Response to Review 1

General Comments

This manuscript investigates how alpine wetland degradation affects soil nitrogen (N) cycling through the lens of enzyme activities in the Gahai Wetland on the Qinghai-Tibet Plateau. The authors employ a three-year field study across four degradation stages, measuring four key N-cycling enzymes (urease, protease, nitrate reductase, nitrite reductase) and developing a novel Nitrogen Cycling Functional Balance (NCFB) index to assess the coordination between hydrolytic and reductive functions.

General Comment: Novelty and Contribution

This manuscript addresses an important knowledge gap concerning how alpine wetland degradation restructures soil nitrogen cycling, moving beyond descriptive assessments of individual enzyme activities to evaluate functional coordination between complementary N-transformation pathways. The novelty lies in three key aspects: (1) the development of a Nitrogen Cycling Functional Balance (NCFB) index that integrates hydrolytic and reductive enzyme functions into a single metric, providing a framework for quantifying functional reorganization rather than simply measuring activity levels; (2) the demonstration that degradation induces enzyme-specific divergent responses—urease and nitrate reductase increasing while protease and nitrite reductase decline—revealing that soil N cycling undergoes pathway-specific restructuring rather than uniform suppression; and (3) the multi-year temporal design that captures how interannual hydroclimatic variability, particularly drought, amplifies degradation-induced functional decoupling. The contribution is significant in providing a mechanistic understanding of how hydrological drying and resource depletion drive N-cycling destabilization in alpine wetlands, with implications for restoration priorities in these vulnerable ecosystems. The work is suitable for publication in SOIL, as it addresses soil biogeochemical cycling in a globally important ecosystem under anthropogenic pressure, though substantial revision is needed to address limitations related to potential enzyme assays, space-for-time substitution assumptions, and causal inference. With careful revision addressing these issues, the manuscript would make a valuable contribution to SOIL. The multi-year design, enzyme-specific responses, and functional balance approach are significant strengths that merit

publication after substantial revision.

Response: We sincerely thank the reviewer for the careful and constructive evaluation of our manuscript. The comments highlighted several important issues concerning the interpretation of potential enzyme activities, the assumptions underlying the space-for-time design, the formulation and ecological interpretation of the N-cycling functional index, and the statistical support for temporal responses. We have substantially revised the manuscript to address these concerns and have restricted our interpretations to conclusions directly supported by the study design and data. The Methods, Results, Discussion, figures, and Supporting Information have been revised accordingly. Our point-by-point responses are provided below.

Major Comments

1. Potential vs. in-situ enzyme activities (Section 2.5 and Discussion)

The authors measure potential enzyme activities under optimized laboratory conditions (24-hour incubation at constant temperature with excess substrate). However:

- Redox sensitivity: Nitrate reductase and nitrite reductase are particularly sensitive to redox conditions. Measuring them under aerobic laboratory conditions may not reflect in-situ activities in wetland soils, where anaerobic microsites and fluctuating redox conditions are critical for denitrification-related processes.

- Substrate availability: The NCFB index is calculated from potential activities measured under optimal conditions, not from actual rates constrained by in-situ substrate availability. This may overstate functional imbalances if the actual rates are co-limited by substrates.

- Enzyme stability: Some soil enzymes can remain active after extraction or in stored samples, potentially overestimating activity.

Required revisions:

- Add a dedicated subsection in the Discussion addressing the limitations of potential enzyme assays for inferring in-situ N cycling

Response: We fully agree with the reviewer. The original manuscript did not sufficiently distinguish potential enzyme activities measured under standardized laboratory conditions from realized enzyme activity and N-transformation rates under field conditions. The four enzyme assays in this study were primarily intended to compare the potential enzymatic abilities of soils

among degradation levels under standardized assay conditions. We have therefore revised the terminology throughout the manuscript and now consistently refer to these measurements as potential soil N-cycling enzyme activities.

We have also added a dedicated discussion of the limitations associated with extrapolating potential enzyme activities to in-situ N cycling. Specifically, we clarify that realized field rates additionally depend on substrate availability, soil moisture and temperature, oxygen and redox conditions, substrate diffusion, microbial physiological state, and enzyme production and stabilization.

- Discuss how potential activities might differ from in-situ rates, particularly for redox-sensitive enzymes

Response: We completely agree that this distinction is particularly critical for nitrate reductase (NR) and nitrite reductase (NIR). Accordingly, we have revised the manuscript to explicitly distinguish between potential enzyme activities and realized soil nitrogen transformation rates.

Methodologically, standard soil enzyme assays conducted under controlled temperature and substrate-saturating conditions are widely interpreted as proxies for potential enzyme capacity rather than direct measurements of in-situ catalytic rates. The realized expression of this potential under field conditions is additionally governed by substrate availability, soil moisture, ambient temperature, oxygen regimes, enzyme stability, and microscale physicochemical conditions. In the specific cases of NR and NIR, although strictly anaerobic conditions were ensured during laboratory assays, their actual activities in the field are heavily regulated by oxygen supply, redox potential, substrate diffusion, nitrate/nitrite concentrations, and the development of anaerobic microsites. These environmental constraints are often partially alleviated or bypassed under standardized laboratory incubation conditions.

To reflect this nuance, we have added an explicit discussion of study limitations in the revised Discussion section, highlighting that enzyme persistence, sample storage, substrate saturation, and standardized incubation conditions can all drive discrepancies between potential enzyme activities and actual in-situ rates.

- Consider whether the NCFB index should be interpreted as "functional potential" rather than "functional balance"

Response: We agree that the term “functional balance” could imply that the index directly quantifies an optimal or realized ecosystem N-cycling state. To avoid this overinterpretation, we replaced the original NCFB with a nitrogen cycling functional potential (NCFP) index. The revised index quantifies the relative configuration of aggregate hydrolytic and reductive potential enzyme activities and is not interpreted as a direct measure of ecosystem functional balance.

- If possible, compare potential activities with any available in-situ rate measurements or cite literature on the relationship between potential and actual enzyme activities in similar systems

Response: Thank you for this important suggestion. Although potential enzyme activities should not be equated with realized field rates, previous studies indicate that they can nevertheless provide ecologically meaningful information about variation in N transformation capacity. For example, Darby et al. (2020) simultaneously quantified potential extracellular enzyme activities and gross and net N transformations and found significant associations between gross N mineralization and enzymes involved in the degradation of relatively labile substrates. In coastal wetland soils, Jia et al. (2020) reported parallel treatment responses of urease and arylamidase activities and net N mineralization and nitrification. Conversely, studies of wetland denitrification enzyme activity emphasize that potential assays are most appropriately used to compare relative functional capacities among sites or treatments rather than as direct measurements of in-situ denitrification (Hartzog et al., 2017).

Particularly relevant to the present study, our previous work in the same Gahai wetland independently quantified field-based N transformations during 2019–2021 (Du et al., 2025). Across degraded wetlands, net ammonification decreased by 22.09–97.10% and net N mineralization by 9.49–16.25%, whereas net nitrification in the heavily degraded wetland increased by 45.38% relative to the non-degraded wetland. These field measurements therefore

also indicate pathway-specific changes rather than uniform suppression of soil N cycling.

We emphasize, however, that this correspondence does not imply a one-to-one relationship between potential enzyme activity and realized N-transformation rates. We now use these independent process-level data as complementary ecological evidence rather than as validation of the enzyme assays.

References:

Darby, B. A., Goodale, C. L., Chin, N. A., Fuss, C. B., Lang, A. K., Ollinger, S. V., and Lovett, G. M.: Depth patterns and connections between gross nitrogen cycling and soil exoenzyme activities in three northern hardwood forests, Soil Biol. Biochem., 147, 107836, https://doi.org/10.1016/j.soilbio.2020.107836, 2020.

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.

Hartzog, P. E., Sladek, M., Kelly, J. J., and Larkin, D. J.: Bottle effects alter taxonomic composition of wetland soil bacterial communities during the denitrification enzyme activity assay, Soil Biol. Biochem., 110, 87–94, https://doi.org/10.1016/j.soilbio.2017.03.006, 2017.

Jia, J., Bai, J., Gao, H., Wang, W., Zhang, G., and Wang, X.: Different effects of NaCl and Na2SO4 on soil net nitrogen mineralization in coastal wetlands, Ecotoxicol. Environ. Saf., 199, 110678, https://doi.org/10.1016/j.ecoenv.2020.110678, 2020.

Liu, M., Shi, J., and Zhang, X.: Responses of Ecological Stoichiometry of Plants and Soils to Degradation Levels in Alpine Wetlands of the Qinghai-Tibet Plateau, Environ. Manage., 75, 2258–2271, https://doi.org/10.1007/s00267-025-02152-y, 2025.

2. Space-for-time substitution assumptions (Section 2.2)

The authors use space-for-time substitution but do not adequately address its limitations. The four degradation stages are described as representing a degradation gradient, but:

- Were sites matched for parent material, topography, and historical land use?

- Is there evidence that the sites represent a true chronosequence (e.g., historical aerial photos, local knowledge of degradation timing)?

- Could differences among sites reflect inherent spatial heterogeneity rather than degradation stage?

Required revisions:

- Provide more information on how degradation stages were delineated (Table S1 is mentioned but not shown in the main text)

Response: Thank you for this important comment. We have revised Section 2.2 to clarify how the four degradation levels were defined. The sites were not classified solely according to their distance from Gahai Lake. Instead, degradation levels were identified before enzyme analysis using an integrated set of independent field indicators, including dominant-species composition, vegetation cover, plant height, aboveground biomass, and groundwater depth, following the regional alpine-wetland degradation criteria cited in the revised manuscript. These diagnostic characteristics are now summarized explicitly in Table S1 and described in the main text.

"We referred to the classification criteria for alpine wetland degradation specified in the "Classification of degradation grade of alpine marshes" (DB63/T 1359–2015) and the "Ecosystem health assessment of Alpine Marsh wetland" (DB63/T 1544–2017) and further considered differences in vegetation characteristics and hydrological conditions within the study area (Liu et al., 2025). Using a space-for-time framework, we established a belt transect extending outward from the relatively intact wetland surrounding Gahai Lake. Degradation levels were identified using an integrated set of diagnostic indicators, including dominant-species composition, vegetation cover, plant height, aboveground biomass, and groundwater depth (Table S1). Sites were selected from relatively flat terrain at comparable elevations and classified into four degradation levels: non-degraded (ND), lightly degraded (LD), moderately degraded (MD), and heavily degraded (HD)."

Liu, M., Shi, J., and Zhang, X.: Responses of Ecological Stoichiometry of Plants and Soils to Degradation Levels in Alpine Wetlands of the Qinghai-Tibet Plateau, Environ. Manage., 75, 2258–2271, https://doi.org/10.1007/s00267-025-02152-y, 2025.

- Discuss the assumptions of space-for-time substitution and how the study design mitigates potential confounding factors

Response: We also clarified how potential environmental confounding was minimized during site selection. All four degradation levels were located within the same local Gahai wetland landscape, on relatively flat terrain, and within a narrow elevation range of 3477–3486 m, corresponding to a maximum elevation difference of only 9 m. These criteria were used to reduce broad-scale differences in topography and geomorphic setting unrelated to degradation. Groundwater depth and vegetation characteristics were independently recorded and are now reported in Table S1.

Nevertheless, plot-specific parent-material characterization and detailed historical land-use records were not available for all plots. We therefore explicitly acknowledge that residual spatial heterogeneity and historical disturbance legacies cannot be completely excluded.

- If available, provide evidence that the sites represent a degradation trajectory (e.g., historical data, local knowledge)

Response: Thank you for this constructive comment. We fully agree that the ecological rationale underlying the ordering of these four sites within a space-for-time substitution framework warranted a clearer justification.

In the absence of long-term continuous monitoring data, a space-for-time substitution (chronosequence) approach was employed. To substantiate the validity of this approach, local soil survey records of the Gahai Wetland demonstrate that the broader wetland landscape primarily comprises meadow soils and swamp soils. Soil pedogenesis in this region is predominantly governed by hydrological and sedimentary processes. Specifically, the marsh soils consistently feature a typical diagnostic profile: a root-mat layer (mattic horizon), followed by a humus or peat layer, and an underlying gleyed horizon. These regional pedological characteristics confirm that the investigated sites share a fundamentally comparable wetland depositional setting and hydro-pedological baseline.

Biologically, the cover of key plant species exhibited consistent and monotonic shifts

across the gradient, reinforcing the premise that these four states represent progressive stages along a unified degradation continuum. Furthermore, we conducted a Principal Component Analysis (PCA) using independent soil physicochemical properties to assess whether the four degradation levels are environmentally distinguishable (Figure S3). The ordination revealed an orderly, sequential separation from ND to HD along the first principal component (PC1), thereby lending robust, independent environmental evidence to corroborate our field-based classification.

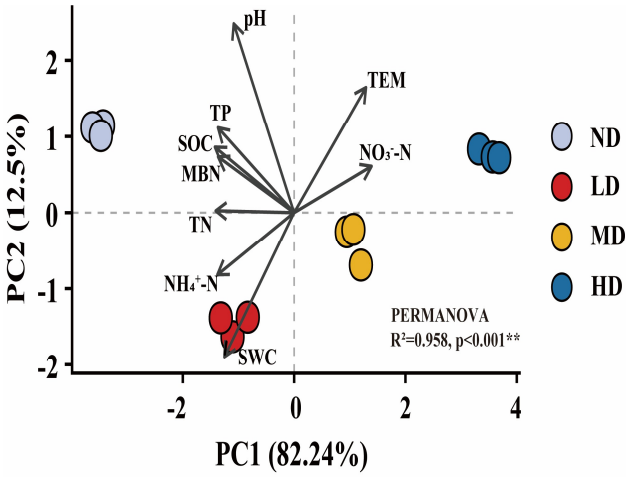

Figure S3. Multivariate differentiation of soil physicochemical conditions among four alpine wetland degradation levels based on principal component analysis (PCA). ND, non-degraded; LD, lightly degraded; MD, moderately degraded; HD, heavily degraded.

3. Definition and interpretation of the NCFB index (Section 2.6, and Discussion)

The NCFB index is a central contribution, but its interpretation requires clarification:

- Why is the balance defined as hydrolytic minus reductive functions? This assumes that higher values are "better" (more coordinated), but this is a value judgment that may not be universally valid.

- What is the ecological significance of the shift from negative to positive correlation between hydrolytic and reductive functions (Figure S4)? The authors note this change but do not fully explain its meaning.

- Could the NCFB shift simply reflect changes in the relative abundance of different microbial groups rather than functional reorganization?

Required revisions:

- Provide a more explicit justification for the NCFB index formulation

Response: We fully agree that the original formulation required greater conceptual justification. The original NCFB was constructed as a standardized directional contrast between hydrolytic and reductive potential enzyme activities, but its subsequent interpretation as a measure of "functional balance" introduced an unjustified value judgment.

In response to this concern, we replaced the original z-score-based NCFB with a ratio-based nitrogen cycling functional potential (NCFP) index:

$$\mathrm{NCFP} = \ln\left[\frac{URE + PRO}{NR + NIR}\right]$$

which is mathematically equivalent to

$$\mathrm{NCFP} = \ln(URE + PRO) - \ln(NR + NIR)$$

Importantly, NCFP is now interpreted as a directional functional-potential index, not as a measure of ecosystem quality or an optimal N-cycling state. NCFP = 0 corresponds to a raw hydrolytic-to-reductive ratio of 1; positive values indicate relatively greater aggregate hydrolytic potential, whereas negative values indicate relatively greater aggregate reductive potential. Neither positive nor negative values are considered intrinsically "better". Instead, the sign and magnitude of NCFP describe the direction and strength of the relative functional configuration between the two measured components.

- Explain what a "balanced" vs. "imbalanced" N-cycling system means in terms of ecosystem function

Response: We have therefore removed the assumption that values closer to, above, or below zero necessarily represent a "better" or "worse" ecosystem state. A value near zero simply indicates that the aggregate hydrolytic and reductive potential activities are of similar magnitude on the assay scale. Whether a given functional configuration is beneficial or detrimental depends on ecosystem context, including substrate supply, plant N demand, hydrological conditions, and potential N losses. We now use terms such as functional reorganization, relative functional configuration, and hydrolytic-to-reductive shift instead of "functional balance" and "functional imbalance".

- Discuss alternative interpretations of the NCFB shift (e.g., microbial community composition changes, not just functional reorganization)

Response: We agree that changes in NCFP may arise not only from physiological or biochemical regulation within a stable microbial community but also from shifts in the abundance or composition of microbial functional groups involved in different N transformations. Previous work in the same Gahai wetland has documented degradation-associated changes in N-cycling functional genes (Du et al., 2024), including *nifH*, *amoA-AOA*, *amoA-AOB*, and *nirK*, supporting microbial functional-group turnover as one plausible contributor to the enzyme-level patterns observed here.

However, potential enzyme activity can also change through altered enzyme expression, microbial physiological regulation, substrate availability, soil moisture and temperature, and enzyme stabilization without proportional changes in taxonomic composition. Because microbial community composition was not measured concurrently for all samples in the present study, we cannot quantitatively separate these mechanisms. We now explicitly acknowledge this alternative explanation and limitation.

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.

4. Carbon limitation as a driver (Section 4.1, Figure 5)

The SEM shows that SOC has a direct negative effect on NCFB (Figure 5b), which seems counterintuitive—higher SOC is associated with lower NCFB. This requires explanation:

- Is this a statistical artifact or a real ecological relationship?

- Does the negative relationship between SOC and NCFB reflect the fact that SOC includes recalcitrant carbon that does not support microbial activity?

- The authors focus on hydrological limitation but the SEM suggests more complex interactions with carbon availability.

Required revisions:

- Provide a more detailed interpretation of the negative SOC-NCFB relationship

Response: Thank you for this important comment. We agree that the negative direct effect of SOC on the original NCFB index reported in the previous version appeared counterintuitive and required careful re-examination. During revision, we rechecked the calculation of the functional index and the SEM path effects and identified an error in the previously reported SOC–NCFB effect. In addition, following the reviewer's comments on the formulation of NCFB, we replaced the original z-score-based NCFB with the revised log-ratio-based nitrogen cycling functional potential (NCFP) index and repeated the structural analysis.

In the revised model, the direct effect of SOC on NCFP was positive (standardized direct effect = 0.62) rather than negative. The summed indirect effect was -0.19. Therefore, the previously reported negative direct relationship between SOC and NCFB was not supported by the corrected analysis and should not be interpreted as evidence that higher SOC directly suppresses N-cycling functional potential. We have corrected Figure 5 and the corresponding Results and Discussion accordingly.

The revised result nevertheless indicates that the relationship between soil C status and N-cycling functional potential is not necessarily represented by a single pathway. The positive direct effect suggests that, after accounting for the other variables included in the model, higher bulk SOC was associated with a relatively higher NCFP. In contrast, the negative indirect effect indicates that part of the SOC–NCFP association was transmitted through the mediating pathways included in the SEM in the opposite direction. We therefore interpret these results as evidence of potentially contrasting direct and indirect associations rather than as a simple unidirectional effect of soil C availability.

- Consider whether the measure of SOC (total organic carbon) adequately captures the labile carbon fraction that drives microbial enzyme production

Response: We also agree with the reviewer that total SOC is not equivalent to microbially available or labile C. SOC integrates chemically and physically heterogeneous C

pools with markedly different turnover rates and microbial accessibility. Unfortunately, labile C fractions such as dissolved organic carbon (DOC), particulate organic carbon (POC), and microbial biomass carbon (MBC) were not measured in the present study. Consequently, these variables cannot be incorporated into the current SEM, and we cannot determine whether the observed SOC related pathways reflect changes in readily available C, protected C pools, or their covariance with other degradation-related environmental factors.

- Discuss whether the model should be refined to include a labile carbon pool

Response: We have therefore revised the Discussion to avoid interpreting SOC as a direct proxy for labile C availability and now refer to it as bulk soil organic C status or the total soil C pool. We have also added the absence of labile-C measurements as a limitation and note that future studies incorporating DOC, POC, MBC, or other biologically available C fractions will be necessary to resolve the specific role of C availability in regulating N-cycling functional potential.

5. Hydroclimatic variability and drought effects (Section 3.3, 4.3)

The authors identify year 2020 as dry and 2021 as wet (implied), but the precipitation data are in the supplement (Figure S1) and the specific values are not presented in the main text:

- What were the actual precipitation amounts for 2019, 2020, and 2021?

- Are these departures from long-term averages significant?

- The conclusion that "drier conditions amplifying degradation effects" is important but needs quantitative support.

Required revisions:

- Present key precipitation statistics in the main text or clearly reference the supplement

Response: Thank you for this important comment. We agree that the original interpretation of drought-related amplification was insufficiently supported because the manuscript did not quantitatively describe interannual precipitation differences. We have now added annual precipitation and precipitation anomalies for the three study years. Annual precipitation was 816.4 mm in 2019, 666.2 mm in 2020, and 1216.4 mm in 2021 (Figure S2).

Relative to the long-term mean annual precipitation of 782 mm, these corresponded to anomalies of +4.4%, -14.8%, and +55.6%, respectively. We therefore describe 2019 as a near-normal precipitation year, 2020 as relatively dry, and 2021 as exceptionally wet.

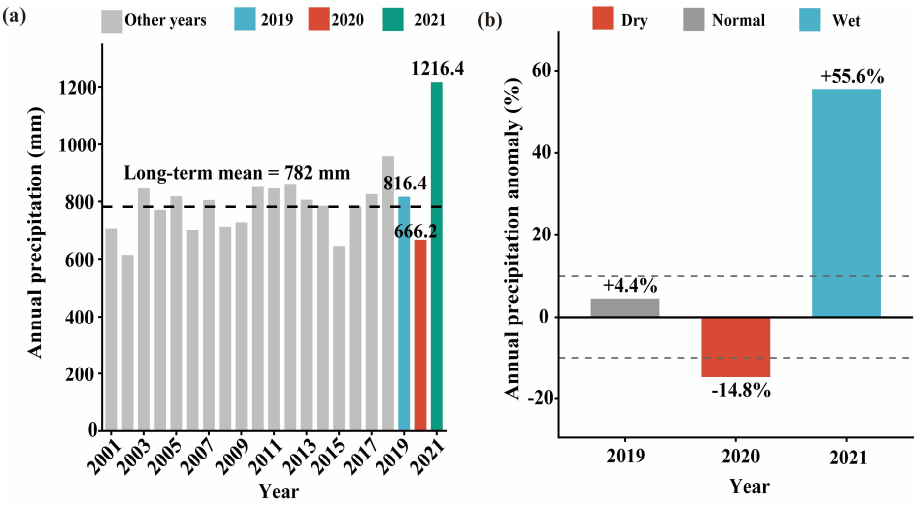

Figure S2. Interannual variation in annual precipitation and precipitation anomalies at the Gahai Wetland. Annual precipitation during 2001–2021. The horizontal dashed line indicates the long-term mean annual precipitation (782 mm) calculated from the 1991–2020 climatological normal (a). Annual precipitation anomalies for 2019–2021 relative to the long-term mean annual precipitation (b). Dashed horizontal lines in panel indicate the ±10% thresholds used to distinguish dry, normal, and wet conditions.

- If possible, quantify the interaction between year (wet/dry) and degradation stage using statistical tests (the mixed models in Table 2 do not include year × degradation interactions)

Response: We also reanalyzed the 0–40 cm enzyme activity data using linear mixed-effects models including degradation level, year, and their interaction as fixed effects, with sampling month and plot identity included as random intercepts. The degradation × year interaction was significant for Urease ($F = 3.91$, $P = 0.001$), Nitrate reductase ($F = 4.23$, $P < 0.001$), and Nitrite reductase ($F = 141.06$, $P < 0.001$), but not for Protease ($F = 0.42$, $P = 0.868$).

Importantly, these interactions did not indicate a consistent amplification of degradation effects in the relatively dry year of 2020. URE showed its strongest among-level differentiation in 2021, whereas the degradation-related decline in NIR was most pronounced in 2019. NR showed a pronounced degradation gradient in both 2019 and 2020 but not in the

exceptionally wet year of 2021. We therefore removed the generalized statement that drought amplified degradation effects and now conclude that interannual hydroclimatic variability modulated degradation responses in an enzyme-specific manner.

Table 2. Fixed effects of degradation level, year, and their interaction on potential soil N-cycling enzyme activities based on linear mixed-effects models.

	df	Urease	Protease	Nitrate reductase	Nitrite reductase
Degradation	(3,129)	15.53**	4.17**	18.41**	349.08**
Year	(2,129)	0.94	98.72**	2.14	382.88**
Degradation × Year	(6,129)	3.91**	0.42	4.23**	141.06**

Note: Degradation level, year, and their interaction were included as fixed effects, whereas sampling month and plot identity were included as crossed random intercepts. F tests for fixed effects were evaluated using Satterthwaite-approximated denominator degrees of freedom. Values shown are F statistics; *P < 0.05 and P < 0.01.

- Discuss the implications of these findings for projections of future climate change effects on alpine wetlands

Response: We have expanded the Discussion section to explicitly elucidate the implications of our findings for forecasting the ecological consequences of climate change in alpine wetlands.

In the revised manuscript, we emphasize that future climate warming and altered precipitation regimes are likely to govern soil nitrogen (N) dynamics predominantly by modulating wetland hydrology, soil aeration, substrate diffusion, and redox-sensitive microbial processes. Consequently, we argue that the impacts of future climatic shifts on alpine wetland N cycling must be evaluated within the context of both degradation status and individual N transformation pathways. Specifically, degraded wetlands suffering from reduced soil water content may possess lower hydrological buffering capacity, rendering their biogeochemical responses to climatic anomalies fundamentally distinct from those of relatively intact wetlands. Furthermore, the divergent interannual responses observed across URE, NR, and NIR underscore that simplistic linear projections based solely on annual precipitation are inadequate.

Minor Comments

1. Line 8: "three-year field investigation" - the methods describe sampling from 2019-2021, but the dates are not specified in the abstract. Consider adding "across 2019-2021" to the abstract.

Response: We agree and have revised the Abstract to explicitly state that the three-year field investigation was conducted during **2019–2021**.

"We conducted a three-year field investigation from 2019 to 2021 across four alpine wetland degradation levels."

2. Lines 135-140: The NCFB index is defined using z-scores. However, z-score standardization across all samples may be sensitive to outliers and assumes normal distributions. Please discuss this and consider alternative standardization approaches if appropriate.

Response: Thank you for this important comment. We agree that the original z-score-based formulation was sensitive to extreme observations because conventional z-scores depend on the sample mean and standard deviation. We note that calculation of a z-score does not mathematically require the raw variable to be normally distributed; nevertheless, the resulting standardized value is dataset-dependent.

To address this issue, we removed z-score standardization from the functional index and replaced the original NCFB with the ratio-based NCFP:

$$\mathrm{NCFP} = \ln\left[\frac{URE + PRO}{NR + NIR}\right] = \ln(URE + PRO) - \ln(NR + NIR)$$

Rather than adopting another dataset-dependent normalization procedure, we considered the log-ratio more appropriate because our objective was to quantify the relative magnitude of hydrolytic and reductive potential activities. All NCFP values and associated analyses were recalculated using this revised formulation.

3. Section 2.7: The statistical methods are generally appropriate, but:

- Why were Tukey's HSD tests used for multiple comparisons rather than, say, Dunnett's test (which compares each treatment to a control)?

Response: Thank you for this suggestion. Dunnett's procedure is particularly suitable

when the primary objective is to compare several treatments with a single reference group. In the present study, however, we were interested not only in comparisons between each degraded level and ND, but also in differences among LD, MD, and HD. We therefore retained pairwise comparisons among all degradation levels and controlled the family-wise error rate using a Tukey adjustment.

- Were data transformation and the assumptions of the mixed models (normality, homogeneity of variance) checked and reported?

Response: Thank you for this constructive suggestion. We recognize that the data transformation procedures and the verification of key assumptions for linear mixed-effects models (specifically, normality and homogeneity of variance) were not sufficiently articulated in the original manuscript. In the revised version, we have explicitly incorporated diagnostic evaluations for homogeneity of variance. For variables that violated model assumptions, a log-transformed was applied as appropriate to ensure normality and variance stability prior to downstream analyses.

"Data normality was assessed using the Kolmogorov–Smirnov test and for homogeneity of variance with Levene's test. Variables that did not meet the assumption of normality were log-transformed before subsequent analyses."

- The linear mixed models are described but the model specification (random intercepts only? random slopes?) is not fully detailed.

Response: Thank you for pointing this out. We have clarified the random-effects structure of the linear mixed-effects models. Degradation level, year, and their interaction were included as fixed effects, whereas sampling month and plot identity were included as random intercepts. No random slopes were fitted. The final model was:

$$\text{enzyme activity} \sim \text{degradation level} \times \text{year} + (1 \mid \text{month}) + (1 \mid \text{plot})$$

Plot identity accounts for repeated observations from the same permanent plots, whereas month accounts for temporal variation shared among plots. We did not fit more complex random-slope structures because of the limited number of levels, particularly for sampling

month, and to avoid overparameterization.

"Linear mixed-effects models were used to evaluate the effects of degradation stage and interannual variation on potential soil N-cycling enzyme activities. Degradation stage, year, and their interaction were included as fixed effects, whereas sampling month and plot identity were included as crossed random intercepts to account for temporal variation and repeated measurements within permanent plots, respectively. No random slopes were included. The model was specified as enzyme activity ~ degradation × year + (1 | month) + (1 | plot)."

4. Figure 1: The enzyme-specific responses are clearly presented. However:

- The y-axis scales differ substantially among enzymes, making comparisons difficult. Consider adding a supplementary figure with standardized scales or including the ranges in the caption.

Response: Thank you for this insightful comment. We fully agree that substantial differences in the absolute activity ranges among the distinct potential soil nitrogen (N)-cycling enzymes make it challenging to directly evaluate and compare their response magnitudes in Figure 1. Because the four enzymes differ markedly in their orders of magnitude and dynamic ranges, we retained independent vertical axes in Figure 1 to preserve the original biological values, but have now explicitly clarified this choice in the figure caption. Nevertheless, to facilitate cross-enzyme comparisons, we have added a new supplementary figure (Figure S4), in which the activity of each enzyme was individually standardized via z-scores prior to plotting. This approach aligns all four enzymes on a shared, dimensionless scale, thereby enabling a straightforward visual comparison of their relative trajectories across the degradation gradient. We would like to clarify that this z-score transformation was employed strictly for visualization purposes and was not involved in the calculation of the revised NCFP index.

Furthermore, to prevent inflating apparent sample size (pseudoreplication) from repeated measurements across multiple sampling years, months, and soil depths, the standardized enzyme activities were first averaged within each independent field plot. These plot-level means were then summarized across the three true replicate plots per degradation stage.

Consequently, the error bars in the revised Figure S4 reflect genuine variation among independent spatial replicates (plots) rather than within-plot variation across temporal or depth observations.

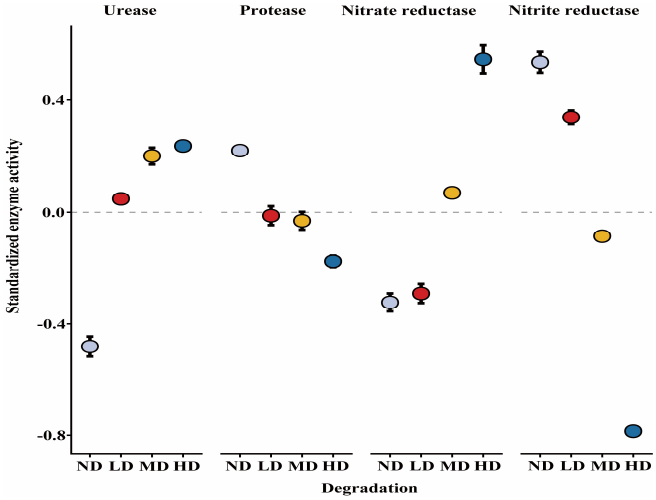

Figure S4. Standardized responses of potential N-cycling enzyme activities to wetland degradation. Enzyme activities were individually standardized within each enzyme assay using z-scores to enable direct comparisons among enzymes spanning different ranges of absolute activity. Data points represent means, and error bars indicate standard errors (SE). ND, non-degraded; LD, lightly degraded; MD, moderately degraded; HD, heavily degraded.

- The statistical comparisons are based on data aggregated across years and depths. Consider whether this aggregation masks important variation.

Response: We agree that aggregation across years and soil depths can obscure important temporal and vertical variation. We have therefore clarified that Figure 1 is intended only to summarize the overall degradation-related pattern and should not be interpreted as indicating temporally invariant responses. Interannual variation is now evaluated explicitly using the linear mixed-effects model including the degradation × year interaction, and year-dependent responses are reported separately in the revised Results and Table 2.

5. Figure 4: The RDA and correlation analyses are useful but:

- The RDA explains 66.2% and 8.62% of variation on axes 1 and 2, but the interpretation of axis 2 is not discussed.

Response: We thank the reviewer for this constructive and insightful comment. We fully agree that while the variance explained by RDA2 was reported in the original manuscript, its underlying ecological implications were not sufficiently discussed. Accordingly, we have revised the Results and Discussion sections to provide a more explicit, nuanced, and cautious interpretation of the environmental gradients captured by the first two RDA ordination axes.

Specifically, RDA1 accounted for 66.20% of the total variance, capturing the dominant environmental gradient driven by alpine wetland degradation. SOC、TN、TP、MBN、SWC and NH_4^+-N loaded strongly on the negative side of RDA1, whereas soil temperature (TEM) and NO_3^--N were positively associated with this axis. Consequently, we interpret RDA1 primarily as a hydrological–resource gradient, which reflects a clear transition from a relatively wet, carbon- and nutrient-rich soil state toward a warmer, drier, and comparatively nitrate-enriched regime.

RDA2 explained a smaller yet non-negligible proportion of variance (8.62%), representing a secondary environmental contrast that is largely orthogonal to the primary RDA1 axis. RDA2 was most prominently and positively associated with soil pH, followed by moderate positive loadings of SOC, TP, and TN, whereas TEM and NO_3^--N exhibited negative associations. Thus, we interpret RDA2 as reflecting secondary variations in soil chemical and nutrient properties that were not fully captured by the overarching hydrological–resource continuum.

Given that the explanatory power of RDA2 is markedly lower than that of RDA1, we deliberately refrain from framing it as an independent, primary driver. Instead, this secondary axis suggests that potential soil N-cycling enzyme activities are predominantly governed by the degradation-induced hydrological and resource gradient, while also being subtly modulated by superimposed edaphic chemical heterogeneity. We have incorporated this balanced interpretation into the revised Results and Discussion sections and updated the description in the caption of Figure 4 accordingly.

- The "circle size" in Figure 4c is visually informative but the legend should clearly explain what it represents.

Response: We have revised the legend of Figure 4c to explicitly define the graphical encoding.

"Circle size represents the relative contribution/importance (%) estimated from the optimal regression model, and color indicates the Spearman correlation coefficient."

6. Lines 202-203: "the timing of maximum activity differed among enzymatic pathways" - this is an interesting observation that merits more discussion. What controls these differences in seasonal timing? Consider discussing this in relation to plant phenology, substrate availability, and environmental conditions.

Response: Thank you for highlighting this interesting feature of our results. We agree that the asynchronous seasonal maxima of the four N-cycling enzymes deserve further ecological interpretation. We have therefore expanded the Discussion to consider the combined effects of plant phenology, substrate availability, and seasonal hydrothermal conditions.

URE, PRO, and NIR generally reached their highest activities during July–August, whereas NR peaked later in September. We interpret this temporal separation as evidence that individual N-cycling pathways respond to different seasonal controls rather than varying synchronously throughout the growing season. During rapid vegetation growth, enhanced root activity, rhizodeposition, microbial activity, and N demand may increase the supply and processing of organic and urea-derived N substrates and thereby favor hydrolytic N-acquisition potential. Favorable mid-growing-season moisture and temperature conditions may also promote NIR potential by influencing microbial activity and oxygen-limited microsites. In contrast, the later NR maximum may reflect declining plant N demand after peak growth together with seasonal changes in nitrate availability and soil environmental conditions.

We also clarify that these seasonal maxima represent changes in potential biochemical capacity under standardized assay conditions and should not be interpreted as direct evidence that the corresponding in-situ N-transformation rates peaked in exactly the same months.

7. Lines 207-213: The generalized additive mixed models (GAMMs) are mentioned briefly but the results are not shown. Please either present the GAMM results or remove the reference. If results are in the supplement, please cite the relevant figure.

Response: We agree. The GAMM results were included in the Supporting Information, but the original manuscript did not clearly refer readers to the corresponding figure. We have now added the appropriate citation in the Results. The revised sentence reads:

"As shown in Figure S6, generalized additive mixed model (GAMMs) indicated that the responses of potential soil N-cycling enzyme activities to degradation were enzyme-specific and not uniformly linear across the degradation gradient."

8. Table 1: The table shows means ± standard deviation but does not indicate sample size. Please add n values to the table or footnote.

Response: We agree and have added the sample size and independent replication information to the table note. The revised note reads:

"Values are means ± SE based on three independent replicate plots (n = 3) within each degradation level."

9. Table 2: The table shows F-values but not degrees of freedom. Please add df values or indicate that these are approximate F-tests from mixed models. Also, note that the table shows only main effects, but the text discusses interactions between degradation and year. Please clarify whether interactions were tested and, if not, why not.

Response: We agree that the original Table 2 was inconsistent with the statistical analysis because it reported only main effects while the manuscript also discussed the degradation × year interaction. We have revised Table 2 so that it now reports the fixed effects of degradation level, year, and their interaction together with the corresponding numerator and denominator degrees of freedom, F statistics, and P values.

Table 2. Fixed effects of degradation level, year, and their interaction on potential soil

N-cycling enzyme activities based on linear mixed-effects models.

	df	Urease	Protease	Nitrate reductase	Nitrite reductase
Degradation	(3,129)	15.53**	4.17**	18.41**	349.08**
Year	(2,129)	0.94	98.72**	2.14	382.88**
Degradation × Year	(6,129)	3.91**	0.42	4.23**	141.06**

Note: Degradation level, year, and their interaction were included as fixed effects, whereas sampling month and plot identity were included as crossed random intercepts. F tests for fixed effects were evaluated using Satterthwaite-approximated denominator degrees of freedom. Values shown are F statistics; *, $P < 0.05$ and **, $P < 0.01$.

Editorial/Technical Comments

1. Line 37: "wetland health and soil functional status" - consider defining "health" more precisely

Response: We agree. Because the term “wetland health” implies a broader, normative ecosystem assessment that was not performed in the present study, we replaced it with more specific terminology. The revised sentence reads: “Soil enzymes have been widely considered sensitive biochemical indicators of soil functional status and nutrient-transformation potential.”

2. Line 27 and 96: "overgrazing" - the introduction mentions overgrazing as a driver but the methods do not describe how grazing intensity was assessed or whether grazing was controlled across sites. Please clarify.

Response: Thank you for this important comment. We agree that the original wording could be interpreted as implying that grazing intensity was explicitly quantified and evaluated as a causal driver in this study. This was not our intention. Overgrazing was mentioned only as a previously recognized regional driver of alpine wetland degradation in the Gahai area.

The present study was not designed to quantify grazing intensity, compare grazing regimes, or isolate the specific contribution of grazing to differences among degradation levels. We have therefore revised the manuscript to avoid treating grazing as a measured causal factor. To minimize direct grazing disturbance during the study period, all experimental plots were fenced and livestock access was excluded, including during the local winter

grazing season from October to the following May.

However, plot-specific historical grazing intensity and duration before fencing were not quantitatively reconstructed. We therefore acknowledge that legacy effects of previous grazing cannot be completely excluded as a potential contributor to differences among contemporary degradation states.

3. Line 101: "space-for-time substitution" - this approach is used but the justification for assuming that sites represent a degradation trajectory needs more clarification.

Response: This issue has been addressed in conjunction with our response to Major Comment 2. Please refer to that response for full details.

4. Line 107: The sampling design is described but the number of replicates and the randomization scheme should be clarified.

Response: We agree and have revised Sections 2.2 and 2.3 to clarify the replication and randomization scheme. Three independent 10 × 10 m plots were established within each of the four degradation levels, resulting in 12 plots in total. Because degradation level represented a naturally occurring field condition rather than an experimentally assigned treatment, the degradation levels themselves could not be randomized. Instead, the three plots were randomly positioned within areas meeting the predefined site-selection criteria within each degradation level.

The plot was treated as the independent experimental unit. Within each plot, five soil cores collected from the same soil depth were homogenized to form one composite sample; these five cores were therefore subsamples used to characterize within-plot heterogeneity and were not treated as independent replicates.

5. Lines 172-174: The statement that "non-degraded wetlands maintained substantially higher SWC, NH_4^+-N, and MBN" is supported by the data in Table 1, but the declines are large (SWC from 0.45 to 0.14 $m^3 \cdot m^3$; MBN from 45.26 to 29.45 $mg \cdot kg^{-1}$). The ecological implications of these magnitudes could be discussed more explicitly.

Response: Thank you for this important suggestion. We agree that the original Discussion emphasized statistical significance without sufficiently considering the ecological magnitude of these changes. We have therefore revised Section 4.1 to explicitly discuss their functional implications. SWC declined from approximately 0.45 to 0.14 $m^3\ m^{-3}$ across the degradation gradient, corresponding to a reduction of approximately 69%, whereas MBN decreased from 45.26 to 29.45 mg kg^{-1}, a reduction of approximately 35%. These changes indicate substantial alteration of both the hydrological environment and the microbial N reservoir rather than minor changes in individual soil properties.

In an alpine wetland, such a large reduction in SWC is particularly important because soil moisture regulates oxygen diffusion, redox conditions, solute transport, and microbial access to nutrients. The decline in MBN further indicates contraction of the microbial N pool and may imply reduced microbial N immobilization and retention. Together with the decrease in NH_4^+-N, these changes provide an important environmental context for the pathway-specific reorganization of potential N-cycling activities observed in this study.

We sincerely thank the reviewer for these constructive comments, which have helped us improve the clarity, rigor, and scope of the manuscript.

Sincerely,

Weiwei Ma

E-mail address: 281205236@qq.com