Response to Review 2

This manuscript reports how degradation restructures enzyme-mediated soil nitrogen (N) cycling and its functional balance in alpine wetlands, based on a three-year field investigation across four degradation stages (non-degraded [ND], lightly degraded [LD], moderately degraded [MD], and heavily degraded [HD]) in the Gahai Wetland. The findings are novel and provide useful insights into the mechanisms underlying soil N cycling destabilization in degraded alpine wetlands, offering valuable implications for wetland restoration under a drier future climate. However, several key issues require clarification prior to publication. In particular, the delineation criteria for the four degradation stages remain unclear, and the lack of significant differences among the LD, MD, and HD stages in key figures (e.g., Figure 3) calls into question whether these four stages are distinct or appropriately defined. Furthermore, while the authors introduce a Nitrogen Cycling Functional Balance (NCFB) index, its functional significance and integration throughout the Abstract and main text are insufficiently articulated. I recommend a Moderate Revision to address these points.

Response: We sincerely thank the reviewer for the constructive comments and for recognizing the potential contribution of our study. We agree that the original manuscript did not sufficiently explain the diagnostic basis of the four degradation levels and that the functional index required greater conceptual precision and stronger integration throughout the manuscript. We have therefore substantially revised the Abstract, Methods, Results, Discussion, figures, and Supporting Information. In particular, we clarified the independent vegetation and hydrological criteria used to define degradation levels, replaced the original NCFB with the log-ratio-based NCFP index, and revised the interpretation of the functional responses accordingly. Our point-by-point responses are provided below.

Specific Comments

1. Lines 8–11: The authors state that four degradation stages were selected in the Gahai Wetland; however, the subsequent results are not linked to these individual stages and instead refer broadly to "degradation." What is the importance and rationale for selecting four distinct

degradation stages? Additionally, while the Nitrogen Cycling Functional Balance (NCFB) index is introduced in the Abstract, it is not revisited in the subsequent results presented in the Abstract. What is the main motivation for developing this index?

Response: We agree. The purpose of retaining four degradation levels was to determine whether potential enzyme activities and soil environmental responses changed continuously across increasing degradation severity or whether different functions showed stage-dependent or nonlinear responses. A simple non-degraded versus degraded comparison could obscure such differences. Importantly, the four degradation levels were defined independently from the N-cycling measurements using vegetation and hydrological criteria before enzyme analysis.

We also agree that the functional index was insufficiently integrated into the original Abstract. In response to comments from both reviewers, we replaced the original NCFB with the ratio-based soil nitrogen cycling functional potential (NCFP) index and now report its actual response across degradation levels directly in the Abstract. NCFP remained similar between ND and LD but declined significantly in MD and further in HD, indicating a pronounced shift in the relative hydrolytic-to-reductive functional configuration from moderate degradation onward.

2. Lines 69–70: Please introduce and explicitly define the criteria used to classify the four stages of alpine wetland degradation.

Response: Thank you for this constructive comment. We fully agree that the criteria used to differentiate the four degradation stages should be transparent and immediately clear to readers in the Introduction.

Accordingly, we have revised the Introduction to concisely outline the classification framework and highlight the primary vegetative and hydrological attributes that distinguish each stage. In accordance with regional alpine wetland degradation standards, the study sites were classified into four degradation levels based on an integrated evaluation of dominant plant species composition, vegetation cover, canopy height, aboveground biomass, groundwater table depth, and visible disturbance indicators. A detailed description of these classification criteria has now been incorporated into Section 2.2 and is elaborated fully in our response to Comment 5.

3. Lines 87–88: I suggest replacing the older FAO classification with the World Reference Base for Soil Resources system (WRB, 4th edition, 2022) for soil classification.

Response: We agree and have updated the soil classification according to the fourth edition of the World Reference Base for Soil Resources (WRB). Based on the available soil-profile information, the experimental soils were classified as Histosols under WRB 2022. The corresponding description and citation have been updated in the Study Area section.

"According to the WRB 2022 classification framework (Iuss Working Group Wrb, 2022), wetland soils in the study area include Histosols. The dominant plant species include *Carex tibetikobresia*, *Carex meyeriana*, *Thalictrum aquilegifolium*, *Artemisia subulata*, and *Artemisia linearis* (Chang et al., 2023)."

Iuss Working Group Wrb: World reference base for soil resources: international soil classification system for naming soils and creating legends for soil maps, 4th ed., International Union of Soil Sciences (IUSS), Vienna, Austria, 2022.

4. Lines 84–90: Please add relevant literature references to support these site descriptions and baseline soil characterizations.

Response: Agreed. We have expanded the "Study Area" section to incorporate relevant literature characterizing the climate, vegetation, soils, degradation status, and land-use patterns of the Gahai Wetland.

"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 QTP, China. The region is characterized by a highland continental monsoon climate. The mean annual temperature at the study site is approximately 1.2 °C, with the lowest and highest monthly mean temperatures occurring in January (-9.1 °C) and July (10.5 °C), respectively (Du et al., 2025). 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 WRB 2022 classification framework (Iuss Working Group Wrb, 2022), wetland soils in the study area include Histosols. The dominant plant species include *Carex tibetikobresia*, *Carex meyeriana*, *Thalictrum aquilegifolium*, *Artemisia subulata*, and *Artemisia linearis* (Chang et al., 2023)."

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.

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.

Iuss Working Group Wrb: World reference base for soil resources: international soil classification system for naming soils and creating legends for soil maps, 4th ed., International Union of Soil Sciences (IUSS), Vienna, Austria, 2022.

5. Lines 100–105: It is critical to explain how the four degradation stages were delineated in the field. However, this information is missing from both the main text and the Supporting Information. Please provide the explicit diagnostic indicators (e.g., vegetation cover, water table depth, soil physical properties).

Response: Thank you for this important comment. We have substantially expanded Section 2.2 and Table S1 to provide the diagnostic characteristics used to distinguish the four degradation levels. The sites were classified using multiple independent field indicators, including dominant-species composition, vegetation cover, plant height, aboveground biomass, and groundwater depth. These indicators showed coordinated changes with increasing degradation severity.

Importantly, degradation level was not defined using soil enzyme activity, NCFP, or the subsequent soil physicochemical results. The vegetation and hydrological criteria were established independently before the N-cycling analyses, thereby avoiding circular classification.

"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). Within each degradation stage, three 10 × 10 m plots were randomly established, resulting in a total of 12 plots. All sampling sites are located within the same local wetland landscape. To minimize potential edge effects, a buffer distance of at least 10 m was maintained between adjacent plots. All experimental plots were fenced throughout the study period to exclude livestock grazing, including the local winter grazing season from October to the following May. Detailed information has been previously reported in our earlier studies (Chang et al., 2023; Du et al., 2024)."

Table S1. Vegetation characteristics of the four wetland degradation levels in the study area.

Degradation	Altitude (m)	Groundwater level (cm)	Cover (%)	Height (cm)	Aboveground biomass (g cm^{-2})	Dominant species
ND	3477	20-40	96.25±5.32a	16.71±2.98a	355.90±174.64a	*Kobresia tibetica*+*Potentilla anserine*+ *Poa annua*
LD	3478	40-70	86.34±7.36b	13.02±2.24b	293.02±143.93b	*Carex* sp.+*Potentilla anserine*+*Artemisia frigida Willd* +*Kobresia capilifolia*
MD	3484	>70	45.33±13.34c	7.43±0.97c	185.73±134.90c	*Artemisia frigida Willd* +*Artemisia sacrorum*.var. + *Kobresia capilifolia*
HD	3486	>100	Severe rodent damage resulted in sparse vegetation, with only little *Artemisia frigida*.and *Polygonum viviparum*			

Note: ND, non-degraded; LD, lightly degraded; MD, moderately degraded; HD, heavily degraded. The different letters above the table represent significant differences among the treatments at P<0.05. Value = mean ± SE.

6. Lines 135–139: The NCFB index was calculated as the standardized difference between hydrolytic and reductive enzyme functions. Why was a difference metric chosen rather than a ratio? A difference metric can generate negative values depending on standardization, whereas a ratio would not be affected by baseline shifts and may offer easier cross-study comparability.

Response: Thank you for this important comment. We agree that the original z-score-based difference formulation was dataset-dependent and that a ratio provides a more direct representation of the relative magnitude of hydrolytic and reductive functional potentials. We have therefore replaced the original NCFB with the ratio-based NCFP:

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

Although this expression is mathematically equivalent to $\ln(URE + PRO) - \ln(NR + NIR)$, it is explicitly interpreted as the natural logarithm of the hydrolytic-to-reductive potential activity ratio. The revised index no longer depends on z-score centering or scaling. NCFP = 0 corresponds to a raw H:R ratio of 1, positive values indicate relatively greater hydrolytic potential, and negative values indicate relatively greater reductive potential.

Importantly, negative NCFP values no longer arise from standardization and are not interpreted as intrinsically unfavorable. A log-ratio formulation may also improve comparability among datasets when the same enzyme activities are measured using comparable protocols and units.

7. Lines 167–176 and Table 1: The manuscript lacks a map of the study area and sampling sites. Furthermore, can the physical and chemical properties listed in Table 1 statistically distinguish the ND, LD, MD, and HD plots? If so, I recommend adding an ordination diagram (e.g., PCA or PCoA) to illustrate the separation among the four degradation stages. If not, consider integrating these properties into the enzyme-specific response sections.

Response: We agree that additional spatial and multivariate environmental information improves the transparency of the field design. We have therefore added a study area map showing the location of the Gahai wetland.

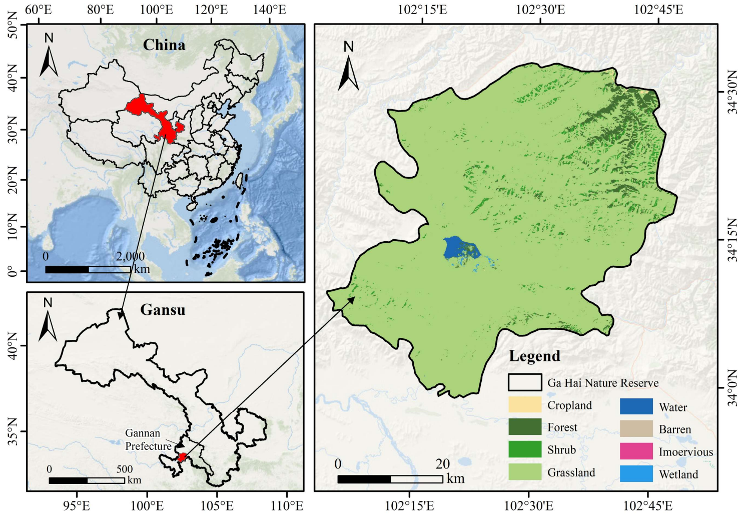

Figure 1. The location of the study area.

We also conducted a supplementary PCA using independent plot-level soil physicochemical data to evaluate whether the four predefined degradation levels occupied different multivariate environmental space. The PCA was used only as independent environmental support for the field classification and was not used to define degradation level, which had already been determined from vegetation and hydrological criteria.

Principal component analysis (PCA) revealed a clear environmental gradient across the four degradation levels (Figure S3). When all soil-depth observations were included, the first two principal components explained 77.4% of the total variation in soil physicochemical properties, with PC1 and PC2 accounting for 82.24% and 12.5%, respectively. The four degradation levels showed an ordered shift primarily along PC1, although some depth-related variation remained within each level. Plot scores showed clear ND, LD, MD, and HD progression along PC1. PERMANOVA further confirmed significant multivariate differentiation in soil physicochemical conditions among degradation levels ($P < 0.001$)

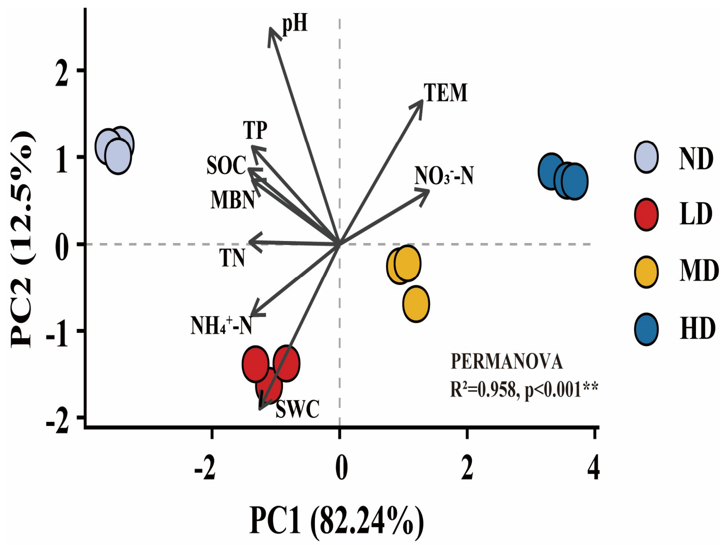

Figure S3. Multivariate differentiation of soil physicochemical conditions among four alpine wetland degradation levels based on principal component analysis (PCA). ND, non-degraded; LD, lightly degraded; MD, moderately degraded; HD, heavily degraded.

8. Line 218: Are only mean enzyme activities plotted in Figure 2? Please state this explicitly in the figure caption.

Response: We agree and have revised the caption of Figure 2 to clarify exactly what each point represents. For each degradation level and sampling month, the plotted value represents the mean potential enzyme activity of three independent replicate plots for the integrated 0–40 cm soil layer. The x-axis represents sampling month, and the panels distinguish enzyme type and year (2019, 2020, and 2021).

"**Figure 2**. Temporal changes in potential soil N-cycling enzyme activities across degradation levels during 2019, 2020, and 2021. Values are means of three independent replicate plots (n = 3) for the integrated 0–40 cm soil layer. URE, urease; PRO, protease; NR, nitrate reductase; NIR, nitrite reductase. ND, non-degraded; LD, lightly degraded; MD, moderately degraded; HD, heavily degraded."

9. Line 233: Figure 3 is key to this work, yet there are no statistically significant differences among LD, MD, and HD. Does this lack of differentiation suggest that the four-stage degradation framework may be over-resolved or improperly categorized for these soils?

Response: Thank you for raising this important point. Following the reviewers' comments, we reconstructed the functional index using the revised log-ratio-based NCFP formulation and repeated the statistical analysis. The revised results differ from those obtained with the original z-score-based NCFB.

NCFP did not differ significantly between ND and LD. However, both ND and LD had significantly higher NCFP values than MD and HD, and HD was also significantly lower than MD. The corresponding means were 0.101 for ND, 0.050 for LD, -0.221 for MD, and -0.484 for HD.

Thus, the revised results indicate that the relative hydrolytic-to-reductive functional configuration remained broadly similar between ND and LD but changed markedly from moderate degradation onward, with progressively greater relative reductive potential in MD and HD.

Importantly, the absence of a significant NCFP difference between ND and LD does not imply that these degradation categories are invalid. The four degradation levels were independently defined using vegetation and hydrological criteria, whereas NCFP represents only one specific dimension of N-cycling functional potential.

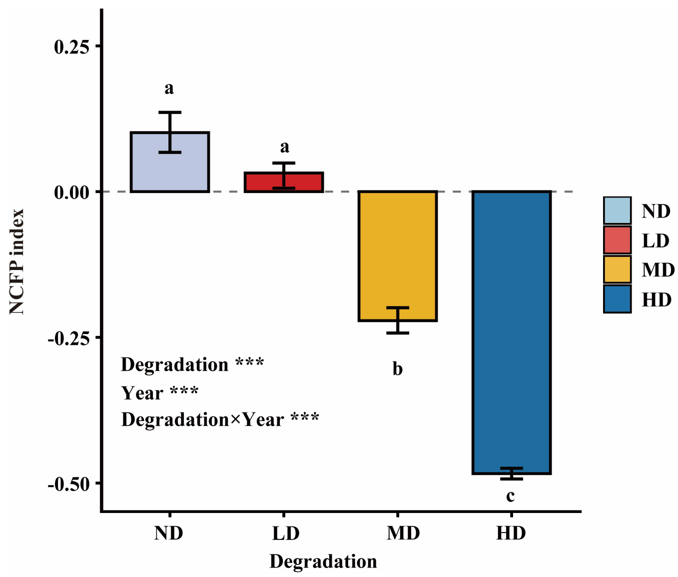

Figure 3. Response of N-cycling functional potential (NCFP) index to wetland degradation. ND, non-degraded; LD, lightly degraded; MD, moderately degraded; HD, heavily degraded. Different lowercase letters indicate significant differences among degradation levels based ($P < 0.05$).

10. Line 265: In Figure 5a, the distinction between "Degradation" and "NCFB" as independent variables/paths is unclear. Please clarify how "Degradation" was operationalized quantitatively versus "NCFB."

Response: We agree that the original manuscript did not clearly distinguish these two variables. In the structural model, Degradation represents the externally defined ordinal degradation gradient based on independent vegetation and hydrological criteria. In the analysis, the four levels were coded ordinally as ND = 0, LD = 1, MD = 2, and HD = 3. In contrast, NCFP is a continuous response variable calculated from the log-ratio of aggregate hydrolytic and reductive potential enzyme activities:

Thus, Degradation and NCFP are conceptually and mathematically distinct. Because numerical coding of degradation assumes an ordered gradient, we now describe the corresponding structural paths as statistical associations with increasing degradation severity rather than as strictly demonstrated causal effects.

We believe this revision brings clearer structure to the Manuscript and effectively addresses your comment. Thank you once again for your insightful suggestion.

Yours sincerely,

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