Agriculture, Ecosystems and Environment 393 (2025) 109837

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

Impacts of seasonal grazing on soil enzymes, physicochemical properties, and vegetation in alpine meadows

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ARTICLE INFO

Keywords:
Enzymatic activity
Plant biomass
Alpine meadows
Grazing effects
Seasonal changes

ABSTRACT

Alpine grasslands of the Qinghai–Tibet Plateau (QTP) are vital for regional biodiversity and nutrient cycling but are increasingly affected by grazing-induced degradation. This study examined the effects of seasonal grazing (spring, summer, autumn, winter) on soil extracellular enzyme activities, key physicochemical properties, and plant biomass in alpine meadows of the QTP. Over two consecutive years (2021–2022), pre- and post-grazing soil and vegetation samples were collected from replicated seasonal grazing plots. Enzyme activities were analyzed in relation to biomass of dominant plant functional groups: *Cyperaceae, Poaceae, Leguminosae*, and miscellaneous grasses. Enzymes were categorized by function into nutrient cycling (phosphatase, hydroxylamine reductase), organic matter degradation (amylase, cellulase, chitinase, glucosidase, polyphenol oxidase), and proteolysis (alkaline proteases). The results revealed distinct seasonal patterns in enzymatic activity and plant biomass. Amylase (3066 $\mu gd^{-1}g^{-1}$) and hydroxylamine reductase (466 $\mu gd^{-1}g^{-1}$) activities were significantly higher during winter grazing ($p < 0.05$), while chitinase (40 $\mu gd^{-1}g^{-1}$) and cellulase (675 $\mu gd^{-1}g^{-1}$) peaked during spring. Glucosidase activity was highest under summer grazing, while alkaline proteases exhibited minimal seasonal variation. Plant biomass varied by season: *Cyperaceae* dominated in winter, *Poaceae* in autumn, and *miscellaneous* grasses in summer, whereas *Leguminosae* biomass remained consistently low. Pearson correlation analysis showed significant correlations between elevated amylase ($p < 0.05$) and hydroxylamine reductase ($p < 0.01$) activities with increased Cyperaceae biomass in winter. Similarly, higher chitinase activity ($p < 0.01$) during spring was positively correlated with the growth of miscellaneous grasses. These findings reveal that seasonal grazing alters soil enzymatic activity, influencing plant growth and species composition, and underscore the need for adaptive grazing strategies to sustain soil health and ecosystem resilience in alpine meadows.

1. Introduction

Grasslands cover over 40 % of the Earth's terrestrial land surface, and grazing is the most intensive land-use activity in these ecosystems (Bicharanloo et al., 2022; Flombaum et al., 2024). These ecosystems play a critical role in biodiversity conservation and provide essential services, including carbon sequestration, erosion control, and livestock production (Stavi, 2019). Grazing influences grassland functioning through direct effects (e.g., trampling, defoliation, nutrient deposition via excreta) and indirect effects mediated by shifts in plant communities and soil microbial activity (Nannipieri et al., 2017; Li et al., 2023). Excessive grazing pressure can degrade vegetation, alter soil properties, and lead to desertification (Argenti et al., 2020), threatening both ecological balance and pastoral livelihoods (Huber et al., 2022).

The Qinghai–Tibet Plateau (QTP), known as the "rooftop of the world," lies at an average elevation above 4000 m in southwest China. Approximately 70 % of this region is covered by diverse alpine grasslands, including desert steppes, alpine steppes, and alpine meadows. Grazing by livestock, primarily yaks, sheep, and goats, has been a traditional practice on the QTP for centuries, sustaining the livelihoods of nomadic pastoral communities. The intensity and management of grazing on the QTP have significant implications for grassland health, biodiversity conservation, and the provision of ecosystem services. The current vegetation of alpine grasslands has been shaped by a combination of natural ecological factors and human activities, mainly represented by animal grazing and agricultural practices (Argenti et al., 2020). In recent decades, the QTP has experienced significant environmental changes, including rising temperatures, altered precipitation

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https://doi.org/10.1016/j.agee.2025.109837
Received 18 December 2024; Received in revised form 18 June 2025; Accepted 25 June 2025
Available online 1 July 2025

patterns, and increased human activities, all of which have exacerbated the impacts of grazing on grassland ecosystems. Changes in land use, deforestation, species invasion, and a lack of sustainable management have led to rapid changes in ecosystem balance (Bhattarai, 2013). Therefore, understanding the complex interactions between grazing, climate change, and human activities is crucial for developing sustainable grassland management strategies that can maintain the ecological integrity of the QTP and support the livelihoods of local communities.

Soil extracellular enzymes, produced largely by microbes, are key indicators of nutrient cycling and ecosystem functioning (Daunoras et al., 2024; Bhaduri et al., 2022). Bacterial enzymes drive carbon-nitrogen cycling, while fungal enzymes decompose complex polymers like lignin and cellulose (Neemisha and Sharma, 2022; Shamshitov et al., 2024). Specific enzymes reflect distinct processes: urease (N mineralization), phosphatase (P cycling), and cellulase (C release) (Adetunji et al., 2017; Khare and Yadav, 2017; Yu et al., 2021). In most soil ecosystems, the activity ofa single enzyme cannot reflect the rate of entire metabolic processes, that's why a multi-enzyme approach is essential (Ren et al., 2016). On the other hand, soil enzyme activities are sensitive to alterations in soil organic matter stability, nutrient availability, and microbial community composition, providing early warnings of soil physicochemical and biological changes (Wang et al., 2023). Soil enzyme activities vary according to changing environmental factors (e.g., pH, water content, temperature, and different geographical locations), which drive temporal variability and spatial differences (Ren et al., 2016; Bashir et al., 2021; Gałązka et al., 2017). These impacts result from reduced plant residue inputs, changes in the transformation rates of soil nitrogen, carbon, and phosphorus, and alterations in the distribution of enzyme activities among soil aggregates of varying particle sizes (Fan et al., 2021). For instance, the activity of β-glucosidase, an enzyme responsible for breaking down carbohydrate polymers such as cellulose, is significantly reduced in moderately and lightly grazed large aggregates (Wang et al., 2023). Interestingly, certain studies suggest that increased grazing intensity can boost microbial biomass-C and the abundance of heterotrophic microbes in the open spaces between plant canopies (Wang et al., 2023). Some studies suggest that introducing labile nitrogen from urine and dung might somewhat offset the negative impacts of grazing on soil enzyme and microbial activities (Prieto et al., 2011). However, enzyme activities tend to decline under extreme temperatures and moisture stress during summer and winter (Bell et al., 2008; Zuccarini et al., 2020). Therefore, monitoring and comprehension of soil enzyme activities are imperative for the sustainable management of soil and the preservation of ecosystems (Joshi et al., 2018).

Despite advances in understanding grazing impacts, few studies have examined how long-established seasonal grazing regimes, synced with plant phenology and climatic fluctuations, regulates soil enzyme dynamics in alpine meadows. In this study, we utilized a grazing platform established in 2010 to investigate seasonal effects during 2021–2022. Understanding whether different grazing seasons promote or suppress specific enzyme pathways remains unclear. Moreover, it is important to examine how enzyme dynamics interact with plant biomass and nutrient availability throughout seasonal cycles. Recent studies highlight that the timing of grazing, particularly in relation to plant phenology and seasonal climatic changes, critically modulates soil enzyme activity in alpine ecosystems (Petermann and Buzhdygan, 2021). Seasonal grazing regimes distinctly affect soil enzymatic processes in alpine grasslands (Jing et al., 2022; Chen et al., 2025). This study aimed to bridge this gap by examining how long-term seasonal grazing affects soil physicochemical properties, enzymatic activities, and plant biomass in alpine meadows of the QTP. Specifically, we compared pre- and post-grazing plant biomass of major functional groups (*Cyperaceae*, *Poaceae*, *Leguminosae*, and miscellaneous grasses) across seasons. We assessed relationships among soil nutrients, enzyme activities, and vegetation responses. Based on previous research and the objectives of this study, we hypothesize the following: (i) Soil enzyme activities will vary significantly across grazing seasons, with distinct patterns in nitrogen and carbon-related enzymes. This hypothesis is grounded in prior studies that show that soil enzymes, particularly those involved in nitrogen and carbon cycling (such as urease and amylase), are influenced by seasonal changes in environmental factors like temperature, moisture, and nutrient availability (Ren et al., 2016; Zhang et al., 2022). We expect grazing to further modulate these enzyme activities, especially in alpine meadows, where grazing intensity and seasonality directly impact microbial activity. (ii) Seasonal grazing will significantly influence the biomass of dominant plant species, including *Cyperaceae*, *Poaceae*, miscellaneous grasses, and *Leguminosae*. This hypothesis is driven by the known interactions between grazing pressures and plant growth, which differ depending on the season. For example, *Poaceae* species typically show higher sensitivity to grazing, especially during specific growing seasons (Li et al., 2023), while species such as Cyperaceae may show more resilience under certain grazing regimes (Zhao et al., 2019). We anticipate that seasonal variations in grazing will lead to fluctuations in the biomass of these functional groups, depending on both grazing intensity and environmental conditions. (iii) Soil enzymatic activities will correlate with plant biomass production, with specific enzyme groups mediating seasonal variations in plant growth. This hypothesis arises from the established role of soil enzymes in nutrient cycling and their potential to influence plant growth by regulating nutrient availability (Gilmullina et al., 2020; Bhaduri et al., 2022). We expect a clear link between enzyme activities, such as phosphatase and cellulase, and plant biomass, with these correlations being modulated by soil nutrient levels, pH, and moisture content, which fluctuate with grazing and seasonal shifts.

2. Materials and methods

2.1. Study site

The study was conducted in the Maqu Pratacultural Research Station of Lanzhou University, located in Maqu County, Gansu Province, China (101°53′E, 33°58′N) in northeastern Qinghai–Tibet Plateau (QTP) (Fig. 1). This region lies on the eastern edge of the QTP, in the upper reaches of the Yellow River Basin, and near the intersection of the QTP, Loess Plateau, and Northwest Inland Arid Zone.

According to the comprehensive classification system for grasslands, this area falls under the category of cold and humid alpine meadow grasslands (Ren et al., 2008). The vegetation is a typical alpine meadow comprising sedges, grasses, and forbs. Dominant plant species include *Kobresia graminifolia*, *Elymus nutans*, *Agrostis spp*., *Poa pratensis*, *Saussurea spp*., and *Anemone spp*. The soil type in the region is subalpine meadow soil (Zhang et al., 2014). The main grazing animals in the study area are yaks (*Bos grunniens*) and Oura Tibetan sheep (Yuan and Hou, 2015), while the primary small mammal species are plateau pika (*Ochotona curzoniae*) and plateau zokor (*Myospalax baileyi*).

The climate is cold and humid, with no absolute frost-free period throughout the year. The annual average temperature during 2010–2019 was approximately 1.2 °C. The warmest months occur from June to August (average ∼12 °C), while the coldest period spans from December to February (average ∼ –10 °C). The region receives an average annual rainfall of ∼620 mm, most of which occurs during the forage growing season from May to September (Wang and Hou, 2021; Wei et al., 2022).

During the study period (2021–2022), monthly temperature and precipitation data followed a typical alpine meadow climate pattern, with peak precipitation from June to August and the lowest temperatures observed from December to February (Fig. 2). Notably, total precipitation in 2022 was slightly lower than the decadal average, which may have influenced seasonal soil moisture and microbial activity. These interannual differences provide context for interpreting seasonal dynamics in soil enzymatic activity and plant biomass.

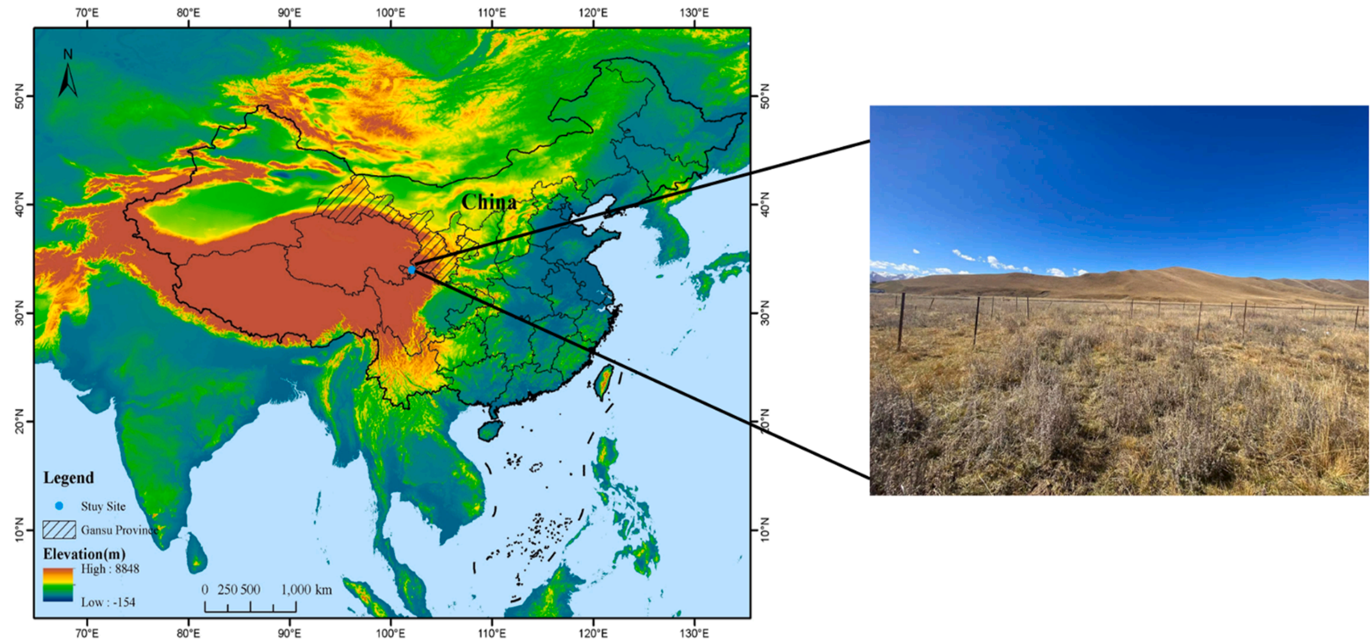


Fig. 1. Location of the study site in Maqu County, Gansu Province, China. The map on the left shows the study site (blue circle) within the Qinghai-Tibet Plateau, with elevation ranges across the region. The image on the right shows the study area, showcasing the alpine meadow landscape.

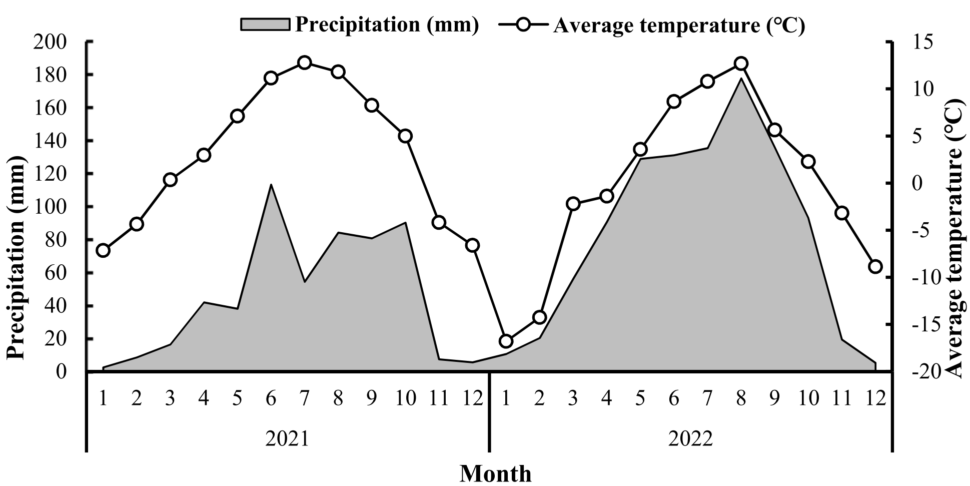


Fig. 2. Monthly temperature (°C) and precipitation (mm) at the study site during 2021 and 2022. Data show the seasonal patterns in temperature and precipitation over the two-year period, providing context for the environmental conditions at the study site.

2.2. Experimental design and sample collections

The seasonal grazing experiment was conducted in at Maqu Pratacultural Station of Lanzhou University, located in Maqu County on the eastern Qinghai–Tibetan Plateau (QTP). This long-term grazing research platform was established in 2010, and since then, seasonal grazing trials have been conducted consistently using controlled yak grazing. To minimize environmental heterogeneity and ensure comparability across treatments, the experimental plots were selected based on uniform vegetation type, adequate water availability, and relatively flat terrain. Prior to the start of seasonal treatments, the designated plots were stabilized and assessed to ensure similarity in soil and plant conditions. During the year 2021–2022 study period, the grassland was divided into four seasonal grazing plots corresponding to spring (April), summer (July), autumn (September), and winter (December). Each seasonal plot was subdivided into three replicated subplots ($n = 3$ per season) to allow for rotational grazing and statistical replication. The plot sizes were 0.5 ha (50 × 100 m), providing a balance between tractable experimental area and ecological relevance, and they were chosen to accommodate yak movement while maintaining manageable heterogeneity. Preliminary surveys indicated low within-treatment variability due to careful site selection, as reported earlier by Wang et al. (2020). The number of yaks in each treatment varied slightly across seasons to account for differences in plant biomass and regrowth capacity. The stocking density was calibrated to 3.3 yaks per hectare, ensuring consistent grazing pressure across all treatments. For each 0.5 ha plot, this resulted in approximately 2 yaks per plot. Grazing pressure was carefully monitored and adjusted based on seasonal plant growth cycles and regrowth potential, ensuring that it remained consistent and representative of natural conditions, effectively mimicking grazing patterns while avoiding overgrazing. Similar trends have been followed in previous experiments at the Maqu Research Station (Yang et al., 2019). Healthy, well-conditioned adult 3–4 years yaks were selected for grazing. Within each seasonal plot, controlled rotational grazing was implemented using temporary fencing to confine yaks to one subplot at a time. Grazing was initiated when the average vegetation height reached

20 cm and concluded when it declined to 5–8 cm, ensuring comparable forage availability and stubble heights across all treatments. Grazing was conducted daily from 08:00–18:00 in spring and winter and 07:00–19:00 in summer and autumn, with yaks housed in pens overnight. To assess treatment effects at both fine and community-wide scales, two types of vegetation sampling plots were used. In each grazing subplot, three fixed sample plots of 0.25 m^2 were established for consistent monitoring of species composition, vegetation structure, and soil conditions. These fixed plots enabled precise, repeated measurement of localized responses to grazing. In parallel, one 1 m^2 quadrat was randomly placed in each subplot before and after grazing to measure total aboveground biomass and to assess plant functional group contributions at a broader scale. This dual-scale sampling strategy ensured both high-resolution spatial data and robust community-level biomass estimates, as the effects of grazing on plant resource stoichiometry can be highly variable, as discussed by Li et al. (2022).

Soil and plant samples were collected pre- and post-grazing in each season. Within each grazing sub-area (subplot), three fixed sample plots of 0.25 m^2 were established to assess treatment effects at finer spatial resolution. For soil analysis two composite soil cores (0–15 cm depth) per subplot were collected using a 10 cm auger and sieved (1 mm, 0.25 mm) for analysis. Soil enzyme activities and soil physicochemical parameters were measured. These parameters were chosen for their importance in assessing microbial activity, nutrient cycling, and overall soil fertility under grazing stress. Plant biomass was evaluated from 1 m^2 quadrats randomly placed in each subplot before and after grazing. Aboveground biomass was clipped, cleaned, and oven-dried at 65°C for 48 h to constant weight. Species-level dry biomass was recorded for four major plant groups: *Poaceae*, *Cyperaceae*, *Leguminosae*, and miscellaneous grasses.

2.3. Soil physicochemical characterization

Soil samples were analyzed for key physicochemical properties using standard laboratory procedures. Soil pH was measured in a 1:2.5 (w/v) soil-to-deionized water suspension using a calibrated Delta 320 pH meter (Mettler-Toledo Instruments, Columbus, OH, USA). Soil total nitrogen (TN) was quantified with a continuous-flow auto-analyzer (AA3, SEAL Analytical GmbH, Norderstedt, Germany), and total phosphorus (TP) was determined using a UV–VIS spectrophotometer (UV-2700, Shimadzu, Kyoto, Japan) following acid digestion.

Inorganic nitrogen species, including ammonium (NH_4^+-N) and nitrate (NO_3^--N), were extracted with 2 M KCl (soil: solution ratio of 1:5) and measured using the same auto-analyzer (AA3, SEAL Analytical). Soil moisture content was determined gravimetrically by oven-drying fresh samples at 105 °C to a constant weight and calculating the water loss relative to dry mass. In situ soil moisture was also monitored using a time-domain reflectometry (TDR) probe (TDR100, Campbell Scientific Inc., Logan, UT, USA), which measures dielectric permittivity to estimate volumetric water content.

Soil electrical conductivity (EC) was measured using a portable EC meter (DDS-307A, INESA Scientific Instrument Co., Shanghai, China). The probe was inserted into a 1:5 soil–water extract and recorded the electrical resistance.

2.4. Soil enzymes analysis

Soil enzyme activities were quantified using standardized colorimetric and spectrophotometric assays adapted from established protocols (Guan et al., 1986; Neemisha and Sharma, 2022). All measurements were performed in triplicate using air-dried, sieved (<2 mm) soil samples stored at 4 °C. Each assay included substrate-free and soil-free blanks to correct for non-enzymatic reactions, and absorbance readings were recorded using a UV–VIS spectrophotometer (UV-2700, Shimadzu, Kyoto, Japan).

Urease activity was determined following the method of Guan et al. (1986). Briefly, 2.0 g of soil was incubated with 10 ml of 10 % urea, 20 ml of 1 M citrate buffer (pH 6.7), and 1 ml of toluene at 37 °C for 24 h. After incubation, 1 ml of the filtrate was mixed with 20 ml of distilled water, 4 ml of sodium phenolate, and 3 ml of sodium hypochlorite. After 20 min, the ammonium concentration (NH_4^+) was measured at 578 nm. Urease activity was expressed as mg NH_4^+-N per gram of soil per 24 h. Amylase activity was assessed using the 3,5-dinitrosalicylic acid (DNS) method. Soil (2.0 g) was incubated with 1 % soluble starch solution and DNS reagent at 37 °C for 24 h. The amount of reducing sugars released, expressed as glucose equivalents, was measured at 540 nm and reported as mg glucose g^{-1} soil 24 h^{-1} . Catalase activity was evaluated via titration. A 2.0 g soil sample was mixed with 40 ml of distilled water and 5 ml of 3 % hydrogen peroxide, shaken for 30 min, and filtered. A 25 ml aliquot of the filtrate was titrated with 0.1 M potassium permanganate until a stable pink color appeared. Catalase activity was expressed as ml $KMnO_4$ consumed per gram of soil per 30 min. β-glucosidase activity was determined by incubating 1.0 g of soil with 4 ml of p-nitrophenyl-β-D-glucopyranoside (pNPG) solution and 1 ml of toluene at 37 °C for 1 h. The reaction was stopped with 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.1 M Tris buffer (pH 12), and the release of p-nitrophenol (pNP) was measured at 400 nm. Results were expressed as μg pNP g^{-1} soil h^{-1} . Acid phosphatase activity was measured using p-nitrophenyl phosphate (pNPP) as a substrate. Soil samples were incubated at 37 °C, and the liberated p-nitrophenol was measured at 410 nm. Polyphenol oxidase activity was analyzed by incubating 2.0 g of soil with 25 ml of 0.1 M pyrogallol at 30 °C for 2 h, followed by absorbance measurement at 430 nm. Protease activity was measured by incubating 1.0 g of soil with 5 ml of 2 % casein solution at 37 °C for 2 h. The reaction was terminated with trichloroacetic acid (TCA), and the absorbance of hydrolyzed peptides was determined at 700 nm. Cellulase activity was assayed by incubating 1.0 g of soil with 1 % carboxymethyl cellulose (CMC) at 50 °C for 24 h. Reducing sugars released were quantified using the DNS method at 540 nm. Chitinase activity was measured using colloidal chitin as a substrate; 1.0 g of soil was incubated at 37 °C for 24 h, and N-acetylglucosamine released was determined at 585 nm. Hydroxylamine reductase activity was assessed based on the reduction of hydroxylamine to ammonium, with the final NH_4^+ concentration determined colorimetrically using Nessler's reagent at 420 nm. All enzyme activities were expressed as micrograms of product released per gram of soil per hour (μg g^{-1} h^{-1}), following the protocols described by Neemisha and Sharma (2022).

2.5. Statistical analysis

Data was analyzed using two-way ANOVA to assess significant differences in soil physicochemical properties and enzymatic activities across seasons. A three-way ANOVA was conducted for plant biomass analysis to evaluate the effects of grazing treatment (pre/post), season, and year (2021/2022). Pearson correlation analysis was conducted to examine relationships between enzymatic activity and soil physicochemical parameters and between plant biomass and both enzymatic activity and soil physicochemical parameters. A Sankey analysis was performed to visualize the comparative analysis of soil enzyme activities between 2021 and 2022 across seasons, highlighting the flow and distribution of changes in enzyme activity. Principal Component Analysis (PCA) was performed to identify seasonal clustering patterns among enzymatic activities and soil properties, revealing variability in soil and plant dynamics across 2021 and 2022. All ANOVA analyses, PCA, and Sankey analysis were conducted using GraphPad Prism (version 10.4.2) and OriginPro 2021 software. Statistical significance was set at ($p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$).

3. Results

3.1. Physicochemical parameters of soil

Seasonal and interannual variations in soil physicochemical parameters were evaluated across the years 2021 and 2022. The results from the two-way ANOVA revealed significant seasonal effects for most parameters, with minimal differences between years (Fig. 3).

Available Phosphorus showed significant seasonal variation ($p < 0.0001$), with winter consistently having the highest levels of phosphorus. In 2022, available phosphorus increased by 29.52 % compared to 2021. Summer 2022, however, saw a 31.35 % decrease compared to summer 2021, while no significant difference was found between years ($p = 0.70$). This indicates that seasonality was the primary factor influencing available phosphorus levels. Soil pH significantly increased in 2022 ($p < 0.0001$), particularly in summer, where it rose by 96.92 %, and in spring, where it increased by 39.20 %. These changes reflect a shift towards more alkaline conditions in 2022, especially during the summer and spring, compared to more acidic conditions in 2021. Alkaline Hydrolyzable Nitrogen (AHN) levels were highest in winter, with a 45.24 % increase in winter 2022 compared to winter 2021. AHN concentrations significantly decreased in other seasons, with a 54.67 % decrease observed in autumn 2022 compared to autumn 2021. Despite these seasonal variations, no significant differences were found between years ($p = 0.23$), indicating that seasonality, rather than interannual climate differences, primarily influenced AHN dynamics. Soil Electrical Conductivity (EC) exhibited significant seasonal variation ($p < 0.0001$), with winter showing the highest conductivity levels. In 2022, EC increased by 35.13 % compared to winter 2021, and spring 2022 showed a 41.81 % increase compared to the previous year, reflecting seasonal shifts in soil conductivity. Total Nitrogen (TN) concentrations varied seasonally ($p < 0.0001$), with the highest values observed in winter. Winter 2022 showed an 18.22 % increase in TN compared to winter 2021, while summer 2022 exhibited a 49.54 % decrease compared to summer 2021. Year-to-year differences were not significant ($p = 0.81$), highlighting that seasonal fluctuations were the dominant influence on TN concentrations. Total Phosphorus (TP) concentrations were significantly higher in the spring and autumn seasons of 2022 compared to 2021 ($p < 0.0001$). Autumn 2022 saw a 43.91 % increase compared to autumn 2021, and spring 2022 showed a 39.20 % increase compared to spring 2021. However, no significant differences were observed between years during the summer and winter seasons.

3.2. Enzymatic activity

Enzymatic activities were assessed across four seasons (Spring, Summer, Autumn, and Winter) in 2021 and 2022, with a focus on key soil enzymes involved in nutrient cycling. A two-way ANOVA was performed to analyze the effects of Season and Year on the activity of each enzyme (Fig. 4).

Amylase activity displayed significant seasonal variation. Winter 2022 showed a 15 % increase compared to Winter 2021 ($p < 0.0001$), and Spring 2022 exhibited a 12 % increase compared to Spring 2021. Conversely, Summer 2022 showed a 16 % decrease compared to Summer 2021, and Autumn 2022 demonstrated a 9 % decrease compared to Autumn 2021. No significant differences were observed between the years ($p = 0.81$), indicating that seasonal factors, rather than year-to-year variation, primarily influenced amylase activity. Glucosidase activity followed a similar seasonal trend, with the highest activity observed in Spring 2021, followed by Summer 2021, and the lowest in Autumn 2021. In Spring 2022, there was a 20 % decrease in activity compared to Spring 2021, and Summer 2022 saw a 15 % decrease compared to Summer 2021. However, glucosidase activity remained consistent between the years ($p = 0.92$), suggesting no significant year-to-year differences. Urease activity remained stable throughout the year, with slight increases in Summer 2021 compared to Summer 2022 (7 % higher). No significant seasonal ($p = 0.82$) or yearly ($p = 0.60$) differences were observed, suggesting that urease activity was not influenced by seasonal or year-to-year variations. Alkaline phosphatase showed significant seasonal variation ($p < 0.0001$), with Winter 2022 showing a 30 % increase compared to Winter 2021 and Spring 2022 showing an 18 % increase compared to Spring 2021. The Autumn months in both years exhibited lower activity. However, no significant yearly effect was found ($p = 0.99$), reinforcing the role of seasonal factors as the primary

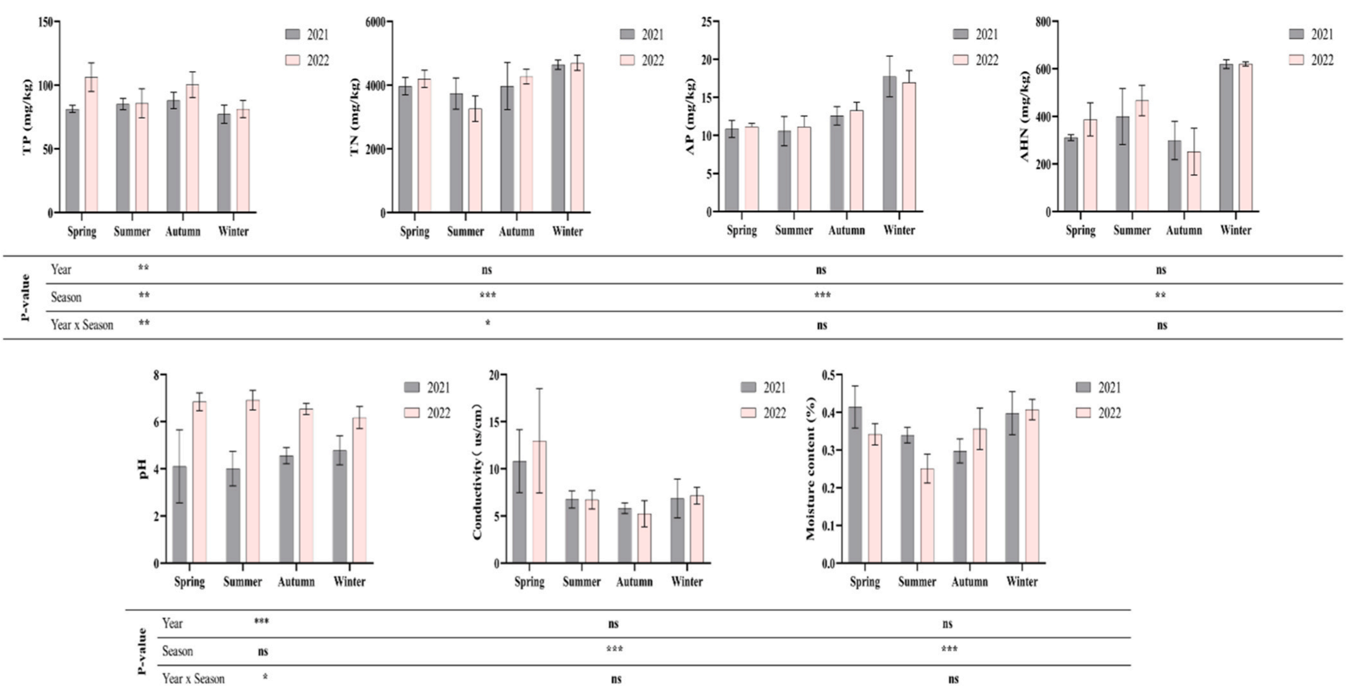


Fig. 3. Seasonal and yearly variations in soil physicochemical parameters. Error bars represent the standard error (SE). A two-way ANOVA was used to evaluate the effects of Season and Year on each parameter, with statistical significance indicated by asterisks (*$p < 0.05$; **$p < 0.01$ and ***$p < 0.001$).

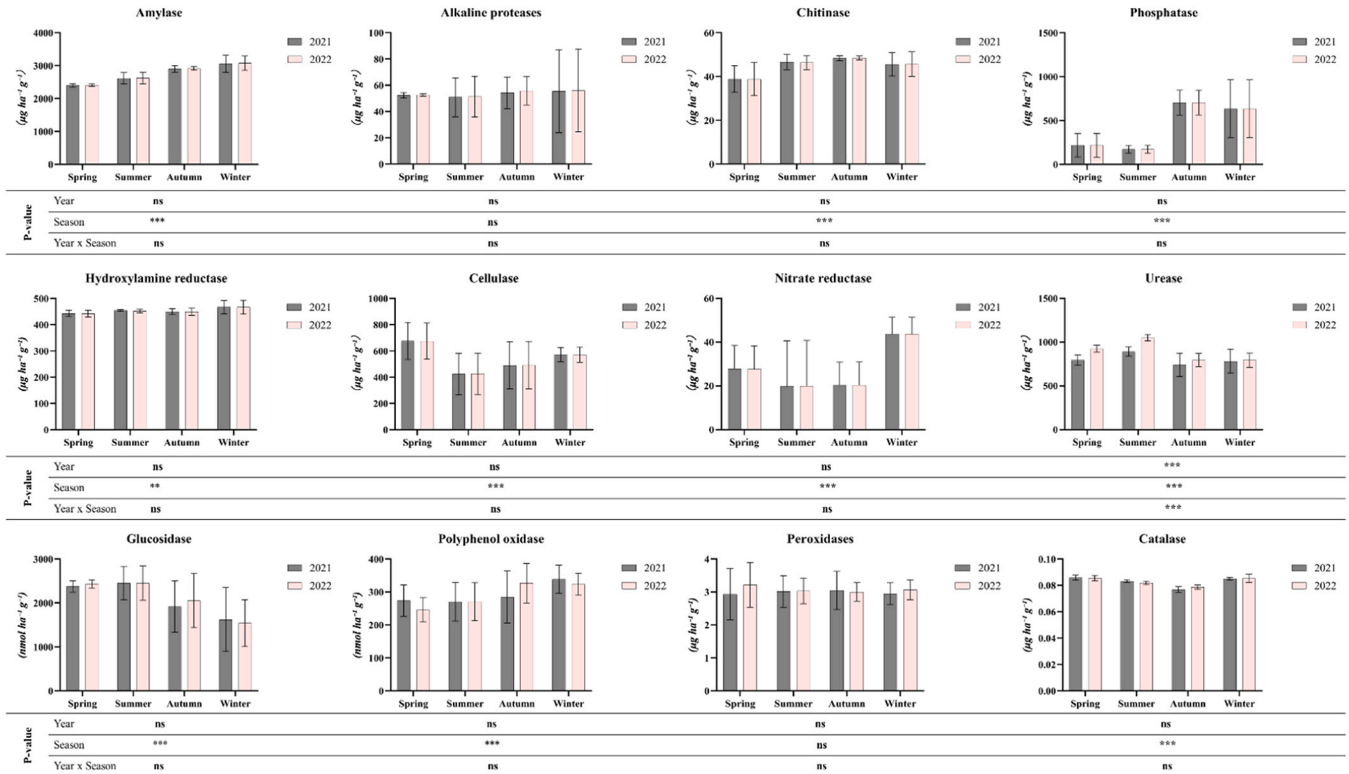


Fig. 4. Seasonal and yearly variations in enzymatic activity for 2021 and 2022. Error bars represent the standard error. A two-way ANOVA was used to assess the effects of Season and Year on enzymatic activity, with statistical significance indicated by asterisks (*p < 0.05; **p < 0.01 and ***p < 0.001).

influence on alkaline phosphatase activity. Cellulase activity exhibited moderate seasonal fluctuations, with Winter 2022 showing an 18 % increase compared to Winter 2021, and Summer 2022 showing a 10 % decrease compared to Summer 2021. The two-way ANOVA showed no significant differences between seasons ($p = 0.70$) or years ($p = 0.60$), indicating that cellulase activity remained stable over time. Alkaline protease and soil chitinase both exhibited consistently low activity levels throughout the year, with minimal seasonal variation. There were no significant differences between 2021 and 2022 for these enzymes ($p = 0.99$ for protease, $p = 0.65$ for chitinase), indicating that their activity remained stable across both years.

Polyphenol oxidase showed slight seasonal fluctuations ($p = 0.81$), with a small decrease in Summer 2022 compared to Summer 2021 ($p = 0.76$). However, no significant differences were observed between the years, with its activity consistently lower than that of other enzymes. Catalase and peroxidase activity remained stable throughout both seasons and years, with no significant seasonal ($p = 0.68$ for catalase, $p = 0.89$ for peroxidase) or yearly differences ($p = 0.82$ for catalase, $p = 0.54$ for peroxidase), indicating that these enzymes were not influenced by seasonal or inter-annual factors. Nitrate reductase activity was consistently low across all seasons and years, with no significant differences observed either seasonally ($p = 0.74$) or yearly ($p = 0.59$), suggesting that nitrate reductase activity remained stable throughout the study period.

3.3. Correlation analysis between enzyme activity and soil physicochemical properties

To understand the dynamics of soil nutrient cycling, we investigated how the relationships between soil enzyme activities and physicochemical properties vary across different seasons. This seasonal variation highlights how key environmental factors such as moisture, pH, and nutrient availability influence enzymatic processes critical to soil health and nutrient cycling, as shown in Fig. 5.

In spring, we found a significant positive correlation between total phosphorus (TP) and urease activity ($p < 0.01$), suggesting that increased phosphorus availability supports nitrogen cycling early in the growing season. This was the only significant correlation in spring, indicating that phosphorus may be a key factor influencing nitrogen transformation during this time. During summer, electrical conductivity (EC) was positively correlated with chitinase activity ($p < 0.01$), indicating that higher ionic strength in the soil may promote enzyme activity linked to nutrient breakdown. Urease activity also showed a positive correlation with soil moisture content (SMC) ($p < 0.01$) and a negative correlation with soil pH ($p < 0.001$), suggesting that slightly acidic and moist conditions are conducive to nitrogen cycling. In autumn, SMC was positively correlated with glucosidase activity ($p < 0.05$), indicating that increased moisture enhances carbohydrate degradation. Conversely, alkaline hydrolyzable nitrogen (AHN) showed a negative correlation with glucosidase activity ($p < 0.01$), suggesting that higher nitrogen availability may suppress this enzymatic activity. In winter, we found that available phosphorus (AP) was positively correlated with both nitrate reductase and urease activity ($p < 0.05$), indicating that phosphorus availability supports nitrogen transformation processes under colder conditions. However, SMC exhibited negative correlations with cellulase and nitrate reductase ($p < 0.05$), suggesting that excessive moisture in winter may inhibit the activity of enzymes involved in carbon and nitrogen cycling.

3.4. Grazing effects on plant biomass

The pre- and post-grazing effects on plant biomass across the four seasons in 2021 and 2022 were assessed. Three-way ANOVA results revealed a significant reduction in biomass after grazing in all seasons for both years, with varying effects across species and seasons (Fig. 6).

Poaceae showed the most pronounced reduction in biomass across both years, especially in Spring 2022 and Summer 2021 ($p = 0.015$ for *Poaceae* Year × Grazing State Interaction). In Summer 2021, *Poaceae*

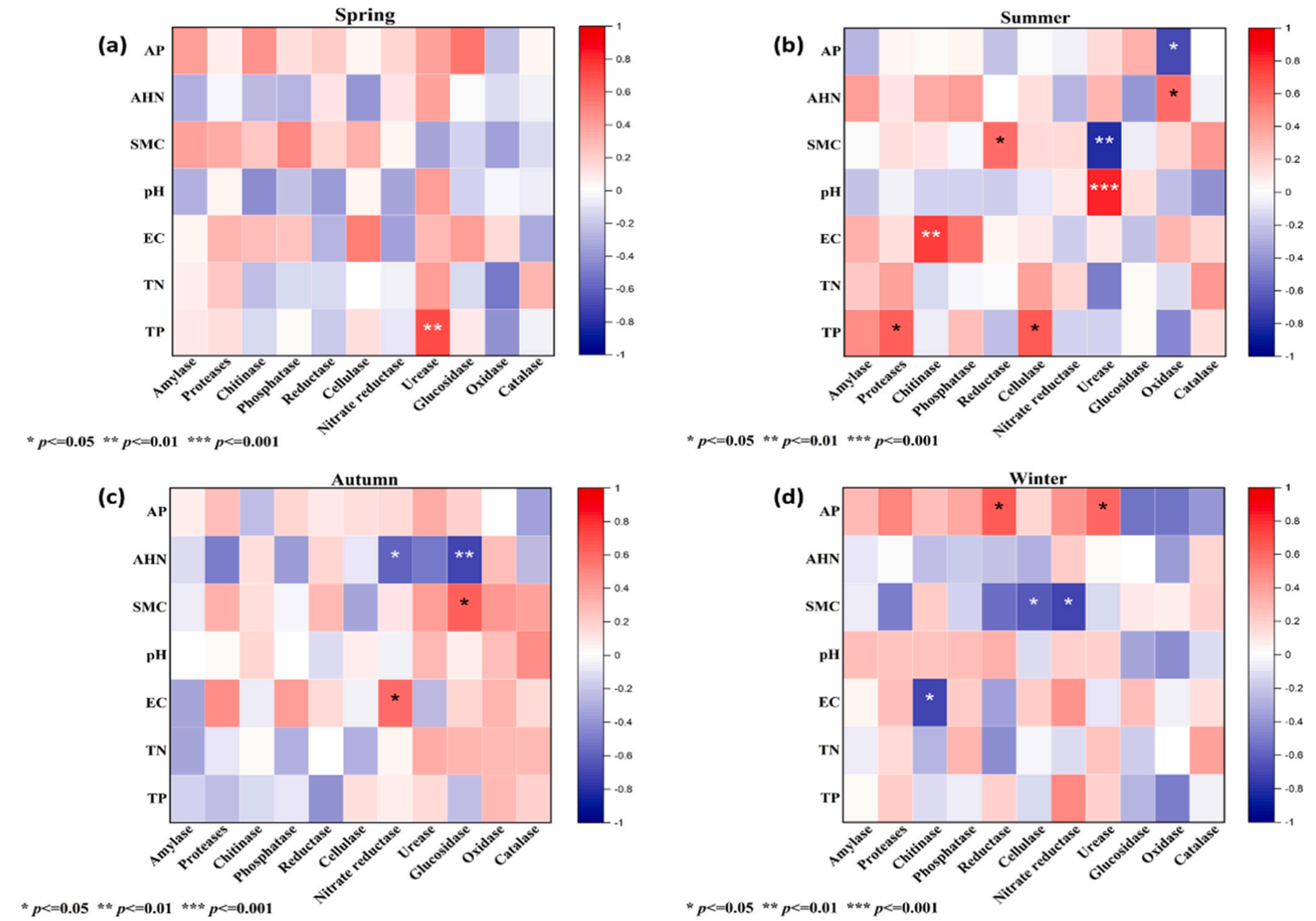
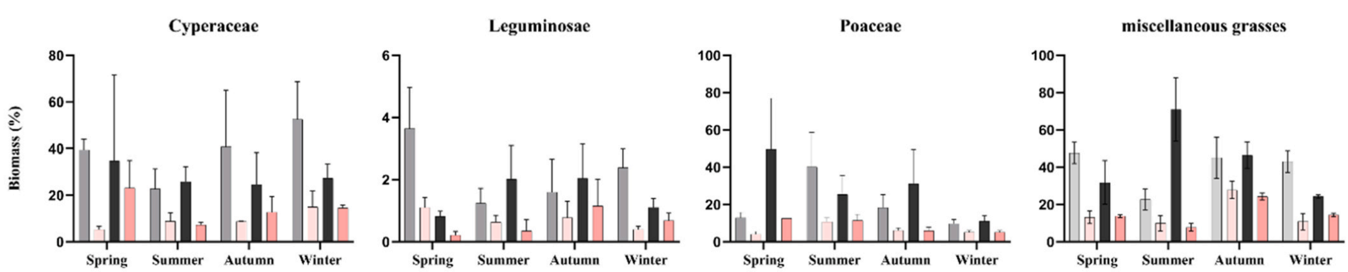


Fig. 5. Correlation matrix showing the relationships between enzyme activities and physicochemical parameters (Available phosphorus (AP), Alkaline hydrolyzable nitrogen (AHN), Soil moisture content (SMC), Soil pH, Electrical conductivity (EC), Total nitrogen (TN), Total phosphorus (TP)) across four seasons. Pearson correlation coefficients (r) are color-coded, with red indicating positive correlations and blue indicating negative correlations. Asterisks denote statistical significance (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$).



P- values	Cyperaceae	Leguminosae	Poaceae	miscellaneous grasses
Years	ns	ns	**	ns
Seasons	***	ns	***	***
Grazing states	***	**	***	***
Y × S	***	***	***	*
Y × G	*	*	**	ns
S × G	ns	ns	ns	ns
Y × S × G	ns	***	***	**

Fig. 6. Seasonal variation in plant biomass (%) under pre- and post-grazing conditions across 2021 and 2022. Error bars represent the standard error (SE). A three-way ANOVA was performed to evaluate the effects of grazing treatment (pre/post), year (2021/2022), and season on plant biomass, with statistical significance indicated by asterisks (*$p < 0.05$, **$p < 0.01$, *** $p < 0.001$). Tukey's HSD test was used for pairwise comparisons between groups.

exhibited the largest biomass decrease, followed by *Cyperaceae* and Miscellaneous Grasses. While the reduction pattern was similar in Summer 2022, the grazing effect was less pronounced ($p < 0.0001$ for Grazing State Interaction). In Autumn, Miscellaneous Grasses and *Cyperaceae* dominated pre-grazing biomass, with significant reductions post-grazing in both years, particularly in Autumn 2022 ($p = 0.002$ for Autumn Year × Grazing State Interaction). *Leguminosae*, however, showed consistently low biomass and little response to grazing across all seasons. In Winter, *Poaceae* showed minimal biomass reduction ($p = 0.73$), indicating limited grazing impact during winter conditions. However, Miscellaneous Grasses and *Cyperaceae* experienced moderate reductions in both Winter 2021 and Winter 2022 ($p = 0.03$ for Winter Year × Grazing State Interaction).

Poaceae and *Cyperaceae* exhibited the largest biomass reductions, especially in the Summer and Spring seasons, while *Leguminosae* showed minimal response to grazing. The greatest recovery after grazing occurred in Autumn 2022 for miscellaneous Grasses ($p = 0.03$ for miscellaneous grasses grazing effect).

3.5. Correlation between vegetation composition and soil physicochemical parameters

Seasonal Pearson correlation analyses conducted with data from 2021 and 2022 revealed distinct relationships between plant functional groups and soil physicochemical parameters, emphasizing how soil conditions regulate vegetation composition and seasonal dynamics in alpine grassland ecosystems (Fig. 7).

In spring, *Poaceae* was positively correlated with both alkaline hydrolyzable nitrogen (AHN) ($p < 0.01$) and soil pH ($p < 0.05$), indicating a preference for nitrogen-rich, moderately alkaline conditions during early growth. *Cyperaceae* exhibited a positive correlation with total nitrogen and total phosphorus ($p < 0.05$), suggesting that nutrient-enriched soils promote their growth. Conversely, *Leguminosae* showed a significant negative correlation with total phosphorus ($p < 0.01$), suggesting a sensitivity to higher phosphorus availability. During summer, *Poaceae* was negatively correlated with soil moisture ($p < 0.05$), indicating that wetter conditions may limit its growth. *Leguminosae* demonstrated a strong negative correlation with available phosphorus ($p < 0.01$), while miscellaneous grasses were negatively associated with AHN ($p < 0.01$), reflecting specific nutrient responses that vary across plant groups. In autumn, *Cyperaceae* was positively correlated with total phosphorus and negatively correlated with conductivity ($p < 0.05$), indicating its preference for phosphorus-rich, low-salinity soils. Miscellaneous grasses were also positively correlated with total phosphorus ($p < 0.05$), suggesting that phosphorus availability drives their abundance in the late season. In winter, *Leguminosae* showed a positive correlation with conductivity ($p < 0.05$), indicating that soil ionic concentration may influence its persistence during colder months.

These results highlight the seasonally dependent responses of plant functional groups to soil nutrient and chemical conditions. *Poaceae* consistently responded positively to nitrogen availability and pH but showed reduced growth under higher moisture in summer. *Leguminosae* and *Cyperaceae* were more influenced by phosphorus and conductivity, especially outside the main growing season. Miscellaneous grasses displayed varying nutrient responsiveness, with sensitivity to nitrogen in summer and a positive association with phosphorus in autumn.

3.6. Correlation between enzymes and vegetation across the seasons

Seasonal Pearson correlation analyses of data from 2021 and 2022 revealed significant associations between soil enzyme activities and plant functional groups, underscoring the relationship between soil

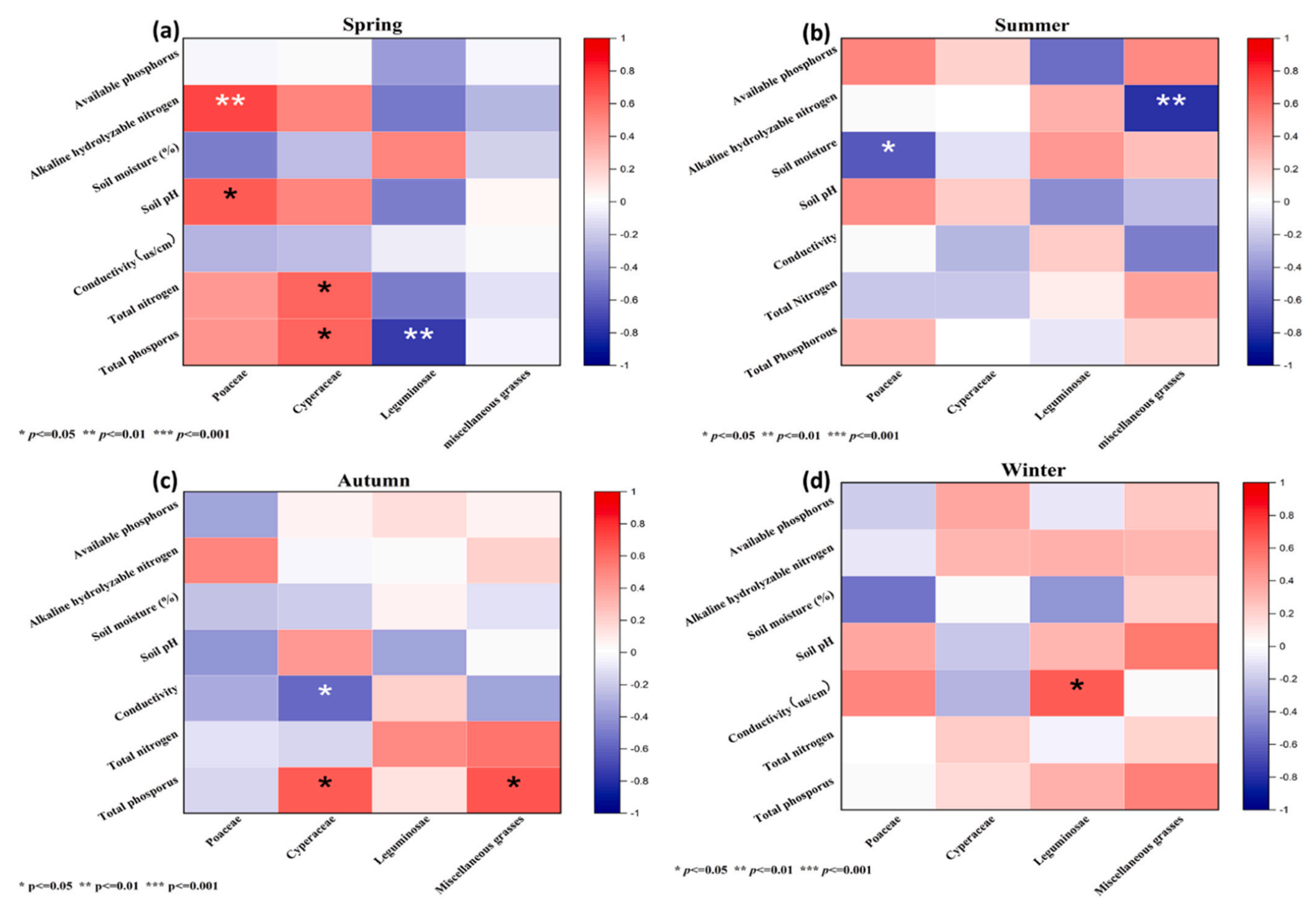


Fig. 7. Correlation matrix showing the relationships between physicochemical parameters and plant biomass across four seasons (Spring, Summer, Autumn, Winter), of 2021 and 2022. Each cell represents the Pearson correlation coefficient (r), with red indicating positive correlations and blue indicating negative correlations. Asterisks denote statistical significance (*$p \le 0.05$, **$p \le 0.01$, ***$p \le 0.001$). The data represents the combined values from 2021 and 2022, with physicochemical parameters placed on the Y-axis and plant types on the X-axis to highlight overall trends across the two years.

biochemical processes and vegetation dynamics in alpine grasslands (Fig. 8).

In spring, urease activity was positively correlated with *Poaceae* ($p < 0.05$) and negatively correlated with *Leguminosae* ($p < 0.01$), indicating that nitrogen mineralization preferentially supports *Poaceae* while inhibiting *Leguminosae*. Polyphenol oxidase was negatively correlated with *Cyperaceae* ($p < 0.05$), suggesting that oxidative processes may hinder the growth of this group during spring. In summer, *Poaceae* showed negative correlations with both hydroxylamine reductase and nitrate reductase ($p < 0.05$), implying that intensified nitrogen transformations may reduce *Poaceae* dominance in this season. *Leguminosae* displayed a negative correlation with glucosidase ($p < 0.05$), indicating that carbohydrate decomposition could suppress *Leguminosae* growth during the summer months. In autumn, *Poaceae* was negatively correlated with both alkaline phosphatase and nitrate reductase ($p < 0.05$), suggesting that phosphorus and nitrogen cycling limitations may restrict *Poaceae* growth during the late growing season. In contrast, in winter, *Leguminosae* exhibited positive correlations with both alkaline protease and nitrate reductase ($p < 0.05$), highlighting the role of protein and nitrogen cycling in supporting its persistence under colder conditions. Polyphenol oxidase was negatively correlated with miscellaneous grasses ($p < 0.05$), indicating that oxidative soil conditions may reduce the growth and survival of these grasses during winter.

These findings demonstrate how enzyme-vegetation interactions vary seasonally, with soil biochemical processes shaping plant community composition throughout the year. This underscores the critical role of nutrient cycling in determining vegetation dynamics in alpine grasslands.

3.7. Comparative analysis of soil enzyme activities

The comparative analysis of soil enzyme activities between 2021 and 2022 revealed consistent seasonal patterns along with notable interannual variability. The Sankey diagrams (Fig. 9) illustrate shifts in the dominant enzyme activities across seasons, highlighting the year-to-year differences in soil biochemical functioning.

In spring, both years exhibited diverse enzyme activity profiles, with 2022 showing a stronger representation of urease and chitinase. This suggests a heightened emphasis on nitrogen mineralization and organic matter degradation compared to 2021. In summer, 2021 was characterized by higher hydroxylamine reductase and cellulase activities, reflecting active nitrogen cycling and organic material decomposition. In contrast, the profile for 2022 was dominated by nitrate reductase and polyphenol oxidase, pointing to a shift in nitrogen transformation and potential lignin breakdown. In autumn, amylase and alkaline phosphatase were the dominant enzymes in 2021, highlighting carbohydrate hydrolysis and phosphorus cycling. However, in 2022, glucosidase activity took precedence, indicating increased breakdown of complex sugars and cellulose. During winter, both years consistently showed high amylase and alkaline phosphatase activity, indicating stable carbohydrate and phosphate metabolism under colder conditions. Moreover, 2022 displayed an enhanced presence of nitrate reductase, suggesting

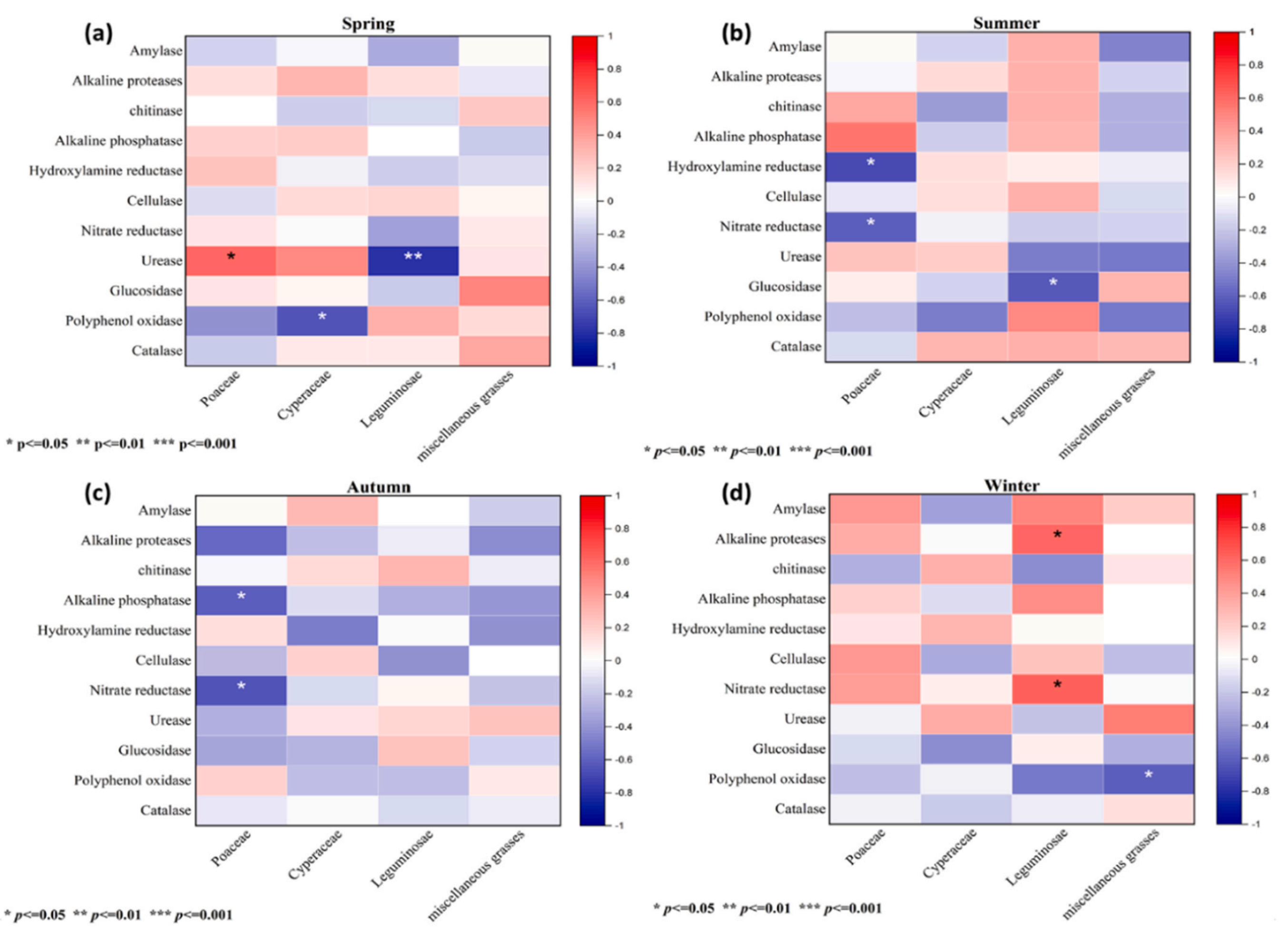


Fig. 8. Pearson correlation matrix showing the relationships between enzyme activities and vegetation types across four seasons. Correlation coefficients (r) are color-coded, with red indicating positive correlations and blue indicating negative correlations. Asterisks indicate statistical significance ($*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$).

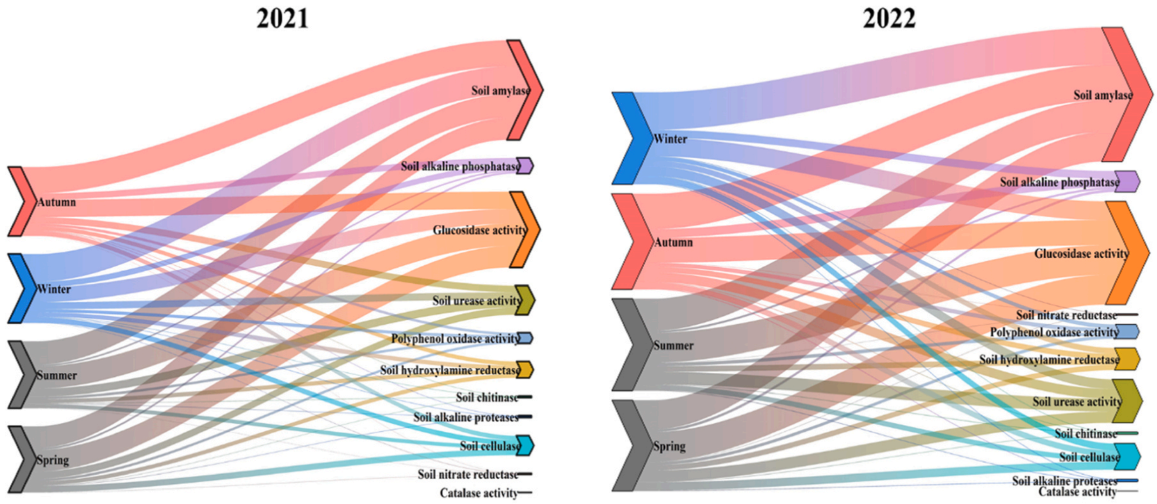


Fig. 9. Sankey diagram showing the comparative analysis of soil enzyme activities in 2021 and 2022 across seasons. The diagram illustrates the seasonal variation and inter-annual differences in enzyme activity patterns. The flow widths represent the relative prominence of each enzyme activity within a given season, with thicker flows indicating higher enzyme activity. The diagram highlights shifts in enzyme activity between the two years, reflecting changes in environmental conditions such as temperature and moisture availability.

increased nitrogen processing in winter soils.

3.8. Assessing seasonal differences in soil enzymatic activity

Principal Component Analysis (PCA) was conducted to assess overall patterns of compositional variation across the seasons. The biplot presented in Fig. 10 shows that the first two principal components explained 90.37 % of the total variance (79.98 % by PC1 and 10.39 % by PC2). The high variance captured by PC1 indicates a strong underlying gradient driving seasonal variation. Samples clustered distinctly by season, with winter samples (WN21 and WN22) forming a tight cluster along the negative axis of PC1, suggesting unique profiles during colder months. Autumn samples (AU21 and AU22) also separated from the spring and summer groups, indicating clear seasonal differentiation. The 95 % confidence ellipses further supported these findings, confirming the robustness of seasonal structuring across the dataset. These results highlight the dominant role of seasonal dynamics in driving variation in soil enzymatic activity, complementing the univariate comparisons and reinforcing the importance of seasonality in shaping microbial and environmental processes.

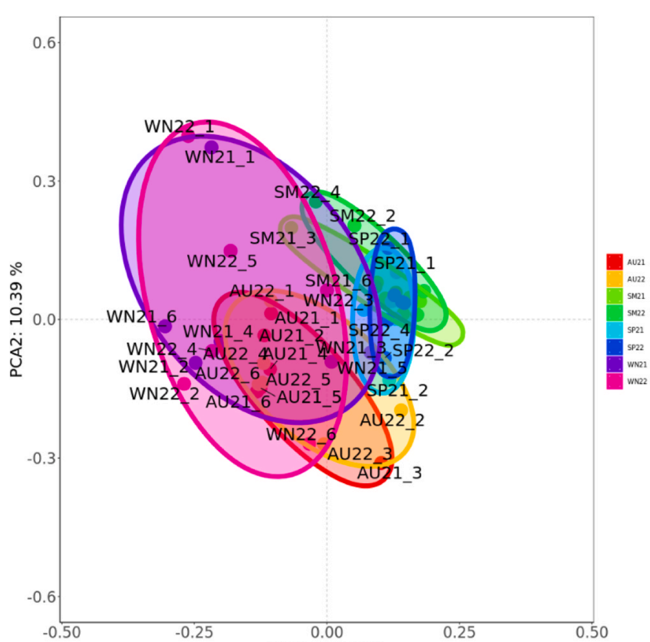


Fig. 10. PCA plot of seasonal variation in soil properties and enzymatic activities across 2021 and 2022. The plot shows the clustering of seasonal groups based on the first two principal components (PC1 and PC2), which explain 79.98 % and 10.39 % of the variance, respectively. The colors represent the four seasons (Winter, Spring, Summer, Autumn) for 2021 and 2022. Ellipses indicate the grouping of data points for each season, highlighting seasonal shifts and the consistency in enzymatic processes over the two years.

4. Discussion

This study investigates the impacts of seasonal grazing on soil enzymatic activities, physicochemical properties, and plant biomass dynamics in alpine meadows of the Qinghai-Tibet Plateau (QTP). Our findings reveal significant seasonal and interannual variability in these processes, with grazing pressure interacting with temperature, moisture, and nutrient availability to shape soil nutrient cycling and plant responses. In this discussion, we focus on the underlying mechanisms driving these changes, particularly how seasonal grazing influences soil enzymatic activities and biomass dynamics, and we propose management strategies to optimize land use for ecosystem health.

4.1. Mechanisms behind enzyme dynamics under seasonal grazing

Our first hypothesis theorized that seasonal grazing would significantly alter soil enzymatic activity. The results support this hypothesis, showing distinct seasonal peaks in enzymes like amylase, glucosidase, alkaline phosphatase, and urease. These findings indicate that grazing pressure, combined with temperature and moisture, drives enzyme activity across seasons.

The observed peak in amylase activity during winter is likely due to low temperatures and reduced plant growth in winter, which limits microbial decomposition of plant material (Deng et al., 2019). Amylase plays an important role in carbohydrate metabolism, critical during periods when plant residues are more abundant, but microbial decomposition processes are slowed due to cold (Chen et al., 2024). The decline in spring amylase activity can be explained by transitional conditions, which limit enzyme activity after the cold months (Zuccarini et al., 2020). Similarly, glucosidase activity peaks during the summer, likely due to higher temperatures and increased microbial activity, which accelerate carbon cycling. The decline in winter is due to cold stress, which limits microbial metabolic rates, affecting the enzyme activity involved in the breakdown of complex carbohydrates (Kang and Freeman., 1999; Luo et al., 2020). Phosphatase activity peaks during autumn and winter, likely because of increased demand for phosphorus when plant growth slows down, reducing plant phosphorus uptake. This suggests that soil enzymes help mobilize nutrients in response to reduced plant activity during these colder months, consistent with findings that microbial phosphorus acquisition processes are intensified during these seasons (Saha et al., 2008; Acosta-Martínez and Tabatabai, 2011; Reddy et al., 2020). Urease activity remained stable across seasons, displaying its consistent role in nitrogen cycling and maintaining its relevance for plant nitrogen uptake, especially in response to grazing disturbances and soil nutrient dynamics (Margalef et al., 2017; Ali et al., 2020). The persistence of this activity, coupled with its positive correlation with total phosphorus, highlights the coupling of nitrogen and phosphorus cycling in grazed ecosystems.

While environmental factors such as temperature and precipitation are known to affect soil biochemical activity, our statistical models included "year" as a fixed factor to control for interannual climatic variability. The year effect was not statistically significant ($p > 0.05$) for most key enzymatic and nutrient parameters, including amylase, glucosidase, alkaline hydrolyzable nitrogen (AHN), and total nitrogen (TN), as reported in Sections 3.1 and 3.2. These findings confirm that the seasonal variations in enzymatic activity observed in this study were primarily associated with grazing seasonality rather than differences in climate between 2021 and 2022.

4.2. Seasonal grazing and its influence on soil enzymes: insights from PCA

Our second hypothesis proposed that seasonal grazing would modulate soil physicochemical properties. The results support this hypothesis, revealing significant seasonal changes in total nitrogen (TN), total phosphorus (TP), available phosphorus, electrical conductivity (EC), and soil pH. These findings suggest that grazing pressure, combined with seasonal climatic factors, regulates nutrient availability in the soil. Below, we explain the potential mechanisms behind these seasonal fluctuations.

We observed that available phosphorus was significantly higher in winter compared to other seasons. This increase is likely due to reduced plant uptake and lower microbial immobilization during colder periods when plant growth slows down (Bünemann et al., 2018). With reduced biological activity in winter, phosphorus accumulates in the soil, especially as grazing pressure diminishes plant cover and nutrient cycling slows. This suggests that seasonal grazing and climatic factors interact to influence nutrient availability, particularly in winter, when plant activity is minimal and microbial processes are slower (Giese et al., 2011; Zhu et al., 2016). The observed elevation in soil pH in 2022, particularly in summer, suggests that grazing pressure, combined with long-term environmental changes, may contribute to a more alkaline soil environment. Grazing can influence soil chemistry by changing the rate of organic matter decomposition, where microbial communities play a key role. As grazing increases the decomposition of organic materials, this can result in an increase in pH over time, especially in systems with reduced plant cover and lower organic inputs (Wang et al., 2021). Soil moisture was negatively correlated with urease and glucosidase activities during the summer. Excess moisture, especially during the rainy season, can lead to waterlogged soil, reducing oxygen availability and inhibiting enzyme function. The moisture stress suppresses key soil enzymatic activities involved in nitrogen and carbon cycling under grazing pressure, consistent with the role of microbial processes in nutrient turnover and enzyme activity (Sardans et al., 2008; Neemisha and Sharma, 2022). Electrical conductivity (EC) showed positive correlations with chitinase activity in summer, likely due to increased microbial turnover under wetter conditions. In contrast, the negative correlation between EC and chitinase activity in winter suggests that salt stress may reduce enzyme activity in drier and colder conditions, altering nutrient dynamics in soil (Mayel et al., 2021).

4.3. Seasonal grazing and plant biomass dynamics

Our third hypothesis posited that seasonal grazing would influence plant biomass and species composition. The results confirmed this hypothesis, with *Poaceae* showing significant biomass reduction across all seasons. This reduction is likely due to the grazing sensitivity of *Poaceae* species, which are often characterized by shallow root systems and high palatability, making them more vulnerable to grazing pressure. The loss of biomass in these species can have broader implications for organic carbon inputs to the soil and affect overall soil fertility. In contrast, species like *Cyperaceae* and miscellaneous grasses exhibited greater resilience due to their deeper root systems or lower palatability, which is consistent with the original data suggesting that these species recover more easily after grazing. The relatively stable biomass of *Leguminosae* is explained by its ability to fix nitrogen, which aids its growth even under grazing pressure, supporting the finding that *Leguminosae* is at a competitive disadvantage in grazed environments due to reduced competitive ability (Lin et al., 2010; Fernández-Lugo et al., 2013; Díaz et al., 2007; Yu et al., 2020; Yang et al., 2017).

4.4. Linking principal component analysis (PCA) to grazing mechanisms

The Principal Component Analysis (PCA) results support our hypothesis that seasonal grazing influences soil processes. The tight clustering of winter samples indicates that grazing during winter results in relatively stable enzyme activity, likely due to reduced microbial activity in colder months. In contrast, the separation of summer and autumn groups in the PCA plot reflects greater variability in soil conditions and enzyme activity, which can be attributed to higher grazing intensity, temperature fluctuations, and moisture changes. This suggests that grazing in warmer months increases microbial stress, leading to less stable enzymatic activity. These insights help us understand how grazing intensity, in combination with seasonal climatic conditions, can shape soil nutrient cycling and plant growth (Ochoa-Hueso et al., 2020).

This distinct seasonal clustering of samples across both years, with no grouping or overlap by year, reinforces that the dominant patterns in soil enzymatic and physicochemical variation were driven by seasonal grazing treatments. The absence of interannual separation in multivariate space supports our conclusion that climatic variability during the study period did not confound the treatment effects and that the observed patterns are attributable to consistent seasonal influences.

4.5. Enhancing grazing management for improved soil and plant health

The insights from this study have practical implications for grazing management and land-use strategies aimed at maintaining soil health and promoting plant productivity. Understanding the seasonal dynamics of soil enzymatic activity and nutrient availability can guide the optimization of fertilization schedules and grazing regimes. For instance, winter grazing might be optimal for phosphorus supplementation, as phosphatase and catalase activity peak during this period, suggesting that phosphorus mineralization is most efficient in winter months (Azeem et al., 2015). Similarly, nitrogen applications may be more effective in spring, when urease activity is highest, promoting nitrogen cycling (Zaman et al., 1999; Adetunji, 2019). Moreover, managing grazing intensity to prevent excessive biomass removal, especially for species like the *Poaceae*, could promote soil fertility and sustainable nutrient cycling. Integrating grazing management with nutrient management strategies could help maintain ecosystem balance and promote long-term grassland productivity (Rumpel et al., 2015). A strategic approach to grazing can help improve soil health, mitigate overgrazing risks, and promote plant diversity, contributing to more resilient ecosystems under changing climatic conditions.

5. Conclusion

This study investigated the impact of seasonal grazing on soil enzymatic activities, physicochemical properties, and plant biomass dynamics in the alpine meadows of the Qinghai-Tibet Plateau (QTP). The findings from this study robustly support the three initial hypotheses. We observed significant seasonal shifts in the activity of key soil enzymes, such as amylase, glucosidase, phosphatase, and urease, which were closely linked to grazing pressures and environmental conditions like temperature, moisture, and nutrient availability. These findings underscore the role of seasonal grazing as a primary driver of soil enzymatic activity, with seasonal shifts in enzyme patterns providing insights into nutrient cycling and soil processes in grazed ecosystems. The study also found that seasonal grazing significantly modulated soil properties, including total nitrogen (TN), total phosphorus (TP), available phosphorus, soil pH, and electrical conductivity (EC). The seasonal and interannual variability observed in these properties suggests that grazing pressure, coupled with climatic factors, regulates soil nutrient availability and nutrient cycling in a complex manner. Our analysis confirmed that seasonal grazing alters plant biomass dynamics, particularly for *Poaceae*, which showed significant biomass reduction. Other plant groups, like *Cyperaceae* and *Leguminosae*, exhibited varying resilience to grazing pressure. These findings highlight the importance of species-specific responses to grazing and the need for targeted grazing management strategies to promote sustainable grassland health. The results from Principal Component Analysis (PCA) further support our hypothesis that seasonal grazing, in combination with climatic variations, shapes soil properties and plant dynamics. The PCA clustering revealed that seasonal factors were the dominant drivers of changes in soil properties and enzyme activities, and climatic variability did not significantly confound the treatment effects.

Despite the robust dataset, this study was limited by its two-year field observation period and focus on a single alpine meadow ecosystem in Maqu County. Expanding the study to cover a broader geographic area and a longer time span would improve the generalizability of these findings. Furthermore, while this study explored correlations between enzyme activities and plant biomass, microbial community composition was not directly assessed. Given its critical role in nutrient cycling, future studies should incorporate microbial profiling to gain deeper mechanistic insights.

Future research should also explore how grazing intensity and fertilization treatments influence soil biochemical health. Incorporating metagenomic approaches will help understand the relationship between microbial dynamics and enzymatic shifts under grazing. Expanding the research across multiple elevation gradients or climate zones could enhance the broader applicability of the findings to different alpine grassland systems. While our study accounted for environmental variables such as temperature, moisture, and nutrient availability, further research should aim to disentangle their direct and interactive effects on soil enzymatic dynamics under grazing pressure. This could involve experimental manipulation or long-term monitoring to better isolate environmental versus grazing-driven responses. This area warrants additional research to separate the roles of grazing versus environmental drivers in influencing soil nutrient cycling and enzyme function. This study provides a solid foundation for developing adaptive grazing management practices, aimed at sustaining soil and ecosystem health in grazing-impacted landscapes. By integrating grazing intensity with nutrient management, we can promote sustainable land-use strategies that ensure long-term grassland productivity and ecosystem resilience in the face of changing climatic conditions.

6. CRediT authorship contribution statement

zhao Weikang: Formal analysis, Data curation. **Fujiang Hou:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Zheng Haozhe:** Methodology, Investigation, Formal analysis, Data curation. **Muhammad Usman:** Writing – review & editing, Formal analysis. **Barkat Ali:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors, Barkat Ali, Haozhe Zheng, Muhammad Usman, Weikang Zhao, and Fujiang Hou, declares that there are no conflicts of interest related to the manuscript titled **"Impacts of seasonal grazing on soil enzymes, physicochemical properties, and vegetation in alpine meadows"** The authors affirm that they have no financial or personal relationships with any organizations or individuals that could influence the results or interpretations presented in this study. The authors also confirm that all funding sources and institutional affiliations have been disclosed, and no competing interests could affect the research integrity. This manuscript is an original work and has not been submitted elsewhere for publication.

Acknowledgement

This research was supported by the National Natural Science Foundation of China (U21A20242), the National Key Research and Development Program of China (2021YFD1300504), the Innovative Research Team of the Ministry of Education (IRT_17R50), and scientific research funding from Lanzhou City to Lanzhou University.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.109837.

Data availability

No data was used for the research described in the article.

References

Acosta-Martínez, V., Tabatabai, M.A., 2011. Phosphorus cycle enzymes. Methods Soil Enzym. 9, 161–183.

Adetunji, A.T., 2019. Impact of cover crop species, termination stage and termination method on soil nitrogen, organic carbon and enzyme activities. (Doctoral dissertation, Doctoral dissertation. Cape Peninsula University of Technology, South Africa.

Adetunji, A.T., Lewu, F.B., Mulidzi, R., Ncube, B., 2017. The biological activities of β-glucosidase, phosphatase and urease as soil quality indicators: a review. J. Soil Sci. Plant Nutr. 17, 794–807.

Ali, N., Karkush, M.O., Haideri, H.H.A., 2020. Isolation and identification of local bacteria produced from soil-borne urease. IOP Conf. Ser. Mater. Sci. Eng. 901 (1), 12035.

Argenti, G., Staglianò, N., Bellini, E., Messeri, A., Targetti, S., 2020. Environmental and management drivers of alpine grassland vegetation types. Ital. J. Agron. 15 (1), 1–12.

Azeem, M., Riaz, A., Chaudhary, A.N., Hayat, R., Hussain, Q., Tahir, M.I., Imran, M., 2015. Microbial phytase activity and their role in organic P mineralization. Arch. Agron. Soil Sci. 61, 751–766.

Bashir, O., Owais, T.A., Baba, Z.A., Rather, G.H., Bangroo, S.A., Mukhtar, S.D., Naik, N., Mohiuddin, R., Bharati, V., Bhat, R.A., 2021. Soil organic matter and its impact on soil properties and nutrient status. In: Naik, M.M. (Ed.), Microbiota and Biofertilizers, Volume 2: Ecofriendly Tools for Reclamation of Degraded Soil Environs. Springer, Singapore, pp. 129–159.

Bell, C., McIntyre, N., Cox, S., Tissue, D., Zak, J., 2008. Soil microbial responses to temporal variations of moisture and temperature in a Chihuahuan Desert grassland. Microb. Ecol. 56, 153–167.

Bhaduri, D., Sihi, D., Bhowmik, A., Verma, B.C., Munda, S., Dari, B., 2022. A review on effective soil health bio-indicators for ecosystem restoration and sustainability. Front. Microbiol 13, 938481.

Bhattarai, P., 2013. Threats on grassland ecosystem services: a case from Shuklaphanta Wildlife Reserve. Nepal J. Sci. Technol. 13 (2), 159.

Bicharanloo, B., Shirvan, M.B., Dijkstra, F.A., 2022. Decoupled cycling of carbon, nitrogen, and phosphorus in a grassland soil along a hillslope mediated by clay and soil moisture. Catena 219, 106648.

Bünemann, E.K., Bongiorno, G., Bai, Z., Creamer, R.E., De Deyn, G., De Goede, R., Fleskens, L., Geissen, V., Kuyper, T.W., Mäder, P., Pulleman, M., 2018. Soil quality–a critical review. Soil Biol. Biochem 120, 105–125.

Chen, Y.P., Huang, H.Y., Tsai, C.F., Young, C.C., 2024. Impact of fertilization and seasonal changes on paddy soil: Unveiling the interplay between agricultural practices, enzyme activity, and gene diversity. Agriculture 14, 1424.

Chen, B., Ma, X., Zhou, X., Zhang, X., Wang, X., Li, Z., Yang, X., Lu, S., Du, W., 2025. Medium-term effect of livestock grazing intensities on the vegetation dynamics in alpine meadow ecosystems. Land 14 (3), 591.

Daunoras, J., Kačergius, A., Gudiukaitė, R., 2024. Role of soil microbiota enzymes in soil health and activity changes depending on climate change and the type of soil ecosystem. Biology 13, 85.

Deng, J., Chong, Y., Zhang, D., Ren, C., Zhao, F., Zhang, X., Han, X., Yang, G., 2019. Temporal variations in soil enzyme activities and responses to land-use change in the loess plateau. China Appl. Sci. 9, 3129.

Díaz, S., Lavorel, S., McIntyre, S.U.E., Falczuk, V., Casanoves, F., Milchunas, D.G., Skarpe, C., Rusch, G., Sternberg, M., Noy-Meir, I.M.A.N.U.E.L.L., Landsberg, J., 2007. Plant trait responses to grazing–a global synthesis. Glob. Change Biol. 13, 313–341.

Fan, J., Jin, H., Zhang, C., Zheng, J., Zhang, J., Han, G., 2021. Grazing intensity induced alternations of soil microbial community composition in aggregates drive soil organic carbon turnover in a desert steppe. Agric. Ecosyst. Environ. 313, 107387.

Fernández-Lugo, S., Bermejo, L.A., de Nascimento, L., Méndez, J., Naranjo-Cigala, A., Arévalo, J.R., 2013. Productivity: key factor affecting grazing exclusion effects on vegetation and soil. Plant Ecol. 214, 641–656.

Flombaum, P., Vivanco, L., Cabrera, F., Sala, O.E., 2024. Grassland communities and ecosystems. In: In: Encyclopedia of Biodiversity, 1–7. Elsevier, Amsterdam. V1-382–V1-390.

Gałązka, A., Gawryjołek, K., Perzyński, A., Gałązka, R., Księżak, J., 2017. Changes in enzymatic activities and microbial communities in soil under long-term maize monoculture and crop rotation. Pol. J. Environ. Stud. 26 (1).

Giese, M., Gao, Y.Z., Lin, S., Brueck, H., 2011. Nitrogen availability in a grazed semi-arid grassland is dominated by seasonal rainfall. Plant Soil 340, 157–167.

Gilmullina, A., Rumpel, C., Blagodatskaya, E., Chabbi, A., 2020. Management of grasslands by mowing versus grazing–impacts on soil organic matter quality and microbial functioning. Appl. Soil Ecol. 156, 103701.

Guan, Y.S., 1986. Soil Enzyme and Research Method. Agricultural Press, Beijing, pp. 309–313.

Huber, R., Clec'h, S.L., Buchmann, N., Finger, R., 2022. Economic value of three grassland ecosystem services when managed at the regional and farm scale. Sci. Rep. 12 (1).

Jing, Y., Bai, M., Xu, C., Wang, L., Yang, H., Jiang, J., Wang, H., Yu, X., 2022. Advancing the spring rest-grazing time until the critical period when soil thaws promotes soil recovery and bacterial diversity in alpine meadows. Ecol. Indic. 139, 108929.

Joshi, S., Mohapatra, B., Mishra, J.P.N., 2018. Microbial soil enzymes: implications in the maintenance of rhizosphere ecosystem and soil health. In. Adv. Soil Microbiol. Recent Trends Future Prospects Vol. 1 SoilMicrobe Inter. 179–192.

Kang, H., Freeman, C., 1999. Phosphatase and arylsulphatase activities in wetland soils: annual variation and controlling factors. Soil Biol. Biochem 31, 449–454.

Khare, E., Yadav, A., 2017. The role of microbial enzyme systems in plant growth promotion. Clim. Change Environ. Sustain 5, 122–145.

Li, L., Wang, M., He, X.Z., Wang, Z., Zhang, X., Hu, J., Huang, L., Hou, F., 2023. Plant α-and β-diversity, and soil microbial stoichiometry co-regulate the alterations in ecosystem multifunctionality in response to grazing and N addition in a typical steppe. Land Degrad. Dev. 34, 2849–2861.

Li, L., Zhao, X., He, X., Zhang, X., Hu, J., Wang, M., Wang, Z., Hou, F., 2022. Different effects of grazing and nitrogen addition on ecosystem multifunctionality are driven by changes in plant resource stoichiometry in a typical steppe. Plant Soil 481 (1), 179–194.

Lin, Y., Hong, M., Han, G., Zhao, M., Bai, Y., Chang, S.X., 2010. Grazing intensity affected spatial patterns of vegetation and soil fertility in a desert steppe. Agric. Ecosyst. Environ. 138, 282–292.

Luo, S.P., He, B.H., Zeng, Q.P., Li, N.J., Yang, L., 2020. Effects of seasonal variation on soil microbial community structure and enzyme activity in a Masson pine forest in Southwest China. J. Mt. Sci. 17, 1398–1409.

Margalef, O., Sardans, J., Fernández-Martínez, M., Molowny-Horas, R., Janssens, I.A., Ciais, P., Goll, D., Richter, A., Obersteiner, M., Asensio, D., Penuelas, J., 2017. Global patterns of phosphatase activity in natural soils. Sci. Rep. 7 (1), 1337.

Mayel, S., Jarrah, M., Kuka, K., 2021. How does grassland management affect physical and biochemical properties of temperate grassland soils? A review study. Grass Forage Sci. 76 (2), 215–244.

Nannipieri, P., Ascher-Jenull, J., Ceccherini, M.T., Giagnoni, L., Pietramellara, G., Renella, G., 2017. Microbial diversity and soil functions. Eur. J. Soil Sci. 68 (1), 655–670.

Neemisha, Sharma, S., 2022. Soil enzymes and their role in nutrient cycling. In: Structure and Functions of Pedosphere. Springer Nature Singapore, Singapore, pp. 173–188.

Ochoa-Hueso, R., Arca, V., Delgado-Baquerizo, M., Hamonts, K., Piñeiro, J., Serrano-Grijalva, L., Shawyer, J., Power, S.A., 2020. Links between soil microbial communities, functioning, and plant nutrition under altered rainfall in Australian grassland. Ecol. Monogr. 90, e01424.

Petermann, J.S., Buzhdygan, O.Y., 2021. Grassland biodiversity. Curr. Biol. 31, R1195–R1201.

Prieto, L.H., Bertiller, M.B., Carrera, A.L., Olivera, N.L., 2011. Soil enzyme and microbial activities in a grazing ecosystem of Patagonian Monte, Argentina. Geoderma 162, 281–287.

Reddy, S.B., Nagaraja, M.S., Mallesha, B.C., Kadalli, G.G., 2020. Enzyme activities at varied soil organic carbon gradients under different land use systems of Hassan District in Karnataka. Int J. Curr. Microbiol Appl. Sci. 9 (3), 1739.

Ren, J.Z., Hu, Z.Z., Zhao, J., Zhang, D.G., Hou, F.J., Lin, H.L., Mu, X.D., 2008. A grassland classification system and its application in China. Rangel. J. 30, 199–209.

Ren, C., Kang, D., Wu, J., Zhao, F., Yang, G., Han, X., Feng, Y., Ren, G., 2016. Temporal variation in soil enzyme activities after afforestation in the Loess Plateau, China. Geoderma 282, 103–111.

Rumpel, C., Crème, A., Ngo, P.T., Velásquez, G., Mora, M.L., Chabbi, A., 2015. The impact of grassland management on biogeochemical cycles involving carbon, nitrogen and phosphorus. J. Soil Sci. Plant Nutr. 15, 353–371.

Saha, S., Mina, B.L., Gopinath, K.A., Kundu, S., Gupta, H.S., 2008. Relative changes in phosphatase activities as influenced by source and application rate of organic composts in field crops. Bioresour. Technol. 99, 1750–1757.

Sardans, J., Peñuelas, J., Estiarte, M., 2008. Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland. Appl. Soil Ecol. 39, 223–235.

Shamshitov, A., Kadžienė, G., Supronienė, S., 2024. The role of soil microbial consortia in sustainable cereal crop residue management. Plants 13, 766.

Stavi, I., 2019. Wildfires in grasslands and shrublands: a review of impacts on vegetation, soil, hydrology, and geomorphology. Water 11 (5), 1042.

Wang, S.L., Hou, F.J., 2021. Burrow characteristics and ecological significance of Marmota himalayana in the northeastern Qinghai-Tibetan Plateau. Ecol. Evol. 11, 9100–9109.

Wang, L., Jia, Z., Li, Q., He, L., Tian, J., Ding, W., Liu, T., Gao, Y., Zhang, J., Han, D., Tian, H., 2023. Grazing impacts on soil enzyme activities vary with vegetation types in the forest-steppe ecotone of northeastern China. Forests 14, 2292.

Wang, Y., Sun, Y., Chang, S., Wang, Z., Fu, H., Zhang, W., Hou, F., 2020. Restoration practices affect alpine meadow ecosystem coupling and functions. Rangel. Ecol. Manag 73 (3), 441–451.

Wei, W., Zhang, Y., Tang, Z., An, S., Zhen, Q., Qin, M., He, J., Oosthuizen, M.K., 2022. Suitable grazing during the regrowth period promotes plant diversity in winter pastures in the Qinghai-Tibetan plateau. Front. Ecol. Evol. 10, 991967.

Yang, T., Cao, J., Wang, Y., Liu, Y., 2017. Soil moisture influences vegetation distribution patterns in sand dunes of the Horqin Sandy Land, Northeast China. Ecol. Eng. 105, 95–101.

Yang, Y., Zhang, X., Zhang, L., Li, J., 2019. Effects of rotational grazing on vegetation restoration and biomass production in alpine meadows of the Qinghai-Tibetan Plateau. Rangel. Ecol. Manag 72 (3), 357–366.

Yu, C., Zhang, L., Yang, L., Bai, W., Feng, C., Li, W., Wu, K., Li, D., Wu, Z., 2021. Effect of a urea and urease/nitrification inhibitor combination on rice straw hydrolysis and nutrient turnover on rice growth. Bioresour 16 (2), 3059.

Yu, R., Zhang, W., Yu, Y., Yu, S., Lambers, H., Li, L., 2020. Linking shifts in species composition induced by grazing with root traits for phosphorus acquisition in a typical steppe in Inner Mongolia. Sci. Total Environ. 712, 136495.

Yuan, H., Hou, F., 2015. Grazing intensity and soil depth effects on soil properties in alpine meadow pastures of Qilian Mountain in northwest China. Acta Agric. Scand. B Soil Plant Sci. 65 (3), 222–232.

Zaman, M.D.H.J., Di, H.J., Cameron, K.C., Frampton, C.M., 1999. Gross nitrogen mineralization and nitrification rates and their relationships to enzyme activities and the soil microbial biomass in soils treated with dairy shed effluent and ammonium fertilizer at different water potentials. Biol. Fertil. Soils 29, 178–186.

Zhang, X., Zhang, W., Sai, X., Chun, F., Li, X., Lu, X., Wang, H., 2022. Grazing altered soil aggregates, nutrients and enzyme activities in a Stipa kirschnii steppe of Inner Mongolia. Soil Tillage Res 219, 105327.

Zhang, Y.J., Zhang, X.Q., Wang, X.Y., Liu, N., Kan, H.M., 2014. Establishing the carrying capacity of the grasslands of China: a review. Rangel. J. 36 (1), 1–9.

Zhao, L.-P., Wang, D., Liang, F.-H., Liu, Y., Wu, G.-L., 2019. Grazing exclusion promotes grasses functional group dominance via increasing of bud banks in steppe community. J. Environ. Manage. 251, 109589.

Zhu, G.Y., Deng, L., Zhang, X.B., Shangguan, Z.P., 2016. Effects of grazing exclusion on plant community and soil physicochemical properties in a desert steppe on the Loess Plateau, China. Ecol. Eng. 90, 372–381.

Zuccarini, P., Asensio, D., Ogaya, R., Sardans, J., Peñuelas, J., 2020. Effects of seasonal and decadal warming on soil enzymatic activity in a P-deficient Mediterranean shrubland. Glob. Change Biol. 26 (6), 3698–3714.