Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Degradation exacerbates C and P limitations of soil microorganisms in the wetland ecosystems

Mingli Zhang
Zhengzhou University
Yike Huang
Zhengzhou University
Xinyu Li
Zhengzhou University
Zhiwei Mao
Zhengzhou University
Kexin Chen
Zhengzhou University
Mei Liu
Henan University of Technology
Yadong Xu
Zhengzhou University
Wenkai Chen
Chengdu Botanical Garden
Lin Wang
Department of Natural Resources of Henan Province
Ming Dou
Zhengzhou University
Junxiang Ding
dingjunxiang@zzu.edu.cn

Zhengzhou University

Research Article

Keywords: soil extracellular enzymes, enzymatic stoichiometry, soil microbial metabolic limitations, riparian wetlands, the Yellow River

Posted Date: July 27th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10228261/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Purpose

Wetland soils are critical hotspots for biogeochemical cycling mediated by microbial extracellular enzymes, yet they are severely affected by ongoing wetland degradation. However, the patterns of extracellular enzyme activities, the associated microbial metabolic limitation, and their key drivers along wetland degradation gradients remain unclear.

Methods

We quantified extracellular enzyme activities associated with carbon (C), nitrogen (N), and phosphorus (P) acquisition, together with plant community attributes and environmental variables, across a wetland degradation gradient in the lower Yellow River wetlands, China, to investigate the response of microbial metabolism to habitat degradation.

Results

Wetland degradation significantly decreased the activities of C-, N-, and P-acquiring enzymes, and increased enzymatic C:N and C:P ratios. The enzymatic stoichiometry modeling further revealed a coupled C and P limitation for microbial metabolism in degraded wetlands, which intensified along the degradation gradient. These changes were primarily driven by plant community characteristics (e.g., plant density), soil environment (pH and electrical conductivity), and nutrient availability. Structural equation modeling showed that wetland degradation resulted in C and P limitation primarily via altering soil electrical conductivity and nutrient availability.

Conclusion

Our results suggest that degradation-induced changes in plant community and soil factors jointly drive C and P co-limitation of microbial metabolism in wetland ecosystems. This understanding highlights the importance of integrating plant–soil interactions and their effects on microbial functions and ecosystem processes in the restoration and management of degraded wetlands.

Introduction

Wetlands are widely recognized as the ‘kidneys of the Earth’ and also serve as a critically important ‘Blue Carbon (C) Sink’ (Kangabam et al. 2016). Although covering only a small portion of the world’s land, wetlands play a critical role in soil C sequestration, as their C storage per unit area far exceeds that of other ecosystems (Mitra et al. 2005). The substantial C sequestration potential of wetland soil is mediated by microbial metabolic processes (including growth, respiration, and turnover), which are often

limited by the availability of C and nutrients (e.g., nitrogen and phosphorus) (Crowther et al. 2019; Philippot et al. 2024). However, recent intensification of anthropogenic activities and climate change has accelerated wetland degradation, leading to significant alterations in both the biotic (e.g., plant productivity) and abiotic components (e.g., soil physicochemical properties) of wetland ecosystems (Fluet-Chouinard et al. 2023; Wang et al. 2022). These changes can alter the activities and stoichiometry of soil extracellular enzymes involved in C, nitrogen (N), and phosphorus (P) acquisition, consequently influencing microbial metabolic limitations (Burns et al. 2013; Cui et al. 2022). Given the critical role of microbial metabolic processes in wetland soil C and nutrient cycling, it is essential to elucidate the resource limitation characteristics of microbial growth and activity in wetland ecosystems under degradation (Burns et al. 2013; Mooshammer et al. 2014).

According to the resource allocation theory, microorganisms invest more resources in producing extracellular enzymes to acquire relatively limited elements (Sinsabaugh et al. 2009; Sinsabaugh and Shah 2012; Yang et al. 2020). Therefore, enzymatic stoichiometry theory, which reflects the balance between microbial biomass stoichiometry and the elemental composition of soil organic matter, has been widely used to assess microbial resource limitation across ecosystems (Cao et al. 2022; Cui et al. 2025; Sinsabaugh 2010). For instance, enzymatic stoichiometric analyses have revealed prevalent P limitation in tropical forest and peatland ecosystems, whereas microbial communities in ultra-oligotrophic desert ecosystems are often co-limited by C and N (Camenzind et al. 2018; Sun et al. 2024). At a global scale, the ratio of C:N:P-acquiring enzymes in topsoil has been observed to average approximately 1:1:1. This convergent enzymatic stoichiometry has been confirmed by local and regional studies, though notable deviations from the 1:1:1 ratio have also been reported (Auwal et al. 2023; Liu et al. 2023). These divergent findings suggest that the ecoenzymatic stoichiometry could be regulated by multiple factors, such as vegetation (e.g., biomass and substrate quality), soil resource availability (Gomez et al. 2020; Stock et al. 2019; Ullah et al. 2023). Vector analysis has further extended the application of ecological stoichiometry theory by offering a quantitative framework to assess microbial nutrient acquisition and metabolic limitation under environmental change (Cui et al. 2025; Moorhead et al. 2016). For example, it revealed a shift from P to N limitation in soil microbes during grassland restoration, despite initial N and P co-limitation (Yang et al. 2020). Together, enzymatic stoichiometry and vector analysis provide mechanistic insights into microbial resource limitation, linking microbial processes to plant succession and ecosystem functioning. However, due to the high environmental complexity and heterogeneity of wetland ecosystems, microbial resource limitations and their underlying drivers have received less attention in these systems than in other ecosystems (Cui et al. 2022; Shahariar et al. 2021; Yang et al. 2024).

Wetland degradation alters plant productivity and the quantity and quality of litter. For instance, as degradation intensifies, aboveground biomass decreases (Song et al. 2024), and the species richness of plants with higher-quality leaves (e.g., nutrient-rich) also declines significantly (Ding et al. 2025a; Xu et al. 2024). These degradation-induced changes imply a reduction in soil resources available to microorganisms, particularly labile C, and are likely to exacerbate microbial C limitation (Zhang et al. 2025; Zhou et al. 2020). Additionally, soil organic C has been found to decline significantly with

increasing wetland degradation, likely reducing C availability for microorganisms and further exacerbating microbial C limitation (Ding et al. 2025b; Xu et al. 2024). Another critical feature of wetland degradation is soil nutrient deficiency and stoichiometric imbalance. While some studies report decreases in total N, total P, C:P, and N:P with increasing degradation (Song et al. 2024; Wang et al. 2022; Xu et al. 2024); others find that changes in C:N and N:P are relatively small despite declining N and P contents (Ding et al. 2025b; Wan et al. 2024). These nutrient changes suggest that microbial N and P limitations are likely to intensify with degradation, but empirical validation is needed. Furthermore, soil pH and salinity have also been shown to significantly influence microbial activity and community structure (including composition and diversity) (Acosta-Martínez et al. 2007; Cao et al. 2022; Gomez et al. 2020). Therefore, degradation-induced changes in soil environments (e.g., soil pH, electrical conductivity [EC]) and resource availability are expected to drive changes in microbial resource limitation patterns.

The lower reaches of the Yellow River in China host extensive riparian wetlands due to its unique hydrological conditions. In recent decades, however, intensified human activities and climate change have resulted in continued wetland loss in the region, accompanied by ecological degradation such as nutrient loss and declining biodiversity (Fluet-Chouinard et al. 2023; Wang et al. 2022). These ecological characteristics provide an ideal platform for examining microbial resource limitation patterns and their drivers in wetland ecosystems under changing environmental conditions. Recent studies in the lower Yellow River wetlands report that aboveground biomass, soil moisture, and nutrient availability decline with increasing degradation (Huang et al. 2026). These findings imply that variations in microbial enzyme activities, stoichiometric ratios, and vector characteristics along degradation gradients are likely to be co-regulated by plant productivity and soil resource availability (e.g., moisture and nutrients). However, current research on the lower Yellow River riparian wetlands has focused on hydrological processes (Ji et al. 2021; Meng et al. 2024), vegetation succession (Peng et al. 2023; Zhang et al. 2025), and soil nutrients (Yan et al. 2025), a critical gap remains in understanding microbial metabolic responses to degradation from an enzymatic stoichiometry perspective. In this study, we explored the patterns of microbial metabolic limitations across a well-characterized degradation gradient in the riparian wetlands of the lower Yellow River and identified the key environmental drivers underlying these changes. Specifically, we measured four hydrolases associated with microbial acquisition of C, N, and P (i.e., β-1,4-glucosidase, N-acetyl-β-D-glucosaminidase, leucine aminopeptidase and alkaline phosphatase), and calculated their stoichiometric ratios, vector angles, and lengths to assess the variations in microbial metabolic limitation along the wetland degradation gradient. We then performed redundancy analysis (RDA) and variation partitioning analysis (VPA) to test the effects of biotic and abiotic factors on soil extracellular enzyme activities and their stoichiometric ratios. Based on the above analyses, we aimed to test the following three hypotheses: (1) Wetland degradation would suppress the activities of soil extracellular enzymes related to C, N, and P acquisition, because reduced moisture and nutrient availability in degraded wetlands limit microbial metabolism and enzyme synthesis (Song et al. 2024; Turner et al. 2024); (2) microbial C and P limitation would intensify along the degradation gradient;

and (3) Changes in microbial metabolic limitation are primarily regulated by soil physicochemical constraints (e.g., soil pH, moisture, and available nutrients).

1 Materials and Methods

1.1 Study area and site selection

The study area is located in the Henan section of the lower Yellow River, extending from 113.65° to 114.70°E, 34.90° to 34.91°N, with an elevation of 74 to 93 meters. The region is characterized by a warm-temperate continental monsoon climate, with a mean annual temperature of 12–16°C and mean annual precipitation between 500 and 800 mm, which is predominantly concentrated from June to September. Periodic flooding during the Yellow River's flood season (July to October) has facilitated the development of extensive riparian wetland ecosystems along the riverbanks. The dominant soil type is alluvial soil, and the common wetland vegetation mainly includes *Phragmites australis* (Cav.) Trin. ex Steud., *Aster subulatus* Michx., *Tamarix chinensis* Lour., *Cynodon dactylon* (L.) Pers., *Imperata cylindrica* (L.) Raeusch., *Salix matsudana* Koidz., *Conyza canadensis* (L.) Cronquist., and *Potentilla supina* L., consisting primarily of annual and perennial herbaceous species.

The study area encompasses extensive riparian wetlands along the lower Yellow River, many of which are undergoing varying degrees of degradation due to natural and anthropogenic factors, particularly the annual water and sediment regulation scheme initiated in 2002 (Chu 2014; Ding et al. 2025a). To assess wetland distribution and health, we conducted a three-year plant community survey (2021–2023). Based on the collected data for vegetation, soil properties, and nutrients, we then selected nine wetlands representing different degradation levels—light (n = 3), moderate (n = 3), and severe (n = 3)—as our experimental sites (Song et al. 2024; Wang et al. 2022). Specifically, lightly degraded wetlands were characterized by higher vegetation coverage (> 54%) and soil water content (32.96%–37.16%). Moderately degraded wetlands had vegetation coverage of 32%-45%, with soil moisture (17.40%–24.38%) being lower than that in lightly degraded wetlands. Severely degraded wetlands showed the lowest vegetation coverage (17%–23%) and soil water content (15.37%–20.81%). Detailed environmental characteristics and community characteristics are provided in Table S1 and Table S2.

1.2 Field sampling

During the peak growing season, three to five experimental plots (10 m × 10 m each) were established at each selected sampling site, with geographic coordinates (i.e., latitude, longitude, and altitude) recorded. Within each plot, three 1 m × 1 m subplots were arranged along the diagonal for community surveys and sample collection. The surveyed plant community characteristics included mean plant height, plant coverage, species composition, and individual plant counts. For each species present in the plot, five healthy and representative individuals were selected, and their heights were measured using a measuring tape to determine the average height. Plant coverage was estimated using the visual estimation method. Five soil cores were randomly collected from each plot using a soil auger (5 cm

inner diameter, 20 cm length) and thoroughly mixed to form a composite sample. All collected samples were placed in a low-temperature storage box and transported to the laboratory for subsequent analysis.

1.3 Determination of soil physicochemical properties

The collected soil samples were sieved through a 2-mm mesh and divided into two aliquots. One aliquot was stored at -20°C for subsequent analysis of soil moisture and inorganic N. The other aliquot was air-dried, ground, and analyzed for pH, EC, total C, total N, and total P. Soil water content was determined by measuring the weight loss after drying at 65°C for 72 hours to a constant weight. Inorganic N was extracted with 2 M KCl and measured using a continuous flow analyzer (AutoAnalyser 3; Seal Analytical GmbH, Germany). Soil pH and EC were measured with a glass electrode (model PHS-2; INESA Instrument, China). Total C and total N were analyzed using an elemental analyzer (Vario EL III; Elementar, Langenselbold, Germany). Total P was digested with H_2SO_4 and $HClO_4$ and measured by inductively coupled plasma optical emission spectrometry (ICP-OES; Optima 5300 DV, PerkinElmer). Available P was extracted with 0.5 M $NaHCO_3$ and determined by the molybdenum blue colorimetric method.

1.4 Determination of soil microbial extracellular enzyme activities and biomass

The activities of C-acquiring enzyme (β-1,4-glucosidase), N-acquiring enzymes (N-acetyl-β-D-glucosaminidase and leucine aminopeptidase), and P-acquiring enzyme (alkaline phosphatase) were measured using a fluorometric assay (German et al. 2011). The functions and substrates of these enzymes are summarized in Table S3. Briefly, 1.0 g of fresh soil was homogenized in 40 mL of 50 mM acetate buffer, with the buffer pH adjusted to match the soil pH. The soil slurry was sonicated (300 W, 60 s) to ensure thorough dispersion. A 200 µL aliquot of the slurry was transferred to a black 96-well microplate, followed by the addition of 50 µL of the corresponding 200 µM fluorescently labeled substrate solution. Each soil sample was analyzed with three technical replicates. Standard curves were developed by adding 200 µl acetate buffer to different concentration gradients of standards (4-methylumbelliferone for β-1,4-glucosidase, N-acetyl-β-D-glucosaminidase and alkaline phosphatase and 7-amino-4-methylcoumarin for leucine aminopeptidase). The microplates were incubated at 25°C for 2 hours for N-acetyl-β-D-glucosaminidase and alkaline phosphatase, and for 4 hours for β-1,4-glucosidase and leucine aminopeptidase. Fluorescence intensity was measured with a multimode microplate reader (Tecan Spark, Männedorf, Switzerland) at an excitation wavelength of 360 nm and an emission wavelength of 460 nm. Soil enzyme activities were expressed in nmol h^{-1} g^{-1} soil. Soil microbial biomass C was determined by the chloroform fumigation–extraction method (Vance et al. 1987).

1.5 Statistical analysis

To characterize microbial nutrient metabolic limitations, this study utilized the activities and stoichiometric patterns of extracellular enzymes associated with C, N, and P acquisition as indicators. Specifically, we calculated the ratios of four key enzyme activities: (1) Enzyme C:N = ln(β-1,4-glucosidase): ln(N-acetyl-β-D-glucosaminidase + leucine aminopeptidase); (2) Enzyme C:P = ln(β-1,4-

glucosidase): ln(alkaline phosphatase); and (3) Enzyme N:P = ln(N-acetyl-β-D-glucosaminidase + leucine aminopeptidase): ln(alkaline phosphatase). To quantify the relative degree of microbial C, N, and P limitations, we further applied a vector analysis model based on enzymatic stoichiometry, calculating vector length and vector angle to represent the relative microbial nutrient limitations (Moorhead et al. 2016). The vector length and angle were calculated using Equations (1) and (2):

Vector length = SQRT (X^2 + Y^2) (1)

Vector angle = Degrees [ATAN2 (X, Y)] (2)

where X = β-1,4-glucosidase / (β-1,4-glucosidase + alkaline phosphatase) and Y = β-1,4-glucosidase / (β-1,4-glucosidase + N-acetyl-β-D-glucosaminidase + leucine aminopeptidase). Vector length represents the relative C limitation, with greater length indicating stronger C limitation. Vector angle indicates relative N or P limitation: an angle greater than 45° suggests relative P limitation, while an angle less than 45° indicates relative N limitation (Moorhead et al. 2016).

To investigate variations in microbial metabolic limitations along the degradation gradient, a one-way analysis of variance was employed to examine differences in the activities and stoichiometric ratios of C-, N-, and P-acquiring enzymes across light, moderate, and severe degradation levels. Spearman correlation analysis was conducted to identify relationships among extracellular enzyme activities, enzymatic stoichiometric ratios, plant community characteristics, and soil physicochemical properties. RDA and VPA were performed to explore the effect of biotic and abiotic factors on enzyme activities and enzymatic stoichiometry and to identify the key driving factors. Furthermore, linear regression was used to examine the responses of soil enzyme activities to key influencing factors. To address multicollinearity among the soil nutrient-related variables (total C, total P, inorganic N, C:N and available P), principal component analysis (PCA) was conducted prior to the regression analysis. The first principal component (PC1), which explained 54.80% of the total variance, was used as a comprehensive indicator of soil nutrient availability (Fig. S1). Further, structural equation modelling (SEM) was used to determine the relationship among soil environment, plant community characteristics, soil nutrient availability and microbial C, P limitation. Plant community characteristics, including plant height, plant density, and species richness, were integrated using PCA to avoid multicollinearity. The PC1, accounting for 58.20% of the total variance, was retained and used as a composite variable representing plant community characteristics in the SEM. All variables were Z-score standardized prior to analysis to allow comparison of parameter estimates on a common scale. All statistical analyses and figure generation were conducted using SPSS 26.0 and R 4.4.3.

2 Results

2.1 Soil enzyme activities and stoichiometry in degraded wetlands

The activities of C-, N-, and P-acquiring enzymes decreased significantly with increasing degradation intensity (Fig. 1). In contrast, the soil C:N and C:P enzyme activity ratios showed increasing trends along the degradation gradient (Fig. 2a, 2b). The soil N:P enzyme activity ratio did not differ significantly among degradation levels, but exhibited a declining trend along the degradation gradient (Fig. 2c). Vector analysis showed that vector length increased significantly along the degradation gradient, with vector angles exceeding 45°, indicating co-limitation of microbial metabolism by C and P in degraded wetlands (Fig. 2d, 3).

2.2 Effects of abiotic and biotic factors on microbial metabolic limitation

RDA showed that plant community characteristics and soil physicochemical factors together explained 85.63% of the variance in enzyme activities and stoichiometric ratios, with RDA1 and RDA2 accounting for 64.35% and 21.28% of the variance, respectively (Fig. 4a). Soil variables (e.g., EC, soil water content and C) and community characteristics (e.g., plant density, plant average height) had significant influences on enzyme activities and stoichiometric ratios (Fig. 4b). Furthermore, the activities of C-, N-, and P-acquiring enzymes were positively correlated with soil moisture, nutrient availability (i.e., total C, inorganic N, available P), and plant density, but negatively correlated with soil pH, EC, and the soil C:N. In contrast, the enzymatic C:N and C:P ratios, along with vector length, showed inverse relationships with the same soil and plant variables (Fig. S3).

VPA identified soil EC, plant density, pH, soil total C and water content as key factors influencing soil enzyme activities and stoichiometric ratios, explaining 13.34%, 12.85%, 11.75%, 11.75% and 11.49% of the variance, respectively. Among these, soil nutrient-related variables were key abiotic factors affecting soil enzyme activities, accounting for 38.49% of the variation (Fig. 4b). The activities of C-, N-, and P-acquiring enzymes decreased significantly with declining soil nutrient availability (Fig. 5). The SEM further showed that wetland degradation significantly increased soil EC, while reducing soil nutrient availability and inhibiting plant growth (Fig. 6a). In turn, soil nutrient availability exerted a strong direct negative effect on C limitation, whereas EC was the main negative predictor of P limitation (Fig. 6c, b).

3 Discussion

3.1 Responses of soil enzyme activities and microbial metabolic limitations to wetland degradation

Our results showed a consistent decline in the activities of C-, N-, and P-acquiring enzymes along the wetland degradation gradient (Fig. 1), supporting our first hypothesis. The decreased enzyme activities suggest a reduced microbial capacity for organic matter decomposition and nutrient acquisition in degraded wetlands (Ding et al. 2025b; Ullah et al. 2023). This pattern is consistent with the established theory in terrestrial ecosystems that habitat degradation constrains microbial activity by limiting the

availability and quality of organic carbon substrates (Sun et al. 2024; Wang et al. 2022). It is also notable that the mean enzyme activities reported here were lower than the global average for 40 ecosystems (Sinsabaugh et al. 2008) and the average for 181 Chinese forest sites (Cui et al. 2022), and even dipped below levels observed in desert ecosystems (Table S4) (Wang et al. 2023). The extremely low enzyme activities are likely attributed to the unique edaphic and hydrological conditions of the riparian wetlands in the lower Yellow River. Unlike forest or grassland soils, which have higher nutrient contents and lower pH, riparian wetlands are frequently subjected to flooding and pronounced wet-dry cycles (Camenzind et al. 2018; Waring et al. 2014). This dynamic hydrological regime promotes salt accumulation, leading to elevated soil pH and EC. Such saline-alkaline conditions inhibit microbial growth and suppress enzyme synthesis, primarily by disrupting enzyme conformation, stability, and substrate binding (Gao et al. 2021; Rath et al. 2019). The resulting suppression of C-, N-, and P-acquiring enzyme activities under this saline-alkaline stress thus supports observations from other wetland systems, such as coastal wetlands, where similar conditions have been shown to suppress enzyme activities (Servais et al. 2021; Zhang et al. 2025).

The ratio of soil enzyme activities related to C, N, and P acquisition in this study was 1:0.73:0.86, which deviated significantly from the global 1:1:1 C:N:P stoichiometry (Sinsabaugh et al. 2008). This deviation reflects a systemic imbalance between microbial nutrient demand and soil resource availability along the degradation gradient, specifically revealing a relatively higher microbial demand for C and P over N (Abay et al. 2024; Auwal et al. 2023; Sinsabaugh and Shah 2012). Such ecosystem-specific stoichiometric imbalances are well-documented; for instance, forest soils often exhibit a greater microbial demand for N and P relative to C (Pang et al. 2025; Zhou et al. 2020). Additionally, enzymatic stoichiometry analysis indicated a progressive shift toward stronger C limitation along the degradation gradient (Fig. 3a). This shift was also quantified by vector analysis, which showed a significant increase in vector length with increasing degradation, a trend that directly reflects enhanced microbial C limitation (Fig. 3) (Moorhead et al. 2016; Yang et al. 2024). The intensifying C limitation can be attributed to the concurrent decline in both the quantity and quality of soil organic C along the degradation gradient (Ding et al. 2025b; Malik et al. 2019; Song et al. 2024). This mechanism is also reflected in processes occurring in forest and grassland soils, where a decline in labile C coupled with an accumulation of recalcitrant forms increases the metabolic cost of C acquisition for microbes, ultimately exacerbating C limitation (Ramin and Allison 2019; Zhou et al. 2020). The decrease in the N:P enzyme activity ratio, reinforced by vector analysis, indicates a shift toward stronger microbial P limitation (Fig. 2, 3b), which aligns with observations in coastal wetlands (Peng et al. 2023; Xu et al. 2024). The pervasive P limitation in wetlands could stem from the inherent scarcity of bioavailable P, as its supply depends on slow geological weathering and it is readily immobilized by adsorption to soil minerals (Cui et al. 2025; Liu et al. 2023; Vitousek et al. 2010). In the riparian wetlands of the lower Yellow River, the unique hydrological regime, characterized by periodic flooding and recession, may exacerbate this limitation by promoting P leaching, further constraining microbial P availability (Cui et al. 2025; Minick et al. 2022). Overall, wetland degradation induces a pronounced stoichiometric imbalance that suppresses extracellular enzyme activities and ultimately leads to a co-limitation pattern of C and P on microbial metabolism.

3.2 Driving factors of soil extracellular enzyme activity and stoichiometric ratios under degradation gradients

The RDA identified soil environments (i.e., soil EC, pH and water content) as the primary factors regulating microbial metabolic limitations, accounting for 36.58% of the explained variance (Fig. 4b). Specifically, soil pH was a key driver of the variations in extracellular enzyme activities and their stoichiometry (Fig. 4b), and a consistent decrease in the activities of C-, N-, and P-acquiring enzymes was observed with increasing pH (Fig. S3). Soil pH can influence enzyme activity both directly, by altering enzyme conformation and substrate affinity, and indirectly, by modulating microbial community structure and function (Cao et al. 2022; Jones et al. 2019). For instance, in coastal wetlands, elevated pH shifts microbial community composition towards copiotrophic bacteria, raising community-level C demand and intensifying microbial C limitation (Jones et al. 2019; Peng et al. 2023; Xu et al. 2024). The significant increase in both C:N and C:P enzyme ratios with rising pH also suggests that degradation-induced alkalinization directly exacerbated microbial C limitation (Abay et al. 2024; Ding et al. 2025a; Yang et al. 2025). Additionally, the positive response of C-, N-, and P-acquiring enzyme activities to nutrient availability reflects enhanced microbial metabolism under richer resource conditions (Fig. 5) (Abay et al. 2024; Murúa and Gaxiola 2023). In line with this resource-driven enhancement, nutrient scarcity in ecosystems such as tundra permafrost consistently suppresses both microbial activity and enzyme synthesis (Allison and Vitousek 2005; Stark et al. 2014). Furthermore, the SEM revealed that degradation significantly exacerbated microbial C limitation primarily by reducing soil nutrient availability (Fig. 6a). However, a notable exception was the weak link between enzyme metrics and total P under the overall P limitation (Fig. S3), likely because total P contains substantial proportions of occluded or organically complexed P that are poorly accessible to microbes (Cui et al. 2025; Vitousek et al. 2010). We also observed divergent responses of enzyme metrics to nutrient decline, with decreased activities of C-, N-, and P-acquiring enzymes but a significantly increase in the soil C:N and C:P enzyme activity ratio (Fig. S3, 5). This pattern highlights that nutrient reduction disproportionately exacerbates microbial C limitation (Allison and Vitousek 2005; Vivanco et al. 2024).

Increased soil EC, a proxy for salinity, could suppress enzyme activities by inhibiting microbial growth via osmotic and ionic stress (Rath et al. 2019; Servais et al. 2021; Yang et al. 2024). In line with this mechanism, we observed significant negative correlations between EC and C-, N-, and P-acquiring enzyme activities (Fig. 4a). This finding aligns with previous reports that salinization suppresses microbial function, such as the concomitant declines in soil organic carbon, microbial activity, and diversity observed in the Yellow River Delta (Herbert et al. 2015; Li et al. 2019; Rath and Rousk 2015). The significant increase in the soil C:N and C:P enzyme activity ratio with rising EC indicates that salinization intensified microbial C limitation (Fig. S3). Simultaneously, soil EC critically influences P availability. In saline soils such as those in the Yellow River Delta, increased EC promotes phosphate precipitation (e.g., with Ca^{2+} and Mg^{2+}) and adsorption, thereby reducing the pool of bioavailable P (Herbert et al. 2015; Hinsinger 2001; Peñuelas et al. 2026). Correspondingly, we observed concurrent decreases in available P concentrations and the soil N:P enzyme activity ratio with increasing EC, suggesting that salinization

also exacerbates microbial P limitation relative to N. SEM further revealed that degradation increased EC, which negatively affected soil nutrient availability and subsequently enhanced P limitation (Fig. 6a, c). These results suggest that salinization not only directly imposes osmotic and ionic stress on microbial activity, but also indirectly alters nutrient limitation through reduced nutrient availability. Beyond ionic stress, soil water content serves as another key regulator by providing the aqueous medium essential for microbial metabolism (Gomez et al. 2020; Philippot et al. 2024; Schimel 2018). We observed that declining soil water content decreased enzyme activities but increased the soil C:N and C:P enzyme activity ratios, indicating that water scarcity further exacerbates microbial C limitation.

In summary, the carbonate- and chloride-rich sediments of the Yellow River underlie the profound geochemical shifts observed during wetland degradation. Declining soil water content drives surface salt accumulation and alkalinization (Herbert et al. 2015; Rath et al. 2019). This process creates saline-alkaline conditions that disrupt enzyme stability and reduce nutrient availability (Allison and Vitousek 2005; Taggart et al. 2025). Concurrently, the depletion of soil nutrients directly constrains substrate supply for microbial metabolism (Rebi et al. 2024; Waring et al. 2014). Together, saline-alkaline stress (inhibiting microbial activity) and resource deficiency (constraining substrate supply) synergistically drive the C and P co-limitation that characterizes these degraded riparian wetlands.

4 Conclusions

This study provides a comprehensive perspective on the patterns and drivers of microbial resource limitation in degraded wetland ecosystems. Our results showed that wetland degradation significantly suppressed the activities of microbial C-, N-, and P-acquiring enzymes, and that the degree of C and P limitation intensified along the degradation gradient. Moreover, our findings revealed that degradation-induced changes in soil EC and nutrient availability were the primary factors driving the shifts in extracellular enzyme activities and C, P co-limitation, likely by influencing microbial growth and metabolic activity. This finding has important implications, as the restoration of wetland functions should not only emphasize vegetation recovery but also consider soil processes mediated by microbial metabolic activity, which play an equally critical role in maintaining wetland functions. Our study suggests that optimizing soil conditions and resource availability, such as reducing soil salinity and enhancing nutrient availability, will help enhance microbial metabolic activity and thereby promote the functional recovery of degraded wetland ecosystems.

Declarations

Funding

This study was supported jointly by Henan Postdoctoral Foundation, the National Natural Science Foundation of China (32201517), Sichuan Science and Technology Program (2023ZYD0102), and China Postdoctoral Science Foundation (2023M743206).

Acknowledgements

We thank Jinbo Guo, Yingshu Cao and Han Wang for their kind help with field and laboratory work.

Statements and Declarations

The authors have no competing interests to declare that are relevant to the content of this article.

Authorship contributions

Mingli Zhang and Junxiang Ding conceived the study. Mingli Zhang, Yike Huang, Kexin Chen and Xinyu Li collected the data, and Mingli Zhang, Xinyu Li and Zhiwei Mao performed the formal analysis. Mingli Zhang wrote the original draft with significant contributions from Yike Huang, Mei Liu, Yadong Xu, Wenkai Chen, Lin Wang, Ming Dou, and Junxiang Ding. Junxiang Ding acquired funding and supervised the project.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Abay P, Gong L, Luo Y, Zhu HQ, Ding ZL (2024) Soil extracellular enzyme stoichiometry reveals the nutrient limitations in soil microbial metabolism under different carbon input manipulations. Sci Total Environ, 913. http://dx.doi.org/10.1016/j.scitotenv.2023.169793.
2. Acosta-Martínez V, Cruz L, Sotomayor-Ramírez D, Pérez-Alegría L (2007) Enzyme activities as affected by soil properties and land use in a tropical watershed. Appl Soil Ecol, 35(1), 35-45. http://dx.doi.org/10.1016/j.apsoil.2006.05.012.
3. Allison SD, Vitousek PM (2005) Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biol. Biochem., 37(5), 937-944. http://dx.doi.org/10.1016/j.soilbio.2004.09.014.
4. Auwal M, Sun H, Adamu UK, Meng J, Van Zwieten L, Singh BP, Luo Y, Xu JM (2023) The phosphorus limitation in the post-fire forest soils increases soil CO2 emission via declining cellular carbon use efficiency and increasing extracellular phosphatase. CATENA, 224. http://dx.doi.org/10.1016/j.catena.2023.106968.
5. Burns RG, DeForest JL, Marxsen J, Sinsabaugh RL, Stromberger ME, Wallenstein MD, Weintraub MN, Zoppini A (2013) Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biol. Biochem., 58, 216-234. http://dx.doi.org/10.1016/j.soilbio.2012.11.009.
6. Camenzind T, Hättenschwiler S, Treseder KK, Lehmann A, Rillig MC (2018) Nutrient limitation of soil microbial processes in tropical forests. Ecol Monogr, 88(1), 4-21. http://dx.doi.org/10.1002/ecm.1279.
7. Cao XW, Shi ZM, Chen J, Liu S, Zhang MM, Chen M, Xu GX, Wu JM, Xing HS, Li FF (2022) Extracellular enzyme characteristics and microbial metabolic limitation in soil of subalpine forest

ecosystems on the eastern Qinghai-Tibetan Plateau. Plant Soil, 479(1-2), 337-353. http://dx.doi.org/10.1007/s11104-022-05521-2.

8. Chu ZX (2014) The dramatic changes and anthropogenic causes of erosion and deposition in the lower Yellow (Huanghe) River since 1952. Geomorphology, 216, 171-179. http://dx.doi.org/10.1016/j.geomorph.2014.04.009.
9. Crowther TW, van den Hoogen J, Wan J, Mayes MA, Keiser AD, Mo L, Averill C, Maynard DS (2019) The global soil community and its influence on biogeochemistry. Science, 365(6455), 772. http://dx.doi.org/10.1126/science.aav0550.
10. Cui YX, Bing HJ, Moorhead DL, Delgado-Baquerizo M, Ye LP, Yu JL, Zhang SP, Wang X, Peng SS, Guo X, Zhu BA, Chen J, Tan WF, Wang YQ, Zhang XC, Fang LCA (2022) Ecoenzymatic stoichiometry reveals widespread soil phosphorus limitation to microbial metabolism across Chinese forests. Commun Earth Environ, 3(1). http://dx.doi.org/10.1038/s43247-022-00523-5.
11. Cui YX, Peng SS, Rillig MC, Camenzind T, Delgado-Baquerizo M, Terrer C, Xu XF, Feng MY, Wang MJ, Fang LC, Zhu B, Du EZ, Moorhead DL, Sinsabaugh RL, Peñuelas J, Elser JJ (2025) Global patterns of nutrient limitation in soil microorganisms. PNAS, 122(20). http://dx.doi.org/10.1073/pnas.2424552122.
12. Ding J, Dou M, Ge W, Cao Y, Guo X, Yin Y, Zhou G, Kong D, Liu M (2025a) Degradation-induced changes in soil nutrient availability alter leaf and root traits and their coordination in a riparian wetland ecosystem. Plant Soil, 514(2), 2779-2795. http://dx.doi.org/10.1007/s11104-025-07544-x.
13. Ding JX, Zhang ML, Zeng XW, Dou M, Ge WJ, Cao YS, Mi QB, Wang JH, Lu FM, Zhao L, Kong DL, Yin HJ, Wang L, Zhou GS (2025b) Soil nutrients drive the spatial variability of wetland productivity along degradation gradients through plant functional traits. J Plant Ecol, 18(2). http://dx.doi.org/10.1093/jpe/rtaf028.
14. Fluet-Chouinard E, Stocker BD, Zhang Z, Malhotra A, Melton JR, Poulter B, Kaplan JO, Goldewijk KK, Siebert S, Minayeva T, Hugelius G, Joosten H, Barthelmes A, Prigent C, Aires F, Hoyt AM, Davidson N, Finlayson CM, Lehner B, Jackson RB, McIntyre PB (2023) Extensive global wetland loss over the past three centuries. Nature, 614(7947), 281-286. http://dx.doi.org/10.1038/s41586-022-05572-6.
15. Gao YF, Liu LL, Zhu PC, Yang SR, Guo WH, Yu XN (2021) Patterns and Dynamics of the Soil Microbial Community with Gradual Vegetation Succession in the Yellow River Delta, China. Wetlands, 41(1). http://dx.doi.org/10.1007/s13157-021-01414-9.
16. German DP, Weintraub MN, Grandy AS, Lauber CL, Rinkes ZL, Allison SD (2011) Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biol. Biochem., 43(7), 1387-1397. http://dx.doi.org/10.1016/j.soilbio.2011.03.017.
17. Gomez EJ, Delgado JA, Gonzalez JM (2020) Environmental factors affect the response of microbial extracellular enzyme activity in soils when determined as a function of water availability and temperature. Ecol Evol, 10(18), 10105-10115. http://dx.doi.org/10.1002/ece3.6672.
18. Herbert ER, Boon P, Burgin AJ, Neubauer SC, Franklin RB, Ardón M, Hopfensperger KN, Lamers LPM, Gell P (2015) A global perspective on wetland salinization: ecological consequences of a growing

threat to freshwater wetlands. Ecosphere, 6(10), art206. http://dx.doi.org/https://doi.org/10.1890/ES14-00534.1.

19. Hinsinger P (2001) Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant Soil, 237(2), 173-195. http://dx.doi.org/10.1023/A:1013351617532.
20. Huang Y, Zhang M, Mao Z, Li X, Xu L, Nie J, Li X, Li M, Chen W, Ding J (2026) Effect of wetland degradation on plant community characteristic and aboveground biomass in the lower reaches of the Yellow River. Front Plant Sci, Volume 16 - 2025. http://dx.doi.org/10.3389/fpls.2025.1719185.
21. Ji W, Liu H, Gao J, Ma H, Song T, Gao Y, Feng C (2021) Spatial-Temporal Variations of Ecological Quality in the Yellow River Basin. Research of Environmental Sciences, 34(7), 1700-1709. http://dx.doi.org/10.13198/j.issn.1001-6929.2021.03.17.
22. Jones DL, Cooledge EC, Hoyle FC, Griffiths RI, Murphy DV (2019) pH and exchangeable aluminum are major regulators of microbial energy flow and carbon use efficiency in soil microbial communities. Soil Biol. Biochem., 138. http://dx.doi.org/10.1016/j.soilbio.2019.107584.
23. Kangabam R, Kanagaraj S, Munisamy G (2016) Assessment of carbon sequestration potential of loktak lake in manipur - a biodiversity hotspot. Int. J. Conserv. Sci., 7, 1145-1152.
24. Li H, Chi ZF, Li JL, Wu HT, Yan BX (2019) Bacterial community structure and function in soils from tidal freshwater wetlands in a Chinese delta: Potential impacts of salinity and nutrient. Sci Total Environ, 696. http://dx.doi.org/10.1016/j.scitotenv.2019.134029.
25. Liu J, Fang L, Qiu T, Bing H, Cui Y, Sardans J, Du E, Chen J, Tan W, Delgado-Baquerizo M, Zhou G, Cui Q, Penuelas J (2023) Disconnection between plant–microbial nutrient limitation across forest biomes. Funct Ecol, 37(8), 2271-2281. http://dx.doi.org/10.1111/1365-2435.14361.
26. Malik AA, Puissant J, Goodall T, Allison SD, Griffiths RI (2019) Soil microbial communities with greater investment in resource acquisition have lower growth yield. Soil Biol. Biochem., 132, 36-39. http://dx.doi.org/10.1016/j.soilbio.2019.01.025.
27. Meng X, Liu M, Meng Z, Wu C, Bao C, Xu B, Wang G, Ma H, Liu Z, Zheng X, Xie X, Cui X (2024) Effects of different restoration stages on soil microbial community composition and diversity in Naolihe Wetland, China. Front Microbiol, Volume 15 - 2024. http://dx.doi.org/10.3389/fmicb.2024.1403849.
28. Minick KJ, Aguilos M, Li XF, Mitra B, Prajapati P, King JS (2022) Effects of Spatial Variability and Drainage on Extracellular Enzyme Activity in Coastal Freshwater Forested Wetlands of Eastern North Carolina, USA. Forests, 13(6). http://dx.doi.org/10.3390/f13060861.
29. Mitra S, Wassmann R, Vlek PLG (2005) An appraisal of global wetland area and its organic carbon stock. Curr. Sci., 88(1), 25-35. http://dx.doi.org/10.1073/pnas.0408999101.
30. Moorhead DL, Sinsabaugh RL, Hill BH, Weintraub MN (2016) Vector analysis of ecoenzyme activities reveal constraints on coupled C, N and P dynamics. Soil Biol. Biochem., 93, 1-7. http://dx.doi.org/10.1016/j.soilbio.2015.10.019.
31. Mooshammer M, Wanek W, Zechmeister-Boltenstern S, Richter A (2014) Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of

microbial adaptations to their resources. Front Microbiol, 5. http://dx.doi.org/10.3389/fmicb.2014.00022.

32. Murúa JM, Gaxiola A (2023) Variability in terrestrial litter decomposition can be explained by nutrient allocation strategies among soil decomposer communities. Funct Ecol, 37(6), 1642-1652. http://dx.doi.org/10.1111/1365-2435.14321.
33. Pang CL, Zhang ZH, Zhu XM, Wei WT, Mustafa A, Chen WB, Mao QG, Mo JM, Li S, Lu XK (2025) Divergent Microbial Metabolic Limitations Across Soil Depths After Two Decades of High Nitrogen Inputs in a Primary Tropical Forest. Global Change Biol, 31(8). http://dx.doi.org/10.1111/gcb.70440.
34. Peng R, Li XY, Lou JB, Song YL, Iqbal B, Tang Y, Xu SX, Cui SS, Zhao XX, Yang ZW, Qin Q, Zhao X (2023) Regulation of the Soil Microbial Metabolism Through Alterations in the Vegetative Community in Wetlands. Pol J Environ Stud, 32(6), 5307-5317. http://dx.doi.org/10.15244/pjoes/169134.
35. Peñuelas J, Zheng B, Tariq A, Sardans J (2026) Microbial phosphorus cycling in terrestrial ecosystems. Nat Rev Microbiol. http://dx.doi.org/10.1038/s41579-026-01296-w.
36. Philippot L, Chenu C, Kappler A, Rillig MC, Fierer N (2024) The interplay between microbial communities and soil properties. Nat Rev Microbiol, 22(4), 226-239. http://dx.doi.org/10.1038/s41579-023-00980-5.
37. Ramin KI, Allison SD (2019) Bacterial Tradeoffs in Growth Rate and Extracellular Enzymes. Front Microbiol, 10. http://dx.doi.org/10.3389/fmicb.2019.02956.
38. Rath KM, Fierer N, Murphy DV, Rousk J (2019) Linking bacterial community composition to soil salinity along environmental gradients. The ISME Journal, 13(3), 836-846. http://dx.doi.org/10.1038/s41396-018-0313-8.
39. Rath KM, Rousk J (2015) Salt effects on the soil microbial decomposer community and their role in organic carbon cycling: A review. Soil Biol Biochem, 81, 108-123. http://dx.doi.org/https://doi.org/10.1016/j.soilbio.2014.11.001.
40. Rebi A, Wang G, Yang T, Kanomanyanga J, Ejaz I, Mustafa A, Rizwan M, Zhou JX (2024) Stoichiometric and bacterial eco-physiological insights into microbial resource availability in karst regions affected by clipping-and-burning. J Environ Manage, 370. http://dx.doi.org/10.1016/j.jenvman.2024.122925.
41. Schimel JP (2018) Life in Dry Soils: Effects of Drought on Soil Microbial Communities and Processes. Annu Rev Ecol Evol Syst, 49(Volume 49, 2018), 409-432. http://dx.doi.org/https://doi.org/10.1146/annurev-ecolsys-110617-062614.
42. Servais S, Kominoski JS, Fernandez M, Morales K (2021) Saltwater and phosphorus drive unique soil biogeochemical processes in freshwater and brackish wetland mesocosms. Ecosphere, 12(8). http://dx.doi.org/10.1002/ecs2.3704.
43. Shahariar S, Helgason B, Soolanayakanahally R, Bedard-Haughn A (2021) Soil Enzyme Activity as Affected by Land-Use, Salinity, and Groundwater Fluctuations in Wetland Soils of the Prairie Pothole Region. Wetlands, 41(2). http://dx.doi.org/10.1007/s13157-021-01431-8.

44. Sinsabaugh RL (2010) Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol. Biochem., 42(3), 391-404. http://dx.doi.org/10.1016/j.soilbio.2009.10.014.
45. Sinsabaugh RL, Hill BH, Follstad Shah JJ (2009) Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature, 462(7274), 795-798. http://dx.doi.org/10.1038/nature08632.
46. Sinsabaugh RL, Lauber CL, Weintraub MN, Ahmed B, Allison SD, Crenshaw C, Contosta AR, Cusack D, Frey S, Gallo ME, Gartner TB, Hobbie SE, Holland K, Keeler BL, Powers JS, Stursova M, Takacs-Vesbach C, Waldrop MP, Wallenstein MD, Zak DR, Zeglin LH (2008) Stoichiometry of soil enzyme activity at global scale. Ecol Lett, 11(11), 1252-1264. http://dx.doi.org/10.1111/j.1461-0248.2008.01245.x.
47. Sinsabaugh RL, Shah JJF (2012) Ecoenzymatic Stoichiometry and Ecological Theory. Annu Rev Ecol Evol Syst, 43, 313-343.
48. Song YZ, Song TJ, An Y, Shan LP, Su XS, Yu SD (2024) Soil ecoenzyme activities coupled with soil properties and plant biomass strongly influence the variation in soil organic carbon components in semi-arid degraded wetlands. Sci Total Environ, 922. http://dx.doi.org/10.1016/j.scitotenv.2024.171361.
49. Stark S, Männistö MK, Eskelinen A (2014) Nutrient availability and pH jointly constrain microbial extracellular enzyme activities in nutrient-poor tundra soils. Plant Soil, 383(1-2), 373-385. http://dx.doi.org/10.1007/s11104-014-2181-y.
50. Stock SC, Köster M, Dippold MA, Nájera F, Matus F, Merino C, Boy J, Spielvogel S, Gorbushina A, Kuzyakov Y (2019) Environmental drivers and stoichiometric constraints on enzyme activities in soils from rhizosphere to continental scale. Geoderma, 337, 973-982. http://dx.doi.org/10.1016/j.geoderma.2018.10.030.
51. Sun HR, Liu JY, Wu JH, Hu HY, Chen QB, Fang HY, Tao K (2024) Effects of alpine grassland degradation on soil microbial community structure and metabolic activity in the Qinghai-Tibet Plateau. Appl Soil Ecol, 200. http://dx.doi.org/10.1016/j.apsoil.2024.105458.
52. Taggart MG, Baah DS, Allen S, Khan Z, Arnscheidt J, Jordan P, O'Hagan BMG, Ibrahim AD, Rao JR, Ternan NG (2025) Fitting soil extracellular enzyme activity into the complex network of abiotic and biotic soil properties often associated with soil health. Front Microbiol, Volume 16 - 2025. http://dx.doi.org/10.3389/fmicb.2025.1638267.
53. Turner BL, Lambers H, Wen ZH, Auer YM, Kandeler E (2024) Microbial nutrient limitation along a 2-million-year dune chronosequence. Soil Biol. Biochem., 192. http://dx.doi.org/10.1016/j.soilbio.2024.109385.
54. Ullah S, Raza MM, Abbas T, Guan X, Zhou W, He P (2023) Responses of soil microbial communities and enzyme activities under nitrogen addition in fluvo-aquic and black soil of North China. Front Microbiol, 14. http://dx.doi.org/10.3389/fmicb.2023.1249471.
55. Vance ED, Brookes PC, Jenkinson DS (1987) An Extraction Method for Measuring Soil Microbial Biomass-C. Soil Biol. Biochem., 19(6), 703-707. http://dx.doi.org/10.1016/0038-0717(87)90052-6.

56. Vitousek PM, Porder S, Houlton BZ, Chadwick OA (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. Ecol Appl, 20(1), 5-15. http://dx.doi.org/10.1890/08-0127.1.
57. Vivanco AM, Sietiö OM, Meyer N, Mganga KZ, Kalu S, Adamczyk S, Huayllasco SC, Orihuela JA, Karhu K (2024) Microbial carbon use efficiency and soil organic carbon stocks across an elevational gradient in the Peruvian Andes. Appl Soil Ecol, 195. http://dx.doi.org/10.1016/j.apsoil.2023.105228.
58. Wan D, Yu P, Kong L, Zhang J, Chen Y, Zhao D, Liu J (2024) Effects of inland salt marsh wetland degradation on plant community characteristics and soil properties. Ecol Indic, 159, 111582. http://dx.doi.org/10.1016/j.ecolind.2024.111582.
59. Wang DJ, Zhou HK, Zuo J, Chen P, She YD, Yao BQ, Dong SK, Wu JS, Li F, Njoroge DM, Shi GX, Mao XF, Ma L, Zhang ZH, Mao Z (2022) Responses of Soil Microbial Metabolic Activity and Community Structure to Different Degraded and Restored Grassland Gradients of the Tibetan Plateau. Front Plant Sci, 13. http://dx.doi.org/10.3389/fpls.2022.770315.
60. Wang XY, Li YQ, Wang LL, Duan YL, Yao B, Chen Y, Cao WJ (2023) Soil extracellular enzyme stoichiometry reflects microbial metabolic limitations in different desert types of northwestern China. Sci Total Environ, 874. http://dx.doi.org/10.1016/j.scitotenv.2023.162504.
61. Waring BG, Weintraub SR, Sinsabaugh RL (2014) Ecoenzymatic stoichiometry of microbial nutrient acquisition in tropical soils. Biogeochemistry, 117(1), 101-113. http://dx.doi.org/10.1007/s10533-013-9849-x.
62. Xu GJ, Kang XM, Wang F, Zhuang WR, Yan WD, Zhang KR (2024) Alpine wetlands degradation leads to soil nutrient imbalances that affect plant growth and microbial diversity. Commun Earth Environ, 5(1). http://dx.doi.org/10.1038/s43247-024-01562-w.
63. Yan C, Li YY, Gao JJ, Wang XY (2025) Characteristics of Soil and Plant Ecological Stoichiometry of Carbon, Nitrogen, and Phosphorus in Different Wetland Types of the Yellow River. Sustainability, 17(7). http://dx.doi.org/10.3390/su17073276.
64. Yang Q, Guo B, Lu M, Liu Y, Kardol P, Reich PB, Bardgett RD, Cornelissen JHC, Kraft NJB, Díaz S, Wright IJ, He N, Hogan JA, Pei Y, Han Q, Li Z, Wang Z, Yang W, Ding J, Yang Z, Wu H, Carmona CP, Valverde-Barrantes OJ, Li D, Cai J, Zeng H, Zhang Y, Ren W, Zhao Y, Yang X, Fan G, Wang J, Li G, Kong D (2025) Arbuscular mycorrhizal association regulates global root–seed coordination. Nat Plants, 11(9), 1759-1768. http://dx.doi.org/10.1038/s41477-025-02089-4.
65. Yang X, Chu DS, Hu HB, Deng WB, Chen JY, Guo SJ (2024) Effects of Land-Use Type and Salinity on Soil Carbon Mineralization in Coastal Areas of Northern Jiangsu Province. Sustainability, 16(8). http://dx.doi.org/10.3390/su16083285.
66. Yang Y, Liang C, Wang YQ, Cheng H, An SS, Chang SX (2020) Soil extracellular enzyme stoichiometry reflects the shift from P- to N-limitation of microorganisms with grassland restoration. Soil Biol. Biochem., 149. http://dx.doi.org/10.1016/j.soilbio.2020.107928.
67. Zhang JL, Zhang Q, Zhang CY, Wang RQ, Wang H, Zheng PM (2025) Vegetation succession enhances microbial diversity, network complexity, and stability in coastal wetlands, underscoring the

pivotal role of soil salinity and key microbial species. J Environ Manage, 388. http://dx.doi.org/10.1016/j.jenvman.2025.125997.

68. Zhou L, Liu S, Shen H, Zhao M, Xu L, Xing A, Fang J (2020) Soil extracellular enzyme activity and stoichiometry in China's forests. Funct Ecol, 34(7), 1461-1471. http://dx.doi.org/10.1111/1365-2435.13555.

Figures

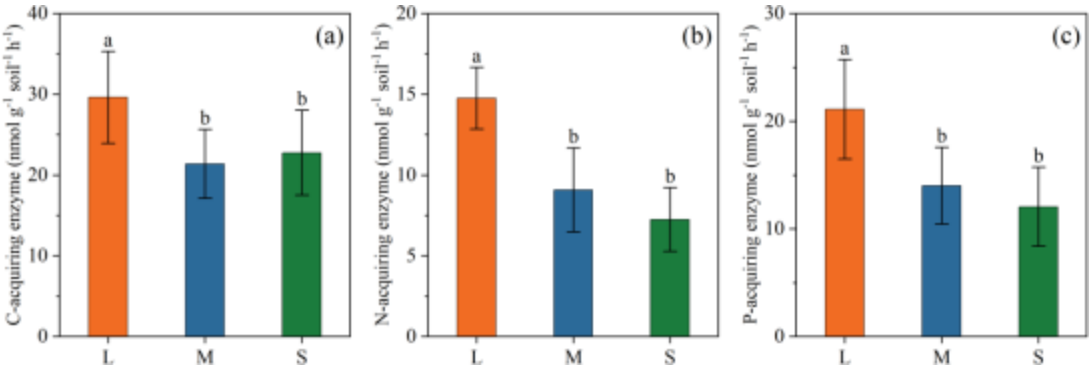


Figure 1

Changes in soil enzyme activities along the wetland degradation gradient. Different lowercase letters indicate significant differences among degradation levels ($p < 0.05$). Data are presented as mean ± SE (n = 3). C-acquiring enzyme, β-1,4-glucosidase; N-acquiring enzymes, N-acetyl-β-D-glucosaminidase + leucine aminopeptidase; P-acquiring enzyme, alkaline phosphatase; L, light degradation; M, moderate degradation; S, severe degradation.

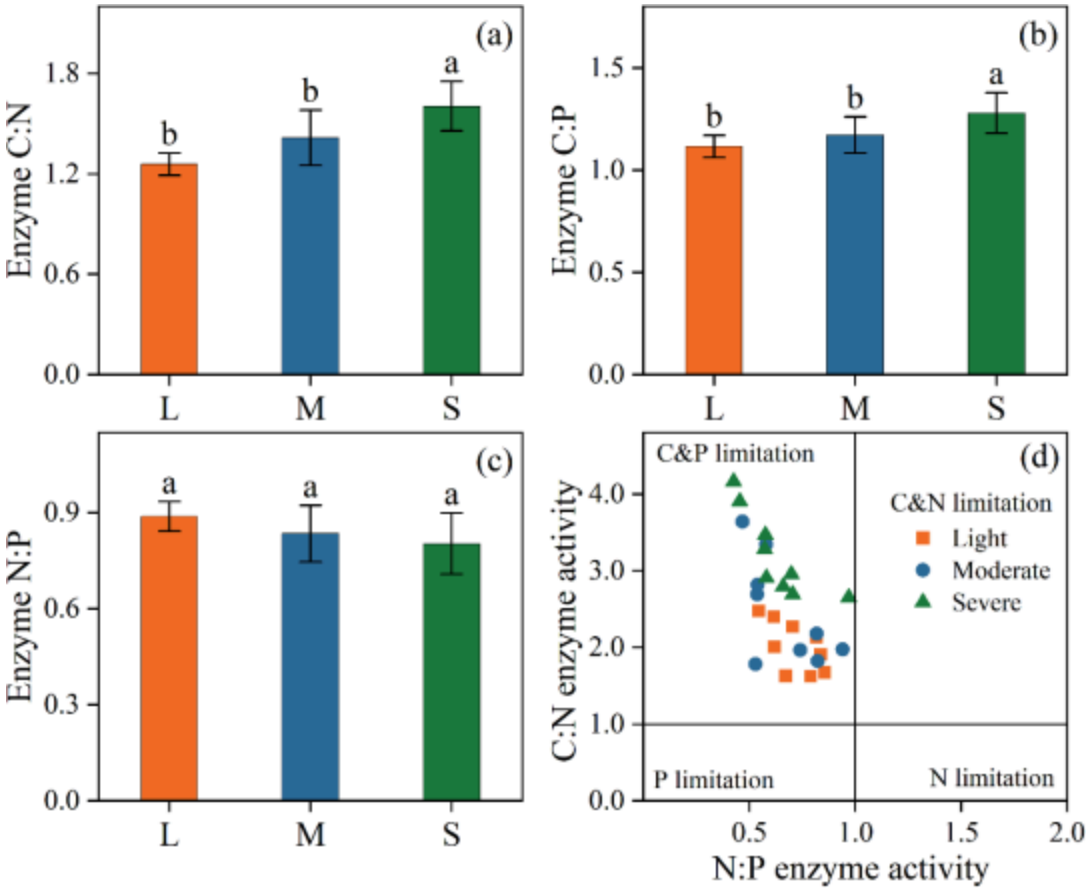


Figure 2

Changes in enzymatic stoichiometric ratios (a–c) and stoichiometric analysis (d) along the wetland degradation gradient. In (a–c), different lowercase letters indicate significant differences among degradation levels ($p < 0.05$). Data are presented as mean ± SE (n = 3). L, light degradation; M, moderate degradation; S, severe degradation.

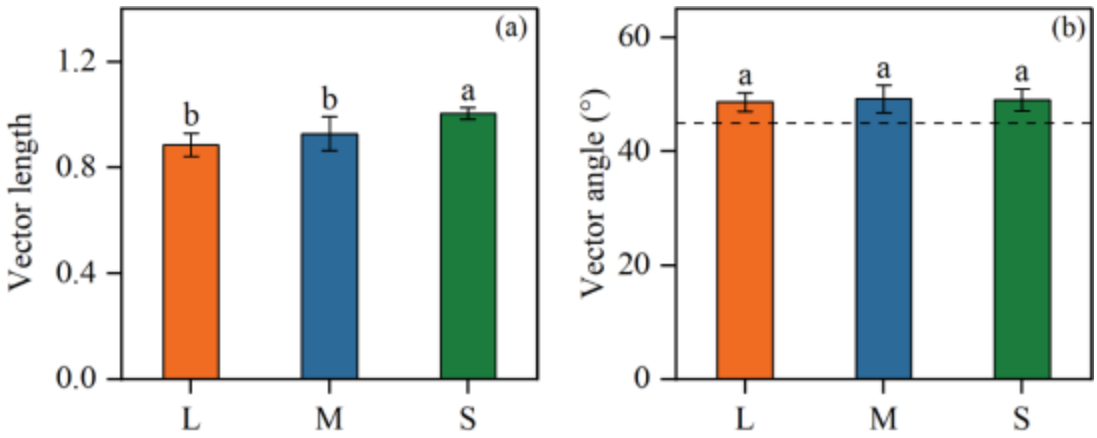


Figure 3

Changes in vector length and vector angle along the wetland degradation gradient. Different lowercase letters indicate significant differences among degradation levels ($p < 0.05$). Data are presented as mean

± SE (n = 3). The dashed line in (b) indicates the reference line of 45°. L, light degradation; M, moderate degradation; S, severe degradation.

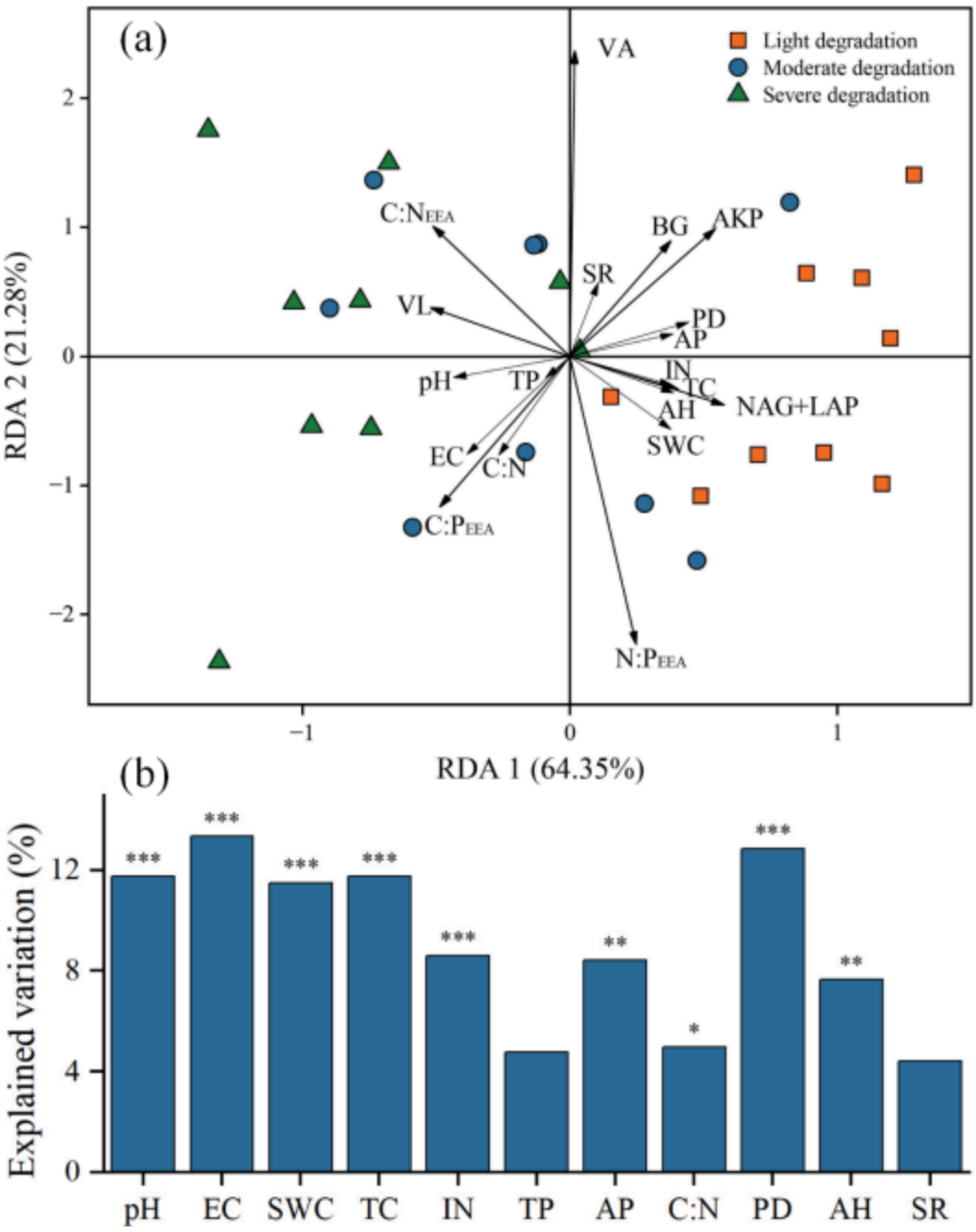


Figure 4

Redundancy analysis of soil enzyme activities, biotic factors, and abiotic factors along the wetland degradation gradient (a). Relative contributions of environmental variables to the variation in response variables (b). SWC, soil water content; pH, soil pH; EC, soil electrical conductivity; TC, soil total carbon; IN, soil inorganic nitrogen; TP, soil total phosphorus; AP, soil available phosphorus; C:N, the ratio of TC to TN; SR, species richness; PD, plant density; AH, average plant height; BG, β-1,4-glucosidase; NAG, N-acetyl-β-D-glucosaminidase; LAP, leucine aminopeptidase; AKP, alkaline phosphatase; $C:N_{EEA}$, the ratio of C- to N-

acquiring enzyme activities; C:P_{EEA}, the ratio of C- to P-acquiring enzyme activities; N:P_{EEA}, the ratio of N- to P-acquiring enzyme activities.

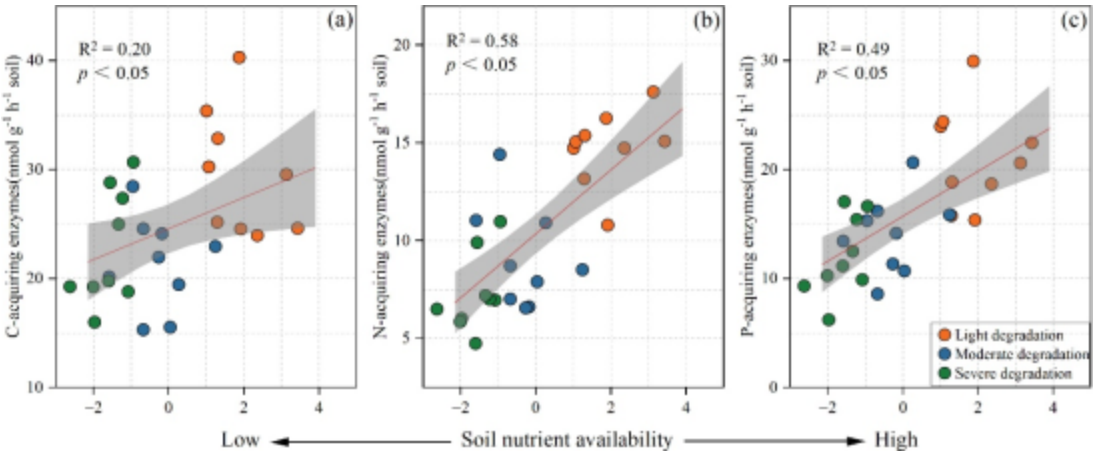


Figure 5

Relationships between soil enzyme activities and soil nutrient availability along the wetland degradation gradient. Soil nutrient availability is represented by the first principal component axis (54.80%) from PCA, which captures the gradient from low to high levels of soil nutrients (i.e., total C, total P, inorganic N, C:N and available P). The gray shaded area represents the 95% confidence interval.

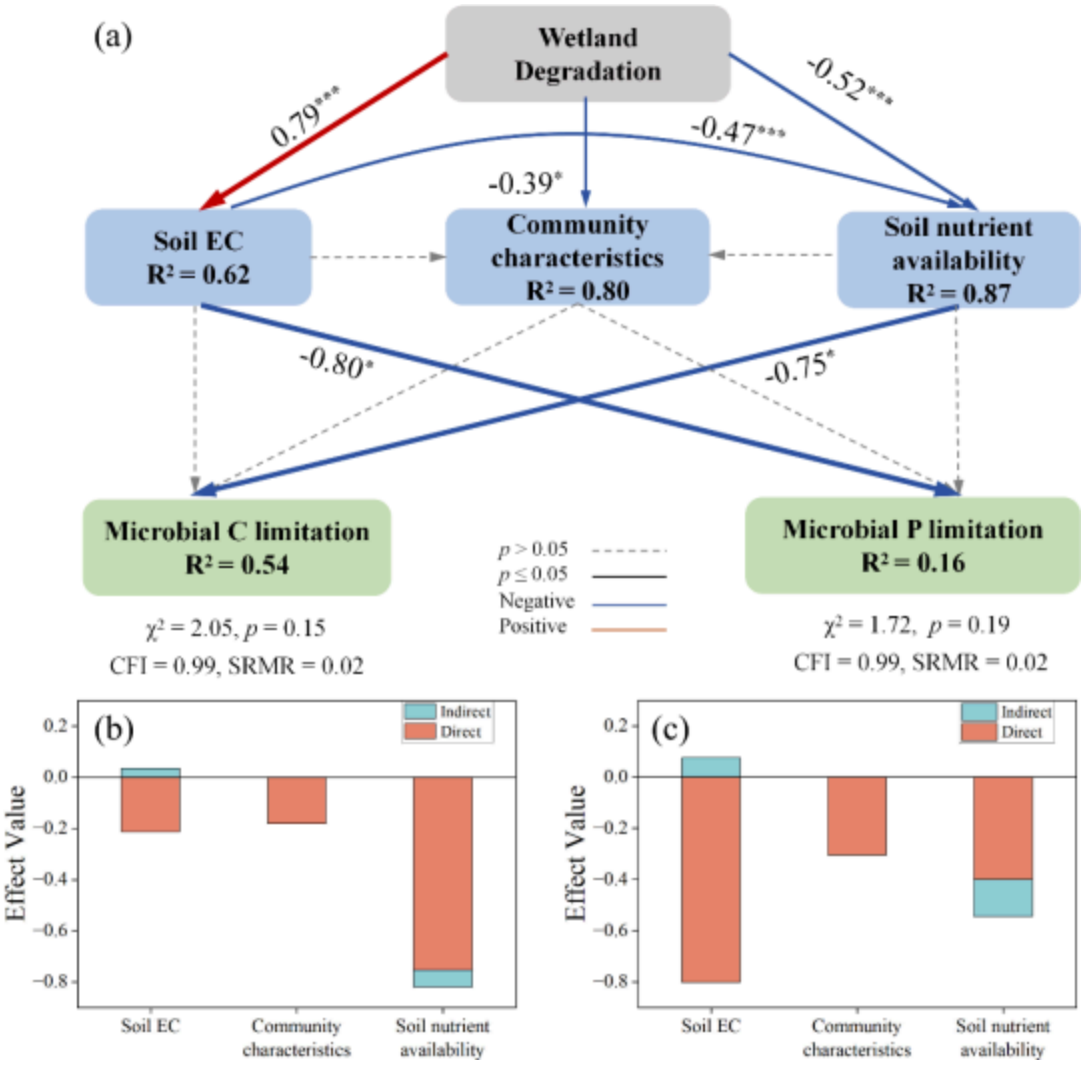


Figure 6

Structural equation models showing the direct and indirect pathways by which degradation degree affects microbial C limitation and P limitation (a), together with the standardized direct and indirect effects of soil EC, community characteristics, and soil nutrient availability on microbial C limitation (b) and P limitation (c). Community characteristics, including plant height, plant density, and species richness, were integrated using PCA to avoid multicollinearity. The PC1, accounting for 58.20% of the total variance, was retained and used as a composite variable representing plant community characteristics in the SEM. Soil nutrient availability is represented by the PC1 (54.80%), which captures soil nutrients (i.e., total C, total P, inorganic N, C:N and available P). Microbial C limitation was represented by vector length, and microbial P limitation was represented by vector angle. Line thickness is proportional to the magnitude of the standardized path coefficients. EC, electrical conductivity; CFI, comparative fit index; SRMR, the standardized root mean square residual. Significance levels are indicated as *$p \le 0.05$, ** $p \le 0.01$, and *** $p \le 0.001$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 2.SupplementaryInformation.docx