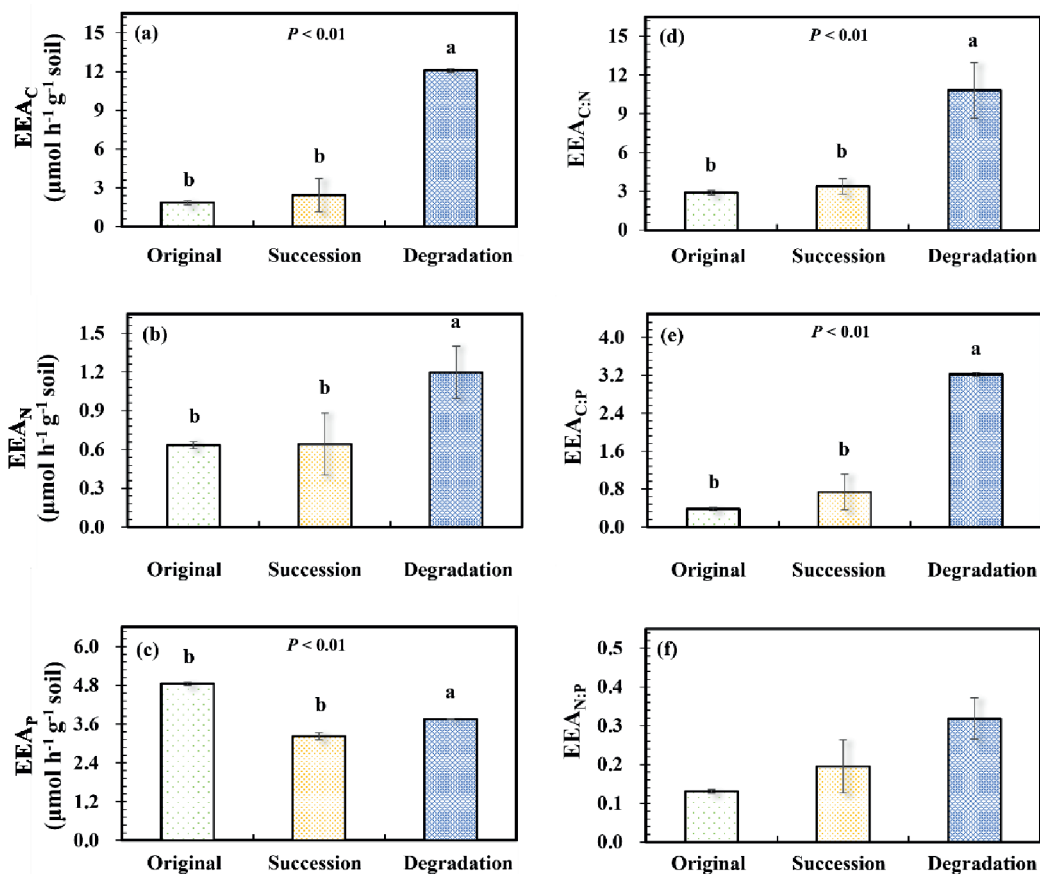


Fig. 1. Soil microbial extracellular enzymic activities and stoichiometry in original, succession, and degradation vegetation community sites. a) Microbial extracellular carbon-acquisition enzymic activity ( $EEA_C$ ); b) Microbial extracellular nitrogen-acquisition enzymic activity ( $EEA_N$ ); c) Microbial extracellular phosphorus-acquisition enzymic activity ( $EEA_P$ ); d) the ratio of  $EEA_C$  to  $EEA_N$  ( $EEA_{C:N}$ ); e) the ratio of  $EEA_C$  to  $EEA_P$  ( $EEA_{C:P}$ ); and f) the ratio of  $EEA_N$  to  $EEA_P$  ( $EEA_{N:P}$ ). Different lower-case letters above column represent significant differences ( $p < 0.050$ ) among the study sites.

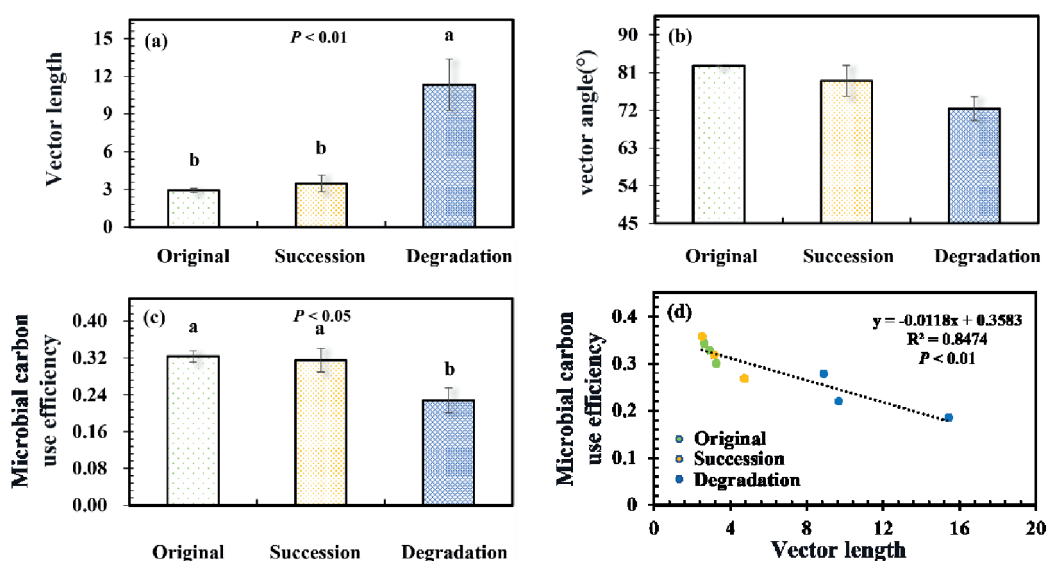


Fig. 2. Soil microbial metabolism in original, succession, and degradation vegetation community sites. a) Soil microbial carbon limitation (Vector length); b) Soil microbial nutrient limitation (Vector angle); c) Soil microbial carbon use efficiency; and d) the correlation between soil microbial carbon limitation and microbial carbon use efficiency. Different lower-case letters above the column represent significant differences ( $p < 0.050$ ) among the study sites.

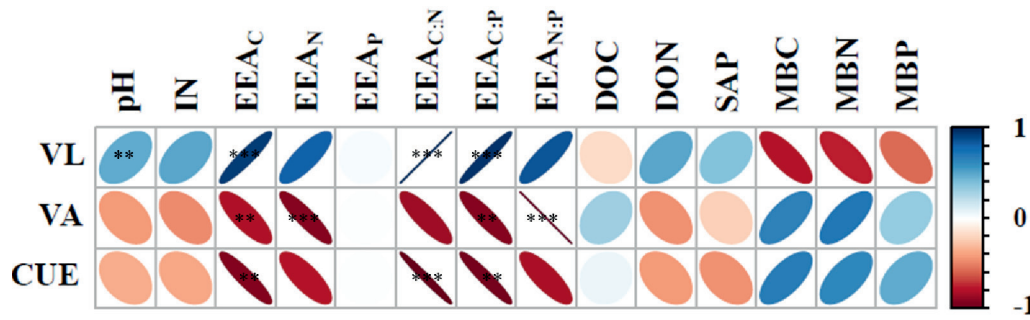


Fig. 3. Heatmap of the results of the Pearson correlation analyses between the soil parameters and microbial metabolic limitations and carbon use efficiency. VL = vector length; VA = vector angle; CUE = microbial carbon use efficiency; IN = inorganic nitrogen;  $EEA_C$  = carbon-acquisition microbial extracellular enzyme activity;  $EEA_N$  = nitrogen-acquisition microbial extracellular enzyme activity;  $EEA_P$  = phosphorus-acquisition microbial extracellular enzyme activity;  $EEA_{C:N}$  = the ratio of  $EEA_C$  to  $EEA_N$ ;  $EEA_{C:P}$  = the ratio of  $EEA_C$  to  $EEA_P$ ;  $EEA_{N:P}$  = the ratio of  $EEA_N$  to  $EEA_P$ ; DOC = dissolved organic carbon; DON = dissolved organic nitrogen; SAP = available phosphorus; MBC = microbial biomass carbon; MBN = microbial biomass nitrogen; MBP = microbial biomass phosphorus. \*\*\* = significant at the level of  $p < 0.001$ , \*\* = significant at the level of  $p < 0.010$ , and \* = significant at the level of  $p < 0.050$ .

activities, stoichiometric ratios, and ultimately carbon use efficiency by influencing the metabolic limitation indices of soil microorganisms (Fig. 4). The effect of extracellular enzymes on VL was positive, while the effect of extracellular enzyme stoichiometric ratio on VA was negative. In addition, pH, VA, and VL were the direct drivers of microbial carbon use efficiency changes at all sites.

The patterns of soil microbial metabolic limitations reflected by extracellular eco-enzymatic stoichiometry varied at different sites with vector length (VL) and angle (VA) values of 2.51-15.42 and 69.18-84.54°, respectively. In the degradation site, the VL was significantly ( $p < 0.01$ ) higher than that in the succession and original vegetation sites (226.22% and 287.67%, respectively). The VA was 9.14% and 14.43% lower than that in the succession and original vegetation sites, respectively, but was insignificant ( $p > 0.05$ ). In addition, all values for VA were  $> 45^\circ$ , indicating that the microbial community at all study sites showed phosphorus limitation (Figs 2(a,b)). All VA values were more prominent than  $45^\circ$ , indicating that phosphorus was the limited element for soil microorganisms at all sites. Microbial phosphorus limitation increased with alterations in the vegetation community, but not significantly ( $p > 0.05$ ). Correspondingly, the lower VL and higher VA of the succession and original sites indicate that the microbial carbon limitation at these two sites will gradually transform into phosphorus limitation, which leads to the intensification of phosphorus limitation at the succession sites. Microbial phosphorus limitation can significantly affect microbial growth and metabolism because microorganisms must maintain a balance of nutrient access to survive. Therefore, a high-intensity phosphorus limitation will intensify the phosphorus competition between plants and soil microorganisms, resulting in the survival of native plants and the decrease of microbial activity, causing the succession of original vegetation communities to specific communities. This

is one explanation for why *Spartina alterniflora* has gradually invaded the *Phragmites australis* native plant in coastal wetlands. Previous studies have shown that if phosphorus becomes relatively limited, microorganisms might consume more carbon and nitrogen to produce and exfiltrate enzymes associated with phosphorus metabolism [51]. Most of the phosphorus required by microorganisms originates from the decomposition of soil organic matter, which is the primary substrate for enzymatic activity [52-53]. Hence, a large amount of soil

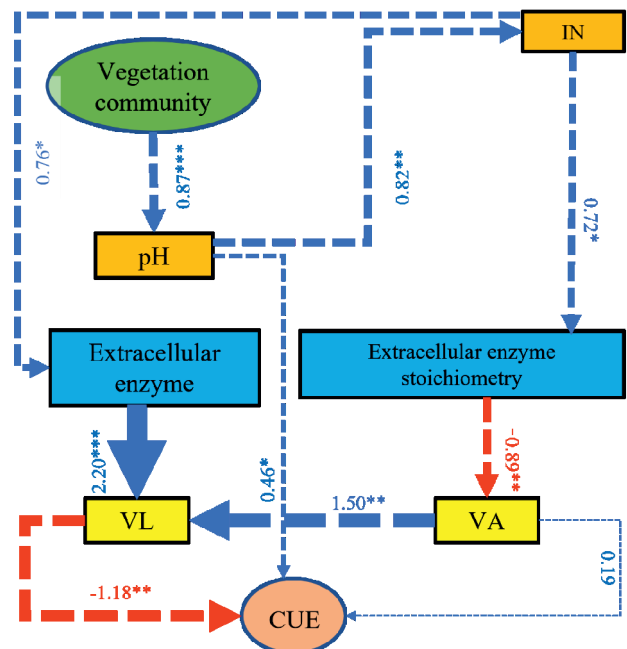


Fig. 4. Partial least squares path modeling result shows potential influence paths of vegetation community alteration on soil microbial metabolic limitations and carbon use efficiency. Blue and red arrows indicate positive and negative causal flows. \*\* = significant at the level of  $p < 0.010$ , and \* = significant at the level of  $p < 0.050$ .

organic matter decomposition leads to increased carbon and nitrogen in the soil, which may partially explain the decreasing carbon restriction of soil microorganisms. In addition, it offers one explanation for the low extracellular enzyme activity of soil microorganisms at the succession site. In conclusion, vegetation community succession had no significant effect on microbial metabolic limitation, but vegetation community degradation significantly affected microbial metabolic limitation.

Soil microbial carbon use efficiency significantly varied between 18.54% and 35.81% among all study sites ( $p < 0.05$ ). Soil microbial carbon use efficiency in the degradation site was 34.78% and 39.13% lower than in the succession and original vegetation sites, respectively (Fig. 2c). Soil microbial carbon use efficiency is significantly negatively correlated with VL ( $R^2 = 0.85$ ,  $p < 0.01$ ; Fig. 2d).

In this study, the soil microbial carbon use efficiency had an average value of 0.30, which aligns with the results of previous research conducted by [54-55]. The soil microbial carbon use efficiency in the vegetation community degradation site was significantly lower than in the other study sites (Fig. 2). Microbial carbon use efficiency describes the proportion of carbon allocated to the growth of total carbon source uptake by microorganisms [14, 18, 20]. The considerably lower microbial carbon use efficiency at the degradation site indicated relatively more carbon consumption via microbial respiration and less carbon sequestration in the microbial biomass, which suggests a tremendous potential to release carbon dioxide into the atmosphere, promoting global warming.

In addition, partial least squares path modeling exhibited that soil pH can not only directly affect microbial carbon use efficiency but also change the activities and stoichiometric balance of extracellular enzymes (Fig. 4). These results indicate that soil pH is a crucial factor that affects soil microbial carbon use efficiency by changing the structure and function of the microbial community [17]. The strong correlation observed between soil pH and microbial carbon limitation was in line with previous studies that reported that altered soil pH could directly or indirectly alter the composition, structure, and function of the microbial community, thereby inducing changes in carbon use efficiency (Fig. 3) [17]. However, the variation pattern of microbial carbon use efficiency was roughly opposite the microbial carbon limitation pattern during the vegetation community alteration process (Figs 1 and 2) [49]. Furthermore, partial least squares path modeling and relationship analysis confirmed that microbial carbon limitation has a strong negative effect on microbial carbon use efficiency (Fig. 4), supporting the third hypothesis. The pH-induced reduction in microbial carbon limitation led to the corresponding enhancement in carbon use efficiency, which could be attributed to a trade-off between microbial carbon limitation and carbon use efficiency, reducing the investment

in microbial activities (such as enzyme production) and enhancing microbial growth to maintain the consumption of carbon as decomposition progresses [27].

As discussed, the changes in the substrate (i.e., litter, residues, and root exudates) input into the soil from the plant may be another crucial mechanism that shifts carbon use efficiency during the process of vegetation community alteration (e.g., succession or degradation). The quality and quantity of plant substrate input are important carbon sources for microorganisms, which can directly affect the uptake and utilization of carbon by microorganisms. Therefore, the relatively low vegetation cover in the vegetation community degradation site would lead to lower quality and quantity of plant substrate input than other sites, thereby inducing resource competition between plants and microbes and within the microbial community. The competition may further alter the physiology and behavior of individual microorganisms and affect the microbial community's structure and function, ultimately affecting microorganisms' carbon use efficiency [56]. Fungal interaction can reduce carbon use efficiency because interspecific interaction could change the microbial respiration rate, decomposition ability, and extracellular enzyme production [53-56]. Thus, to balance microbial carbon limitation and offset the adverse effects of pH and substrate changes caused by vegetation community alteration, microorganisms would take a trade-off and allocate less energy to new biomass biosynthesis and more energy to produce related extracellular enzymes.

#### Ecological Effects of Vegetation Community Alteration

Despite coastal wetlands providing essential ecosystem functions, they are ecologically sensitive and fragile zones due to their unique ecotone environment located between aquatic and terrestrial ecosystems. They are incredibly vulnerable to the effects of global climate change and human activities, such as warming, sea level rise, and land use change, leading to the destruction of ecosystem structure and loss of ecosystem function. In recent decades, due to human interference and invasive species, China's coastal ecosystems have been considerably damaged by the replacement of native vegetation with invasive plants (e.g., *Spartina alterniflora*), a steep decline in vegetation community diversity, and the degradation of the vegetation community.

Microbial metabolism has also been used to infer organic matter decomposition in soil [1]. This organic matter decomposition releases most nutrients to the soil profiles, which are available to the plants and primarily used for crop productivity [57, 58]. Additionally, invasive alien plants have a harmful direct and indirect impact on the soil's nutritional composition, which lowers crop yield and promotes biological invasion in the agroecosystem [59]. Therefore, the present study found

that the negative influence of vegetation community degradation on microbial metabolism, as reflected by microbial carbon limitation and carbon use efficiency, was more drastic than that of the vegetation community replaced by *Spartina alterniflora* (Figs 1 and 2). The decrease in microbial carbon use efficiency could represent greater carbon allocation to microbial activity (e.g., enzyme production) and the potential to promote microbial respiration and organic matter decomposition. Synthesizing the findings from previous studies with the results of the present study, it is evident that changes in vegetation communities will undoubtedly impede the turnover and storage of carbon. These alterations have a direct impact on microbial metabolism, leading to an increase in carbon emissions into the atmosphere. Moreover, they also facilitate microbial respiration and the decomposition of organic matter, further exacerbating the issue and contributing to climate change.

### Conclusions

This study provides a unique assessment of microbial extracellular enzyme activities, metabolic limitations, and carbon use efficiency in wetland soils across various vegetation communities. The study found that vegetation community degradation led to considerable variations in  $EEA_C$ ,  $EEA_p$ , and ecological enzyme stoichiometric ratios, such as  $EEA_{C:N}$  and  $EEA_{C:P}$ . Therefore, microorganisms in degraded vegetation communities experienced higher carbon limitation and lower carbon use efficiency compared to microorganisms in other sites. Moreover, microbial carbon use efficiency was negatively correlated with microbial carbon limitation and highly correlated with soil pH. The underlying mechanism behind these results is the trade-off between microbial carbon limitation and microbial carbon use efficiency in response to environmental stress. To balance microbial carbon limitation and offset the adverse effects of pH changes caused by vegetation community alteration, microorganisms allocate less energy to new biomass biosynthesis and more energy to producing related extracellular enzymes. This study demonstrated that microbial metabolism could be an effective and sensitive indicator and provided a preliminary drawing of the responses of microbial metabolism to vegetation community alteration in coastal wetlands. Therefore, microbial metabolism holds the potential for monitoring future carbon turnover and storage in coastal wetlands, as well as their feedback to climate change.

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### Conflict of Interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary Material

Table S1. Soil properties among study sites, presented as means  $\pm$  standard errors (n = 3).

Parameters	F	<i>p</i>	Original	Succession	Degradation
pH	15.34	**	8.57 $\pm$ 0.08b	8.45 $\pm$ 0.05b	9.00 $\pm$ 0.09a
IN ( $\mu\text{g}^{-1}$ N g soil)	4.28	*	2.15 $\pm$ 0.15b	2.37 $\pm$ 0.65b	7.14 $\pm$ 1.81a
DOC (mg C g <sup>-1</sup> soil)	2.63	ns	0.21 $\pm$ 0.01	0.25 $\pm$ 0.02	0.22 $\pm$ 0.01
DON ( $\times 10$ mg N g <sup>-1</sup> soil)	5.54	*	0.13 $\pm$ 0.01b	0.11 $\pm$ 0.01ab	0.17 $\pm$ 0.02a
SAP (mg P g <sup>-1</sup> soil)	0.55	ns	0.29 $\pm$ 0.06	0.22 $\pm$ 0.08	0.21 $\pm$ 0.01
MBC (mg C g <sup>-1</sup> soil)	3.10	ns	0.45 $\pm$ 0.01	0.69 $\pm$ 0.17	0.35 $\pm$ 0.01
MBN ( $\times 10$ mg N g <sup>-1</sup> soil)	3.55	ns	0.37 $\pm$ 0.01	0.49 $\pm$ 0.11	0.25 $\pm$ 0.01
MBP (mg P g <sup>-1</sup> soil)	5.00	ns	0.07 $\pm$ 0.02	0.20 $\pm$ 0.06	0.04 $\pm$ 0.02

IN = inorganic nitrogen; DOC = dissolved organic carbon; DON = dissolved organic nitrogen; SAP = available phosphorus; MBC = microbial biomass carbon; MBN = microbial biomass nitrogen; MBP = microbial biomass phosphorus. \* = significant at the level of  $p < 0.050$ , and \*\* = significant at the level of  $p < 0.010$ . Different lower-case letters above column represent significant differences among the study sites.