https://doi.org/10.5194/egusphere-2026-3406
Preprint. Discussion started: 27 July 2026

Alpine wetland degradation shifts enzyme-mediated soil nitrogen cycling from functional coordination to imbalance

Wenhua Chang, Weiwei Ma*, Jianfang Wang, Liangcui Song, Jianan Du, Wanpeng He, Guang Li

College of Forestry, Gansu Agricultural University, Lanzhou 730070, China

Correspondence to: Weiwei Ma, (281205236@qq.com)

Abstract. Alpine wetlands on the Qinghai–Tibet Plateau are increasingly degraded, threatening soil nitrogen (N) cycling and ecosystem functioning. However, how degradation restructures enzyme-mediated soil N cycling and its functional balance remains poorly understood. To address this gap, we conducted a three-year field investigation across four degradation stages (non-degraded, lightly degraded, moderately degraded, and heavily degraded wetlands) in the Gahai Wetland. We quantified four key soil N-cycling enzymes, namely urease, protease, nitrate reductase, and nitrite reductase, and developed a nitrogen cycling functional balance (NCFB) index to assess shifts in the coordination between hydrolytic and reductive functions. Degradation-induced drying and nutrient depletion reduced soil water content, soil organic carbon, total nitrogen, ammonium availability, and microbial biomass N, while increasing soil temperature and nitrate accumulation. Degradation increased urease and nitrate reductase activities but decreased protease and nitrite reductase activities, indicating enzyme-specific restructuring of soil N cycling. Most enzyme activities peaked in mid-growing season, whereas nitrate reductase peaked later. Soil N cycling shifted from a relatively coordinated state in non-degraded wetlands toward greater functional imbalance in degraded wetlands. This reorganization was strongly modulated by hydroclimatic variability, with drier conditions amplifying degradation, induced divergence in enzyme activities. Redundancy analysis and piecewise structural equation modeling showed that soil water content, organic carbon, inorganic N availability, and microbial biomass N were the primary regulators of enzyme variation and N-cycling functional imbalance. Overall, this study provides new insight into mechanisms of soil N-cycling destabilization in degraded alpine wetlands and informs wetland restoration under a drier future climate.

1 Introduction

Alpine wetlands are among the most important ecosystem types on the Qinghai–Tibet Plateau (QTP), which is often referred to as the “Third Pole of the Earth”, and play indispensable roles in biodiversity conservation, regional climate regulation, water retention, and global carbon (C) and nitrogen (N) biogeochemical cycling (Li et al., 2021; Wei et al., 2015; Yin et al., 2022). Over recent decades, however, alpine wetlands across the QTP have undergone widespread degradation under the combined pressures of climate change and human disturbance, particularly overgrazing (Li et al., 2021, 2019; Yuan et al., 2025). This degradation typically manifests as vegetation succession, biodiversity loss, soil water depletion, and nutrient impoverishment (Alhassan et al., 2018; Du et al., 2024; Ma et al., 2020a; Zhao et al., 2025). Because N cycling is a central process regulating primary productivity, microbial activity, and ecosystem resilience in alpine systems, degradation-induced shifts in soil N

https://doi.org/10.5194/egusphere-2026-3406
Preprint. Discussion started: 27 July 2026

cycling are likely to have profound consequences for ecosystem functioning and restoration potential (Chen et al., 2022). Clarifying how soil N cycling responds to alpine wetland degradation is therefore essential for understanding and predicting ecosystem feedback to ongoing global change.

Soil enzymes, which are mainly produced by soil microorganisms and plant roots, mediate essential biogeochemical processes involved in carbon, nitrogen, and phosphorus cycling (Allison and Vitousek, 2005; Ma et al., 2020b). Owing to their rapid responses to environmental perturbations, soil enzymes have been widely recognized as sensitive biochemical indicators of wetland health and soil functional status (Sinsabaugh et al., 2009; Sinsabaugh and Follstad Shah, 2012; Wang et al., 2022). In N-limited alpine ecosystems, N-cycling enzymes regulate key steps of organic N depolymerization and inorganic N transformation, thereby influencing N mineralization, denitrification, and potential N loss pathways (Xiang et al., 2024; Zuccarini et al., 2023). In particular, hydrolytic enzymes, such as urease and protease increase the bioavailability of organic N, whereas reductive enzymes such as nitrate reductase and nitrite reductase regulate nitrate and nitrite reduction processes, thereby influencing whether inorganic N is retained within the soil system or further transformed and lost through denitrification-related pathways (Brzostek et al., 2012; Jin et al., 2023; Ma et al., 2021; Priemé et al., 2002; Rajsz et al., 2019; Tabatabai et al., 2010). Despite their central role in regulating soil N transformations, the responses of N-cycling enzymes to alpine wetland degradation remain insufficiently resolved.

The activities of soil N-cycling enzymes are jointly regulated by soil physicochemical properties, plant community composition, temperature, moisture, nutrient availability, and substrate concentrations (Sardans et al., 2008; Song et al., 2019; Tan et al., 2023). Wetland degradation can substantially reshape these controls by altering vegetation composition, hydrological conditions, soil properties, and microbial habitat quality (Abulaizi et al., 2023; Du et al., 2024; Li et al., 2022). As degradation intensifies, the replacement of hygrophytic species by mesophytic or xerophytic plants generally reduces plant-derived C and nutrient inputs (Wu et al., 2017), leading to declines in soil organic carbon and total nitrogen pools (Liu et al., 2018). Meanwhile, hydrological drying and associated microclimatic shifts may constrain enzyme diffusion, suppress microbial activity, and reduce catalytic efficiency (Breidenbach et al., 2022; Song et al., 2024). However, the effects of degradation are not uniformly negative across all stages. Although many studies have reported reduced enzyme activity under degraded conditions, others have observed transient stimulation during early degradation, indicating strong context dependence associated with degradation intensity, resource availability, and grazing pressure (Ma et al., 2020a). Such complexity is especially relevant in the fragile alpine wetlands of the QTP, where degradation may irreversibly alter soil conditions and microbial functioning (Alhassan et al., 2018; Du et al., 2024).

This knowledge gap is particularly important in alpine wetlands because enzyme-mediated N transformations are highly sensitive not only to degradation intensity but also to strong seasonal and interannual hydroclimatic variability (Deng et al., 2025). On the Qinghai–Tibet Plateau, short growing seasons, large fluctuations in soil moisture, and increasingly frequent drought events can substantially modify microbial activity (Sorensen et al., 2018), substrate diffusion, and redox conditions, thereby altering both the magnitude and direction of soil N-cycling processes (Asensio et al., 2024; Gao et al., 2021). Although seasonal variation in individual soil enzymes has been reported in alpine and wetland ecosystems (Akinyemi et al., 2020; Bai

https://doi.org/10.5194/egusphere-2026-3406
Preprint. Discussion started: 27 July 2026

et al., 2023; Brzostek et al., 2012), most existing studies have been based on single sampling periods, short-term observations, or isolated enzyme responses. Consequently, we still lack a process-based understanding of whether wetland degradation simply suppresses soil N cycling or instead reorganizes it by differentially affecting distinct enzymatic pathways under contrasting hydroclimatic conditions.

To address this knowledge gap, we conducted a three-year field investigation across four stages of alpine wetland degradation in the Gahai Wetland on the northeastern QTP. Rather than treating soil N-cycling enzymes as independent indicators, we selected four representative enzymes involved in N hydrolysis and N reduction to test whether wetland degradation drives a functional reorganization of soil N cycling under hydrological drying and nutrient depletion. We further developed a nitrogen cycling functional balance (NCFB) index to quantify shifts in the relative coordination between hydrolytic and reductive functions, and linked these shifts to changes in soil water status, carbon and nitrogen availability, and microbial biomass. Specifically, this study asked: (1) whether degradation alters soil N cycling through enzyme-specific rather than uniform responses; (2) whether hydrological limitation and substrate depletion are the main mechanisms underlying these divergent responses; and (3) whether interannual climatic variation, particularly drought, amplifies degradation-induced functional decoupling. By explicitly integrating degradation intensity, hydroclimatic variability, and enzymatic functional balance, this study moves beyond descriptive assessments of enzyme activity and provide a mechanistic framework for understanding how alpine wetland degradation reshapes belowground N cycling.

2. Materials and methods

2.1 Study area

This study was conducted in the Gahai–Zecha National Nature Reserve (102°26′ E, 34°16′ N; 3450 m a.s.l.), located in the northeastern Qinghai–Tibet Plateau (QTP), China. The region is characterized by a highland continental monsoon climate. The mean annual temperature of the Gahai wet meadow is 1.2 °C, with the lowest and highest monthly mean temperatures occurring in January (-9.1 °C) and July (10.5 °C), respectively. The mean annual precipitation is approximately 782 mm, of which nearly 80% occurs during the growing season from May to September (**Figure S1**). According to the Food and Agriculture Organization of the United Nations (FAO) soil classification system, the soil is classified as typical swamp meadow soil with a sandy loam texture. The dominant plant species include *Carex tibetikobresia*, *Carex meyeriana*, *Thalictrum aquilegifolium*, *Artemisia subulata*, and *Artemisia linearis*.

Over the past half-century, temperatures in this region have increased by 0.4 °C per decade, while precipitation has decreased by 2 mm per decade (Masson-Delmotte et al., 2021; Wang et al., 2024). This climatic shift has triggered progressive drying within the Gahai Wetland, significantly diminishing its water storage capacity. Concurrently, under pressure from population growth and socioeconomic activities, a substantial portion of the region's wetlands were drained for conversion to pastureland. From October to April, the Gahai wet meadows are routinely utilized as winter pasture for yaks, sheep, and goats. Long-term overgrazing coupled with inappropriate grazing management practices have greatly exacerbated wetland degradation (Du et

https://doi.org/10.5194/egusphere-2026-3406
Preprint. Discussion started: 27 July 2026

al., 2025). Furthermore, high densities of *Ochotona curzoniae* are observed in this area. Consequently, this site presents an ideal setting for investigating the response of N-cycle enzyme activities to wet meadow degradation.

2.2 Experimental design

Based on differences in wetland vegetation characteristics and hydrological conditions in the study area, a space-for-time substitution approach was used to delineate four degradation stages: non-degraded (ND), lightly degraded (LD), moderately degraded (MD), and heavily degraded (HD) wetlands (**Table S1**). Within each degradation stage, three 10 m × 10 m sampling plots were established, resulting in a total of 12 sampling plots. To minimize potential edge effects, a buffer distance of at least 10 m was maintained between plots belonging to different degradation stages. Detailed information on the vegetation characteristics of the sampling plots has been reported in our previous studies (Chang et al., 2023; Du et al., 2024).

2.3 Soil samples and analysis

Soil samples were collected during the growing seasons from June to September in 2019, 2020, and 2021. Within each of the 12 established plots, composite soil samples were collected using a five-point sampling method. At each sampling point, soil cores with a diameter of 5 cm were collected from three depths: 0–10, 10–20, and 20–40 cm. Soil samples from the same depth within each plot were thoroughly homogenized to form one composite sample, yielding a total of 432 composite soil samples across the study period: 12 sampling events × 4 degradation stages × 3 soil depths × 3 replicates.

In addition, undisturbed soil cores were collected from each plot using stainless-steel cutting rings with a volume of 100 cm^3 to determine soil bulk density. Visible roots, plant residues, and organic debris were carefully removed by hand. Fresh soil samples were then passed through a 2-mm mesh sieve to ensure sample homogeneity. Each sieved sample was divided into two subsamples. One subsample was air-dried at room temperature for the analysis of basic soil physicochemical properties, whereas the other was stored at 4 °C in a portable cooler and transported to the laboratory for the analysis of microbial biomass and related biochemical properties. These analyses were completed within two weeks of sample collection.

2.4 Soil physicochemical property analysis

Soil pH was determined in a 1:2.5 soil-to-water suspension using a pH meter (Bao, 2000). Soil organic carbon (SOC) was measured using the potassium dichromate oxidation method. Total nitrogen (TN) was determined using the semi-micro Kjeldahl distillation–titration method (Lu, 2000). Total phosphorus (TP) was measured using the molybdenum–antimony colorimetric method after acid digestion. Soil ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) contents were determined by KCl extraction followed by distillation–titration (Min et al., 2011). Microbial biomass nitrogen (MBN) was measured using the chloroform fumigation–extraction method with K_2SO_4 as the extractant (Brookes et al., 2002). Soil water content (SWC) and soil temperature (TEM) were measured using soil moisture/temperature sensors (5TM and EC-TM sensors) connected to a data logger (Em50G, Decagon Devices, Pullman, WA, USA).

2.5 Soil N-cycle enzyme activity analysis

The activities of four soil N-cycling enzymes, namely urease, protease, nitrate reductase, and nitrite reductase, were determined colorimetrically using urea, casein, nitrate, and nitrite as substrates, respectively (Ma et al., 2021; Ou et al., 2019; Pu et al., 2019). After a 24-h incubation at a constant temperature, the absorbance of each enzyme assay was measured using a

https://doi.org/10.5194/egusphere-2026-3406
Preprint. Discussion started: 27 July 2026

spectrophotometer at the corresponding wavelength: 578 nm for urease, 560 nm for protease, and 520 nm for both nitrate reductase and nitrite reductase. Detailed procedures for these enzyme assays have been described previously (Chang et al., 2023).

2.6 Soil nitrogen cycling functional balance

To assess degradation-induced shifts in the internal organization of soil N cycling, a nitrogen cycling functional balance (NCFB) index was calculated as the standardized difference between hydrolytic and reductive enzyme functions: NCFB = z(urease) + z(protease) − [z(nitrate reductase) + z(nitrite reductase)], where z denotes z-score standardization across all samples. Higher NCFB values indicate a relative predominance of hydrolytic over reductive functions, whereas lower values indicate a shift toward reductive dominance and greater functional imbalance within soil N cycling.

2.7 Statistical analyses

All statistical analyses were performed in R version 4.2.2 (R Core Team, 2022). Data normality was assessed using the Kolmogorov–Smirnov test. Variables that did not meet the assumption of normality were log-transformed before subsequent analyses. One-way analysis of variance (ANOVA) was used to test for differences in soil physicochemical properties and soil N-cycling enzyme activities among degradation stages, followed by Tukey's honestly significant difference (HSD) test for multiple comparisons.

To quantify the effects of degradation stage, sampling year, and sampling month on soil N-cycling enzyme activities, linear mixed-effects models were fitted using the lme4 package (Bates et al., 2014). Degradation stage, sampling year, and sampling month were treated as fixed effects, whereas sampling block and soil depth were included as random effects. To estimate the relative importance of the main fixed factors, hierarchical partitioning was further performed using the glmm.hp package (Lai et al., 2022), and the independent contributions of degradation stage, sampling year, and sampling month to variation in each enzyme activity were calculated. To evaluate the temporal sensitivity of degradation effects, enzyme responses in degraded wetlands relative to non-degraded wetlands were additionally expressed as logarithmic response ratios (Hedges et al., 1999). Because enzyme responses along the degradation gradient were not expected to be strictly linear, generalized additive mixed models (GAMMs) were fitted to test for potential nonlinear relationships between degradation intensity and each enzyme activity (Wood, 2011).

Redundancy analysis (RDA) was performed to examine the relationships between soil N-cycling enzyme activities and soil physicochemical and microbial variables (Oksanen et al., 2007). Prior to ordination, highly collinear explanatory variables were removed based on collinearity diagnostics. The significance and relative importance of the remaining explanatory variables were then evaluated using Monte Carlo permutation tests with 999 permutations. Because some potentially important variables were excluded during collinearity screening, Spearman correlation analysis combined with best-subset multiple regression was further used to quantify the relative contributions of key biotic and abiotic factors to variation in enzyme activities. Finally, based on the key environmental variables identified by the RDA and regression analyses, a piecewise structural equation model was constructed to evaluate the direct and indirect pathways through which wetland degradation regulated the NCFB index (Lefcheck, 2016). Statistical significance was assessed at $P < 0.05$ unless otherwise stated.

https://doi.org/10.5194/egusphere-2026-3406
Preprint. Discussion started: 27 July 2026

3. Results

3.1 Wetland degradation reshaped the soil hydrological and nutrient environment

Wetland degradation was accompanied by pronounced shifts in soil hydrological and biogeochemical conditions (**Table 1**). Across the degradation gradient, soil water content (SWC), pH, soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), ammonium nitrogen (NH_4^+-N), and microbial biomass nitrogen (MBN) generally declined, whereas soil temperature (TEM) and nitrate nitrogen (NO_3^--N) increased. Soil bulk density was also higher in degraded wetlands than in non-degraded wetlands, with the highest value observed under moderate degradation. Among these variables, the strongest degradation-related gradients were observed for SWC, MBN, and inorganic N forms. Non-degraded wetlands maintained substantially higher SWC, NH_4^+-N, and MBN, whereas degraded wetlands, particularly moderately degraded and heavily degraded wetlands, showed lower substrate and microbial N availability but higher NO_3^--N. Overall, these patterns indicated that wetland degradation progressively transformed the soil environment from a wetter and C- and N-rich state toward a drier, warmer, and more nutrient-depleted condition.

Table 1 Basic physical and chemical properties of sample plots.

	ND	LD	MD	HD
SWC ($m^3 \cdot m^{-3}$)	0.45±0.12 a	0.31±0.09 a	0.23±0.08 b	0.14±0.03 b
TEM (°C)	9.82±0.10 c	10.35±0.11 b	11.28±0.12 b	13.26±0.05 a
pH	7.92±0.04 a	7.79±0.06 b	7.77±0.08 b	7.76±0.06 b
SOC ($g \cdot kg^{-1}$)	52.55±7.85 a	42.01±5.60 ab	36.45±1.89 ab	32.99±5.99 b
TN ($g \cdot kg^{-1}$)	3.07±0.18 a	2.67±0.20 ab	2.41±0.16 b	1.88±0.21 c
TP ($g \cdot kg^{-1}$)	1.48±0.51 a	1.29±0.30 ab	1.17±0.08 b	1.15±0.22 b
C:N	16.24±0.48 a	15.41±0.72 a	14.75±0.42 b	14.07±0.20 b
C:P	35.53±0.54 a	33.02±0.61 b	31.17±0.51 c	28.67±0.38 d
N:P	2.21±0.02 a	2.16±0.11 a	2.15±0.12 a	1.71±0.07 b
NH_4^+-N ($mg \cdot kg^{-1}$)	6.20±0.51 a	5.87±0.45 b	5.20±0.35 c	4.81±0.21 c
NO_3^--N ($mg \cdot kg^{-1}$)	4.71±0.21 c	5.01±0.18 c	6.32±0.14 b	7.12±0.09 a
MBN ($mg \cdot kg^{-1}$)	45.26±2.25 a	37.35±3.20 b	35.12±3.84 b	29.45±2.24 c

Note: SWC: soil water content, TEM: temperature. BD: bulk density, SOC: soil organic carbon, TN: total nitrogen, TP: total phosphorus, NH_4^+-N: ammonium nitrogen, NO_3^--N: nitrate nitrogen, MBN, microbial biomass nitrogen. ND, non-degraded; LD, lightly degraded; MD, moderately degraded; HD, heavily degraded. Different lowercase letters in the same row indicate significant differences between different degrees of degradation (P<0.05). The data in the table are expressed as average ± standard deviation

3.2 Soil N-cycling enzymes responded divergently to degradation

Wetland degradation induced clear divergence among soil N-cycling enzymatic pathways (**Figure 1**). Urease and nitrate reductase activities generally increased along the degradation gradient, whereas protease and nitrite reductase activities declined. Although nitrate reductase activity did not differ significantly between non-degraded and lightly degraded wetlands, urease activity differed significantly between these two degradation stages ($P < 0.05$). Soil protease activity decreased with

https://doi.org/10.5194/egusphere-2026-3406
Preprint. Discussion started: 27 July 2026

increasing wetland degradation, but the maximum reduction, observed under heavy degradation, was only 13.00% relative to non-degraded wetlands. Accordingly, no significant differences in protease activity were detected among the four degradation stages. In contrast, nitrite reductase activity showed a pronounced decreasing trend, with significant differences observed among degradation stages. In particular, nitrite reductase activity decreased by 95.30% from non-degraded to heavily degraded wetlands.

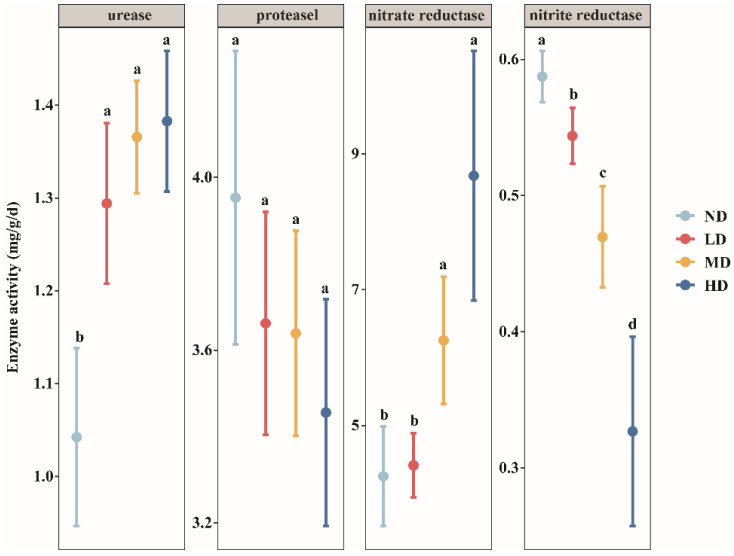

Figure 1. Enzyme-specific responses of four soil nitrogen-cycling enzymes across the alpine wetland degradation gradient. Activities of (a) urease (URE), (b) protease (PRO), (c) nitrate reductase (NR), and (d) nitrite reductase (NIR) are shown for non-degraded (ND), lightly degraded (LD), moderately degraded (MD), and heavily degraded (HD) wetlands. Different lowercase letters indicate significant differences among degradation stages at $P < 0.05$.

3.3 Temporal variation modulated the strength of degradation effects

Soil N-cycling enzyme activities exhibited clear seasonal and interannual variation (**Figure 2**). Urease activity generally increased from the early to the middle growing season and then declined toward September, whereas protease activity showed a similar seasonal trajectory but remained relatively low late in the growing season. Nitrate reductase activity was typically lowest in June and increased thereafter, whereas nitrite reductase activity displayed a more conserved seasonal pattern, with peak values generally occurring in August. These patterns indicated that the timing of maximum activity differed among enzymatic pathways, suggesting different temporal controls on hydrolytic and reductive processes.

Linear mixed-effects models further showed that degradation stage and sampling month significantly affected all four enzymes, whereas the effects of sampling year were enzyme-specific (**Table 2**). Degradation stage explained the largest proportion of variation in urease and nitrite reductase activities, sampling month explained most of the variation in nitrate reductase activity, and sampling year dominated variation in protease activity (**Figure S2**). Interannual contrasts also showed that degradation effects were not constant over time. Urease responses to degradation were weak in 2019 but became stronger in 2020 and 2021, especially under moderate and heavy degradation. Protease activity generally declined with degradation, with the clearest reductions occurring in the drier year. Nitrate reductase and nitrite reductase activities also showed year-dependent differences

https://doi.org/10.5194/egusphere-2026-3406
Preprint. Discussion started: 27 July 2026

in the magnitude, but not the overall direction, of degradation effects. Together, these results showed that temporal variation regulated not only absolute enzyme activity but also the strength with which degradation was functionally expressed. Generalized additive mixed models (GAMMs) further showed that the responses of soil N-cycling enzymes to degradation were enzyme-specific and were not uniformly linear along the alpine wetland degradation gradient (**Figure S3**). Urease and nitrate reductase activities showed stronger nonlinear increases with degradation, whereas protease and nitrite reductase activities declined more gradually or showed year-specific rates of decline.

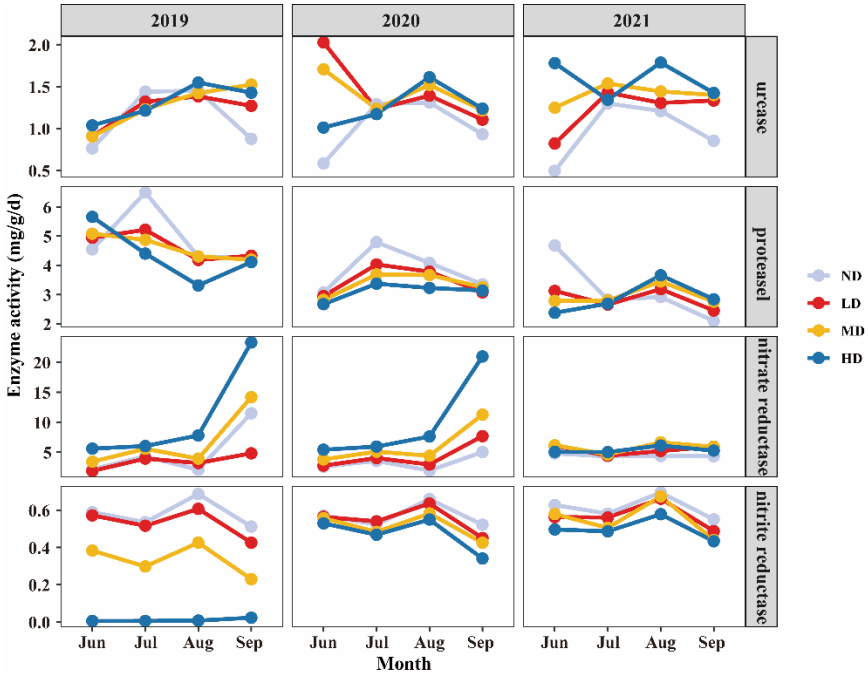

Figure 2. Temporal changes in soil N-cycle enzyme activities at different levels of degradation during 2019, 2020 and 2021. urease (URE), protease (PRO), nitrate reductase (NR), and nitrite reductase (NIR). ND, non-degraded; LD, lightly degraded; MD, moderately degraded; HD, heavily degraded.

Table 2 Effects of degradation stage, year, and sampling time on soil N-cycling enzyme activities based on linear mixed-effects models.

	Urease	Protease	Nitrate reductase	Nitrite reductase
Degradation	14.90**	4.40**	29.13**	87.44**
Year	1.516	196.45**	6.74*	164.11**
Month	12.60**	7.73**	52.03**	30.28**

Note: Summary of linear mixed model analyzing the effects of degradation and time on four soil N-cycle enzyme activities, using degradation, time, and their interaction as fixed effects, depth and block were taken as a random factor. The numeric value represents the size of the F value, * and ** representing $p<0.05$ and $p<0.01$, respectively.

3.4 Wetland degradation induced imbalance in soil N-cycling functions

The nitrogen cycling functional balance (NCFB) index decreased significantly with increasing degradation intensity (**Figure 3**). Relative to ND, the NCFB index declined by 0.465, 0.660, and 0.775 in LD, MD, and HD, respectively. The NCFB index

https://doi.org/10.5194/egusphere-2026-3406
Preprint. Discussion started: 27 July 2026

was positive in ND wetlands (0.432), approached zero under LD, and became negative under MD and HD (-0.18 and -0.30, respectively). ANOVA showed that ND differed significantly from all degraded stages, including LD, MD, and HD, confirming a strong effect of wetland degradation on soil N-cycling functional balance.

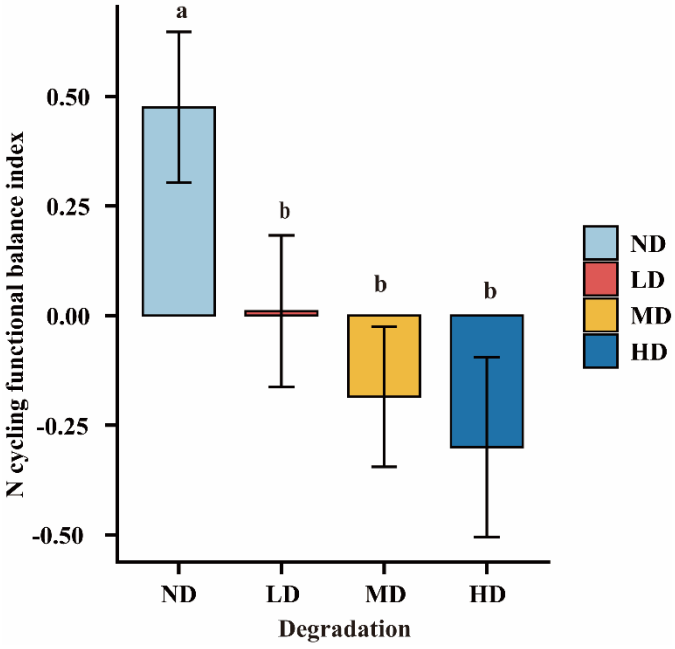

Figure 3. Response of N cycling functional balance (NCFB) index to wetland degradation. ND, non-degraded; LD, lightly degraded; MD, moderately degraded; HD, heavily degraded.

Changes in the relationship between hydrolytic and reductive functions further supported this functional reorganization (**Figure S4**). ND, LD, and MD showed negative associations between hydrolytic and reductive functions, whereas HD showed a positive association. The strongest pattern occurred under light degradation, where the two functional groups were significantly negatively correlated (ρ = -0.534). By contrast, the relationships in ND (ρ = -0.215), MD (ρ = -0.242), and HD (ρ = 0.265) were not significant. Overall, these results indicated that wetland degradation altered not only the magnitude of enzyme activity but also the coupling structure between major soil N-cycling pathways.

3.5 Relationships between soil N-cycling enzyme activities and potential environmental drivers

Redundancy analysis (RDA) indicated that soil environmental factors strongly structured variation in soil N-cycling enzyme activities, with RDA1 and RDA2 explaining 66.20% and 8.62% of the total variation, respectively (**Figure 4**). Urease and protease activities were mainly associated with SOC, TN, TP, MBN, SWC, and NH_4^+-N, whereas nitrate reductase activity was more closely associated with TEM and NO_3^--N. Nitrite reductase activity showed weaker associations with individual environmental variables. Permutation tests with 999 permutations identified NH_4^+-N as the most important independent explanatory variable, followed by TN, MBN, NO_3^--N, and SOC. Correlation analysis combined with best-subset multiple regression further confirmed that TN, MBN, NO_3^--N, and SOC were the major contributors to variation in enzyme activities, with greater explanatory power for urease and protease than for nitrate reductase and nitrite reductase.

https://doi.org/10.5194/egusphere-2026-3406
Preprint. Discussion started: 27 July 2026

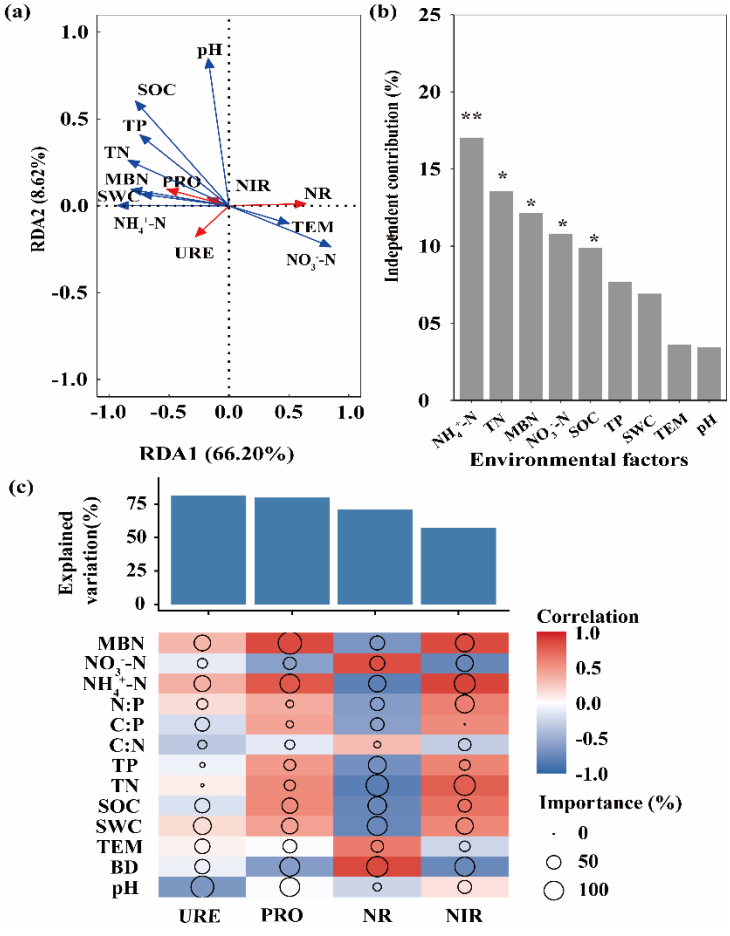

Figure 4. Contributions of soil biotic and abiotic factors to soil N-cycling enzyme activities. (a) Redundancy analysis (RDA) ordination of enzyme activities and environmental variables. (b) Permutation-based ranking of the independent contributions of environmental factors identified by RDA. (c) Relative importance of soil variables for enzyme activities based on correlation analysis and optimal multiple regression modeling. Circle size indicates the relative importance of each variable, and color indicates the Spearman correlation coefficient. URE, urease; PRO, protease; NR, nitrate reductase; NIR, nitrite reductase; BD, bulk density; TEM, temperature; SWC, soil water content; SOC: soil organic carbon; TN, total nitrogen; TP: total phosphorus; NH_4^+-N, ammonium nitrogen; NO_3^--N, nitrate nitrogen; MBN, microbial biomass nitrogen.

Based on the key environmental factors identified by the RDA and regression analyses, we constructed a piecewise structural equation model to elucidate how wetland degradation affected the NCFB index through hydrological limitation, carbon resource status, soil N availability, and microbial biomass (**Figure 5**). Wetland degradation had significant negative effects on SWC and SOC, and SWC showed positive pathways to TN, NH_4^+-N, and NO_3^--N. NH_4^+-N exerted a significant positive effect on NCFB, suggesting that greater ammonium availability was associated with a relative predominance of hydrolytic over reductive N-cycling functions. In contrast, SOC had a direct negative effect on NCFB. Overall, the path analysis identified hydrological limitation as a dominant control on NCFB imbalance, with SWC exerting both direct and indirect effects through associated changes in soil N availability and microbial biomass.

https://doi.org/10.5194/egusphere-2026-3406
Preprint. Discussion started: 27 July 2026

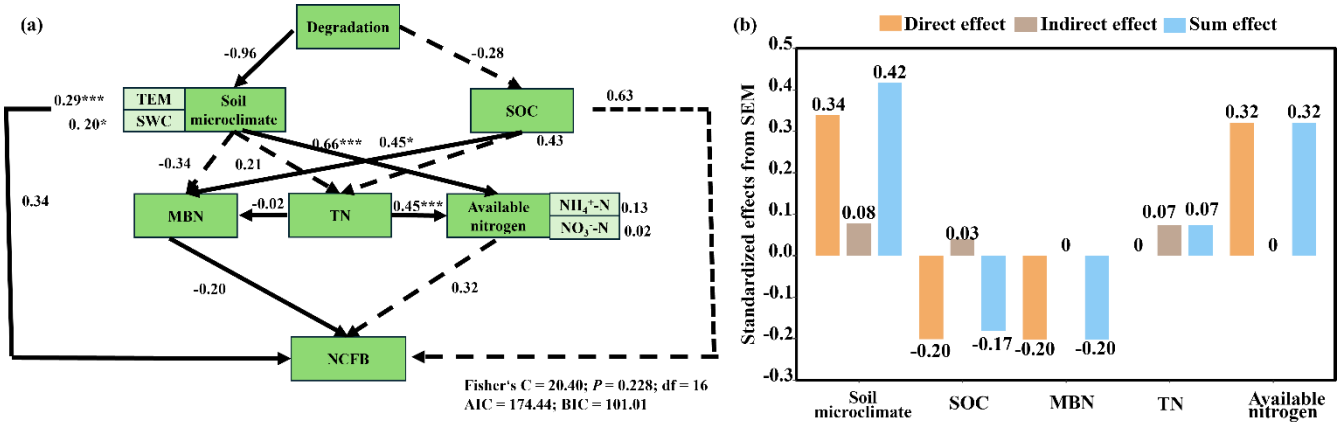

Figure 5. Piecewise structural equation model showing how wetland degradation influences nitrogen-cycling functional imbalance through hydrological drying, substrate depletion, and microbial biomass constraints. a) Structural equation modeling shows the influences of N cycling functional balance. b) Direct effects, indirect effects and standardized total effect values of structural equation models. TN, total nitrogen; NH_4^+-N, ammonium nitrogen; NO_3^--N, nitrate nitrogen; MBN, microbial biomass nitrogen; SWC, soil water content; TEM, temperature.

4. Discussion

4.1 Wetland degradation redefines the hydrological and resource environment of soil N cycling

From a process-based perspective, hydrological drying appears to be the primary trigger linking vegetation degradation to belowground functional change. As wetland soil loses water and becomes more aerated, substrate diffusion, oxygen availability, and nutrient accessibility are altered (**Table 1**). At the same time, declines in SOC and NH_4^+-N suggest a progressive reduction in readily available C and N substrates, whereas lower MBN indicates reduced microbial biomass and potentially weakened microbial N retention capacity (Cai et al., 2024; Li et al., 2024). Together, these changes create a resource-limited and increasingly oxic soil environment in which microorganisms may need to reallocate enzymatic investment to maintain N acquisition and transformation (Li et al., 2025). Therefore, the first mechanistic consequence of alpine wetland degradation is the restructuring of the environmental context in which soil N cycling operates, rather than a uniform weakening of all microbial functions.

4.2 Differential responses of soil N-cycling enzymes to alpine wetland degradation

Against this altered environmental background, the four soil N-cycling enzymes did not respond synchronously. Instead, wetland degradation promoted a clear divergence among enzymatic pathways, indicating that soil N cycling was reorganized rather than uniformly suppressed (**Figure 1**). Urease and nitrate reductase activities increased with degradation, whereas protease and nitrite reductase activities declined. The increase in urease activity can be interpreted as a shift toward faster access to relatively simple N compounds under resource-constrained conditions (Burns et al., 2013). As soil drying and nutrient depletion progress, microorganisms may rely more heavily on enzymatic routes that rapidly release inorganic N from accessible substrates, especially when the decomposition of complex organic matter becomes increasingly constrained (Geisseler et al., 2010). As the enzyme that directly catalyzes the hydrolysis of urea to ammonium(Krajewska, 2009), urease

https://doi.org/10.5194/egusphere-2026-3406
Preprint. Discussion started: 27 July 2026

is closely linked to substrate turnover, pH conditions, and soil aeration (Pan et al., 2020; Wang et al., 2023). In contrast, the decline in protease activity indicates a reduced capacity to depolymerize more complex organic N pools, which is consistent with lower substrate supply, reduced microbial activity, and poorer diffusion conditions in degraded soils(Song et al., 2014; Vranova et al., 2013). At the same time, declines in SOC, TN, NH_4^+-N, and MBN suggest progressive depletion of both substrate resources and microbial metabolic capacity (Greenfield et al., 2021), thereby reducing microbial investment in the acquisition of complex organic N (Brockett et al., 2011; Chen et al., 2018; Ma et al., 2021; Vranova et al., 2013). This interpretation is consistent with findings from studies of alpine wetland degradation in Zoige (Huo et al., 2013). Thus, within the hydrolytic component of soil N cycling, degradation appears to shift function away from complex organic N acquisition and toward rapid-turnover pathways.

A similar internal divergence occurred within the reductive component of N cycling. Nitrate reductase activity increased, whereas nitrite reductase activity declined sharply with degradation (**Figure 1**). This decoupling is consistent with a drying-induced shift in soil redox status: lower water tables and greater aeration can stimulate nitrification and nitrate accumulation (Szajdak and Gaca, 2010; Li et al., 2015), thereby supporting the initial step of nitrate reduction while simultaneously suppressing the anaerobic microsites required for sustained downstream nitrite reduction (Hefting et al., 2004). In other words, degradation appears to create a functional bottleneck in the N-reduction pathway, in which upstream substrate supply is maintained or enhanced (Ayiti and Babalola, 2022) whereas downstream oxygen-sensitive reduction is constrained (Jin et al., 2019) This interpretation is also supported by the observed increase in NO_3^--N and by the stronger association of nitrate reductase activity with TEM and NO_3^--N in the RDA results. Therefore, wetland degradation did not simply weaken soil N cycling overall, but reorganized it by enhancing some rapid-turnover processes while suppressing enzymes associated with complex organic N depolymerization and oxygen-sensitive reduction steps.

4.3 Hydroclimatic variability modulated the magnitude of degradation-induced functional divergence

Our multi-year analysis from 2019 to 2021 revealed pronounced seasonal and interannual patterns in soil N-cycling enzyme activities (**Figure 2**). At the seasonal scale, urease, protease, and nitrite reductase, reached peak activity during the middle of the growing season, particularly in July and August, whereas nitrate reductase activity showed a delayed peak in September. The midsummer maxima were consistent with the period of relatively favorable environmental conditions on the QTP, when higher temperatures, adequate soil moisture, and intensified plant growth jointly stimulate microbial metabolism and enzyme production (Kivlin and Treseder, 2014). In particular, July and August correspond to the peak period of plant vegetative growth, during which enhanced root exudation supplies labile C to the rhizosphere, thereby stimulating microbial activity and promoting enzyme synthesis (Brzostek et al., 2011; Meier et al., 2017). By contrast, the delayed peak in nitrate reductase activity in September likely reflected a seasonal shift in plant–microbe competition for N. As plant senescence progresses toward the end of the growing season, plant N uptake declines, allowing residual nitrate (NO_3^-) to accumulate in soil (Hefting et al., 2004; Keiluweit et al., 2017). This substrate accumulation, together with increased soil moisture and the formation of localized anaerobic microsites under cooler autumn conditions, may create a favorable microenvironment for nitrate reductase

https://doi.org/10.5194/egusphere-2026-3406
Preprint. Discussion started: 27 July 2026

induction (Kotroczó et al., 2014). These seasonal differences highlight the complexity of soil N-cycling enzyme regulation under wetland degradation and indicate that individual enzymes respond to distinct substrate and microenvironmental controls. More importantly, the effects of wetland degradation on soil N-cycling enzymes were not static, but were strongly modulated by interannual hydroclimatic variation, particularly under drought conditions. The marked differences in annual precipitation during the study period appeared to determine the extent to which structural degradation was translated into functional divergence. Urease and nitrate reductase activities generally showed positive responses to degradation relative to ND, indicating that these two enzymes remained consistently higher in degraded wetlands than in non-degraded wetlands, with the contrast being especially pronounced in MD and HD during the dry year of 2020 (**Figure S2**). This pattern suggests that drought may amplify the relative advantage of rapid N hydrolysis and the initial step of nitrate reduction in degraded wetlands, likely because degradation-enhanced aeration, stronger redox differentiation, and altered N transformation pathways become more evident under water deficit (Asensio et al., 2024; Steinweg et al., 2012). In contrast, protease and nitrite reductase activities showed consistently negative responses across the three years, indicating that degraded wetlands persistently exhibited lower capacities for complex organic N depolymerization and downstream nitrite reduction than ND wetlands (Priemé et al., 2002). Notably, the negative response of protease activity was strongest in 2020 but became weaker under the wetter conditions of 2021, suggesting that this enzyme was particularly sensitive to soil moisture and resource availability. Drought likely intensified degradation-induced constraints on substrate diffusion, microbial activity, SOC availability, and MBN (**Figure 5**). By comparison, the negative response of nitrite reductase activity was more directionally stable across years, despite variation in magnitude, implying that this process was intrinsically more sensitive to degradation and could not readily recover even under wetter conditions (Giles et al., 2012). Together, these findings suggest that degradation created a vulnerable soil environment, whereas drought intensified the same constraints that pushed the system toward greater functional divergence. This hydroclimatic dependence also helps explain why enzyme responses should not be interpreted as fixed, trait-like outcomes of degradation stage alone. In wetter years or wetter periods of the growing season, partial rewetting may buffer some differences among degradation stages by improving substrate diffusion and microbial activity. However, this buffering should not be mistaken for functional recovery. Rather, it indicates that the expression of degradation effects depends strongly on the hydrological background. The key implication is that future climatic drying is likely to increase, rather than reduce, the ecological importance of wetland degradation for soil N cycling on the QTP.

4.4 Functional decoupling of soil N cycling and its implications for wetland stability and restoration

The progressive decline in the nitrogen cycling functional balance (NCFB) index provides an integrative summary of these enzyme-specific responses. As degradation intensified, the NCFB index shifted from positive values in non-degraded wetlands toward near-zero or negative values in degraded wetlands (**Figure 3**), indicating that the relative coordination between hydrolytic and reductive functions was progressively weakened. This interpretation is consistent with the broader ecoenzymatic framework, in which the relative activities of enzymes involved in nutrient acquisition and transformation reflect shifts in microbial resource allocation and coupled biogeochemical functioning (Luo et al., 2017; Moorhead et al., 2023). However, this shift should not be interpreted as a uniform weakening of all hydrolytic processes or a uniform strengthening of

https://doi.org/10.5194/egusphere-2026-3406
Preprint. Discussion started: 27 July 2026

all reductive processes. Rather, it reflects pathway-specific reallocation, whereby increased urease and nitrate reductase activities were accompanied by declines in protease and nitrite reductase activities. Such reallocation is consistent with the concept of microbial economic allocation, whereby microorganisms adjust enzymatic investment according to resource demand, substrate accessibility, and environmental constraints (Malik et al., 2019). Thus, the decline in NCFB indicates that the internal balance of enzyme-mediated soil N cycling became increasingly skewed under degraded conditions.

This interpretation is further supported by the stage-dependent changes in the relationship between hydrolytic and reductive functions (**Figure S4**). The association between these two functional groups shifted in both strength and direction along the degradation gradient, suggesting that wetland degradation altered not only enzyme activity levels but also the coupling structure among major N-cycling pathways. In particular, the strongest negative relationship occurred in lightly degraded wetlands, indicating that mild degradation may generate a pronounced functional mismatch between hydrolytic and reductive processes. This mismatch may arise because early-stage degradation differentially affects substrate availability, soil moisture, and microbial allocation strategies before the system shifts toward a more uniformly constrained state under severe degradation (Song et al., 2024). By the heavily degraded stage, soil N cycling no longer appeared to be organized around a relatively balanced internal turnover regime; instead, its functional configuration became increasingly skewed toward specific stress-responsive pathways. Given that alpine wetland degradation can induce soil nutrient deficiencies, alter stoichiometric balance, and reshape microbial diversity and function, such stage-dependent decoupling is ecologically plausible and provides a mechanistic explanation for the observed decline in NCFB.

From a land degradation and restoration perspective, these findings suggest that the principal consequence of alpine wetland degradation is not merely a reduction in belowground biological activity, but a reorganization of soil N-cycling functions toward a less balanced and potentially less stable state. Restoration efforts should therefore focus not only on aboveground vegetation recovery but also on re-establishing hydrological conditions that support substrate diffusion, microbial biomass maintenance, and the internal coordination of N cycling. In practical terms, rewetting and the reduction of chronic disturbance are likely to be more important for restoring biogeochemical functioning than simply increasing nutrient inputs. At the same time, several limitations should be acknowledged. This study was based on potential enzyme activities rather than direct measurements of gross N transformation rates, and microbial functional mechanisms were inferred rather than directly measured. Future work should integrate hydrological manipulation experiments, direct measurements of N transformation processes, and microbial functional gene or metagenomic analyses to more explicitly test how degradation restructures the soil N-cycling network. Even so, the current study provides a mechanistic basis for understanding how alpine wetland degradation and climatic drying may interact to destabilize belowground nutrient cycling.

5. Conclusions

Alpine wetland degradation did not uniformly suppress soil nitrogen cycling; instead, it reorganized soil N cycling by differentially affecting enzyme-mediated transformation pathways. With increasing degradation intensity, soil N cycling shifted from a relatively coordinated state in non-degraded wetlands toward a functionally decoupled configuration,

https://doi.org/10.5194/egusphere-2026-3406
Preprint. Discussion started: 27 July 2026

characterized by enhanced rapid-turnover processes but weakened complex organic N depolymerization and downstream oxygen-sensitive reduction. This functional reorganization was primarily linked to degradation-induced hydrological drying and resource depletion. Declines in soil water content and carbon and nitrogen availability altered the biochemical environment in which microorganisms allocated enzymatic investment, thereby favoring some N transformation pathways while constraining others. Interannual hydroclimatic variability further modulated this process, with drier conditions amplifying the functional divergence between degraded and non-degraded wetlands. These findings suggest that the ecological consequence of alpine wetland degradation is not merely a reduction in soil biochemical activity, but a shift toward a less balanced and potentially less stable belowground N-cycling system. Restoration of degraded alpine wetlands should therefore prioritize hydrological recovery and microbial functional capacity, rather than focusing solely on vegetation cover or nutrient status.

Data availability

The data used in this paper can be accessed via the Zenodo: https://zenodo.org/records/20739760 (Chang et al., 2026).

Author contributions

Conceptualization, Methodology, Data curation, Software, Formal analysis, Writing - original draft: WC. Supervision, Project administration, Funding acquisition, Writing - review & editing: WM. Writing - review & editing: JW. Investigation: LS. Methodology: JD. Investigation: WH. Resources, Writing - review: GL.

Competing interests

The contact author has declared that none of the authors has any competing interests.

Disclaimer

Publisher's note: Copernicus Publications remains neutral with regard to jurisdictional claims made in the text, published maps, institutional affiliations, or any other geographical representation in this paper. While Copernicus Publications makes every effort to include appropriate place names, the final responsibility lies with the authors. Views expressed in the text are those of the authors and do not necessarily reflect the views of the publisher.

Acknowledgements

We thank all the people involved in this work.

https://doi.org/10.5194/egusphere-2026-3406
Preprint. Discussion started: 27 July 2026

Financial support

The study was supported by the National Natural Science Foundation of China (31860143); Gansu Provincial Science and Technology Plan (Major Special Project) Grant in 2026 (26ZDFG001); Innovation Star Project for Graduate Students in Universities in Gansu Province in 2026 (2026CXZX-793); Priority Talent Project of Gansu Province in 2024: Research on Carbon Sequestration and Sink Enhancement Technology in Degraded Wetlands in the Upper Reaches of The Yellow River in Gannan and Talent Cultivation (2024RCXM82); The Central Government Guide Local Science and Technology Development Funds In 2024 (24ZYQA023); and Gansu Provincial Department of Education Industrial Support Program Project (2021CYZC-15).

References

Abulaizi, M., Chen, M., Yang, Z., Hu, Y., Zhu, X., and Jia, H.: Response of soil bacterial community to alpine wetland degradation in arid Central Asia, Front. Plant Sci., 13, 990597, https://doi.org/10.3389/fpls.2022.990597, 2023.

Akinyemi, D. S., Zhu, Y., Zhao, M., Zhang, P., Shen, H., and Fang, J.: Response of soil extracellular enzyme activity to experimental precipitation in a shrub-encroached grassland in Inner Mongolia, Global Ecology and Conservation, 23, e01175, https://doi.org/10.1016/j.gecco.2020.e01175, 2020.

Alhassan, A.-R. M., Ma, W., Li, G., Jiang, Z., Wu, J., and Chen, G.: Response of soil organic carbon to vegetation degradation along a moisture gradient in a wet meadow on the Qinghai-Tibet Plateau, Ecol. Evol., 8, 11999–12010, https://doi.org/10.1002/ece3.4656, 2018.

Allison, S. D. and Vitousek, P. M.: Responses of extracellular enzymes to simple and complex nutrient inputs, Soil Biol. Biochem., 37, 937–944, https://doi.org/10.1016/j.soilbio.2004.09.014, 2005.

Asensio, D., Zuccarini, P., Sardans, J., Marañón-Jiménez, S., Mattana, S., Ogaya, R., Mu, Z., Llusià, J., and Peñuelas, J.: Soil biomass-related enzyme activity indicates minimal functional changes after 16 years of persistent drought treatment in a Mediterranean holm oak forest, Soil Biol. Biochem., 189, undefined-undefined, https://doi.org/10.1016/j.soilbio.2023.109281, 2024.

Ayiti, O. E. and Babalola, O. O.: Factors Influencing Soil Nitrification Process and the Effect on Environment and Health, Front. Sustainable Food Syst., 6, https://doi.org/10.3389/fsufs.2022.821994, 2022.

Bai, W., Wang, G., Shang, G., Xu, L., and Wang, Z.: Effects of experimental warming on soil enzyme activities in an alpine swamp meadow on the Qinghai-Tibetan Plateau, Pedobiologia, 101, 150910, https://doi.org/10.1016/j.pedobi.2023.150910, 2023.

Bao, S. D.: Soil and agricultural chemistry analysis, China Agriculture Science and Technique Press, beijing, 2000.

Bates, D., Mächler, M., Bolker, B., and Walker, S.: Fitting Linear Mixed-Effects Models using lme4, https://doi.org/10.48550/arXiv.1406.5823, 23 June 2014.

https://doi.org/10.5194/egusphere-2026-3406
Preprint. Discussion started: 27 July 2026

Breidenbach, A., Schleuss, P.-M., Liu, S., Schneider, D., Dippold, M. A., de la Haye, T., Miehe, G., Heitkamp, F., Seeber, E., Mason-Jones, K., Xu, X., Huanming, Y., Xu, J., Dorji, T., Gube, M., Norf, H., Meier, J., Guggenberger, G., Kuzyakov, Y., and Spielvogel, S.: Microbial functional changes mark irreversible course of Tibetan grassland degradation, Nat. Commun., 13, 1–10, https://doi.org/10.1038/s41467-022-30047-7, 2022.

Brockett, B. F. T., Prescott, C. E., and Grayston, S. J.: Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada, Soil Biol. Biochem., 44, 9–20, https://doi.org/10.1016/j.soilbio.2011.09.003, 2011.

Brookes, P. C., Landman, A., Pruden, G., and Jenkinson, D. S.: Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil, Soil Biol. Biochem., 17, 837–842, https://doi.org/10.1016/0038-0717(85)90144-0, 2002.

Brzostek, E., Greco, A., Drake, J., and Finzi, A.: Root carbon inputs to the rhizosphere stimulate extracellular enzyme activity and increase nitrogen availability in temperate forest soils, Biogeochemistry, 115, https://doi.org/10.1007/s10533-012-9818-9, 2011.

Brzostek, E. R., Blair, J. M., Dukes, J. S., Frey, S. D., Hobbie, S. E., Melillo, J. M., Mitchell, R. J., Pendall, E., Reich, P. B., Shaver, G. R., Stefanski, A., Tjoelker, M. G., and Finzi, A. C.: The effect of experimental warming and precipitation change on proteolytic enzyme activity: positive feedbacks to nitrogen availability are not universal, Glob. Change Biol., 18, 2617–2625, https://doi.org/10.1111/j.1365-2486.2012.02685.x, 2012.

Burns, R. G., DeForest, J. L., Marxsen, J., Sinsabaugh, R. L., Stromberger, M. E., Wallenstein, M. D., Weintraub, M. N., and Zoppini, A.: Soil enzymes in a changing environment: Current knowledge and future directions, Soil Biol. Biochem., 58, 216–234, https://doi.org/10.1016/j.soilbio.2012.11.009, 2013.

Cai, M., Zhang, Y., Zhao, G., Zhao, B., Cong, N., Zhu, J., Zheng, Z., Wu, W., and Duan, X.: Excessive climate warming exacerbates nitrogen limitation on microbial metabolism in an alpine meadow of the Tibetan Plateau: Evidence from soil ecoenzymatic stoichiometry, Science of The Total Environment, 930, 172731, https://doi.org/10.1016/j.scitotenv.2024.172731, 2024.

Chang, W: Alpine wetland degradation shifts enzyme-mediated soil nitrogen cycling from functional coordination to imbalance-data, Zenodo [data set], https://doi.org/10.5281/zenodo.20739760, 2026.

Chang, W., Ma, W., Song, L., Tang, Y., Long, Y., Xu, G., and Yuan, J.: Responses of soil N-cycle enzyme activities to vegetation degradation in a wet meadow on the Qinghai-Tibet Plateau, Front. Ecol. Evol., 11, 1210643, https://doi.org/10.3389/fevo.2023.1210643, 2023.

Chen, H., Li, D., Zhao, J., Xiao, K., and Wang, K.: Effects of nitrogen addition on activities of soil nitrogen acquisition enzymes：A meta-analysis, Agric. Ecosyst. Environ., 252, 126–131, https://doi.org/10.1016/j.agee.2017.09.032, 2018.

Chen, H., Ju, P., Zhu, Q., Xu, X., Wu, N., Gao, Y., Feng, X., Tian, J., Niu, S., Zhang, Y., Peng, C., and Wang, Y.: Carbon and nitrogen cycling on the Qinghai–Tibetan Plateau, Nat. Rev. Earth Env., 3, 701–716, https://doi.org/10.1038/s43017-022-00344-2, 2022.

https://doi.org/10.5194/egusphere-2026-3406
Preprint. Discussion started: 27 July 2026

Deng, Y., Li, X., Yang, C., Shi, F., Zhao, S., Zhou, S., Miao, C., Ding, R., Fu, Y. H., Yang, X., and Wang, Z.: Rapid degradation of frozen soil environments in thermokarst-affected alpine grasslands on the Qinghai-Tibet Plateau under climate change, Catena, 254, 108936, https://doi.org/10.1016/j.catena.2025.108936, 2025.

Du, J., Ma, W., Li, G., Chang, W., and Chun, L.: Soil nitrogen-related functional genes undergo abundance changes during vegetation degradation in a Qinghai-Tibet Plateau wet meadow, Appl. Environ. Microbiol., 90, e00813-24, https://doi.org/10.1128/aem.00813-24, 2024.

Du, J., Ma, W., Li, G., Wu, J., and Chang, W.: Vegetation degradation and its progressive impact on soil nitrogen mineralization in the Qinghai-Tibet Plateau's alpine wetlands: Insights from a three-year study, J. Environ. Manage., 373, 123668, https://doi.org/10.1016/j.jenvman.2024.123668, 2025.

Gao, W., Reed, S. C., Munson, S. M., Rui, Y., Fan, W., Zheng, Z., Li, L., Che, R., Xue, K., Du, J., Cui, X., Wang, Y., and Hao, Y.: Responses of soil extracellular enzyme activities and bacterial community composition to seasonal stages of drought in a semiarid grassland, Geoderma, 401, 115327, https://doi.org/10.1016/j.geoderma.2021.115327, 2021.

Geisseler, D., Horwath, W. R., Joergensen, R. G., and Ludwig, B.: Pathways of nitrogen utilization by soil microorganisms – A review, Soil Biol. Biochem., 42, 2058–2067, https://doi.org/10.1016/j.soilbio.2010.08.021, 2010.

Giles, M. E., Morley, N. J., Baggs, E. M., and Daniell, T. J.: Soil nitrate reducing processes – drivers, mechanisms for spatial variation, and significance for nitrous oxide production, Front. Microbiol., 3, https://doi.org/10.3389/fmicb.2012.00407, 2012.

Greenfield, L. M., Puissant, J., and Jones, D. L.: Synthesis of methods used to assess soil protease activity, Soil Biol. Biochem., 158, 108277, https://doi.org/10.1016/j.soilbio.2021.108277, 2021.

Hedges, L. V., Gurevitch, J., and Curtis, P. S.: The meta-analysis of response ratios in experimental ecology, Ecology, 80, 1150–1156, https://doi.org/10.1890/0012-9658(1999)080%5B1150:TMAORR%5D2.0.CO;2, 1999.

Hefting, M., Clément, J. C., Dowrick, D., Cosandey, A. C., Bernal, S., Cimpian, C., Tatur, A., Burt, T. P., and Pinay, G.: Water table elevation controls on soil nitrogen cycling in riparian wetlands along a European climatic gradient, Biogeochemistry, 67, 113–134, https://doi.org/10.1023/B:BIOG.0000015320.69868.33, 2004.

Huo, L., Chen, Z., Zou, Y., Lu, X., Guo, J., and Tang, X.: Effect of Ruoerge alpine wetland degradation on the density and fractions of soil organic carbon, Ecol. Eng., 51, 287–295, https://doi.org/10.1016/j.ecoleng.2012.12.020, 2013.

Jin, P., Chen, Y., Yao, R., Zheng, Z., and Du, Q.: New insight into the nitrogen metabolism of simultaneous heterotrophic nitrification-aerobic denitrification bacterium in mRNA expression, J. Hazard. Mater., 371, 295–303, https://doi.org/10.1016/j.jhazmat.2019.03.023, 2019.

Jin, P., Liu, M., Xu, X., Sun, Y., Kuzyakov, Y., and Gunina, A.: Gross mineralization and nitrification in degraded alpine grassland soil, Rhizosphere, 27, 100778, https://doi.org/10.1016/j.rhisph.2023.100778, 2023.

Keiluweit, M., Wanzek, T., Kleber, M., Nico, P., and Fendorf, S.: Anaerobic microsites have an unaccounted role in soil carbon stabilization, Nat. Commun., 8, https://doi.org/10.1038/s41467-017-01406-6, 2017.

Kivlin, S. N. and Treseder, K. K.: Soil extracellular enzyme activities correspond with abiotic factors more than fungal community composition, Biogeochemistry, 117, 23–37, https://doi.org/10.1007/s10533-013-9852-2, 2014.

https://doi.org/10.5194/egusphere-2026-3406
Preprint. Discussion started: 27 July 2026

Kotroczó, Z., Veres, Z., Fekete, I., Krakomperger, Z., Tóth, J. A., Lajtha, K., and Tóthmérész, B.: Soil enzyme activity in response to long-term organic matter manipulation, Soil Biol. Biochem., 70, 237–243, https://doi.org/10.1016/j.soilbio.2013.12.028, 2014.

Krajewska, B.: Ureases I. Functional, catalytic and kinetic properties: A review, J. Mol. Catal. B: Enzym., 59, 9–21, https://doi.org/10.1016/j.molcatb.2009.01.003, 2009.

Lai, J., Zou, Y., Zhang, S., Zhang, X., and Mao, L.: glmm.hp: an R package for computing individual effect of predictors in generalized linear mixed models, J. Plant Ecol., 15, 1302–1307, https://doi.org/10.1093/jpe/rtac096, 2022.

Lefcheck, J. S.: piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics, Methods Ecol. Evol., 7, 573–579, https://doi.org/10.1111/2041-210X.12512, 2016.

Li, C. Y., Li, X. L., Su, X. X., Yang, Y. W., and Li, H. L.: Effects of Alpine Wetland Degradation on Soil Microbial Structure and Diversity on the Qinghai Tibet Plateau, Eurasian. Soil. Sci+., 54, S33–S41, https://doi.org/10.1134/S1064229322030097, 2022.

Li, H., Li, T., Sun, W., Zhang, W., Zhang, Q., Yu, L., Qin, Z., Guo, B., Liu, J., and Zha, X.: Degradation of wetlands on the Qinghai-Tibetan Plateau causing a loss in soil organic carbon in 1966–2016, Plant Soil, 467, 253–265, https://doi.org/10.1007/s11104-021-05086-6, 2021.

Li, X., Su, L., Jing, M., Wang, K., Song, C., and Song, Y.: Nitrogen addition restricts key soil ecological enzymes and nutrients by reducing microbial abundance and diversity, Sci. Rep., 15, 5560, https://doi.org/10.1038/s41598-025-87327-7, 2025.

Li, Y., Hodak, M., and Bernholc, J.: Enzymatic mechanism of copper-containing nitrite reductase, Biochemistry, 54, 1233–1242, https://doi.org/10.1021/bi5007767, 2015.

Li, Y., Jin, J., Li, S., Xia, S., and Wei, J.: A Study of the Effects of Wetland Degradation on Soil-Microbial-Extracellular Enzyme Carbon, Nitrogen, and Phosphorus and Their Ecological Stoichiometry, Agronomy, 14, 3008, https://doi.org/10.3390/agronomy14123008, 2024.

Li, Z., Gao, J., Wen, L., Zou, C., Feng, C., Li, D., and Xu, D.: Dynamics of soil respiration in alpine wetland meadows exposed to different levels of degradation in the Qinghai-Tibet Plateau, China, Sci. Rep., 9, 1–14, https://doi.org/10.1038/s41598-019-43904-1, 2019.

Liu, L., Chen, H., Jiang, L., Hu, J., Zhan, W., He, Y., Zhu, D., Zhong, Q., and Yang, G.: Water table drawdown reshapes soil physicochemical characteristics in Zoige peatlands, Catena, 170, 119–128, https://doi.org/10.1016/j.catena.2018.05.025, 2018.

Lu, R. K.: Methods of soil agricultural chemical analysis, Chinese Agricultural Science and Technology Press, Beijing, 2000.

Luo, L., Meng, H., and Gu, J.-D.: Microbial extracellular enzymes in biogeochemical cycling of ecosystems, J. Environ. Manage., 197, 539–549, https://doi.org/10.1016/j.jenvman.2017.04.023, 2017.

Ma, S., Wang, Z., Chen, Y., Lu, X., China, 1college Of Tourism Henan Normal University Xinxiang Henan 453007, China, 2key Laboratory Of Mountain Surface Processes And Ecological Regulation Institute Of Mountain Hazards And Environment Chinese Academy Of Sciences Chengdu 610041, China, 3university Of Chinese Academy Of Science Beijing 100049, and China, 4wuhan Botanical Garden Chinese Academy Of Sciences Wuhan 430074: Effect of soil organic matter chemical

https://doi.org/10.5194/egusphere-2026-3406
Preprint. Discussion started: 27 July 2026

compositions on soil protease and urease activity in alpine grassland soils in Northern Xizang, China, Chinese Journal of Plant Ecology, 45, 516–527, https://doi.org/10.17521/cjpe.2020.0169, 2021.

Ma, W., Li, G., Wu, J., Xu, G., and Wu, J.: Response of soil labile organic carbon fractions and carbon-cycle enzyme activities to vegetation degradation in a wet meadow on the Qinghai–Tibet Plateau, Geoderma, 377, 114565, https://doi.org/10.1016/j.geoderma.2020.114565, 2020a.

Ma, W., Li, J., Gao, Y., Xing, F., Sun, S., Zhang, T., Zhu, X., Chen, C., and Li, Z.: Responses of soil extracellular enzyme activities and microbial community properties to interaction between nitrogen addition and increased precipitation in a semi-arid grassland ecosystem, Sci. Total Environ., 703, 134691, https://doi.org/10.1016/j.scitotenv.2019.134691, 2020b.

Malik, A. A., Puissant, J., Goodall, T., Allison, S. D., and Griffiths, R. I.: Soil microbial communities with greater investment in resource acquisition have lower growth yield, Soil Biol. Biochem., 132, 36–39, https://doi.org/10.1016/j.soilbio.2019.01.025, 2019.

Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S. L., Péan, C., Chen, Y., Goldfarb, L., Gomis, M. I., Matthews, J. B. R., Berger, S., Huang, M., Yelekçi, O., Yu, R., Zhou, B., Lonnoy, E., Maycock, T. K., Waterfield, T., and Leitzell, K.: Working Group I Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change, 16, 2021.

Meier, I. C., Finzi, A. C., and Phillips, R. P.: Root exudates increase N availability by stimulating microbial turnover of fast-cycling N pools, Soil Biology and Biochemistry, 106, 119–128, https://doi.org/10.1016/j.soilbio.2016.12.004, 2017.

Min, K., Kang, H., and Lee, D.: Effects of ammonium and nitrate additions on carbon mineralization in wetland soils, Soil Biol. Biochem., 43, 2461–2469, https://doi.org/10.1016/j.soilbio.2011.08.019, 2011.

Moorhead, D., Cui, Y., Sinsabaugh, R., and Schimel, J.: Interpreting patterns of ecoenzymatic stoichiometry, Soil Biol. Biochem., 180, 108997, https://doi.org/10.1016/j.soilbio.2023.108997, 2023.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., and Wagner, H.: The vegan package, Community ecology package, 10, 1–295, 2007.

Ou, Y., Rousseau, A. N., Wang, L., Yan, B., Gumiere, T., and Zhu, H.: Identification of the alteration of riparian wetland on soil properties, enzyme activities and microbial communities following extreme flooding, Geoderma, 337, 825–833, https://doi.org/10.1016/j.geoderma.2018.10.032, 2019.

Pan, T., Hou, S., Liu, Y., Tan, Q., Liu, Y., and Gao, X.: Influence of degradation on soil water availability in an alpine swamp meadow on the eastern edge of the Tibetan Plateau, Sci. Total Environ., 722, 137677, https://doi.org/10.1016/j.scitotenv.2020.137677, 2020.

Priemé, A., Braker, G., and Tiedje, J. M.: Diversity of Nitrite Reductase (nirK andnirS) Gene Fragments in Forested Upland and Wetland Soils, Appl. Environ. Microbiol., 68, 1893–1900, https://doi.org/10.1128/AEM.68.4.1893-1900.2002, 2002.

Pu, Y., Zhu, B., Dong, Z., Liu, Y., Wang, C., and Ye, C.: Soil N2O and NOx emissions are directly linked with N-cycling enzymatic activities, Appl. Soil Ecol., 139, 15–24, https://doi.org/10.1016/j.apsoil.2019.03.007, 2019.

R Core Team: R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2022.

https://doi.org/10.5194/egusphere-2026-3406
Preprint. Discussion started: 27 July 2026

Rajsz, A., Wojtuń, B., Mróz, L., Żołnierz, L., and Kempers, A. J.: Nitrate reductase activity in high-mountain plants: a test across species, growth form and habitat type, J. Plant Ecol., 12, 519–530, https://doi.org/10.1093/jpe/rty044, 2019.

Sardans, J., Peñuelas, J., and Estiarte, M.: Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland, Appl. Soil Ecol., 39, 223–235, https://doi.org/10.1016/j.apsoil.2007.12.011, 2008.

Sinsabaugh, R. L. and Follstad Shah, J. J.: Ecoenzymatic stoichiometry and ecological theory, Annu. Rev. Ecol. Evol. S., 43, 313–343, https://doi.org/10.1146/annurev-ecolsys-071112-124414, 2012.

Sinsabaugh, R. L., Hill, B. H., and Follstad Shah, J. J.: Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment, Nature, 462, 795–798, https://doi.org/10.1038/nature08632, 2009.

Song, Y., Song, C., Tao, B., Wang, J., Zhu, X., and Wang, X.: Short-term responses of soil enzyme activities and carbon mineralization to added nitrogen and litter in a freshwater marsh of Northeast China, Eur. J. Soil Biol., 61, 72–79, https://doi.org/10.1016/j.ejsobi.2014.02.001, 2014.

Song, Y., Song, C., Shi, F., Wang, M., Ren, J., Wang, X., and Jiang, L.: Linking plant community composition with the soil C pool, N availability and enzyme activity in boreal peatlands of Northeast China, Appl. Soil Ecol., 140, 144–154, https://doi.org/10.1016/j.apsoil.2019.04.019, 2019.

Song, Y., Song, T., An, Y., Shan, L., Su, X., and Yu, S.: Soil ecoenzyme activities coupled with soil properties and plant biomass strongly influence the variation in soil organic carbon components in semi-arid degraded wetlands, Science of The Total Environment, 922, 171361, https://doi.org/10.1016/j.scitotenv.2024.171361, 2024.

Sorensen P. O., Finzi A. C., Giasson M.-A., Reinmann A. B., Sanders-DeMott R., and Templer P. H.: Winter soil freeze-thaw cycles lead to reductions in soil microbial biomass and activity not compensated for by soil warming, Soil Biol. Biochem., 116, 39–47, https://doi.org/10.1016/j.soilbio.2017.09.026, 2018.

Steinweg, J. M., Dukes, J. S., and Wallenstein, M. D.: Modeling the effects of temperature and moisture on soil enzyme activity: Linking laboratory assays to continuous field data, Soil Biol. Biochem., 55, 85–92, https://doi.org/10.1016/j.soilbio.2012.06.015, 2012.

Szajdak, L. W. and Gaca, W.: Nitrate reductase activity in soil under shelterbelt and an adjoining cultivated field, Chem. Ecol., 26, 123–134, https://doi.org/10.1080/02757540.2010.501028, 2010.

Tabatabai, M. A., Ekenler, M., and Senwo, Z. N.: Significance of Enzyme Activities in Soil Nitrogen Mineralization, Commun. Soil Sci. Plan., 41, 595–605, https://doi.org/10.1080/00103620903531177, 2010.

Tan, X., He, J., Nie, Y., Ni, X., Ye, Q., Ma, L., Megharaj, M., He, W., and Shen, W.: Climate and edaphic factors drive soil enzyme activity dynamics and tolerance to Cd toxicity after rewetting of dry soil, Sci. Total Environ., 855, 158926, https://doi.org/10.1016/j.scitotenv.2022.158926, 2023.

Vranova, V., Rejsek, K., and Formanek, P.: Proteolytic activity in soil: A review, Appl. Soil Ecol., 70, 23–32, https://doi.org/10.1016/j.apsoil.2013.04.003, 2013.

https://doi.org/10.5194/egusphere-2026-3406
Preprint. Discussion started: 27 July 2026

Wang, G., Gao, Q., Yang, Y., Hobbie, S. E., Reich, P. B., and Zhou, J.: Soil enzymes as indicators of soil function: A step toward greater realism in microbial ecological modeling, Glob. Change Biol., 28, 1935–1950, https://doi.org/10.1111/gcb.16036, 2022.

Wang, H., Wu, J., Li, G., Yan, L., and Liu, S.: Effects of extreme rainfall frequency on soil organic carbon fractions and carbon pool in a wet meadow on the Qinghai-Tibet Plateau, Ecol. Indic., 146, 109853, https://doi.org/10.1016/j.ecolind.2022.109853, 2023.

Wang, P., Wang, J., Elberling, B., Ambus, P., Li, Y., Pan, J., Zhang, R., Guo, H., and Niu, S.: Regional emissions of soil greenhouse gases across Tibetan alpine grasslands, Geoderma, 443, 116843, https://doi.org/10.1016/j.geoderma.2024.116843, 2024.

Wei, D., Ri, X., Tarchen, T., Dai, D., Wang, Y., and Wang, Y.: Revisiting the role of CH4 emissions from alpine wetlands on the Tibetan Plateau: Evidence from two in situ measurements at 4758 and 4320 m above sea level, J. Geophys. Res-Biogeo., 120, 1741–1750, https://doi.org/10.1002/2015JG002974, 2015.

Wood, S. N.: Fast Stable Restricted Maximum Likelihood and Marginal Likelihood Estimation of Semiparametric Generalized Linear Models, J. R. Stat. Soc. Ser. B. Stat. Methodol., 73, 3–36, https://doi.org/10.1111/j.1467-9868.2010.00749.x, 2011.

Wu, P., Zhang, H., Cui, L., Wickings, K., Fu, S., and Wang, C.: Impacts of alpine wetland degradation on the composition, diversity and trophic structure of soil nematodes on the Qinghai-Tibetan Plateau, Sci. Rep., 7, 837, https://doi.org/10.1038/s41598-017-00805-5, 2017.

Xiang, Y., Yao, B., Peñuelas, J., Sardans, J., Nizzetto, L., Li, R., Liu, Y., Luo, Y., Räty, M., Long, J., and Li, Y.: Microplastic effects on soil nitrogen cycling enzymes: A global meta-analysis of environmental and edaphic factors, J. Hazard. Mater., 484, 136677, https://doi.org/10.1016/j.jhazmat.2024.136677, 2024.

Yin, T., Feng, Y., Liang, S., Wang, G., Yuan, N., Yan, D., Wu, P., Kuang, X., and Wan, L.: A suitable method for alpine wetland delineation: An example for the headwater area of the yellow river, Tibetan Plateau, Front. Environ. Sci., 10, 1062954, https://doi.org/10.3389/fenvs.2022.1062954, 2022.

Yuan, M., Wang, X., Li, Y., Niu, Z., Li, J., Zhao, Y., Xia, J., Zhang, X., and Wang, F.: Alpine wetland degradation affects carbon cycle function genes but does not reduce soil microbial diversity, Catena, 249, 108637, https://doi.org/10.1016/j.catena.2024.108637, 2025.

Zhao, W., Yin, Y., Wang, Y., Zheng, W., Gao, H., Zhao, Y., and Li, S.: No-Tillage Reseeding Enhances the Restoration of Degraded Alpine Meadows by Regulating Plant Soil and Bacterial Communities, Land Degrad Dev, ldr.70368, https://doi.org/10.1002/ldr.70368, 2025.

Zuccarini, P., Sardans, J., Asensio, L., and Peñuelas, J.: Altered activities of extracellular soil enzymes by the interacting global environmental changes, Glob. Change Biol., 29, 2067–2091, https://doi.org/10.1111/gcb.16604, 2023.