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Linkages Among Vegetation Structure, Nutrient Availability, and Soil Enzyme Activities in Alpine Wetlands of the Qinghai–Tibet Plateau

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Abstract

Alpine wetlands are highly sensitive to climate warming and anthropogenic disturbances such as grazing, highlighting the urgent need to identify operational indicators for monitoring soil functional changes. In this study, the Zequ National Wetland Park on the Qinghai–Tibet Plateau was selected as the study area. At the plot scale (n = 66), vegetation structure (aboveground biomass, vegetation height, and coverage), soil nutrient properties (soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), ammonium nitrogen (NH_4^+–N), nitrate nitrogen (NO_3^-–N), available phosphorus (AP)), soil enzyme activities (β-glucosidase (BG), N-acetylglucosaminidase (NAG), and acid phosphatase (ACP)) were measured simultaneously. Spearman correlation analysis and redundancy analysis (RDA) were applied to examine their statistical relationships. Descriptive statistics revealed pronounced variability among plots, with aboveground biomass ranging from 115.43 to 1505.27 g·m^{-2}, AP from 0.75 to 70.23 mg·kg^{-1}, and BG activity from 0.25 to 14.71 μmol·g^{-1}·h^{-1}, indicating strong spatial heterogeneity in alpine wetlands. Both correlation and RDA results consistently showed that nutrient availability—particularly inorganic nitrogen and AP—was more closely associated with soil enzyme activities, whereas total nutrient contents exhibited a relatively limited ability to explain short-term variations in soil functional processes. These findings suggest that a combined indicator framework integrating nutrient availability and soil enzyme activities has strong potential for the early detection of soil quality changes and degradation in alpine wetlands, thereby providing quantitative support for sustainable wetland management and restoration assessment.

Keywords: extracellular enzymes; nutrient fractions; inorganic nitrogen; available phosphorus; microbial nutrient acquisition; plot-scale heterogeneity; wetland degradation; soil functioning

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Academic Editor: Jan Hopmans

Received: 24 January 2026
Revised: 5 March 2026
Accepted: 10 March 2026
Published: 11 March 2026

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1. Introduction

Wetlands are among the most productive ecosystems on Earth and play critical roles in regulating biogeochemical cycles, maintaining biodiversity, and providing a wide range of ecosystem services [1–3]. In alpine regions, wetlands are particularly important for carbon sequestration and nutrient retention [4]. However, they are also highly sensitive to climate change and anthropogenic disturbances, including grazing pressure [5], hydrological alterations [6], and vegetation degradation [7]. These disturbances can substantially modify key

soil processes such as organic matter decomposition and nutrient cycling, thereby altering ecosystem functioning and posing serious challenges to the sustainability of alpine wetland ecosystems [8]. Consequently, identifying indicators that can sensitively reflect changes in soil quality and ecosystem functioning is essential for the sustainable management and conservation of alpine wetlands.

Soil enzymes are key mediators of organic matter decomposition and nutrient cycling, as they catalyze biochemical reactions involved in carbon, nitrogen, and phosphorus transformations and play a central role in soil ecosystem functioning [9]. Compared with conventional physicochemical soil properties, soil enzyme activities respond more rapidly to environmental changes and are widely recognized as effective indicators of soil biological activity and soil quality [10,11]. Variations in soil enzyme activities can integratively reflect changes in microbial metabolic processes and nutrient availability, making them particularly valuable for assessing soil functional responses and ecosystem sustainability under different environmental conditions and management regimes.

Vegetation exerts a fundamental influence on soil processes by regulating litter inputs, root exudation, and soil microclimatic conditions, thereby shaping soil microbial community structure and functional activity [12]. Structural characteristics of vegetation, such as aboveground biomass, plant height, and coverage, are closely linked to ecosystem productivity and are commonly used as indicators of ecosystem management status and degradation [13]. These vegetation structural traits can influence soil enzyme activities by altering soil organic carbon inputs and nutrient availability [14]. However, most existing studies have focused on low-altitude or temperate ecosystems, and understanding of how vegetation structure regulates soil enzyme activities through changes in nutrient availability in alpine wetland ecosystems remains limited. This knowledge gap is particularly important because alpine wetlands differ markedly from low-altitude ecosystems in terms of temperature constraints, growing season length, freeze–thaw dynamics, and nutrient cycling rates.

Although previous studies have demonstrated that vegetation characteristics and soil nutrient conditions can influence soil enzyme activities [15], most research has concentrated on temperate ecosystems or alpine meadow systems. Comprehensive assessments of the relationships among vegetation structure, soil nutrient properties, and multiple soil enzyme activities at the plot scale in alpine wetland ecosystems are still lacking. In particular, under the context of sustainable wetland management, it remains unclear whether nutrient availability is more sensitive than total nutrient content in reflecting soil functional changes and potential degradation [16] risks, and empirical evidence addressing this issue is scarce.

In alpine wetland ecosystems, soil processes are highly sensitive to climate change and human disturbances, yet soil changes are often cryptic and lagged [17]. Reliance solely on traditional physicochemical indicators, such as total soil nutrient contents, may therefore be insufficient to detect early changes in ecosystem functioning. Identifying biological and chemical indicators that respond rapidly, are operationally feasible, and effectively reflect soil functional changes represents a key scientific challenge for improving monitoring efficiency and supporting sustainable management decisions in alpine wetlands [18]. Although soil enzyme activities and nutrient availability have been shown to more directly reflect microbial metabolic processes and nutrient supply status, existing evidence is largely derived from temperate or alpine meadow ecosystems [19]. For alpine wetlands—characterized by distinct hydrological regimes and nutrient cycling processes—the relative informational value of different indicators in capturing plot-scale spatial heterogeneity in soil functioning remains poorly evaluated.

Against this background, the present study focuses on a typical alpine wetland ecosystem on the Qinghai–Tibet Plateau. By integrating vegetation structural characteristics, soil

total and available nutrient indicators, and soil enzyme activity data at the plot scale, this study aims to evaluate the relative performances of different indicators in characterizing spatial variability in soil functioning. Specifically, the objectives were as follows: (1) to examine variations in soil enzyme activities under different vegetation structural conditions; (2) to compare the relative importance of nutrient availability versus total nutrient content in explaining soil enzyme activity patterns; and (3) to explore potential pathways through which vegetation structure influences soil enzyme activities by altering soil physicochemical properties. We hypothesize that, at the plot scale in alpine wetlands, nutrient availability and soil enzyme activities serve as more sensitive indicators of soil quality than total nutrient contents, thereby providing a scientific basis for long-term monitoring and sustainable management of alpine wetland ecosystems.

2. Materials and Methods

2.1. Study Area

Zequ National Wetland Park is located in the southwestern part of Zeku County, Qinghai Province, China, with geographic coordinates ranging from 34°50′04″ to 35°07′32″ N and from 101°00′06″ to 101°27′58″ E. The park covers an area of approximately 72,303.44 ha and lies on the northeastern edge of the Qinghai–Tibet Plateau, representing a typical alpine wetland ecosystem [7].

The study area is characterized by high elevation and a cold climate, with generally low annual temperatures, long winters, and a short growing season. Water and thermal conditions are strongly constrained, which exerts pronounced influences on vegetation growth and soil processes. The vegetation is dominated by alpine-adapted plant species, particularly sedges (*Cyperaceae*), and exhibits distinct spatial differentiation across the landscape. The combined effects of complex topography, hydrological conditions, and heterogeneous vegetation distribution have resulted in characteristic alpine soil types and ecological processes within the wetland system [7].

Representative field photographs of typical alpine wetland biotopes sampled in this study are shown in Figure 1.

2.2. Vegetation Survey

2.2.1. Sampling Design

Vegetation and soil were sampled synchronously in Zequ National Wetland Park using a hierarchical sampling design integrating transects, plots, and quadrats. To capture the major environmental gradients across the study area, six transects were established, consisting of three oriented in the east–west direction and three in the north–south direction. A total of 66 plots were selected along these transects. Field surveys and soil sampling were conducted during the peak growing season (July–August 2025).

Each plot covered an area of 100 m × 100 m and served as the basic independent sampling unit in this study. The average distance between adjacent plots was approximately 1500 m. This spacing was chosen to ensure fieldwork feasibility while effectively capturing plot-scale spatial heterogeneity across the main environmental gradients of the study area [20].

Within each plot, three vegetation quadrats (1 m × 1 m) were randomly established, with a minimum distance of 20 m between quadrats to reduce spatial autocorrelation. Plot-level vegetation variables were obtained by averaging measurements from the three quadrats and were subsequently used for statistical analyses [20]. The spatial distribution of transects and the locations of sampling plots are shown in Figure 2.

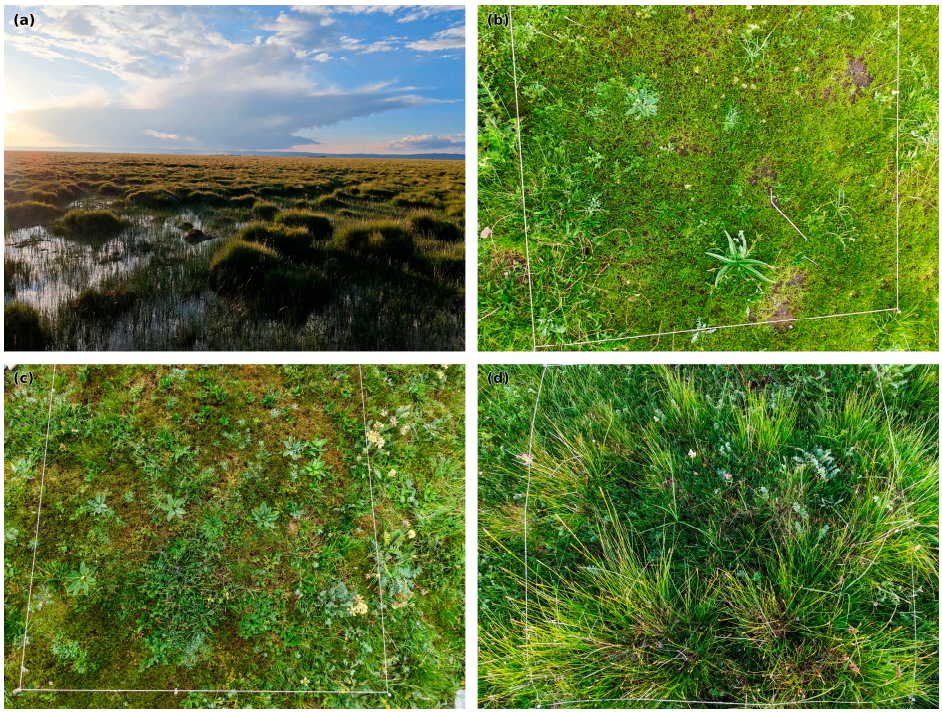


Figure 1. Representative photographs of typical alpine wetland biotopes and quadrat-scale vegetation communities sampled in this study: (**a**) landscape view of a typical wet meadow showing hummock–hollow microtopography and shallow surface water; (**b**) quadrat-scale community with high vegetation cover typical of wet meadow soils; (**c**) quadrat-scale community illustrating moderate cover and visible within-quadrat patchiness; (**d**) quadrat-scale community dominated by tussock-forming vegetation, highlighting microhabitat heterogeneity within the wet meadow. Photographs were taken during the sampling campaign (July–August 2025).

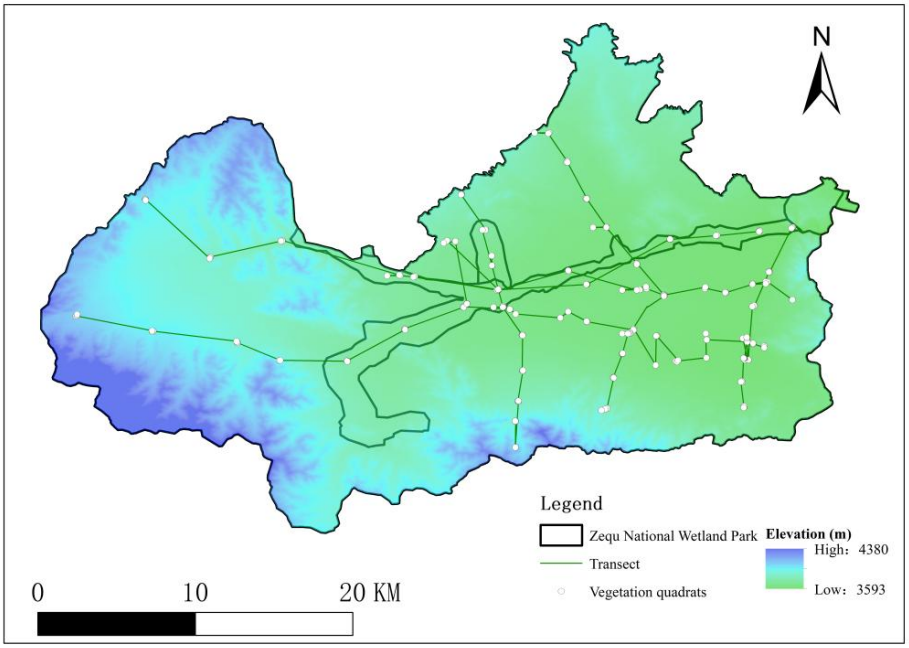


Figure 2. Location of the study area and schematic diagram of the sampling design in Zequ National Wetland Park, Qinghai Province, China. The background shading represents elevation derived from a digital elevation model (DEM). Green lines indicate sampling transects, and open circles denote vegetation quadrats (subsamples). Three quadrats were established within each plot, and plot-level values were calculated as the mean of quadrat measurements and used for subsequent statistical analyses.

2.2.2. Vegetation Measurements

Within each vegetation quadrat, aboveground biomass, mean vegetation height, and vegetation coverage were measured. Vegetation coverage was estimated using the point-intercept method [21]. Plant height was measured as natural standing height using a measuring tape, with at least 10 individuals randomly selected within each quadrat; when fewer than 10 individuals were present, all individuals were measured [20].

Aboveground biomass was determined by clipping all plant material at ground level within each quadrat and measuring fresh mass in the field. Biomass was expressed as fresh mass ($g \cdot m^{-2}$) and was used primarily for comparing relative differences among plots. Because variation in dry-to-fresh mass ratios may introduce bias in cross-study comparisons, aboveground biomass in this study was not interpreted as an absolute estimate of ecosystem productivity.

Measurements from the three quadrats within each plot were averaged to obtain plot-level vegetation structural variables, which were used in subsequent statistical analyses.

2.3. Soil Sampling and Analysis

2.3.1. Soil Sampling

Soil sampling was conducted synchronously with vegetation surveys. Within each vegetation quadrat, soil samples were collected using a five-point sampling method. Given that plant roots and soil biological processes in alpine wetlands are primarily concentrated in surface soils, only the 0–10 cm soil layer was analyzed to characterize soil processes most directly associated with vegetation activity.

Soil samples were collected using a soil auger with a diameter of 3 cm. The five soil cores obtained from each quadrat were thoroughly mixed to form a composite sample, from which stones, roots, and other debris were removed. Samples were passed through a 2 mm sieve prior to analysis. Subsamples for soil enzyme activity measurements were stored in sterile bags at 4 °C, whereas subsamples for soil physicochemical analyses were air-dried at room temperature. Consistent with vegetation data, soil variables were analyzed at the plot level [22,23].

2.3.2. Soil Chemical Properties

Soil pH was determined using a potentiometric method. Total nitrogen (TN) was measured using the semi-micro Kjeldahl method, total phosphorus (TP) was determined by sodium hydroxide fusion, and soil organic carbon (SOC) was measured using the potassium dichromate oxidation method. Inorganic nitrogen included ammonium nitrogen (NH_4^+–N) and nitrate nitrogen (NO_3^-–N), which were determined using the indophenol blue colorimetric method and ultraviolet spectrophotometry, respectively. Available phosphorus (AP) was extracted using sodium bicarbonate and determined by the molybdenum–antimony colorimetric method. All analyses followed standard procedures described in Soil Agrochemical Analysis and widely accepted international protocols [24].

2.3.3. Soil Enzyme Activities

Soil enzyme activities measured in this study included β-glucosidase (BG), N-acetyl-β-D-glucosaminidase (NAG), and acid phosphatase (ACP) [25,26], which represent the microbial acquisition of carbon, nitrogen, and phosphorus substrates, respectively, during soil nutrient cycling.

An amount of fresh soil equivalent to 2 g dry soil mass was weighed and suspended in 100 mL of sodium acetate buffer (pH = 6.0). The suspension was shaken at 200 rpm for 10 min to obtain a homogeneous soil slurry. Subsequently, 500 µL of the soil suspension was mixed with 500 µL of the corresponding p-nitrophenyl (pNP) substrate solution in a deep-

well plate, resulting in a total incubation volume of 1.0 mL. The substrate concentration was 10 mmol·L^{-1}. After thorough mixing, samples were incubated at 37 °C with shaking at 200 rpm. Incubation times were 2 h for β-glucosidase (BG) and 1 h for N-acetyl-β-D-glucosaminidase (NAG) and acid phosphatase (ACP).

At the end of incubation, 250 µL of 0.5 mol·L^{-1} NaOH was added to terminate the reaction and develop color. After standing, the supernatant was collected, and absorbance was measured at 405 nm using a microplate reader (K6600A, Beijing Kaiao Technology Development Co., Ltd., Beijing, China). A standard curve was generated using a series of pNP standards, and the amount of pNP released was calculated. Enzyme activities were expressed as micromoles of pNP released per gram of dry soil per hour (µmol·g^{-1}·h^{-1}) [26–28].

All assays therefore represent potential enzyme activities measured under standardized laboratory conditions (buffer pH = 6.0; incubation at 37 °C), facilitating comparisons among plots.

2.4. Statistical Analysis

To guide variable selection and interpretation, we developed a conceptual framework (Figure 3) summarizing hypothesized direct and indirect pathways linking vegetation structure, nutrient pools/availability, and potential enzyme activities.

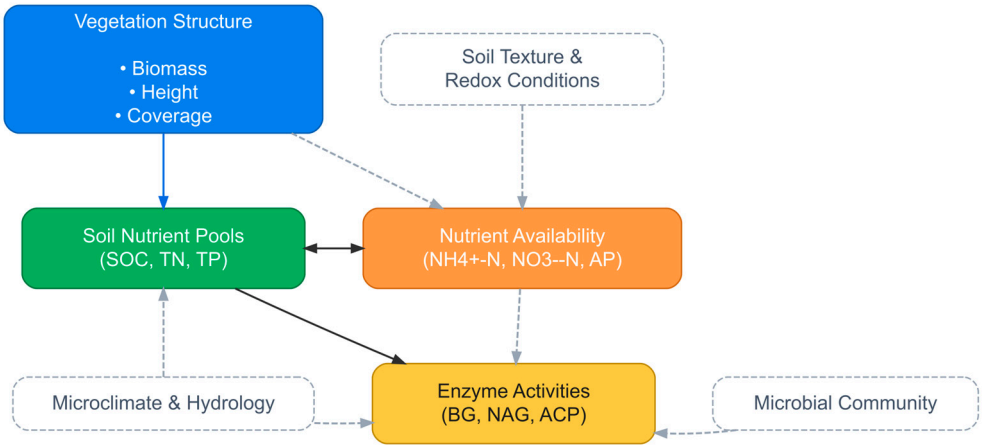


Figure 3. Conceptual framework illustrating hypothesized pathways linking vegetation structure (biomass, height, coverage), soil nutrient pools (SOC, TN, TP), nutrient availability (NH_4^+–N, NO_3^-–N, AP), and potential enzyme activities (BG, NAG, ACP). Dashed boxes indicate unmeasured but potentially important co-drivers (e.g., microclimate/hydrology, soil texture/redox conditions, and microbial community composition) that may shape observed relationships. Solid arrows indicate hypothesized direct linkages among measured variables, whereas dashed arrows indicate indirect, context-dependent, or potentially unmeasured influences.

All statistical analyses were conducted at the plot level (n = 66), which served as the basic independent analytical unit. Descriptive statistics and Spearman correlation analyses were performed using IBM SPSS Statistics 27 (IBM Corp., Armonk, NY, USA). Spearman correlation was applied to assess statistical relationships for variables that did not meet normality assumptions [29].

Redundancy analysis (RDA) was performed using R software (version 4.5.2), with statistical significance evaluated using 999 permutations. Prior to RDA, all explanatory variables were standardized [30,31], and multicollinearity among explanatory variables was assessed using variance inflation factors (VIFs) to avoid the distortion of model interpretation [32]. To reduce potential bias associated with reliance on a single analytical approach, both correlation analysis and RDA were used to comprehensively evaluate relationships among vegetation structure, soil nutrient properties, and soil enzyme activities.

Spearman correlation analysis was employed to identify bivariate relationships between individual vegetation or soil variables and enzyme activities, whereas RDA was used to quantify the relative contributions of multiple explanatory variables to overall variation in soil enzyme activity patterns within a multivariate framework [33].

Plot coordinates (longitude and latitude) were used to evaluate potential spatial dependence. The spatial autocorrelation of enzyme activities and regression residuals was assessed using Moran's I with a k-nearest neighbors spatial weights matrix (k = 6) and 999 permutation tests. To ensure robustness of statistical inference under spatial dependence, key regression models were additionally refitted using spatial block–clustered standard errors.

3. Results

Descriptive statistics of vegetation structure, soil physicochemical properties, nutrient availability, and soil enzyme activities across the 66 plots are presented in Table 1. At the plot scale, all variables exhibited substantial variability, indicating pronounced spatial heterogeneity within the alpine wetland ecosystem. Vegetation structural parameters, including aboveground biomass, vegetation height, and coverage, showed wide ranges among plots. Soil physicochemical properties and nutrient availability also varied markedly, with particularly large variation observed for available nutrient indicators. In addition, soil enzyme activities associated with carbon, nitrogen, and phosphorus cycling displayed considerable plot-scale variability. Together, these results demonstrate strong spatial heterogeneity in vegetation characteristics, soil nutrient conditions, and soil functional attributes, providing a quantitative basis for subsequent analyses of relationships among vegetation structure, nutrient availability, and soil enzyme activities.

Table 1. Descriptive statistics of vegetation structure, soil properties, and soil enzyme activities at the plot scale in alpine wetlands (n = 66).

Category	Variable	Unit	Mean ± SD	Min	Max
Vegetation structure	Aboveground biomass	g m^{-2}	596.59 ± 275.05	115.43	1505.27
	Mean vegetation height	cm	8.81 ± 4.21	2.27	24.86
	Vegetation coverage	%	76.32 ± 13.09	41.33	93
Soil properties	Soil organic carbon (SOC)	g kg^{-1}	109.81 ± 68.31	20.41	341.85
	Total nitrogen (TN)	g kg^{-1}	5.37 ± 2.74	0.97	13.21
	Total phosphorus (TP)	g kg^{-1}	0.96 ± 0.23	0.35	1.43
	pH	–	7.48 ± 0.97	4.35	9.4
Nutrient availability	Ammonium nitrogen (NH_4^+–N)	mg kg^{-1}	8.40 ± 5.65	0.52	24.52
	Nitrate nitrogen (NO_3^-–N)	mg kg^{-1}	2.96 ± 2.65	0.12	14.88
	Available phosphorus (AP)	mg kg^{-1}	16.83 ± 16.81	0.75	70.23
Soil enzyme activities	β-glucosidase (BG)	μmol g^{-1} h^{-1}	4.67 ± 4.17	0.25	14.71
	N-acetyl-β-D-glucosaminidase (NAG)	μmol g^{-1} h^{-1}	7.98 ± 7.77	0.87	32.64
	Acid phosphatase (ACP)	μmol g^{-1} h^{-1}	5.49 ± 5.76	0.19	23.54

3.1. Plot-Scale Variability of Vegetation Structure

At the plot scale, vegetation structural characteristics exhibited pronounced variation across the study area (Figure 4). Aboveground biomass (596.59 ± 275.05 g m^{-2}), mean vegetation height (8.81 ± 4.21 cm), and vegetation coverage (76.32 ± 13.09%) all showed wide distributions among plots, indicating significant spatial heterogeneity in vegetation structure within the alpine wetland ecosystem. This plot-scale variability provides a basis

for subsequent analyses of relationships between vegetation structure, soil properties, and soil enzyme activities.

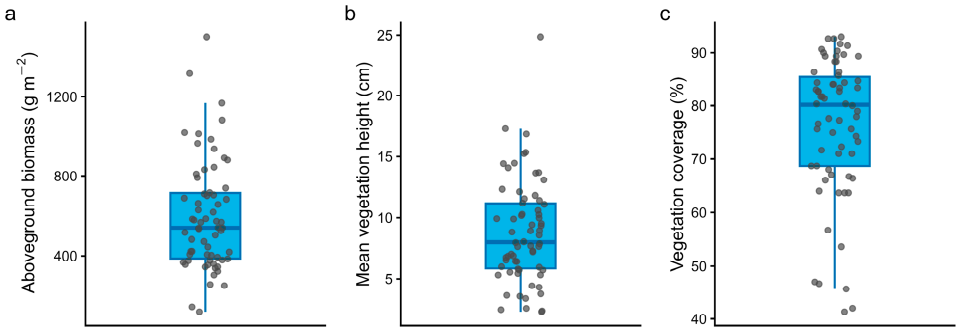


Figure 4. Distribution of vegetation structural characteristics across plots. Subfigures show (**a**) aboveground biomass, (**b**) mean vegetation height, and (**c**) vegetation coverage. The figure illustrates plot-scale variation in aboveground biomass, mean vegetation height, and vegetation coverage across the 66 plots in the study area. Boxplots represent the interquartile range, the central line indicates the median, and dots denote observations from individual plots.

3.2. Plot-Scale Variability of Soil Properties and Nutrient Availability

Soil physicochemical properties and nutrient availability also exhibited pronounced variation across plots (Figure 5). Soil organic carbon (SOC; 109.81 ± 68.31 g kg^{-1}), total nitrogen (TN; 5.37 ± 2.74 g kg^{-1}), and total phosphorus (TP; 0.96 ± 0.23 g kg^{-1}) showed wide distributions among plots, reflecting substantial spatial variability in soil nutrient stocks. In contrast, indicators of nutrient availability, including ammonium nitrogen (NH_4^+–N; 8.40 ± 5.65 mg kg^{-1}), nitrate nitrogen (NO_3^-–N; 2.96 ± 2.65 mg kg^{-1}), and available phosphorus (AP; 16.83 ± 16.81 mg kg^{-1}), exhibited even greater plot-scale variability, indicating pronounced heterogeneity in soil nutrient supply conditions across the study area.

3.3. Plot-Scale Variability of Soil Enzyme Activities

Soil enzyme activities differed markedly among plots (Figure 6). The activities of β-glucosidase (BG; 4.67 ± 4.17 μmol·g^{-1}·h^{-1}), N-acetyl-β-D-glucosaminidase (NAG; 7.98 ± 7.77 μmol·g^{-1}·h^{-1}), and acid phosphatase (ACP; 5.49 ± 5.76 μmol·g^{-1}·h^{-1}) showed wide distributions at the plot scale, indicating pronounced spatial heterogeneity in soil biological processes associated with carbon, nitrogen, and phosphorus cycling in the alpine wetland ecosystem.

3.4. Relationships Between Enzyme Activities and Vegetation/Soil Factors

To reduce the risk of Type I errors associated with multiple comparisons, *p*-values from Spearman correlation analyses were adjusted using the false discovery rate (FDR) correction following the Benjamini–Hochberg procedure. The adjusted results revealed consistent correlation patterns between soil enzyme activities and vegetation structure as well as nutrient availability (Figure 7).

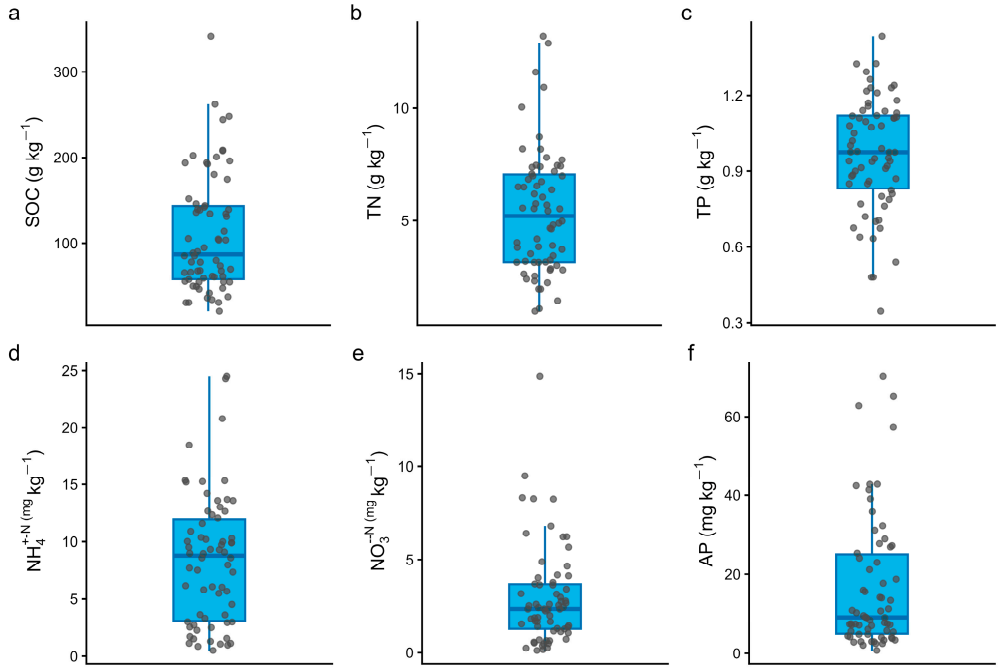


Figure 5. Distribution of soil properties and nutrient availability across plots. Subfigures show (**a**) soil organic carbon (SOC), (**b**) total nitrogen (TN), (**c**) total phosphorus (TP), (**d**) ammonium nitrogen (NH_4^+–N), (**e**) nitrate nitrogen (NO_3^-–N), and (**f**) available phosphorus (AP). The figure shows plot-scale variation in soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), as well as ammonium nitrogen (NH_4^+–N), nitrate nitrogen (NO_3^-–N), and available phosphorus (AP) across the 66 plots in the study area. Boxplots represent the interquartile range, the central line indicates the median, and dots denote observations from individual plots.

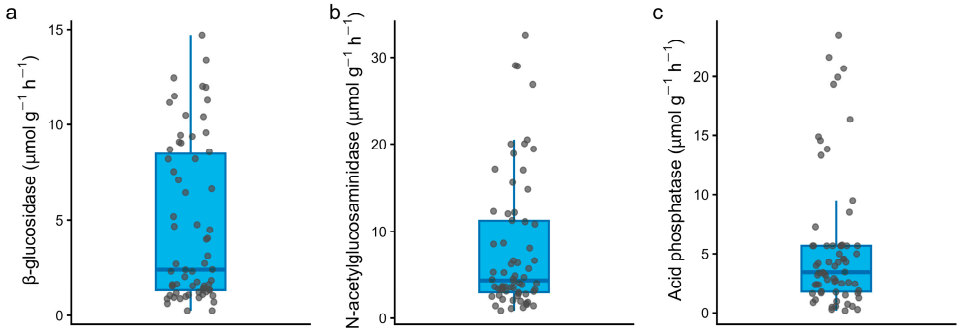


Figure 6. Distribution of soil enzyme activities across plots. Subfigures show (**a**) β-glucosidase (BG), (**b**) N-acetyl-β-D-glucosaminidase (NAG), and (**c**) acid phosphatase (ACP). The figure illustrates plot-scale variation in β-glucosidase (BG), N-acetyl-β-D-glucosaminidase (NAG), and acid phosphatase (ACP) activities across the 66 plots in the study area. Boxplots represent the interquartile range, the central line indicates the median, and dots denote observations from individual plots.

Among vegetation structural variables, vegetation coverage showed significant positive correlations with BG and NAG activities (BG: r = 0.44, FDR-adjusted $p < 0.01$; NAG: r = 0.46, FDR-adjusted $p < 0.001$), whereas the correlation between ACP and vegetation coverage was not significant ($p > 0.05$). In contrast, aboveground biomass and vegetation height exhibited generally low correlation coefficients with all three enzyme activities, and none of these relationships remained significant after FDR correction ($p > 0.05$). These results indicate that, at the plot scale, associations between soil enzyme activities and vegetation structure are primarily reflected by vegetation coverage, which represents canopy cover conditions.

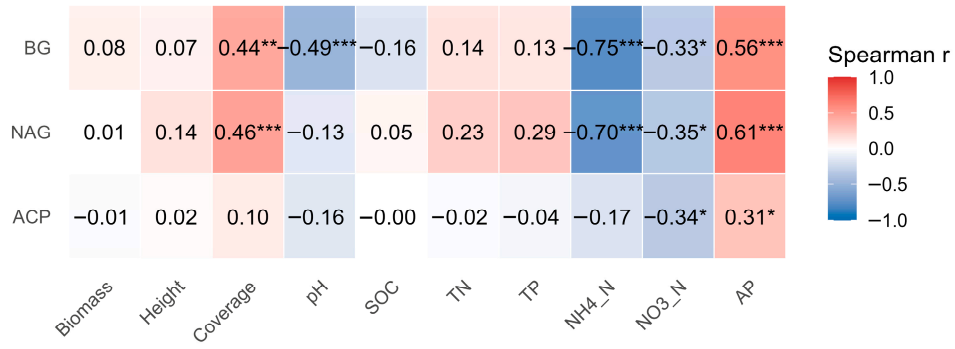


Figure 7. Spearman correlation heatmap showing relationships between soil enzyme activities and vegetation structure, as well as soil factors. The heatmap illustrates correlations between soil enzyme activities (BG, NAG, and ACP) and vegetation structural characteristics and soil physicochemical properties. Colors represent the magnitude of Spearman's correlation coefficients (r), and values indicate the corresponding r values. Asterisks denote significance levels (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

For soil nutrient variables, relationships between soil enzyme activities and nutrient availability were more pronounced. Ammonium nitrogen (NH_4^+–N) showed strong and significant negative correlations with BG and NAG (BG: r = −0.75, FDR-adjusted $p < 0.001$; NAG: r = −0.70, FDR-adjusted $p < 0.001$), with consistently negative associations observed across plots. Nitrate nitrogen (NO_3^-–N) was also negatively correlated with BG and NAG (BG: r = −0.33, FDR-adjusted $p < 0.05$; NAG: r = −0.35, FDR-adjusted $p < 0.05$) and additionally showed a significant negative correlation with ACP (ACP: r = −0.34, FDR-adjusted $p < 0.05$). In contrast, available phosphorus (AP) exhibited significant positive correlations with all three enzyme activities, with stronger relationships for BG and NAG (BG: r = 0.56, FDR-adjusted $p < 0.001$; NAG: r = 0.61, FDR-adjusted $p < 0.001$; ACP: r = 0.31, FDR-adjusted $p < 0.05$). Potential mechanisms underlying these contrasting patterns are discussed in Section 4.2.

Notably, correlations between soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP) and enzyme activities were not significant after FDR correction ($p > 0.05$). This indicates that, at the plot scale, enzyme activities were more consistently associated with nutrient availability—particularly NH_4^+–N and AP—than with carbon stocks or total nutrient pools (SOC, TN, TP).

The complete Spearman correlation matrices and adjusted significance levels are provided in Tables S1–S4.

3.5. Multivariate Drivers of Enzyme Activity Patterns Based on RDA

Redundancy analysis (RDA) indicated that vegetation structure and soil physicochemical factors jointly explained significant variation in soil enzyme activities (BG, NAG, and ACP) (Figure 8). The overall model was significant based on 999 permutation tests (F = 5.53, $p = 0.001$), accounting for 50.1% of the total variation in enzyme activities ($R^2 = 0.501$, adjusted $R^2 = 0.411$). Axis-wise permutation tests showed that both Redundancy analysis axis 1 (RDA1) and Redundancy analysis axis 2 (RDA2) were significant, with RDA1 explaining 43.1% of the variation (F = 47.60, $p = 0.001$) and RDA2 explaining 5.2% (F = 6.56, $p = 0.013$), whereas higher-order axes were not significant.

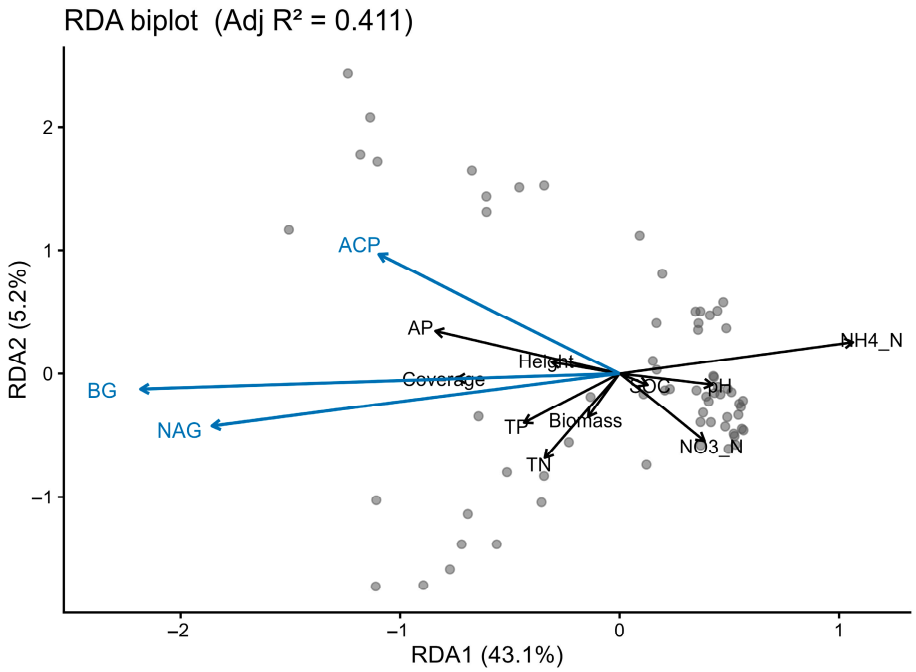


Figure 8. Redundancy analysis (RDA) biplot illustrating the effects of vegetation structure and soil factors on soil enzyme activities (n = 66). Grey points represent individual plots. Blue arrows indicate soil enzyme activities (BG, NAG, and ACP), and black arrows represent explanatory variables (pH, SOC, TN, TP, NH_4^+–N, NO_3^-–N, AP, biomass, height, and coverage). RDA axis 1 (RDA1) and axis 2 (RDA2) explain 43.1% and 5.2% of the total variation in soil enzyme activities, respectively. The overall model was significant based on 999 permutation tests (F = 5.53, p = 0.001; R^2 = 0.501; adjusted R^2 = 0.411).

Marginal permutation tests revealed that ammonium nitrogen (NH_4^+–N), total nitrogen (TN), total phosphorus (TP), nitrate nitrogen (NO_3^-–N), soil pH, available phosphorus (AP), and vegetation coverage significantly contributed to the variation in soil enzyme activities ($p < 0.05$). In contrast, soil organic carbon (SOC), aboveground biomass, and vegetation height did not show significant explanatory effects ($p > 0.1$). Collinearity diagnostics indicated that variance inflation factors (VIFs) for all explanatory variables were below 2.2, suggesting no serious multicollinearity.

Detailed RDA permutation test results (overall model, constrained axes, and marginal effects of each explanatory variable) and VIF diagnostics are reported in Tables S5–S9.

Overall, spatial variation in soil enzyme activities in the alpine wetland ecosystem was primarily associated with differences in nutrient availability rather than being driven by total soil nutrient contents or vegetation structural traits alone.

3.6. Spatial Dependence of Enzyme Activity Patterns and Robustness Checks

Moran's I indicated significant positive spatial autocorrelation for BG (I = 0.152, p = 0.016) and ACP (I = 0.291, p = 0.001), whereas NAG showed a weaker spatial structure (I = 0.109, p = 0.074). Residuals from ordinary least squares models also exhibited spatial autocorrelation, indicating that spatial dependence could affect classical significance tests. Therefore, we performed a robustness check using spatial block–clustered standard errors, and the main relationships remained consistent. These spatial diagnostics are summarized in Table 2.

Table 2. Spatial autocorrelation diagnostics (Moran's I) for enzyme activities and OLS residuals (k-nearest neighbors weights, k = 6; 999 permutations).

Variable	Moran's I	P (Perm)	Notes
BG	0.152115995	0.016	kNN (k = 6), row-standardized; permutation p (999)
NAG	0.109440199	0.074	kNN (k = 6), row-standardized; permutation p (999)
ACP	0.290536665	0.001	kNN (k = 6), row-standardized; permutation p (999)
BG residuals (OLS)	0.352879933	0.001	Residuals from OLS: enzyme~vegetation + nutrients
NAG residuals (OLS)	0.17347554	0.006	Residuals from OLS: enzyme~vegetation + nutrients
ACP residuals (OLS)	0.309554576	0.001	Residuals from OLS: enzyme~vegetation + nutrients

Notes: kNN weights (k = 6), row-standardized; *p*-values from 999 permutations.

4. Discussion

4.1. Effects of Vegetation Structure on Soil Enzyme Activities in Alpine Wetlands

The results of this study demonstrate pronounced differences in vegetation structural characteristics among plots in the alpine wetland ecosystem, and vegetation structure showed measurable associations with soil enzyme activities at the plot scale. Redundancy analysis revealed that vegetation coverage had a significant explanatory effect on soil enzyme activities, whereas aboveground biomass and vegetation height did not show significant effects. This pattern suggests that, compared with plant size or biomass accumulation, vegetation coverage may be a more informative vegetation attribute linked to plot-scale variability in soil enzyme activities, potentially because coverage integrates multiple vegetation-mediated influences on near-surface conditions and substrate inputs [12,13]. However, because soil microclimatic variables (e.g., soil moisture and temperature) were not directly measured in this study, mechanistic interpretations should be considered tentative.

Previous studies have shown that vegetation coverage is often associated with soil moisture regimes, temperature fluctuations, and litter inputs, which can affect soil microbial activity and enzyme-mediated processes [34,35]. In alpine wetland ecosystems, higher vegetation coverage may buffer exposure to extreme climatic conditions and could be linked to reduced sensitivity of microbial processes to freeze–thaw cycles and temperature variability, thereby coinciding with relatively higher soil enzyme activities [36]. Nevertheless, these microclimate-mediated pathways remain hypotheses in the context of our dataset and require explicit measurements to be confirmed.

In contrast, aboveground biomass and vegetation height were not significant predictors of soil enzyme activities in this study, despite their substantial variability across plots (aboveground biomass: 115.43–1505.27 g m^{-2}; mean vegetation height: 2.27–24.86 cm; Table 1). This suggests that plot-scale variation in enzyme activities was not primarily associated with standing biomass or plant stature per se. One plausible explanation is that biomass and height provide limited independent information once vegetation coverage and soil nutrient availability are considered, because these vegetation metrics can covary and may influence soil processes largely through indirect pathways (e.g., litter and root-derived inputs) operating on longer timescales. By contrast, coverage may better capture aspects of vegetation structure related to near-surface microhabitat conditions (e.g., shading and moisture retention), and thus may serve as a proxy linking aboveground vegetation patterns with belowground biological processes [37–39].

4.2. The Key Role of Nutrient Availability in Regulating Soil Enzyme Activities

Both correlation analysis and redundancy analysis consistently indicated that soil enzyme activities were more strongly associated with indicators of nutrient availability (NH_4^+–N, NO_3^-–N, and AP) than with total nutrient contents (SOC, TN, and TP). This pattern supports the view that soil enzyme activities respond more sensitively to nutri-

ent availability than to nutrient stock levels, highlighting the importance of bioavailable nutrients in regulating soil functional processes.

Soil enzymes represent essential biological tools through which microorganisms acquire carbon, nitrogen, and phosphorus, and their activities generally reflect microbial demand for, and access to, available substrates [40]. Soil enzymes reflect microbial investment in acquiring limiting resources, and their activities can increase or decrease depending on nutrient supply and microbial allocation strategies. In this study, the strong negative associations between inorganic N (NH_4^+–N and NO_3^-–N) and BG/NAG suggest that where inorganic N is more abundant, microorganisms may reduce investment in C- and N-acquiring enzymes, consistent with resource-allocation theory. By contrast, the positive relationships between AP and all three enzymes likely indicate that plots with higher P availability also exhibit generally higher microbial activity and substrate turnover, leading to elevated enzyme potentials. Overall, these patterns highlight that "nutrient availability" does not imply a monotonic increase in enzyme activity; rather, different nutrient forms can be linked to contrasting microbial strategies and co-varying environmental conditions. In contrast, total nutrient contents largely reflect long-term accumulated nutrient pools, which tend to change slowly and are therefore less capable of capturing short-term dynamics in soil biological processes [41].

Notably, significant correlations were observed between NH_4^+–N and several soil enzyme activities in this study, underscoring the important role of inorganic nitrogen forms in regulating soil enzyme activity in alpine wetland ecosystems. Similar findings have been reported in other alpine and cold-region ecosystems, where spatial variability in inorganic nitrogen availability has been shown to influence microbial metabolic strategies and substrate investment patterns [42,43]. These results collectively suggest that, in alpine wetlands, nutrient availability rather than total nutrient pools is likely to be the primary factor constraining soil enzyme activities and associated nutrient cycling processes.

The positive ACP–AP association may appear counterintuitive because phosphatase activity is often expected to increase under P limitation. However, ACP is also influenced by microbial biomass, organic matter inputs, and microenvironmental conditions (e.g., aeration/redox status and moisture) that can simultaneously promote P mineralization and elevate extractable AP levels. Therefore, ACP and AP may co-vary as co-regulated outcomes of generally enhanced microbial processing rather than indicating that AP directly stimulates ACP production. Importantly, our results represent spatial correlations and should not be interpreted as direct causality.

4.3. Comparison with Other Alpine Wetlands Across the Qinghai–Tibet Plateau

Comparisons with other alpine wetlands across the Qinghai–Tibet Plateau further support the ecological relevance of the nutrient availability–enzyme linkages observed in this study. In the Zoigê (Zoige) alpine wetland complex, field experiments manipulating N and P supply have reported pronounced shifts in extracellular enzyme activities (including N-acquiring enzymes and phosphatases), consistent with microbial resource-allocation responses under cold, waterlogged conditions [44]. Evidence from other plateau wet meadows similarly indicates that inorganic N fractions and available P are closely associated with enzyme dynamics, and that degradation and grazing can substantially modify nutrient–enzyme coupling by altering substrate inputs, vegetation composition, and local hydrological/redox environments [39,45,46]. Although fewer studies explicitly focusing on wetland soils are available for Nagqu (Naqu) prefecture and the Qilian Mountains, research from high-elevation ecosystems in these regions emphasizes strong hydrothermal constraints on soil biogeochemical responses and highlights the importance of water availability and temperature limitation in regulating nutrient transformations and

microbial activity [47,48]. Accordingly, cross-site similarities and differences are expected to arise from contrasting hydrological regimes (e.g., water table depth and soil moisture), plant community composition (sedge- vs. grass-dominated systems), organic matter inputs, and disturbance intensity, all of which jointly shape nutrient availability and enzyme expression [39,45,46]. In addition, methodological differences among studies (soil depth, assay temperature and buffer pH, and sampling season) can influence reported enzyme magnitudes and should be considered when interpreting inter-site comparisons [39,45]. Overall, our findings are broadly consistent with evidence from Zoigê and other plateau wet meadows, reinforcing the view that bioavailable nutrient fractions provide sensitive indicators of functional change in heterogeneous alpine wetland soils.

4.4. Implications for Soil Quality Assessment and Sustainable Management of Alpine Wetlands

The results of this study indicate that vegetation structural characteristics and soil enzyme activities at the plot scale effectively capture spatial variability in soil processes in alpine wetlands, providing valuable biological information for soil quality assessment. Compared with traditional physicochemical indicators, soil enzyme activities exhibit higher sensitivity to environmental changes and can more directly reflect soil functional status [40,41]. This sensitivity makes soil enzymes particularly suitable for detecting subtle or early changes in soil functioning.

In alpine wetlands—ecosystems that are highly sensitive to climate change and anthropogenic disturbances—reliance solely on total soil nutrient indicators may be insufficient to accurately assess changes in ecosystem functioning. Integrating vegetation structural traits with soil enzyme activities allows for a more comprehensive evaluation of soil quality changes and potential degradation risks [49,50]. In particular, vegetation coverage and indicators of nutrient availability emerge as key variables that can support sustainable management and restoration monitoring in alpine wetland ecosystems.

Overall, this study highlights the feasibility and value of combining vegetation structure and soil enzyme activities in soil quality assessment frameworks for alpine wetlands. Such an integrated indicator system enhances understanding of vegetation–soil interactions and provides a scientific basis for wetland conservation and sustainable management.

From a management and monitoring perspective, the combined use of soil enzyme activities and nutrient availability indicators represents a promising tool for assessing soil quality and ecosystem functional changes in alpine wetlands. Because soil enzyme activities respond rapidly to environmental disturbances, changes in enzyme activity may serve as early-warning signals of altered soil processes, thereby improving the timeliness and effectiveness of ecosystem monitoring [51]. This is particularly important for long-term monitoring of alpine wetlands under ongoing climate warming and increasing grazing pressure [52,53].

Furthermore, the observed association between vegetation coverage and soil enzyme activities underscores the close linkage between aboveground vegetation structure and belowground soil functioning. In practical management, integrating vegetation coverage assessments with soil biological indicators can facilitate a more holistic evaluation of wetland ecosystem condition and inform the design of conservation and restoration strategies. Taken together, the findings of this study support the use of vegetation structural characteristics, nutrient availability, and soil enzyme activities as complementary indicators for soil quality assessment and sustainable management of alpine wetland ecosystems.

4.5. Study Limitations

Although this study provides insights into the relationships among vegetation structure, soil nutrient properties, and soil enzyme activities in alpine wetlands, several limita-

tions should be acknowledged. First, soil sampling was restricted to the 0–10 cm surface layer, and potential vertical variation in soil nutrients and enzyme activities in deeper soil horizons was not assessed. In alpine wetlands, nutrient pools, redox conditions, and microbial activity can vary markedly with depth due to changes in root distribution, oxygen availability, and hydrological connectivity; therefore, relationships observed in surface soils may not directly translate to deeper layers. Second, the analyses were based on measurements collected during a single growing season (July–August 2025), which limits our ability to capture interannual dynamics and seasonal variability in soil processes. In particular, freeze–thaw cycles and snowmelt-driven hydrological pulses can strongly regulate substrate supply, microbial turnover, and enzyme expression in high-elevation wetlands, potentially altering nutrient–enzyme coupling outside the peak growing season. Third, enzyme assays were conducted under standardized laboratory conditions; therefore, the reported values represent potential rather than in situ enzyme activities, and interpretations should emphasize relative spatial differences among plots.

Fourth, although the sampling design was intended to reduce the influence of spatial autocorrelation, potential spatial dependence among plots cannot be entirely excluded. Finally, this study did not directly assess soil microbial community composition, which constrains mechanistic interpretation of observed variations in soil enzyme activities. Future research incorporating multi-season or multi-year observations, deeper soil profiles, in situ monitoring of hydrothermal conditions (e.g., soil moisture and temperature), microbial community analyses, and experimental manipulations would help to further elucidate soil functional processes and their drivers in alpine wetland ecosystems.

5. Conclusions

In this study, we reached the following conclusions:

(1) Plot-scale spatial heterogeneity. Vegetation structure, soil nutrient availability, and soil enzyme activities exhibited pronounced spatial heterogeneity at the plot scale in alpine wetlands of the Qinghai–Tibet Plateau.

(2) Vegetation signal. Among vegetation structural traits, vegetation coverage was the most informative predictor of variation in soil enzyme activities, whereas aboveground biomass and vegetation height showed limited explanatory power.

(3) Nutrient availability dominates enzyme patterns. Both correlation and redundancy analyses consistently indicated that enzyme activities were more strongly associated with indicators of nutrient availability than with total nutrient contents. Inorganic nitrogen forms and available phosphorus emerged as key correlates of enzyme activity patterns, highlighting the sensitivity of soil biological processes to bioavailable nutrient pools rather than long-term nutrient stocks.

(4) Implications for monitoring and management. Integrating vegetation coverage, nutrient availability, and soil enzyme activities provides a practical framework for assessing soil quality and detecting early functional changes in alpine wetlands. This integrated indicator set can improve the efficiency and responsiveness of monitoring programs and support sustainable management and restoration under ongoing climate change and increasing anthropogenic pressures.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/su18062735/s1, Table S1: Spearman correlation coefficient matrix (Spearman_r_matrix.csv). Table S2: raw p value matrix (Spearman_p_matrix_raw.csv). Table S3: FDR-adjusted p value matrix (Spearman_p_matrix_FDR.csv). Table S4: significance annotations after FDR correction (Spearman_sigstar_FDR.csv) and pairwise Spearman results (Supplement_Table_Spearman_pairs_FDR.csv). Table S5: RDA model R^2 and adjusted R^2 (RDA_R2_new.txt).

Table S6: overall permutation test for the RDA model (RDA_anova_all_new.csv). Table S7: axis-wise permutation tests (RDA_anova_axis_new.csv). Table S8: term-wise marginal effects with permutation tests (RDA_anova_term_new.csv). Table S9: multicollinearity diagnostics using variance inflation factors (RDA_VIF_new.csv). Table S10: Spatial diagnostics outputs (spatial_diagnostics_outputs.xlsx).

Author Contributions: Conceptualization, G.J., Z.Y., J.W., J.L. and M.Y.; methodology, G.J., Z.Y., J.W., J.L. and M.Y.; investigation, G.J., Z.Y., J.W., J.L., M.Y. and C.L.; resources, C.L.; project administration, C.L.; formal analysis, G.J., Z.Y. and M.Y.; data curation, G.J., Z.Y. and M.Y.; visualization, G.J., Z.Y. and M.Y.; writing—original draft preparation, G.J.; writing—review and editing, G.J., C.L., Z.Y., J.W., J.L. and M.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Qinghai University Research Ability Enhancement Project, grant number 2025KTST06.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The core statistical outputs supporting the findings of this study are provided in the Supplementary Materials. The underlying dataset is available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

SOC	Soil organic carbon
TN	Total nitrogen
TP	Total phosphorus
NH_4^+–N	Ammonium nitrogen
NO_3^-–N	Nitrate nitrogen
AP	Available phosphorus
BG	β-glucosidase
NAG	N-acetyl-β-D-glucosaminidase
ACP	Acid phosphatase
pNP	p-nitrophenyl
SPSS	Statistical Package for the Social Sciences
RDA	Redundancy analysis
RDA1	Redundancy analysis axis 1
RDA2	Redundancy analysis axis 2
VIF	Variance inflation factor
DEM	Digital elevation model

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