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Grazing Impacts on Soil Enzyme Activities Vary with Vegetation Types in the Forest-Steppe Ecotone of Northeastern China

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Abstract: Grazing impacts soil enzyme activities by changing soil conditions and microbial functions. Yet, the specific effects of grazing on soil enzymes in different northeastern China forest-steppe vegetation types remain poorly understood. To examine this, catalase (CA), urease (UA), and cellulase (CEA) activities were measured in different vegetation types (NS, MF, CP, GL) under both grazing and non-grazing conditions. Soil microbial biomass carbon and nitrogen (MBC and MBN) and other soil factors were also studied to gauge their impact on enzyme activities. The results indicated that enzyme activities were influenced by grazing, soil nutrient levels, mineralization, and microbial biomass carbon and nitrogen content. Grazing exerted the most significant influence on UA. CEA was predominantly affected by the content of biomass nitrogen and soil mineralization. CA, on the other hand, was primarily influenced by soil nutrient levels. Grazing influenced enzyme activities differently based on vegetation type. Under grazing, CA showed higher values in NS, MF, CP, and GL (4.09, 2.42, 3.26, and 3.90 mL 0.1 mol L^{-1} $KMnO_4$ g^{-1} soil 20 min^{-1}, respectively) with increases ranging from 32.52% to 505.00% ($p < 0.05$). Additionally, UA values were significantly higher in MF and CP (0.24 and 0.59 mg NH_4^+-N g^{-1} soil d^{-1}, respectively) with increases of 66.67% and 156.00%, while UA and CEA were lower in GL, showing reductions of 78.79% and 166.67% ($p < 0.05$) (0.33 NH_4^+-N g^{-1} soil d^{-1} and 0.06 mg glucose g^{-1} soil 72 h^{-1}, respectively) under grazing conditions. These findings underscore the importance of vegetation types in the grazing effects on soil enzymes at the forest-steppe ecotone and suggest that further efforts should be made to strengthen grassland grazing management to mitigate negative impacts on soil environmental health.

Keywords: grazing effects; catalase; urease; cellulase; microbial biomass

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1. Introduction

Soil enzymes are vital biological agents that mediate various chemical reactions in soil ecosystems [1,2]. These enzymes have their origins in plant roots, microorganisms, and soil fauna. They play a fundamental role in supporting a multitude of biogeochemical cycles, including those involving carbon, nitrogen, and phosphorus [3–5]. Soil enzyme activities play a vital role in sustaining soil health and productivity. They are responsible for the decomposition of organic matter and the conversion of nutrients into forms readily accessible to vegetation [6]. For example, cellulase degrades cellulose in plant residues, which liberates carbon and other nutrients that can be assimilated by soil microorganisms and plants [7]. The ammonia generated by urease activity can be absorbed by plants or further

converted into other nitrogen forms through nitrification and denitrification processes [8]. Studies have demonstrated that catalase promotes nitrogen fixation and mineralization, ultimately enhancing plant upgrowth by increasing the accessibility of nitrogen and other essential nutrients [9]. Polyphenol oxidase degrades polyphenols, releasing bound phosphorus and enhancing soil phosphorus content and solubility [8]. While soil enzymes may originate from plants, animals, and microorganisms, microorganisms are generally considered the primary source of soil extracellular enzymes [10]. Enzymes produced by soil bacteria and fungi play distinct roles in biochemical processes. Enzymes produced by soil bacteria primarily contribute to soil carbon–nitrogen cycling and biological nitrogen fixation, while enzymes from soil fungi are mainly involved in the decomposition of recalcitrant macromolecular organic compounds like cellulose and lignin, aiding in nutrient release for plant uptake [11–13]. On the other hand, soil enzyme activities are sensitive to alterations in soil organic matter stability, nutrient availability and microbial community composition, providing early warmings of soil physicochemical and biological changes [14–16]. Hence, the monitoring and comprehension of soil enzyme activities are imperative for the sustainable management of soil and the preservation of ecosystems [17].

Various vegetation types have distinct plant species compositions that can influence nutrient inputs into soil [8]. Organic matter serves as a vital substrate for soil enzymes. Consequently, the presence of diverse vegetation types can lead to fluctuations in the activity of specific soil enzymes [8,18]. Furthermore, soil enzyme activity is affected by a range of environmental factors, including soil nitrogen levels, pH, microbial biomass (MB), and physical parameters [19,20]. The extent of these environmental changes is largely attributed to anthropogenic disturbances. In the context of steppe ecosystems, grazing is widely acknowledged to have predominantly negative impacts on soil enzyme and microbial activities [21]. These impacts stem from a decrease in plant residue inputs, modifications in the conversion rates of soil nitrogen, carbon, and phosphorus, and shifts in the distribution of enzyme activities across soil aggregates of different particle sizes [22,23]. 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 [22]. However, certain studies have indicated positive associations between heightened grazing intensity and an increase in microbial biomass-C and counts of heterotrophic microorganisms within inter-canopy regions [21,24]. This finding suggests that the introduction of labile nitrogen from urine and dung might, to some extent, offset the negative impacts of grazing on soil enzyme and microbial activities [21]. In the context of forest ecosystems, studies have revealed that artificial afforestation influences the regulation of soil enzymes by fine roots [25]. The substitution of native forest tree species with plantation trees has resulted in notable reductions in the accounts of acid phosphatase, arylsulphatase, as well as invertase enzymes in the topsoil [26].

The forest-steppe ecotone signifies a transitional region between forest and steppe biomes, distinguished by a diverse mixture of trees, shrubs, and grasses [27,28]. This ecotone is a result of natural habitat fragmentation that occurs due to different topographical, climatic, and hydrological conditions [29]. This occurrence results in fluctuations in plant coverage and composition, thereby offering unique stoichiometric resources for soil biota [27]. Normally, forest soils exhibit thicker humus layers and higher C:N ratios compared to grasslands, whereas steppe soils tend to have higher P quantity [30]. As a result, forest soils demonstrate higher activities of cellulases and ligninases but lower activities of urease and protease than steppe soils [2]. Despite sharing some features with closed forest and treeless steppe, the forest-steppe ecotone exhibits distinct composition, structure, and function [31,32]. Recent studies have extensively documented the heightened susceptibility of this region to climate change and human interventions [33,34]. Importantly, our comprehension of the impact of grazing on soil enzyme activities within various vegetation types in the forest-steppe ecotone of northeastern China is limited, and there is a scarcity of information regarding the relative significance of environmental factors in relation to these variances.

In this study, our primary aim was to conduct a comprehensive assessment of soil enzyme activities in typical vegetation types under both grazing and non-grazing conditions within the forest-steppe ecotone. We sought to identify the factors associated with the observed changes in these activities. Our specific objectives encompassed three key aspects: (1) clarifying the distinctions between grazing and non-grazing scenarios within the forest-steppe ecotone across different vegetation types; (2) pinpointing the primary factors contributing to variations in soil enzyme activities; and (3) quantifying the relative contributions of these factors to the observed alterations in soil enzyme activities. Our underlying hypothesis suggested that the influence of grazing on soil enzyme activities would manifest in diverse patterns across various vegetation types and that this effect would be significantly influenced by soil nutrients and microorganism.

2. Materials and Methods

2.1. Study Area

The study area (Figure 1), a typical forest-steppe ecotone, is situated in the northeastern part of the Gunger grassland, adjacent to the southern foot of the Great Khingan Mountains to the north and the Hunsandak Sands to the west. The sampling sites, encompassing natural secondary forests (NS), mixed forests (MF), coniferous plantations (CP), and grasslands (GL), were located on the sunny slope within the Baiyin Obo Forest farm. These sites spanned from 43°25′ N to 43°37′ N and 117°13′ E to 117°29′ E. The area features a continental cold-temperate semi-arid forest-steppe climate zone, characterized by an average annual temperature ranging from −1 °C to 4 °C. Extreme temperature fluctuations include a minimum of −40 °C and a maximum of 33 °C. The effective accumulated temperature totals 1942 °C. Annual precipitation varies between 300–448 mm, with the majority occurring during June to August, aligning with a concurrent increase in water and heat. Annual evaporation measures 1520–1526 mm, equivalent to 3.4 times the annual precipitation, and the growing season spans 90 days. Sunshine hours average between 2800–3000 h annually, the mean wind speed is 3.8 m/s, and maximum wind speeds peak at 28 m/s. Winds primarily originate from the northwest in winter and the southwest in summer (the data were sourced from the China Meteorological Data Service Center: http://data.cma.cn, accessed on 10 March 2003). Based on the soil classification method employed by the reference system for Chinese soils using the FAO World Reference Base for Soil Resources (WRB), the soil types in this region comprise Haplic Luvisols, Petric Calcisols, Luvic Kastanozems, Gleyic Solonchaks, Calcaric Regosols, Gleyic Chernozems, among others (the data were retrieved from the National Soil Information Service Platform of China, accessible at http://www.soilinfo.cn, accessed on 27 February 2017) [35]. The vegetation species are mainly Mongolian steppe plants, such as *Stipa baicalensis*, *Stipa grandis*, *Achnatherum sibiricum*, *Filifolium sibiricum*, *Leymus chinensis*, *Elymus dahuricus*, *Artemisia frigida*, *Cleistogenes chinensis*, etc. [36].

2.2. Soil Sampling

In July 2021, we established sampling sites in four distinct vegetation types, each with two treatment conditions: grazing and non-grazing (Table 1). Grazing had been carried out at moderate intensity, with 6–9 sheep per hectare [22,37], for more than a decade. Seasonal rotational grazing was implemented, with grazing taking place during the months of June through August. At each sampling site, three 50 m × 50 m plots were randomly designated to ensure spatial independence among the sampling units. To avoid spatial dependence of nutritional and microbial variables, the distance between the sampling plots was kept at a minimum of 15 m [38]. Each plot was subdivided into six 5 m × 5 m quadrats, and soil samples were collected using a five-point method within each quadrat. Soil sampling extended to a depth of 0–10 cm, resulting in a total of 72 soil samples, each weighing approximately 500 g. The soil samples were promptly transported to the laboratory in a portable ice box. Upon arrival, visible roots, gravels, and other extraneous materials were removed from the soil samples. Subsequently, the samples were divided into two

portions: One portion was stored in a refrigerator at 4 °C for assessing soil enzyme activity. The remaining portion of soil samples was sieved through a 2 mm sieve. From this, one part was refrigerated at 4 °C for the evaluation of soil parameters, including ammonium nitrogen, nitrate nitrogen, and microbial biomass. The remainder of these sieved samples was air-dried and utilized for analyzing soil organic carbon, total nitrogen, and pH. The determination of parameters for all refrigerated soil samples was completed within a span of 15 days.

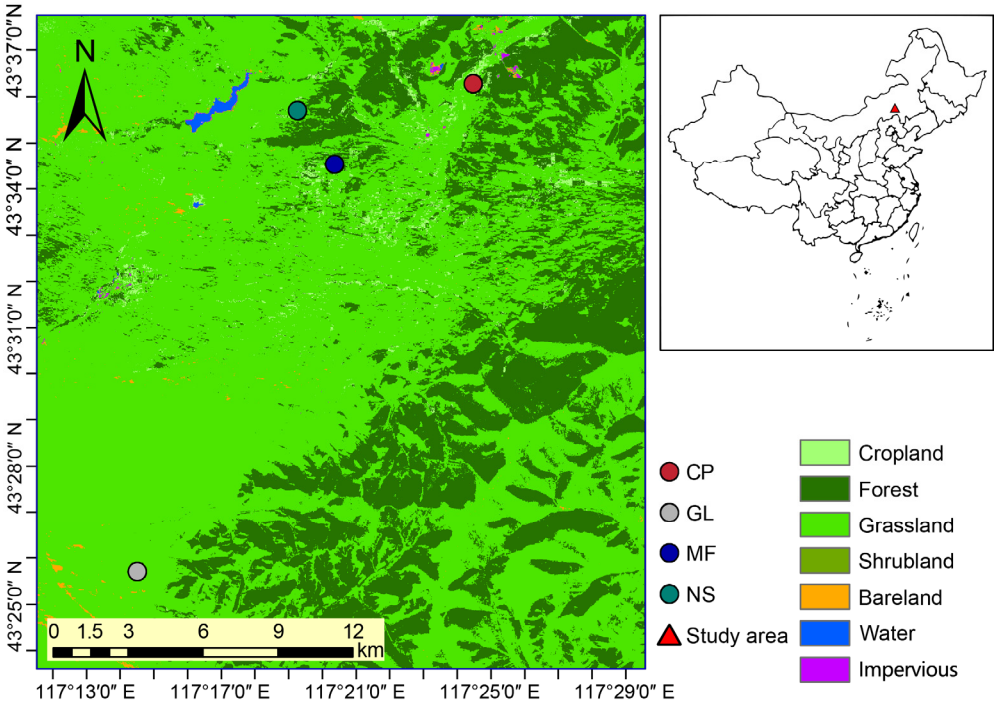

Figure 1. Sampling sites. Notes: NS, MF, CP and GL represent natural secondary forests, mixed forests, coniferous plantations, and grasslands, respectively.

Table 1. Description of the sampling sites.

Sample Site	Treatment	pH	Slope (°)	Aspect	Soil Type	Geographic Coordination	Altitude (m)	Plant Community	Main Community Species
NS1	Grazing	7.71	18	South	Gleyic Chernozems	43.595° E 117.322° N	1490	*Populus davidiana* + *Betula platyphylla*	*Rosa davurica, Galium verum, Deyeuxia angustifolia*
NS2	Non-grazing	6.02	16	South					
MF1	Grazing	6.29	15	South	Gleyic Chernozems	43.575° E 117.341° N	1525	*Betula platyphylla* + *Pinus sylvestris*	*Sanguisorba officinalis, Taraxacum mongolicum, Rosa davurica, Spiraea salicifolia*
MF2	Non-grazing	5.56	10	South					
CP1	Grazing	5.94	11	South	Gleyic Chernozems	43.428° E 117.242° N	1405	*Pinus sylvestris*	*Carex duriuscula, Potentilla bifurca*
CP2	Non-grazing	5.99	13	South					
GL1	Grazing	7.95	17	South	Luvic Kastanozems	43.604° E 117.410° N	1540	*Leymus chinensis*	*Carex duriuscula, Cleistogenes squarros, Agropyron cristatum, Potentilla acaulis*
GL2	Non-grazing	6.79	18	South					

Notes: NS, MF, CP and GL represent natural secondary forests, mixed forests, coniferous plantations, and grasslands, respectively.

2.3. Laboratory Analyses

Soil analysis procedures were conducted as follows: (1) Soil total organic carbon (TOC) was determined using chromic acid titration. (2) Total nitrogen (TN) was measured using an elemental analyzer (FlashSmart CHNS/O, Thermo Fisher Scientific Inc., Waltham, MA, USA). (3) Soil pH was determined by assessing a 1:2.5 (*w*/*v*) soil-to-water suspension through a glass electrode (pH meter PHS-3C, Qiwei Instrument Co., Hangzhou, Zhejiang,

China) [39]. (4) Nitrate nitrogen (NN) was extracted from fresh soil with 0.01 mol L^{-1} $CaCl_2$, acidified with a 1:9 H_2SO_4 solution, and quantified at 210 nm using an ultraviolet spectrometer (UV1901, AUCY Scientific Instrument Co., Ltd., Shanghai, China). (5) Ammonium nitrogen (AN) was extracted with a 2 mol L^{-1} KCl solution, and its concentration was determined by forming a blue chromophore with phenol nitroprusside and buffered hypochlorite reagents, then measuring at 630 nm using an ultraviolet spectrometer. (6) Soil catalase activity (CA) was measured by introducing 0.3% H_2O_2 to fresh soil, stirring for 20 min, filtering, and titrating the filtrate with 0.1 mol L^{-1} $KMnO_4$. The activity was expressed as mL 0.1 mol L^{-1} $KMnO_4$ g^{-1} soil 20 min^{-1} [20]. (7) Urease activity (UA) was measured by incubating fresh soil with a 10% urea solution at 37 °C for 24 h, filtering, treating with a sodium phenol solution and 0.9% sodium hypochlorite solution, and measuring the released ammonium at 578 nm using an ultraviolet spectrometer. The activity was expressed as mg NH_4^+-N g^{-1} soil d^{-1} [20]. (8) Cellulase activity (CEA) was determined by incubating fresh soil with a 1% solution at 37 °C for 72 h and measuring the glucose concentration at 540 nm using an ultraviolet spectrometer. Activity was expressed as mg glucose g^{-1} soil 72 h^{-1} [40]. (9) Microbial biomass carbon and nitrogen (MBC and MBN) were quantified using the chloroform fumigation-extraction method [41].

2.4. Statistical Analysis

We analyzed the data using R version 4.3.0. Normality (Shapiro–Wilk test) and homoscedasticity (Bartlett's test) were assessed for all variables prior to comparing soil enzyme activities and microbial biomass (MB) between vegetation types and performing the following principal component analysis (PCA) and redundancy analysis (RDA). We conducted these comparisons using a one-way ANOVA and Tukey's post hoc test with the "agricolae" package. Linear models were employed to explore the relationship between soil enzyme activities and microbial biomass (MB) under both grazing and non-grazing conditions. PCA was conducted to investigate variations in the soil environment under grazing and non-grazing conditions across different vegetation types. RDA was used to assess the correlations between soil nutrients and microbial biomass (environmental variables) with enzyme activities (species variables). The PCA-MLR method was utilized to estimate the relative influences of environmental factors on soil enzyme activities. This method integrated principal component analysis (PCA) to reduce dimensions and multiple linear regression (MLR) to quantify the impact of environmental factors on soil enzyme activities. In the MLR process, the PCA-derived factor scores of environmental factors were employed as independent variables, while the z-scores of each soil enzyme activity were considered the dependent variable. Sequentially, independent variables were included in a stepwise regression in descending order based on their individual simple correlation. The contributions of each source or group of sources were computed from the coefficients [42]. The z-scores were computed with the following equation:

$$z = (x - \mu)/\sigma \quad (1)$$

where z represents the z-score, x denotes each observed variable, μ signifies the mean, and σ indicates the standard deviation.

We represented vegetation types as ordinal categorical variables, where 1 indicated grassland, 2 indicated natural secondary forest, 3 indicated mixed forest, and 4 indicated coniferous plantation. Additionally, grazing treatments were coded as binary variables, with 0 representing non-grazing and 1 representing grazing. We mapped the sampling sites using ArcGIS 10.5 with the WGS-84 coordinate system, utilizing 10 m resolution global land cover data [43]. We created plots illustrating soil enzyme activities, microbial biomass (MB), and their relationships using the "ggplot2 version 3.4.2" package.

3. Results

3.1. Characteristics of Soil Enzyme Activities and Microbial Biomass

We observed a significant impact of grazing on soil enzyme activities, as illustrated in Figure 2. Specifically, catalase (CA) activities were substantially higher under grazing conditions compared to non-grazing conditions in all four vegetation types (NS, MF, CP, and GL). The values were 4.09, 2.42, 3.26, and 3.90 mL 0.1 mol L^{-1} $KMnO_4$ g^{-1} soil 20 min^{-1}, respectively, representing increases of 209.85%, 505.00%, 32.52%, and 61.16%, respectively ($p < 0.05$) (see Figure 2a). Additionally, urease (UA) activities were significantly higher under grazing conditions than under non-grazing conditions in MF and CP, with values of 0.24 and 0.59 mg NH_4^+-N g^{-1} soil d^{-1}, respectively, showing increases of 156.00% and 66.67%, respectively ($p < 0.05$) (refer to Figure 2b). In contrast, both UA and cellulase (CEA) activities were significantly lower under grazing conditions than under non-grazing conditions in GL. UA had a value of 0.33 NH_4^+-N g^{-1} soil d^{-1}, exhibiting a reduction of 78.79%, while CEA had a value of 0.06 mg glucose g^{-1} soil 72 h^{-1}, showing a reduction of 166.67% ($p < 0.05$) (refer to Figure 2b,c). Moreover, there were no significant changes in UA between grazing and non-grazing conditions in NS, nor were there significant changes in CEA between grazing and non-grazing conditions in NS, MF, and CP ($p > 0.05$) (see Figure 2b,c).

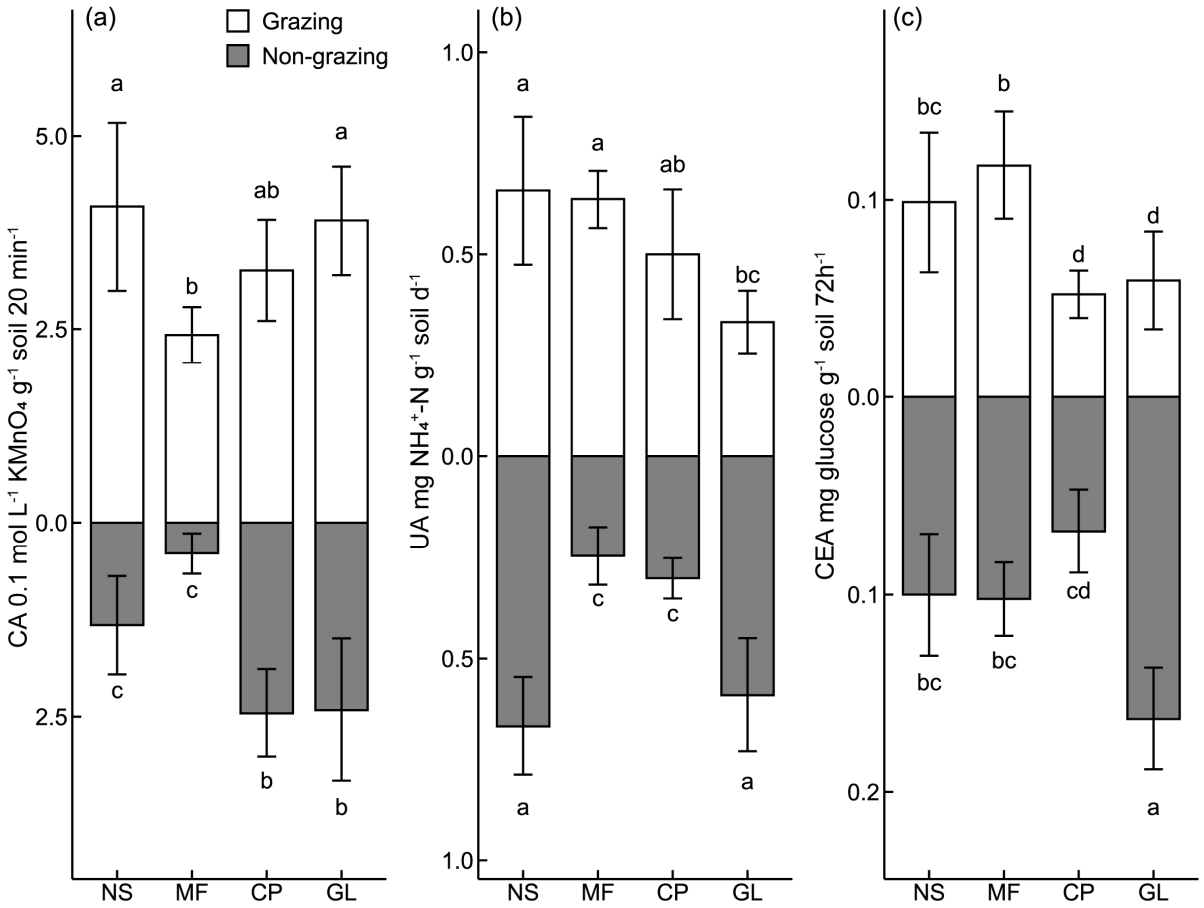

Figure 2. Soil enzyme activities: (**a**) CA, (**b**) UA, (**c**) CEA under the grazing and non-grazing conditions in different vegetation types. Notes: NS, MF, CP, and GL denote natural secondary forests, mixed forests, coniferous plantations, and grasslands, respectively. The abbreviations CA, UA, and CEA represent catalase activity, urease activity, and cellulase activity, respectively. Statistically significant differences ($p < 0.05$) are denoted by distinct lowercase letters. All values are expressed as mean ± SD (with error bars).

Grazing exhibited limited effects on microbial biomass carbon (MBC) and nitrogen (MBN) across all vegetation types, except for GL, as illustrated in Figure 3. Under grazing conditions, MBN in GL experienced a significant reduction to 35.08 mg kg^{-1}, representing a decrease of 46.42% ($p < 0.05$) (Figure 3b). However, there was no significant difference in MBC in GL. In the case of MBC, MF consistently maintained the highest levels, reaching 244.88 mg kg^{-1} under grazing conditions, surpassing CP by 17.27% ($p < 0.05$) and showing no significant difference from NS and GL ($p > 0.05$) (Figure 3a). Similarly, under non-grazing conditions, MF also exhibited the highest MBC levels (258.53 mg kg^{-1}), exceeding CP and GL by 14.75% and 15.37%, respectively ($p < 0.05$). The difference in MBC between MF and NS under non-grazing conditions was not statistically significant ($p > 0.05$). As for MBN among various vegetation communities under grazing conditions, GL displayed a reduction of 36.94%–42.33% when compared to the other vegetation types. Under non-grazing conditions, no significant differences were observed in MBN among NS, MF, CP, and GL ($p > 0.05$).

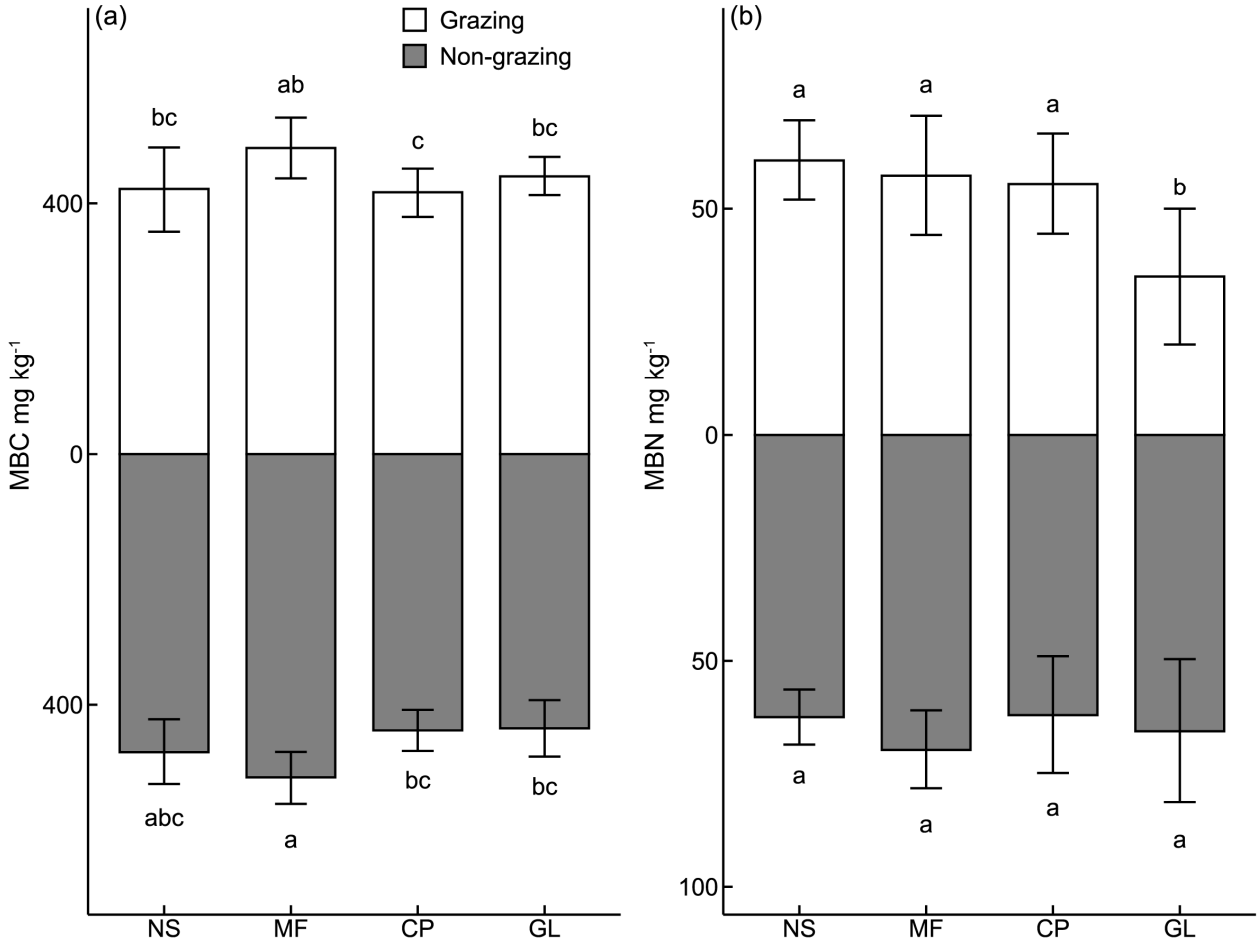

Figure 3. (**a**) Soil microbial biomass carbon and (**b**) soil microbial biomass nitrogen under the grazing and non-grazing conditions in different vegetation types. Notes: NS, MF, CP and GL represent natural secondary forests, mixed forests, coniferous plantations, and grasslands, respectively. MBC and MBN stand for soil microbial biomass carbon and soil microbial biomass nitrogen, respectively. Statistically significant differences ($p < 0.05$) are denoted by distinct lowercase letters. All values are expressed as mean ± SD (with error bars).

3.2. The Associations between Soil Enzyme Activities and Microbial Biomass

In general, microbial biomass carbon (MBC) and nitrogen (MBN) affected soil enzyme activities differently (Figure 4). Specifically, CA activity decreased linearly with MBC (Figure 4a) and non-linearly with MBN (Figure 4b). CEA increased non-linearly with MBN

(Figure 4f), but was independent of MBC (Figure 4e). Moreover, UA showed no significant correlation with either MBC (Figure 4c) or MBN (Figure 4d).

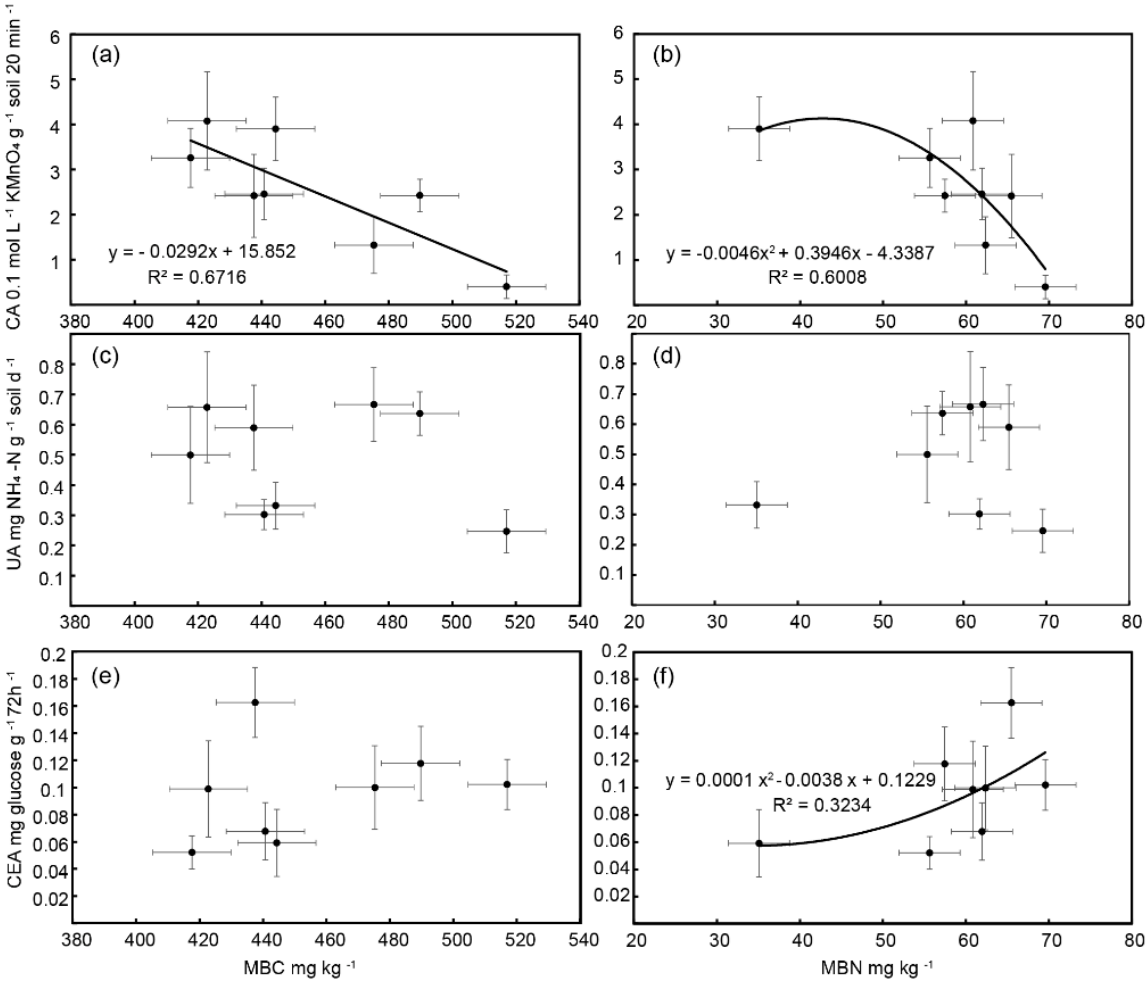

Figure 4. (**a**) The associations between MBC and CA. (**b**) The associations between MBN and CA. (**c**) The associations between MBC and UA. (**d**) The associations between MBN and UA. (**e**) The associations between MBC and CEA. (**f**) The associations between MBN and CEA. Notes: CA, UA, and CEA represent catalase activity, urease activity, and cellulase activity, while MBC and MBN stand for microbial biomass carbon and microbial biomass nitrogen, respectively. All values are expressed as mean ± SD (with error bars).

3.3. Differences and Commonalities in the Soil Environment

The outcomes of the PCA analysis revealed that PCA1 and PCA2 contributed to 65.56% and 16.69% of the total variations, resulting in an accumulated contribution rate of 82.25%. This indicates that these two principal components were sufficient to capture most of the variations in the data. The grazing and non-grazing groups were clearly distinguished, suggesting that grazing significantly affects the soil environment. The distances between grazing and non-grazing conditions were larger in GL, NS, and MF than in CP, indicating that the variations between grazing and non-grazing conditions were more pronounced in these vegetation types. In contrast, the variations in CP tended to be more stable. These findings suggest that grazing can have a significant impact on soil properties in certain types of vegetation, particularly in GL and NS (Figure 5).

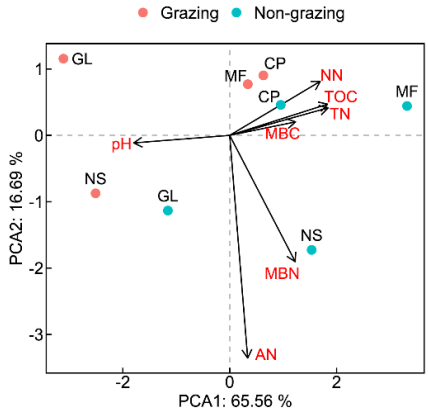

Figure 5. Principal component analysis (PCA) of soil properties within the forest-steppe ecotone's different vegetation types. Notes: NS, MF, CP, and GL represent natural secondary forests, mixed forests, coniferous plantations, and grasslands, respectively. The abbreviations CA, UA, and CEA denote catalase activity, urease activity, and cellulase activity, while MBC and MBN refer to microbial biomass carbon and microbial biomass nitrogen, respectively. Additionally, TOC, TN, NN, and AN stand for soil total organic carbon, total nitrogen, nitrate nitrogen, and ammonium nitrogen, respectively.

3.4. The Impact of Environmental Factors on Enzyme Activities

The findings from the RDA analysis (Figure 6) unveiled connections between soil enzyme activities and soil elements, encompassing soil nutrients and microbial biomass. RDA1 and RDA2, representing the primary two axes, explained 98.12% and 1.83% of the variance, respectively. The relationships between soil enzyme activities and soil factors varied in their strengths. Broadly, CA displayed negative associations with most soil factors but exhibited positive correlations with soil pH. Conversely, CEA and UA demonstrated positive correlations with most soil factors, yet no correlation was observed with soil pH. Notably, TN displayed a negative correlation with CA, while AN and MBN showed positive correlations with UA and CEA.

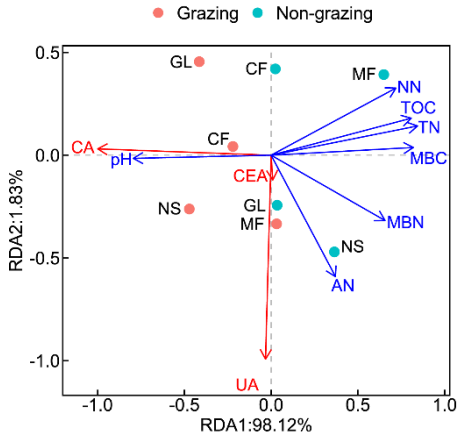

Figure 6. Conducted redundancy analysis (RDA) on soil characteristics within various vegetation types of the forest-grassland ecotone. Notes: NS, MF, CP, and GL represent natural secondary forests, mixed forests, coniferous plantations, and grasslands, respectively. The abbreviations CA, UA, and CEA refer to catalase activity, urease activity, and cellulase activity, while MBC and MBN stand for microbial biomass carbon and microbial biomass nitrogen, respectively. Additionally, TOC, TN, NN, and AN represent soil total organic carbon, total nitrogen, nitrate nitrogen, and ammonium nitrogen, respectively.

The PCA-MLR results demonstrated that five principal components collectively accounted for over 90% of the variance (Table 2). The first four principal components (PC1–PC4) primarily comprised soil-related factors. PC1 exhibited a variance propor-

tion of 52.61% and featured high-loading factors such as TN, TOC, NN, and pH, with loadings of 0.641, 0.647, 0.613, and −0.637, respectively. These factors are vital indicators reflecting soil nutrient levels; hence, PC1 was designated as the soil nutrient factor. PC2, which represented 19.15% of the variance, showed a high-loading factor, AN, at 0.646, a significant indicator reflecting soil nitrogen mineralization [44], and thus was classified as the nitrogen mineralization factor. PC3, accounting for 10.53% of the variance, prominently featured the high-loading factor MBC at 0.864, and was consequently labeled as the microbial biomass carbon factor. PC4, with a variance proportion of 8.34%, showcased the high-loading factor MBN, and hence was identified as the microbial biomass factor. The fifth principal component, PC5, contributing 4.32% of the variance, was notably influenced by grazing (loading: 0.731), and thus classified as the grazing factor.

Table 2. Principal component analysis (PCA) performed on soil properties including MBC (microbial biomass carbon), MBN (microbial biomass nitrogen), TN (total nitrogen), TOC (total organic carbon), NN (nitrate nitrogen), and pH, while considering the impact of grazing and various vegetation types in the forest-steppe ecotone.

Parameters	PC1	PC2	PC3	PC4	PC5
MBC	0.168	0.117	**0.864**	0.406	0.105
MBN	0.207	0.290	−0.428	**0.782**	0.168
TN	**0.641**	−0.037	0.061	−0.115	0.026
TOC	**0.647**	−0.071	−0.004	−0.089	−0.049
NN	**0.613**	−0.125	0.086	−0.239	0.149
AN	0.069	**0.646**	−0.061	−0.358	0.612
pH	**−0.637**	0.027	0.072	0.047	0.009
Grazing	−0.263	−0.502	0.052	0.088	**0.731**
Vegetation types	0.315	−0.459	−0.217	0.088	0.161
Eigenvalue	2.176	1.313	0.973	0.867	0.624
Proportion of variance (%)	52.61%	19.15%	10.53%	8.34%	4.32%
Cumulative proportion (%)	52.61%	71.77%	82.29%	90.64%	94.96%

Notes: MBC and MBN stand for microbial biomass carbon and microbial biomass nitrogen, respectively. TOC, TN, NN, and AN stand for soil total organic carbon, total nitrogen, nitrate nitrogen and ammonium nitrogen, respectively. Bold denotes elements with relatively high loadings (loading > 0.6), indicating the primary constituents of a particular component.

In general, the primary variances observed in the three soil enzyme activities were predominantly explained by soil factors denoted as PC1–PC4, accounting for values ranging from 46.41% to 89.56%. Concerning CA, the most pivotal factor was PC1, explaining 33.35% of the variation. PC2 emerged as the most influential factor for CEA, contributing 36.20% to the variance. Notably, the variability in UA was primarily influenced by PC5, which exhibited a contribution value of 53.59%. Assessing the contributions of these diverse factors to the activities of the four enzymes, alterations in PC5 were notably significant, while changes in PC2 and PC3 demonstrated a more consistent pattern.

4. Discussion

The impacts of grazing disturbance on plant cover, soil physicochemical properties, and microbial activities have been extensively studied [45–47]. Grazing animals consume aboveground vegetation and excrete feces, which can increase bacterial growth [48]. However, in sensitive areas, excessive trampling can lead to soil compaction [49], while reduced plant coverage can result in significant fluctuations in soil moisture and temperature, creating a challenging environment for soil microorganisms [18]. These factors can affect soil enzyme activities directly or indirectly.

In the current research, grazing led to alterations in soil environmental variables, as depicted in Figure 5, potentially impacting soil enzymes [18,50]. Various studies have suggested that grazing notably elevates soil pH and bulk density, consequently boosting catalase activity to alleviate hypoxia stress among aerobic microorganisms [21,24,50]. These findings align with the observations in the present study. Furthermore, grazing has ex-

panded inter-canopy areas, intensifying light exposure on the topsoil, which has shown a positive impact on catalase activity [51].

The impact of grazing on soil urease activity can vary depending on several factors, including grazing intensity, season, and vegetation type [52–54]. Generally, grazing emerges as the primary factor directly affecting soil urease activity in the forest-steppe ecotone, as highlighted by the PCA-MLR (Figure 7). Previous research has consistently shown that grazing can reduce soil urease activity in wet meadows by diminishing soil organic matter input and microbial activity [50]. Studies conducted in the Stipa kirschnii steppe of Inner Mongolia also support these findings, indicating a negative impact of grazing on soil urease activity due to reduced soil nitrogen levels and diminished water stability of aggregates [52]. In our investigation, grazing was noted to decrease soil urease activity in grasslands (Figure 2), aligning with prior research. However, in mixed forests and coniferous plantations, grazing appeared to increase soil urease activity, while no significant difference was observed in natural secondary forests. This variation might be attributed to higher plant diversity and the growth of undergrowth vegetation, such as grasses and shrubs in natural forests, which promote the accumulation of soil organic carbon and root development [55]. The abundance of soil microorganisms within natural forests significantly encourages the adhesion and intertwining of fungi and bacterial mycelia with plant roots, consequently promoting macroaggregate formation and indirectly enhancing soil aggregate stability [56]. This robust soil structure in natural forests demonstrates greater resilience to the impact of moderate grazing. Additionally, the stability of soil aggregates substantially influences soil enzyme activity, contributing to the stability observed in natural forest soil enzyme activity [57]. In contrast, plantations exhibit reduced soil microbial biomass nitrogen, mineral nitrogen, and nitrogen mineralization values, especially evident in coniferous stands like cypress and pine [26]. Consequently, enzyme activities related to soil nitrogen cycling notably decrease as the tree species transition from natural forest to plantations [26]. These findings emphasize a strong correlation between soil urease activity and alterations in soil nitrogen utilization and microbial biomass. This correlation is further supported by the results of the RDA analysis (Figure 6). Grazing primarily augments soil urease activity by introducing labile nitrogen through urine and dung deposition. Furthermore, it influences vegetation structure by spreading annual weeds from adjacent areas via livestock dispersal [21,58]. Seasonally, grazing demonstrates varied effects on soil urease activity, with higher values observed in autumn compared to spring or summer [50]. Future research will focus on exploring the seasonal variations in soil urease within the forest–grassland transition zone and its response to varying grazing intensities.

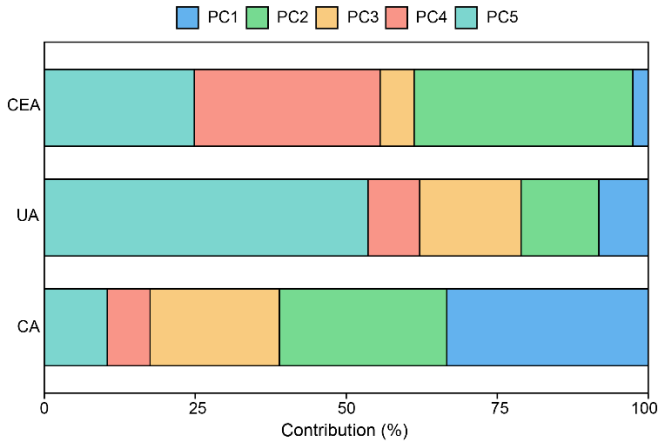

Figure 7. The relative contributions of PC1 (soil nutrient factor), PC2 (soil mineralization factor), PC3 (microbial biomass carbon factor), PC4 (microbial biomass nitrogen factor), and PC5 (grazing factor) to variations observed in soil enzyme activities. CA, UA, and CEA denote catalase, urease, and cellulase activity, respectively.

The grazing of livestock significantly influences soil enzyme activities by altering soil microbial biomass levels [26,59,60]. This impact is primarily observed through the transformation in microbial community composition, inducing a transition from fungi to bacterial dominance and a shift from slow-growing to fast-growing microorganisms [18]. This shift in dominance leads to a change from fungi-dominant food webs, reliant predominantly on recalcitrant soil organic carbon like lignin, lipids, and suberin, to bacteria-dominant food webs that primarily utilize labile soil organic carbon, such as microbial biomass carbon (MBC) [18,61,62]. MBC is a crucial element in most terrestrial ecosystems, and even a slight alteration in microbial biomass can have a substantial impact on plant nutrient availability in the short term [26]. Thus, understanding the effects of grazing on soil microbial biomass across different plant communities is vital. However, our present study suggests that grazing does not significantly impact MBC levels. This might be due to the complex nature of grazing effects, as it not only reduces aboveground biomass and soil organic carbon—diminishing MBC levels [63]—but also augments nutrient input into the soil, partially counterbalancing the negative impacts of grazing [21]. Notably, MBC levels are predominantly influenced by vegetation types, with mixed forests exhibiting the highest MBC levels. This is attributed to their relatively higher coverage, thickness, and stock of forest floor [64,65].

The research highlighted a reduction in soil cellulase levels and microbial biomass nitrogen in the grassland due to grazing. Notably, soil cellulase activity demonstrated a positive correlation with the content of microbial biomass nitrogen and the soil mineralization factor (Figures 4 and 7). This suggests that the ability of soil microorganisms to break down cellulose is linked to their ability to use nitrogen and other nutrients for growth and reproduction [66,67]. When the levels of MBN in the soil are reduced by grazing, there may be fewer microorganisms in the soil that are capable of producing cellulase [68]. Interestingly, grazing had no significant effect on microbial biomass nitrogen in forests (including natural secondary forests, mixed forests and coniferous plantations). On one hand, there may be differences in the plant community composition and nutrient cycling processes between forests and grasslands. Forest ecosystems often exhibit greater species diversity than grasslands, resulting in differences in nutrient requirements and uptake strategies among plant species [34,69]. Therefore, the impact of grazing on nutrient availability in forests may be relatively smaller than that in grasslands [70]. On the other hand, forest soils typically contain higher levels of organic matter than grassland soils, which can serve as a nutrient reservoir [71,72]. As a result, even if grazing does reduce nutrient availability in the short term, there may still be sufficient nutrients stored in the soil to support microbial activity and maintain levels of microbial biomass nitrogen [73].

Grazing exerts complex and diverse effects on soil enzyme activities at the forest-steppe ecotone. The net impact of grazing on these activities is contingent upon the grazing intensity and frequency, the specific type of vegetation cover, and the inherent soil properties [22,74,75]. The research findings demonstrated that grazing at a moderate intensity of 6–9 sheep per hectare elicits distinct effects on soil enzyme activities across diverse vegetation types at the forest-steppe ecotone. Notably, grasslands demonstrate a higher sensitivity to grazing disturbances, while forest soils tend to exhibit stability and, in some cases, enhancements in enzyme activities. Several factors contribute to this phenomenon. Firstly, steppes and forests possess different soil types within the forest–grass transition zone. Steppes predominantly consist of Kastanozems, whereas forests primarily comprise Chernozems [76]. In comparison to Chernozems, Kastanozems exhibit lower organic matter accumulation and lower levels of clay and silt particles [77]. Research indicates that soils with higher concentrations of clay and silt particles, along with microaggregates, harbor increased microbial abundance [22]. Secondly, significant differences exist in the vegetation types between grasslands and forests. Grasslands typically consist of shrubs and herbaceous plants, whereas forests are characterized by an abundance of trees, dense vegetation, and well-developed root systems, thereby providing a richer nutrient influx to the soil, stimulating soil enzyme activity [78,79]. Moreover, environmental conditions

vary between forests and grasslands. Forests, in particular, often possess better moisture conditions, and adequate soil moisture is crucial for biological processes such as enzyme activity within the soil [80,81]. Sufficient soil water can boost the integration of plant and microbial communities, significantly increasing enzyme activities [82]. This emphasizes the necessity for improved management of grazing activities, particularly in grasslands. Additional investigation is crucial to delve into the impacts of various grazing intensities and forms on soil enzyme activities, unravel the mechanisms dictating grazing effects on soil function, and devise management strategies to mitigate adverse grazing impacts [52].

5. Conclusions

This study examined the impact of grazing on soil enzyme activities across various vegetation types within the forest-steppe ecotone. Our results indicated that grazing, soil nutrients, mineralization, and microbial biomass carbon and nitrogen all influenced variations in enzyme activities. Among these enzymes, UA was the most affected by grazing, while CEA was primarily influenced by biomass nitrogen content and the soil mineralization factor, and CA was mainly influenced by the soil nutrient factor. Additionally, the impact of grazing on enzyme activities differed based on vegetation type, resulting in positive effects observed on CA across all vegetation types and on UA in MF and CP. However, grazing did not significantly change UA and CEA in NS and CEA in MF and CP, whereas negative effects were found on UA and CEA in GL.

The findings of this study underscore the importance of considering vegetation types in the grazing effects on soil enzymes at the forest-steppe ecotone. The positive effects of grazing on CA and UA in some vegetation types suggest that appropriate grazing management could be beneficial for soil health. However, the adverse effects on UA and CEA in GL underscore the necessity for implementing effective management strategies to alleviate the negative impacts of grazing on soil environmental health. Overall, this study provides insights into the complex interactions between grazing, vegetation types, and soil enzyme activities, which could inform future studies and management practices in similar ecosystems. Future research will further explore the regulatory mechanisms of grazing activities and ecological restoration measures on soil biochemical properties, based on soil enzyme activity indicators. This will aim to elucidate the most suitable management practices in various vegetation types within the forest-steppe ecotone.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy concerns.

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Abbreviations

CA	catalase activities
UA	urease activities
CEA	cellulase activities
NS	natural secondary forests
MF	mixed forests
CP	coniferous plantations
GL	grasslands
MBC	microbial biomass carbon
MBN	microbial biomass nitrogen
TOC	total organic carbon
TN	total nitrogen
NN	nitrate nitrogen
AN	ammonium nitrogen

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