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RESEARCH ARTICLE

Dynamic rhizosphere C:N:P stoichiometry of plants, soils and microbial biomass under different grazing patterns

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Abstract

1. Analysing grassland ecological stoichiometry is crucial for evaluating ecosystem quality and health via energy flow and elemental equilibrium. Grazing is a dominant human activity shaping alpine grasslands on the Qinghai–Tibet Plateau (QTP), yet few studies have used rhizosphere stoichiometry to assess plant–soil–microbe interaction dynamics under different grazing regimes. We compared rhizosphere stoichiometric changes of the degradation indicator *Medicago ruthenica* and dominant *Kobresia humilis* under yak grazing (YG), sheep grazing (SG) and mixed grazing (MG) with yak-to-sheep ratios of MG(1:2), MG(1:4) and MG(1:6).
2. MG(1:2) increased total N and reduced total P in *M. ruthenica*, significantly elevating its N:P ratio; MG(1:4) increased total N and P in *K. humilis*, lowering its C:N and C:P ratios. Rhizosphere soil of *K. humilis* showed significantly increased total C and P under MG. For *K. humilis*, microbial biomass carbon (MBC) and nitrogen (MBN) initially decreased then increased under MG, while microbial biomass phosphorus (MBP) significantly increased across all grazing patterns, with stable plant and microbial biomass N:P ratios.
3. Regulatory pathways differed by species: *M. ruthenica* affected MBC, MBN and MBP via plant C and N under single grazing, and MBP via soil properties under MG; *K. humilis* influenced these microbial indices via soil properties and plant C:N:P under single grazing, and soil properties under MG.
4. Our findings reveal trait-dependent stoichiometric responses of key plant species to grazing, advance understanding of rhizosphere plant–soil–microbe interactions in alpine grasslands, and provide a scientific basis for sustainable grazing management on the QTP.

KEYWORDS
alpine grassland, grazing patterns, plant species, rhizosphere stoichiometry, soil microbial biomass

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1 | INTRODUCTION

Grazing influences plant community structure and ecosystem processes by altering the composition of species and functional groups, thereby modifying the nutrient cycling patterns of carbon (C), nitrogen (N) and phosphorus (P) pools across different ecosystem components (Nie et al., 2023; Yang et al., 2019). Livestock grazing also affects root traits and soil physicochemical properties through selective feeding or excretion, which directly or indirectly impacts rhizosphere stoichiometry, nutrient storage and nutrient distribution (Dong et al., 2022; Zhang, Wang, et al., 2021). The rhizosphere is the most microbially active habitat in grassland ecosystems, and it plays a key role in driving the cycling of matter, energy and nutrients (Kuzyakov & Razavi, 2019; Post et al., 1982). The presence of plant roots in the rhizosphere can alter nutrient cycles, such as during organic matter decomposition and N mineralization (Hou et al., 2024). During growth, plants also affect soil microbial biomass activity and composition by altering the physical environment (Zhang et al., 2025). Additionally, through transpiration, shade and mulching, they modify moisture regimes, which in turn affect substrate availability through root exudates and nutrient availability through nutrient absorption (Bird et al., 2011; Wang et al., 2022). In the rhizosphere, the stoichiometry of soil, microbes and plants is inherently interconnected. Moreover, the stoichiometric ratios of microbial biomass can indicate how shifts in resource allocation alter nutrient demand (Bell et al., 2013; Xu et al., 2024). For instance, the N:P ratio in plant litter correlates with that of microbial biomass during litter decomposition, which suggests that substrate stoichiometry shapes the stoichiometry of microbial biomass (Carnicer et al., 2015; Fanin et al., 2013).

The Qinghai–Tibet Plateau (QTP), the world's largest alpine grassland ecosystem, plays a pivotal role in global carbon cycling and regional ecological security (Wang et al., 2022; Zhang et al., 2024). Alpine meadows dominated by *Kobresia* species cover about 35% of the QTP's grassland area, with aboveground biomass ranging from 120 to 380 $g\,m^{-2}$ in undisturbed conditions-values highly sensitive to grazing intensity (Hossain & Li, 2024; Ren et al., 2024). Our study site in Haiyan County (eastern QTP) is representative of these *Kobresia*-dominated meadows, where livestock grazing is the dominant land-use practice and a key driver of shifts in plant functional group composition (Dong et al., 2022; Liu et al., 2025). Recent studies have shown that overgrazing in this region reduces the biomass of dominant graminoids while promoting the proliferation of leguminous forbs (e.g. *Medicago ruthenica*)—a functional group shift linked to altered soil nutrient cycling and rhizosphere microbial activity (Zhang, Zhao, et al., 2023). We specifically focused on two functionally distinct and ecologically representative plant species in QTP alpine grasslands: the dominant species *Kobresia humilis* and the degradation indicator species *M. ruthenica*. As the core component of QTP alpine meadow plant communities, *K. humilis* is a perennial graminoid with a dense fibrous root system and strong tolerance to grazing and harsh alpine environments (Yang et al., 2019). Its high abundance and key role in maintaining ecosystem stability make it a critical indicator of grassland health under grazing disturbance. In contrast, *M. ruthenica* is a leguminous forb widely recognized as a degradation indicator in alpine grasslands. Its proliferation often signals grassland degradation induced by overgrazing, and its symbiotic nitrogen-fixation capability allows it to adapt to nutrient-poor soils (Zhang, Zhao, et al., 2023). The contrasting functional traits and ecological roles of these two species make them an ideal pair to dissect species-specific responses of rhizosphere stoichiometry to grazing. By comparing their rhizosphere C:N:P dynamics, we can gain insights into how grazing affects both stable and disturbed grassland states, which is essential for developing targeted grazing management strategies.

Previous studies on grazing management have shown that plant growth may be more limited by nitrogen than by phosphorus at higher soil C:N ratios and lower soil N:P ratios (Yang et al., 2019). Additionally, depending on the type of grazers and grazing patterns, *K. humilis* and *M. ruthenica* exhibit distinct rhizosphere soil bacterial communities and associated soil properties (Hu et al., 2023; Zhang, Zhao, et al., 2023). However, the dynamic characteristics of rhizosphere microbial biomass C, N and P stoichiometry under different grazing patterns have yet to be fully investigated. In this study, a 10-year field control experiment was conducted on grazing in alpine grasslands. To this end, *M. ruthenica*, *K. humilis* and their rhizosphere soil were collected over 3 consecutive years for stoichiometric analysis. The primary goal of this study was to examine the relationships between plant and rhizosphere soil stoichiometry, and rhizosphere microbial biomass under different grazing patterns. We hypothesized that (1) an increase in the proportion of sheep in mixed grazing systems reduces the rhizosphere stoichiometric turnover rate of the alpine grassland dominant species *K. humilis* and degradation indicator species *M. ruthenica*, and that this reduction drives species-specific shifts in plant C:N:P stoichiometric accumulation; and (2) *M. ruthenica* and *K. humilis* regulate rhizosphere microbial biomass C:N:P stoichiometry under different grazing patterns through divergent plant–soil stoichiometric mediation pathways. This work provides an effective scientific basis for managing and maintaining the stoichiometric balance of grazed grassland ecosystems.

2 | MATERIALS AND METHODS

2.1 | Study site and experimental design

The investigation was conducted in Haiyan County, Qinghai Province, China (36°44′–37°39′ N, 100°23′–101°20′ E; Figure 1). The site lies at an altitude of 3100 m, the soil type is clay and the climate is typical of a mountain plateau and characterized by cold and humid conditions. The average annual temperature is 1.4°C, with seasonal fluctuations ranging from −24.8°C in winter to 12.5°C in summer, indicating the lack of a year-round frost-free period. The main plants in the grassland are *K. humilis* and the leguminous *M. ruthenica* and *Astragalus polycladus* (Yang et al., 2019). Since 2014, a grazing field control experiment with local yak and Tibetan sheep animals has

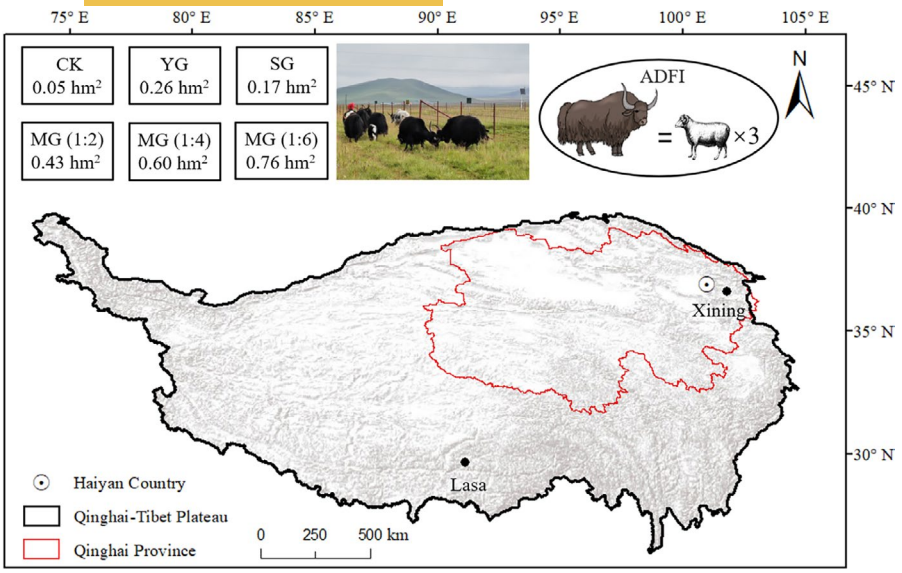


FIGURE 1 Geographical distribution of the studied site on the Qinghai-Tibet Plateau (QTP). The study site was in Haiyan County (36°44–37°39 N, 100°23–101°20 E), Qinghai Province, China. CK, no grazing; YG, single yak grazing; SG, single sheep grazing; MG(1:2), yak to sheep as 1:2; MG(1:4), yak to sheep as 1:4; MG(1:6), yak to sheep as 1:6.

been performed annually from June to late October. Grazing activity continues each month until the livestock consumed roughly 50% of the above-ground biomass (Dong et al., 2015). To ensure that this grazing intensity was maintained throughout the study, the sizes of the experimental plots were calculated based on the biomass of the palatable forage grasses and the feed intake determined for the grazing livestock. The livestock were housed in segregated test plots with no additional feed and were watered every other day. Based on preliminary studies, one yak ate the same amount of feed as three sheep per day ($3.7 \pm 0.7\,kg\,m^{-2}$ per yak and $1.2 \pm 0.2\,kg\,m^{-2}$ per sheep) (Liu et al., 2023). No grazing (CK), yak grazing (YG), sheep grazing (SG) and mixed grazing (MG) with yak-to-sheep ratios of 1:2 (MG1:2), 1:4 (MG1:4) and 1:6 (MG1:6) were all included in the experimental design (Table S1). A thoroughly randomized design was used, and each treatment was carried out in triplicate.

2.2 | Plant and rhizosphere soil characteristics

Plant and rhizosphere soil samples were collected from each plot from 18 to 20 August each year for 3 consecutive years. Rhizosphere soil samples were collected randomly through a 5-point sampling method. The collected *M. ruthenica* and *K. humilis* samples were transported to the laboratory and dried at 70°C for the stoichiometry treatment. After removing soil adhering to roots at depths of 2–5 cm, fresh soil adhered to roots at a depth of 20 cm was shaken off and then transported to the laboratory using refrigerator (4°C) to test its biochemical and stoichiometry properties (Zhang et al., 2024). One portion of the fresh rhizosphere soil was dried at room temperature (20–25°C for 70 days) to determine the biochemical and stoichiometry properties, while the other was utilized to determine the soil microbial biomass. In short, the potassium dichromate ($K_2Cr_2O_7$) volumetric method (Analytik Jena, Jena, Germany) was used to measure the total C (TC) content of plants. (Yang et al., 2022). The sulphuric acid-hydrogen peroxide digestion and micro-Kjeldahl N testing method were used to determine the total N (TN) content (Yang et al., 2022), and molybdenum-antimony resistance colorimetry following microwave digestion (*μPREP-A*; MLS, Leutkirch, Germany) was used to determine the total P (TP) content (Zhang, Li, et al., 2023). A spectrophotometer (FLUOstar Omega, BMG Labtech, Ortenberg, Germany) was used to detect absorbance at 660 nm. Soil organic carbon (SOC) was evaluated by passing 0.3 g of air-dried soil through a 0.150 mm mesh sieve and detecting the oxidation of potassium dichromate ($K_2Cr_2O_7$) (Zuo et al., 2019). Soil bulk density and soil water content (SM) were measured using the ring knife and drying methods, respectively (Zhu et al., 2021). A pH meter (Model IQ150; Spectrum, USA) was used to monitor the soil pH in a 1:5 soil–water solution. All measurements were performed with nine random repetitions.

2.3 | Rhizosphere soil microbial biomass C, N and P

Microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) and microbial biomass phosphorus (MBP) in the soil were assessed using the chloroform fumigation-extraction method (Bell et al., 2013; Brookes et al., 1985; Yao et al., 2021). For MBC and MBN determination: Briefly, fresh soil (8.0 g dry weight equivalent) was weighed into a 100 mL beaker and placed in a desiccator, along with one beaker containing 50 mL of NaOH solution (to absorb CO_2) and another beaker containing ~50 mL of ethanol-free chloroform. The desiccator was vacuum-pumped until the chloroform boiled and held at that state for ≥2 min to ensure complete fumigation. The leak valve was closed, and the desiccator was stored in the dark at 25°C for 24 h. After fumigation, chloroform was completely removed by repeated evacuation (three times, 5 min each). All fumigated soil was transferred to a 250 mL triangular flask, 40 mL of $0.5\,mol\,L^{-1}$ potassium sulfate (K_2SO_4) solution was added and the mixture was shaken at 180 rpm for 30 min, followed by filtration through quantitative filter paper (Whatman

No. 42). Non-fumigated soil samples (8 g) were treated identically without chloroform exposure. The C and N contents of the extracts were directly determined using a C and N analyser (TOC L CPH, Shimadzu Corporation, Japan).

$$MBC = Ec \times 2.2, \quad (1)$$

where 2.2 represents the correction factor for organic C release from microbial cells, and Ec is the difference in organic C content between fumigated and non-fumigated soil extracts.

$$MBN = E_N \times 1.85, \quad (2)$$

where 1.85 represents the adjustment factor for N release from microbial cells, and EN is the difference in total N content between fumigated and non-fumigated soil extracts.

For MBP determination: Fresh soil (3.0 g dry weight equivalent) was weighed into a large aluminium box and fumigated with chloroform (as described above) in the dark at 25°C for 24 h. After removing residual chloroform by evacuation, 50 mL of 0.5 mol L^{-1} sodium bicarbonate ($NaHCO_3$, pH 8.5) was added to each box, and the mixture was shaken at 180 rpm for 30 min, followed by filtration through Whatman No. 42 filter paper. Non-fumigated soil samples (3.0 g) were extracted identically. A 10 mL aliquot of the filtrate was transferred to a digestion tube, and 1 mL of 330 g L^{-1} sulphuric acid (H_2SO_4), 1 mL of potassium sulfate (K_2SO_4) solution and 0.5 mL of magnesium sulfate ($MgSO_4$) solution were added sequentially. After adding 0.2 mL of hydrogen peroxide (H_2O_2), the tube was digested in an oil bath at 120°C for 30 min to convert organic P to inorganic P. The P content in the digested solution was determined via molybdenum-antimony resistance colorimetry using a spectrophotometer (FLUOstar Omega, BMG Labtech, Ortenberg, Germany) at 660 nm.

$$MBP = (\text{P content in fumigated soil} - \text{P content in unfumigated soil}) / 0.4, \quad (3)$$

where 0.4 is the conversion coefficient for MBP in alpine grassland soils, accounting for the proportion of microbial P released and recovered during fumigation-extraction (Brookes et al., 1985; Cui et al., 2024).

2.4 | Data analysis

The plant, rhizosphere soil stoichiometric contents, soil microbial biomass and soil physicochemical properties were processed using GraphPad Prism 8.3.0 (GraphPad Software, Inc., La Jolla, CA, USA) for visualization. All statistical analyses were performed using SPSS 26.0 (IBM Corp., Armonk, NY, USA). Prior to analysis, data were tested for normality (Shapiro–Wilk test) and homogeneity of variances (Levene's test), and all stoichiometric and microbial biomass data met the assumptions of parametric tests. A two-way repeated measures analysis of variance (RM-ANOVA) was used to evaluate the effects of grazing treatment and sampling year on the measured variables. The RM-ANOVA tested for significant main effects of grazing treatment and year, as well as their interaction effect (Grazing × Year). When main effects or interaction effects were significant ($p < 0.05$), Tukey's Honestly Significant Difference (HSD) post hoc test was used for pairwise comparisons to identify specific differences among treatment groups. Pearson's correlation coefficients were calculated for the stoichiometric contents of plants, soil and rhizosphere microbial biomass (significance level set at $\alpha = 0.05$, marked as $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). The 'pheatmap' package in R 4.2.1 was used to generate correlation heatmaps, and the 'igraph' package was used for variation partitioning analysis. The explained variation was derived based on the *p*-value and determination coefficient (R^2; Liao et al., 2023). The link between soil characteristics and microbial biomass distribution in the rhizosphere was explored using canonical correlation analysis (CCA), and the Mantel test was used to establish the significance of correlations (Su et al., 2023). Structural equation modelling (SEM) was performed using AMOS 22.0 (IBM Corp., Armonk, NY, USA) to assess the pathways by which plant and soil stoichiometric contents affect rhizosphere soil microbial biomass under different grazing patterns. The model fit was evaluated using the following indices: $\chi^2/df = 1.82$ ($p = 0.16$), comparative fit index (CFI) = 0.94, root mean square error of approximation (RMSEA) = 0.062 and standardized root mean square residual (SRMR) = 0.048. These indices meet the acceptable model fit criteria ($\chi^2/df < 2$, $p > 0.05$, CFI > 0.90, RMSEA < 0.08, SRMR < 0.08; Shen et al., 2020). The significant pathways identified in the final SEM model not only exhibit large effect sizes but also align with the conclusions derived from Mantel tests, further validating the biological plausibility and statistical reliability of the model pathways.

3 | RESULTS

3.1 | Plant C, N and P stoichiometry of *M. ruthenica* and *K. humilis* under different grazing patterns

Grazing patterns induce significant species-specific variations in the total TC, TN and TP contents of *M. ruthenica* and *K. humilis* over the 3 consecutive years (Figure 2). Compared with CK, *M. ruthenica* TC content was significantly elevated by 25.9%, 23.7% and 23.6% under SG but reduced by 44.2%, 47.4% and 45.2% under MG1:6 across the 3 years (Figure 2a). In contrast, *K. humilis* TC content exhibited a significant decrease under all mixed grazing treatments relative to CK, with the reduction rates ranging from 37.9% to 53.6% during the 3-year period (Figure 2b). TN contents of both species were significantly increased under YG, SG, MG1:2 and MG1:4 compared with CK, with the increment percentages of *M. ruthenica* and *K. humilis* varying from 6.8% to 82.7% across the 3 years (Figure 2c,d). TP content showed opposite response patterns between the two species: *M. ruthenica* TP content decreased by 9.0% to 36.7% under MG1:2 and MG1:4

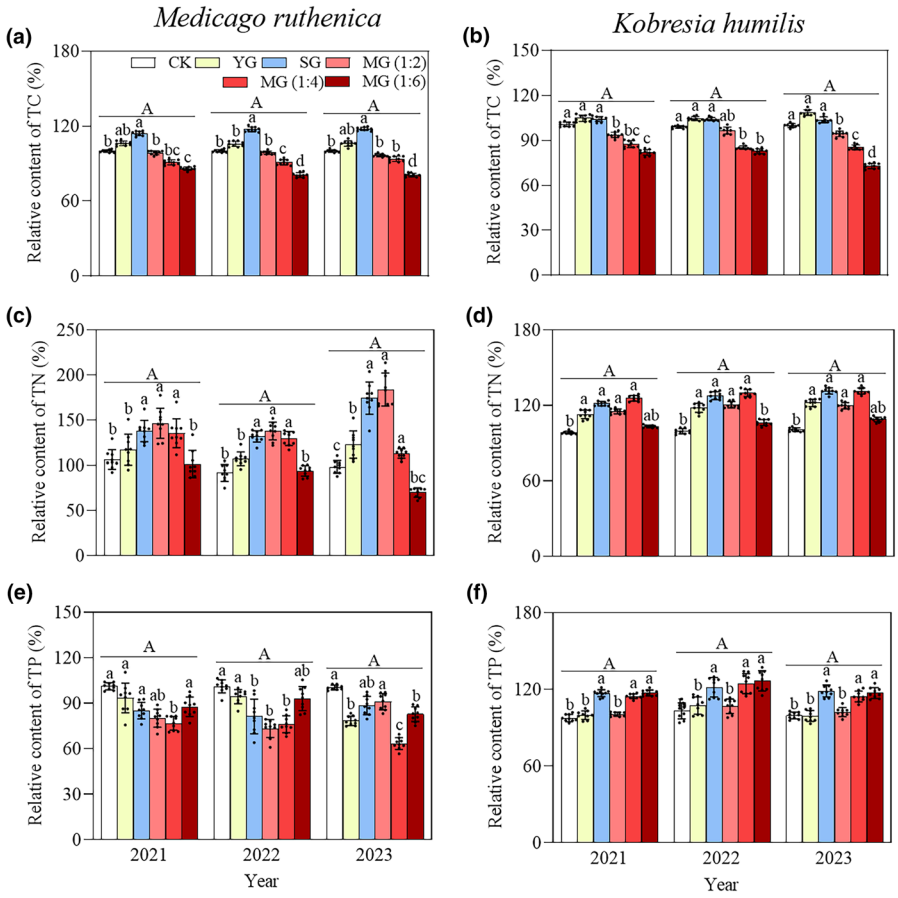


FIGURE 2 Variation in (A, C and E) relative contents of TC (total carbon), TN (total nitrogen), and TP (total phosphorus) of *M. ruthenica* and (B, D and F) relative contents of TC, TN and TP of *K. humilis* over three consecutive years under different grazing patterns. All data are presented as the mean ± SE, $n = 9$, and bars with different letters indicate significant differences at $p < 0.05$. CK, no grazing; YG, single yak grazing; SG, single sheep grazing; MG(1:2), yak to sheep as 1:2; MG(1:4), yak to sheep as 1:4; MG(1:6), yak to sheep as 1:6.

over the 3 years (Figure 2e), while *K. humilis* TP content was significantly elevated by 14.3% to 26.1% under SG, MG 1:4 and MG 1:6 relative to CK (Figure 2f). Grazing patterns also altered the C:N, C:P and N:P stoichiometric ratios of the two species, with notable interspecific differences (Figure S1). The C:N ratio of *M. ruthenica* was reduced by 47.3% under MG1:2 in 2023 compared with CK (Figure S1A), while that of *K. humilis* decreased by 34.4% and 35.1% under MG1:4 in 2022 and 2023, respectively (Figure S1B). For the C:P ratio, *M. ruthenica* showed an increase of 32.6% and 35.7% under SG and MG1:2 in 2022, and a 41.6% rise under MG1:4 relative to CK (Figure S1C); *K. humilis* C:P ratio was significantly reduced by 6.8% to 37.7% under all MG treatments (MG1:2, MG1:4, and MG1:6) across the 3 years (Figure S1D). The N:P ratio of *M. ruthenica* was markedly increased by 61.4% to 155.9% under SG, MG1:2 and MG1:4 compared with CK (Figure S1E), whereas no significant variation in the N:P ratio of *K. humilis* was observed across all grazing treatments during the study period (Figure S1F).

3.2 | Rhizosphere soil C, N and P stoichiometry under different grazing patterns

Grazing exerted distinct effects on the rhizosphere soil TC, TN and TP contents of *M. ruthenica* and *K. humilis*, with significant temporal and interspecific variations (Figure 3). Relative to the 2021 CK, *M. ruthenica* rhizosphere soil TC content decreased by 28.1% to 63.4% under YG, SG, MG1:2 and MG1:4 in 2022 and 2023 (Figure 3a), while the corresponding index of *K. humilis* increased by 27.11% to 66.2% under YG, SG and all MG treatments in the same years (Figure 3b). *M. ruthenica* rhizosphere soil TN content exhibited a significant reduction of 37.8% to 52.6% under YG, SG, MG1:2 and MG1:4 in 2022 and 2023 compared with the 2021 CK (Figure 3c), whereas no significant difference in *K. humilis* rhizosphere soil TN content was detected across all grazing treatments and years (Figure 3d). Rhizosphere soil TP content showed a time-dependent response in *M. ruthenica*: it decreased by 11.1% to 33.8% under YG and SG in 2022 and 2023 and decreased significantly under all grazing patterns in 2023, relative to the 2021 CK (Figure 3e). A slight increase of 1.4% to 1.6% in *K. humilis* rhizosphere soil TP content was also observed in 2023 compared with the 2021 CK (Figure 3f). In contrast, no significant variation in *K. humilis* rhizosphere soil TP content was found across all grazing treatments and the 3 years (Figure 3f). No significant differences in the C:N, N:P stoichiometric ratios of rhizosphere soil for both species were observed across all grazing treatments over the 3 years relative to CK (Figure S2A,B,E,F). The rhizosphere soil C:P ratio of *M. ruthenica* decreased with the increase in the proportion of sheep in MG systems, while that of *K. humilis* was reduced under YG and SG in 2022 compared with CK (Figure S2C,D). The comprehensive biochemical properties of the rhizosphere soil for *M. ruthenica* and *K. humilis* under different grazing patterns over the three consecutive years are presented in Table S2.

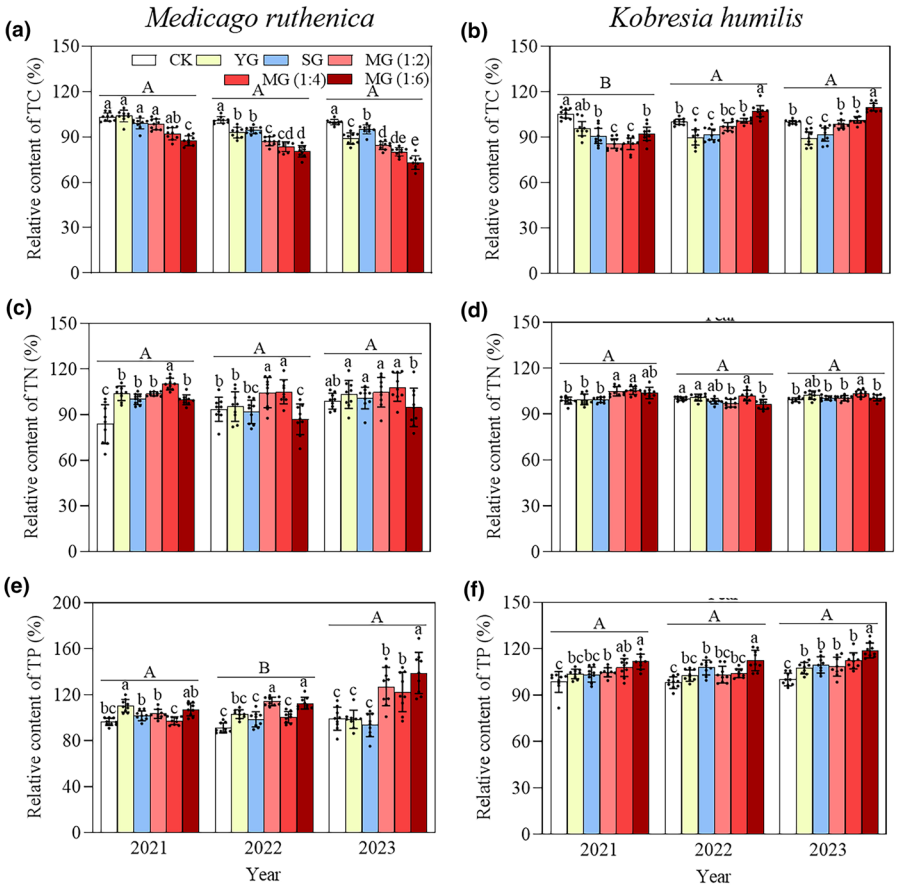


FIGURE 3 Variation in (A, C and E) relative contents of TC (total carbon), TN (total nitrogen) and TP (total phosphorus) of rhizosphere soil of *M. ruthenica* and in (B, D and F) relative contents of TC, TN and TP of rhizosphere soil of *K. humilis* over three consecutive years under different grazing patterns. All data are presented as mean ± SE, $n = 9$, and bars with different letters indicate significant differences at $p < 0.05$. CK, no grazing; YG, single yak grazing; SG, single sheep grazing; MG(1:2), yak to sheep as 1:2; MG(1:4), yak to sheep as 1:4; MG(1:6), yak to sheep as 1:6.

3.3 | Rhizosphere soil microbial biomass C, N and P stoichiometry under different grazing patterns

Grazing patterns significantly affected the MBC, MBN and MBP contents in the rhizosphere soil of *M. ruthenica* and *K. humilis*, with obvious interspecific differences (Figure 4). For *M. ruthenica*, rhizosphere soil MBC content increased by 8.9% to 16.6% under MG1:2 and by 7.1% to 22.1% under SG and MG1:4 across the 3 years compared with CK (Figure 4a,b), and a significant annual increase in MBC content was also detected under all grazing treatments (Figure 4c). The MBN content of *M. ruthenica* rhizosphere soil was elevated by 2.8% to 22.4% under MG1:4 and MG1:6 in 2022 and 2023 relative to CK, while that of *K. humilis* increased significantly under MG1:6, with the highest value recorded in 2023 (Figure 4d). MBP content exhibited a species-specific response to grazing: *M. ruthenica* MBP content increased by 26.2% to 42.6% under MG1:4 over the 3 years (Figure 4e), and *K. humilis* MBP content was significantly elevated under all SG and MG treatments during the study period, relative to CK (Figure 4f). The stoichiometric ratios of rhizosphere soil microbial biomass showed distinct interspecific variations under different grazing patterns (Figure S3). The MBC:MBN ratio of *M. ruthenica* decreased significantly under MG1:4 and MG1:6 across the 3 years (Figure S3A), while that of *K. humilis* was only reduced under SG in 2021 compared with CK (Figure S3B). No significant differences in the MBC:MBP ratio of both species were observed across all grazing treatments and years (Figure S3C,D). The MBN:MBP ratio of *M. ruthenica* decreased significantly under YG for 3 consecutive years (Figure S3E), whereas no notable variation in the MBN:MBP ratio of *K. humilis* was detected across all grazing regimes (Figure S3F).

3.4 | Relationships between rhizosphere stoichiometry and grazing patterns

The stoichiometric ratios of plant TC and soil TC were consistently higher than other C:N:P ratios for both *M. ruthenica* and *K. humilis* across all grazing treatments (Figure S4). The response patterns of rhizosphere stoichiometry to grazing showed high similarity between YG and SG, as well as between MG1:2 and MG1:4 for *M. ruthenica* (Figure S4A); for *K. humilis*, the rhizosphere stoichiometry responses were highly consistent between CK and YG, and between SG and MG1:2 (Figure S4B). Pearson correlation analysis revealed distinct coupling relationships between rhizosphere soil microbial biomass and plant/soil C:N:P stoichiometry for the two species under different grazing patterns (Figure S5). For *M. ruthenica* under YG, MBC, MBN and MBP were both positively and negatively correlated with soil and plant nutrient indices (Figure S5A); under SG, these microbial biomass indices were negatively correlated with plant TC, plant TP, soil TC and SM (Figure S5B); under MG, they were negatively correlated with plant TP and soil TC (Figure S5C). For *K. humilis* under YG, MBC, MBN and MBP were only negatively correlated with plant TC, soil TC and SM (Figure S5D); under SG,

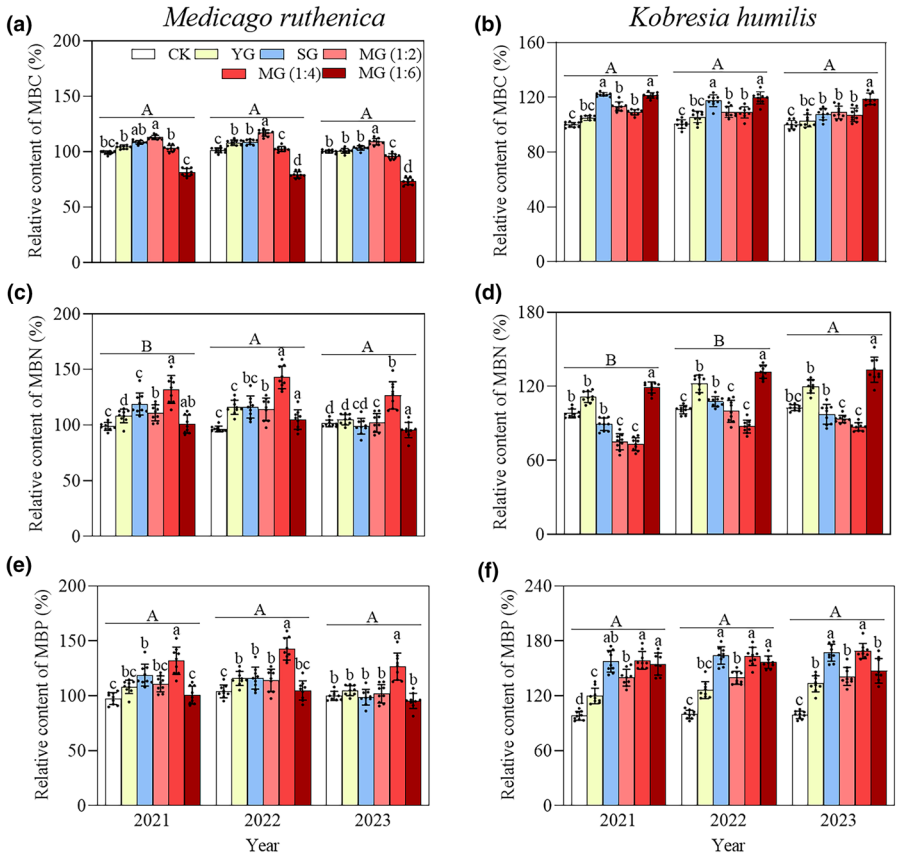


FIGURE 4 Variation in (A, C, and E) relative contents of MBC (microbial biomass carbon), MBN (microbial biomass nitrogen) and MBP (microbial biomass phosphorus) of rhizosphere soil of *M. ruthenica* and (B, D and F) relative contents of MBC, MBN and MBP of rhizosphere soil of *K. humilis* over three consecutive years under different grazing patterns. All data are presented as mean ± SE, $n = 9$, and bars with different letters indicate significant differences at $p < 0.05$. CK, no grazing; YG, single yak grazing; SG, single sheep grazing; MG(1:2), yak to sheep as 1:2; MG(1:4), yak to sheep as 1:4; MG(1:6), yak to sheep as 1:6.

these indices were positively correlated with plant TC, plant TN and plant TP (Figure S5E); under MG, they showed a positive correlation with plant TP, soil TP and soil pH (Figure S5F). Canonical correlation analysis (CCA) indicated that soil properties were regulated by different grazing patterns (Figure S6): YG and SG were mainly driven by SM, while MG was primarily affected by SBD, SOC and soil pH. SEM further revealed the divergent regulatory pathways of grazing patterns on the rhizosphere C:N:P stoichiometry of *M. ruthenica* and *K. humilis*. For *M. ruthenica*, YG and SG affected MBC and MBN by regulating plant C and N contents (Figure 5a,c), MG altered MBC and MBN by modifying soil properties (Figure 5b,d), and all grazing patterns influenced MBP by changing rhizosphere soil TP content (Figure 5e,f). For *K. humilis*, YG and SG affected MBC, MBN and MBP through the combined regulation of soil properties and plant C, N and P contents (Figure 6a,c,e), while MG regulated MBN and MBP via soil properties and plant N content, respectively (Figure 6b,d,f).

4 | DISCUSSION

4.1 | Effects of different grazing patterns on plant stoichiometry

Grazing regulates plant stoichiometry via two key pathways: direct physical disturbances (e.g. trampling, foraging) and indirect alterations to soil nutrient cycling (Kouba et al., 2024). Previous studies have consistently linked grazing intensity to shifts in plant C:N ratios; specifically, Shen, Dong, et al. (2023) demonstrated that plant C:N ratios decline sharply with increasing grazing intensity, a pattern associated with accelerated N cycling and modified plant resource allocation strategies (Du & Gao, 2021). Our results align with this general trend but further reveal grazing pattern-specific effects (single vs mixed grazing) and interspecific divergence in P dynamics that extend beyond previous intensity-based frameworks. For the dominant *K. humilis*, MG1:4 reduced both C:N and C:P ratios, with no significant change in N:P ratios across all grazing treatments. This response is consistent with the 'concentration effect' in graminoids, where defoliation reduces above-ground biomass and concentrates residual N and P in remaining tissues. Li et al. (2016) observed increased plant N and P concentrations alongside decreased N:P ratios under higher grazing intensity, a pattern partially supported by our data for SG: *K. humilis* exhibited elevated TN and TP concentrations under SG, mirroring their findings. Grazing-induced regrowth of defoliated shoots and leaves prioritizes carbohydrate consumption for tissue repair, which in turn reduces plant C concentrations (Hou et al., 2020). This mechanism directly explains the reduced C:N and C:P ratios of *K. humilis* under MG(1:4). In contrast, the leguminous *M. ruthenica* displayed a species-specific response under MG, driven by its functional traits. While its TN concentration remained stable under MG-induced nutrient competition (buffered by its high N-fixation capacity), TP concentration decreased significantly. This divergence led to a marked increase in N:P ratios specifically under MG (1:2), a shift indicating a skewed nutrient balance towards N surplus and

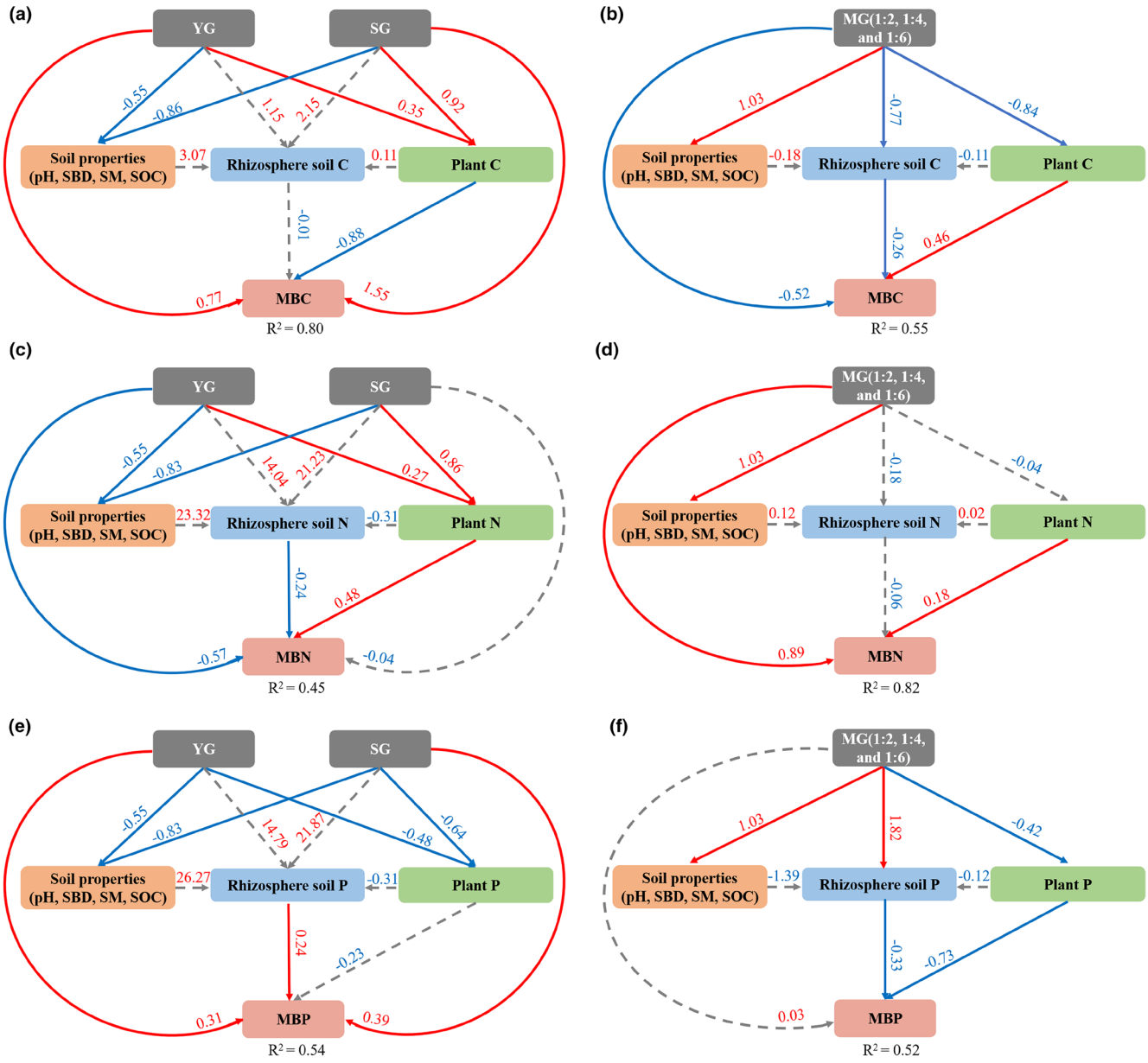


FIGURE 5 Structural equation diagram of the effects of YG and SG on (A, C and E) rhizosphere stoichiometry of C, N and P and the effect of MG on (B, D and D) rhizosphere stoichiometry of C, N and P of *M. ruthenica*. Solid blue lines indicate a significant negative relationship ($p < 0.05$), and solid red lines indicate a significant positive relationship ($p < 0.05$). Dashed lines indicate indirect relationships ($p < 0.05$). CK, no grazing; YG, single yak grazing; SG, single sheep grazing; MG, mixed grazing of yak and sheep.

P limitation. Liang et al. (2019) noted that such imbalances may enhance photosynthetic capacity via higher N accumulation but constrain growth-related processes due to P limitation, ultimately limiting aboveground biomass accumulation. This P limitation in *M. ruthenica* is likely attributable to MG-induced soil compaction (restricting root P uptake) and increased microbial P immobilization in the rhizosphere, processes directly linked to mixed grazing regimes. Collectively, these results confirm that plant functional traits mediate stoichiometric responses to grazing patterns. Specifically, N-fixation capacity and growth strategy are key drivers, with MG driving contrasting nutrient balance outcomes for dominant graminoids and leguminous indicator species.

4.2 | Effects of different grazing patterns on rhizosphere soil stoichiometry

Grazers modulate rhizosphere soil stoichiometry primarily through faecal/urine input, litter decomposition and trampling-induced changes to organic matter mineralization (Hui et al., 2022; Liu et al., 2021). Inconsistencies persist in the literature regarding grazing effects on soil C:N:P cycles: Kytöviita et al. (2012) reported decreased soil C:N:P concentrations with higher grazing intensity, while Hou et al. (2020) observed the opposite trend. Chen et al. (2020) attributed this discrepancy to uncontrolled background factors such as soil texture or grazing patterns, and our results further resolve

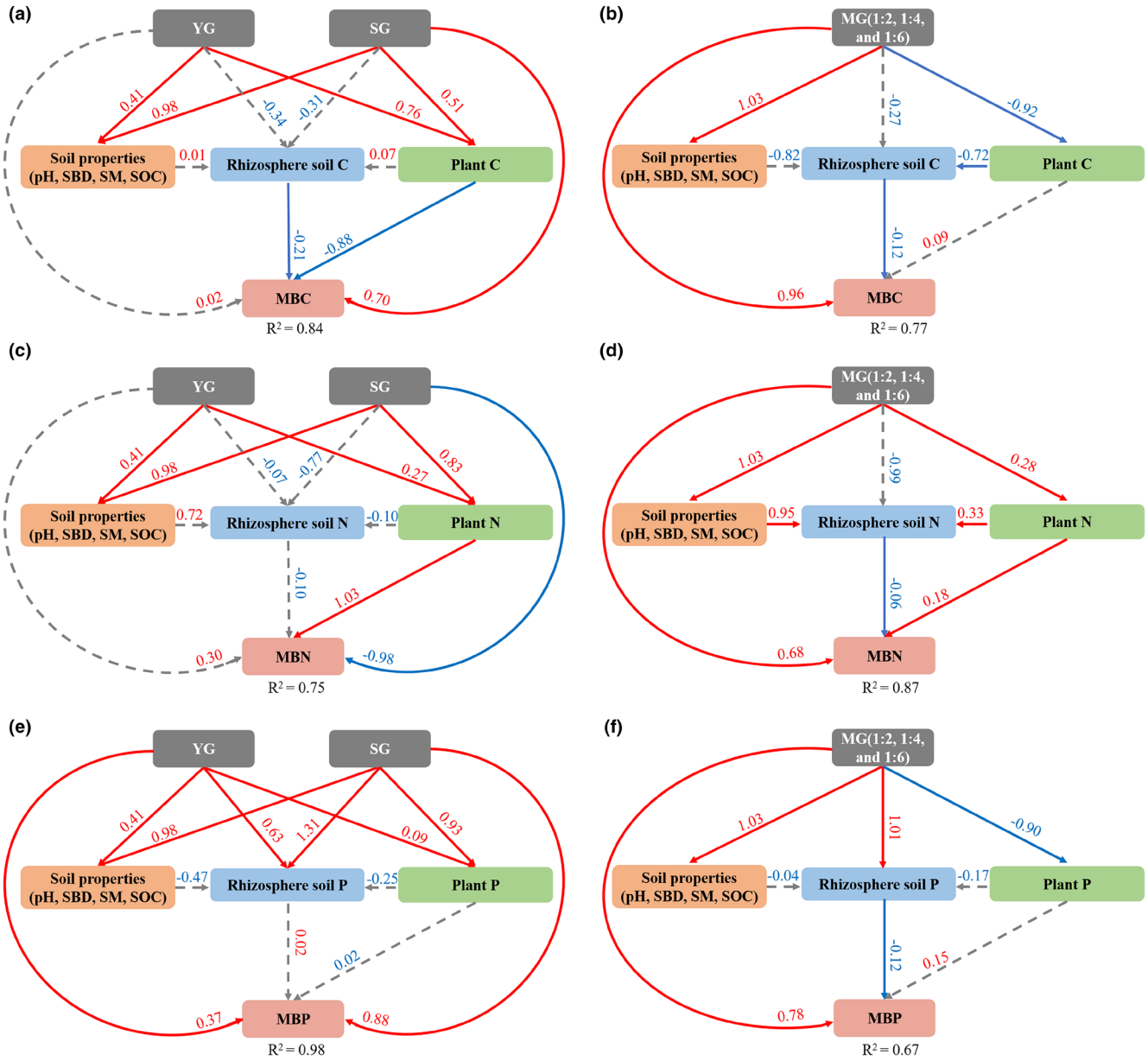


FIGURE 6 Structural equation diagram of the effects of YG and SG on (A, C and E) rhizosphere stoichiometry of C, N and P and the effect of MG on (B, D and D) rhizosphere stoichiometry of C, N and P of *K. humilis*. Solid blue lines indicate a significant negative relationship ($p < 0.05$), and solid red lines indicate a significant positive relationship ($p < 0.05$). Dashed lines indicate indirect relationships ($p < 0.05$). CK, no grazing; YG, single yak grazing; SG, single sheep grazing; MG, mixed grazing of yak and sheep.

this uncertainty by demonstrating strong species-dependent rhizosphere soil stoichiometric responses to grazing. For *M. ruthenica*, rhizosphere soil TN and TP concentrations decreased significantly with increasing grazing duration, driven by two interconnected processes. First, MG-induced trampling accelerated litter decomposition, which increased short-term nutrient release but exhausted long-term soil organic matter pools (Hui et al., 2022). Second, *M. ruthenica*'s stable N uptake (via symbiotic fixation) coupled with reduced P uptake led to net N accumulation in plant tissues, further lowering soil N reserves. In stark contrast, *K. humilis* rhizosphere soil under MG exhibited increased TC and TP concentrations, a divergence rooted in its fibrous root system. Hou et al. (2020) noted that SG and MG promote fine root proliferation in the topsoil, and our results extend this finding by linking fine root growth to enhanced root exudation of labile C and accelerated P mineralization. This pattern aligns with Zhang, Zhao, et al. (2021), who found that grazing boosts the conversion of insoluble soil P to plant-available forms via root exudates. Notably, the C:P ratio of *K. humilis* rhizosphere soil decreased under MG, consistent with recent findings that moderate grazing enhances soil C cycling while accelerating P turnover (Liu et al., 2024; Shen, Fang, et al., 2023). Together, these results confirm that grazing patterns mediate nutrient transport between plants and soil in a species-specific manner. For *M. ruthenica*, MG reduces rhizosphere N and P availability via plant uptake and organic matter

depletion; for *K. humilis*, MG enhances rhizosphere C and P nutrient cycling via root–microbe interactions. This distinction underscores the critical role of plant functional traits (e.g. root architecture, symbiotic N-fixation) in shaping soil stoichiometric responses to grazing regimes.

4.3 | Effects of different grazing patterns on rhizosphere microbial biomass stoichiometry

Soil microbes act as both nutrient stores and core regulators of C:N:P cycling, with their stoichiometric ratios reflecting adaptive responses to grazing-induced changes in resource availability (Kytöviita et al., 2012; Liao et al., 2023). A key distinction in microbial responses lies in their stoichiometric homeostasis: homeostatic microbes maintain their stable C:N:P ratios regardless of environmental changes, while non-homeostatic microbes adjust their ratios to match resource supply (Li et al., 2023). Our data demonstrate that grazing patterns alter microbial homeostasis in a species-specific manner, mediated by rhizosphere resource availability and plant–microbe nutrient competition. In the *M. ruthenica* rhizosphere, mixed grazing significantly decreased MBC but increased MBN, leading to a lower MBC:MBN ratio. This shift indicates clear non-homeostatic behaviour: reduced MBC likely reflects limited labile C input from *M. ruthenica* under MG stress, while increased MBN stems from elevated soil N availability and enhanced microbial N immobilization. Under MG, *M. ruthenica*'s reduced P uptake may alleviate microbial P limitation, allowing microbes to allocate more resources to N immobilization (Wu et al., 2022). This represents a nutrient trade-off that further shapes the microbial stoichiometric profile. For *K. humilis*, SG and MG increased both MBC and MBP, consistent with Yang et al. (2021), who reported that grazing intensity drives higher microbial P demand. Our results extend this finding by demonstrating that MG amplifies MBP accumulation in the *K. humilis* rhizosphere, a pattern likely fuelled by two complementary processes: increased root exudate C supply and enhanced soil P mineralization. These processes provide microbes with sufficient P to match elevated C inputs, maintaining a balanced stoichiometric profile. The SG/MG-induced increase in soil C:P ratios further supports this balance, as it supplies microbes with co-limiting C and P resources (Wu et al., 2022). Collectively, these patterns indicate that mixed grazing accelerates soil nutrient cycling through two species-specific pathways: shifting the *M. ruthenica* rhizosphere microbial community towards N-rich biomass; and boosting C and P stocks in the *K. humilis* rhizosphere microbiome. These changes sustain a steady state of microbial biomass turnover that supports overall ecosystem nutrient cycling.

4.4 | Relationships among the stoichiometry of plant, soil and rhizosphere microbial biomass

Alpine grasslands produce substantial aboveground biomass annually, which supplies soil microorganisms with abundant litter. This litter acts as a natural organic substrate that fuels microbial activity and nutrient cycling (Zhang, Li, et al., 2023). However, grazing modifies this dynamic by reducing the quantity of organic matter entering the soil, leading to insufficient energy for microbial growth and subsequent decreases in MBC and MBN concentrations (Brewer et al., 2023; Zhang, Zhao, et al., 2023). Beyond litter input, vegetation also shapes soil microbial community composition by modulating soil moisture and nutrient availability, creating a tightly linked plant–soil–microbe system (Zhang, Wang, et al., 2021). Gao and DeLuca (2022) reported that livestock grazing significantly reduced MBC and MBN contents in grazed compared to ungrazed grasslands but had no significant effect on topsoil TN concentrations. This discrepancy aligns with our observations and suggests that while grazing disrupts microbial communities, it does not substantially deplete total surface soil TN stocks. This buffering effect may stem from slower cycling soil N fractions or external inputs such as atmospheric deposition (Abdalla et al., 2018). Plant growth relies primarily on soil-derived N and P, and soil nutrient concentrations directly shape plant C:N:P stoichiometry. This relationship creates a reciprocal feedback loop that is further modified by grazing. Our study identifies divergent regulatory pathways linking plant, soil and microbial stoichiometry for the two species under different grazing patterns. For *M. ruthenica*, SG influenced MBC, MBN and MBP contents primarily by modifying plant C and N status, whereas MG indirectly affected MBP content via changes to soil physical and chemical properties. For *K. humilis*, SG modulated MBC, MBN and MBP through both soil C:N:P stoichiometry and plant C:N:P status, while MG shifted to a purely soil-driven pathway, with effects mediated solely by soil C:N:P stoichiometry. These pathway shifts are likely driven by MG-induced soil compaction via trampling, which restricts root penetration and development, reduces soil organic matter input and alters nutrient availability. Importantly, grazing effects on alpine grasslands are context-dependent, varying with grazing intensity and differing across QTP alpine grasslands and other rangeland habitats (Cui et al., 2024; Ren et al., 2024). Our 3-year field study captures plot-scale mechanistic linkages, but large-scale responses of QTP alpine grasslands to varying grazing intensities remain poorly characterized. Consequently, the full complexity of stoichiometric relationships among plant, soil and rhizosphere microbial biomass remains incompletely understood. The unique stoichiometric characteristics of their distinct biological niches also require further investigation. Future research focusing on long-term, large-scale grazing experiments will help identify the optimal grazing intensity and pattern for sustaining the stoichiometric balance and ecological integrity of QTP alpine grassland ecosystems.

5 | CONCLUSION

Grazing induces divergent rhizosphere soil nutrient dynamics in two functionally key species of Qinghai–Tibet Plateau alpine grasslands: the degradation indicator *M. ruthenica* and the dominant *K. humilis*. For *M. ruthenica*, enhanced plant N uptake forms a strong vegetation

N sink. This reduces rhizosphere soil N concentrations and accelerates soil P transformation to compensate for plant P acquisition. For *K. humilis*, mixed grazing at a 1:4 yak-to-sheep ratio reduces plant C:N and C:P ratios while maintaining N:P homeostasis. It also increases rhizosphere microbial biomass carbon and phosphorus, thereby sustaining the stability of microbial-mediated nutrient cycling. Our findings identify a critical ecological constraint. Grazing disrupts the coupling of C, N and P cycles between degradation-indicator and dominant functional groups in alpine plant communities. Practically, moderate mixed grazing regimes, specifically yak-to-sheep ratios of 1:2 and 1:4, represent a sustainable management strategy. This approach preserves core ecosystem functions including nutrient cycling efficiency and vegetation productivity. It also maintains the stoichiometric balance of alpine grassland ecosystems, providing a robust scientific basis for targeted grazing management on the QTP.

AUTHOR CONTRIBUTIONS

Mingxu Zhang: Conceptualization, methodology, data curation, formal analysis and writing—original draft. Jinpeng Hu: Visualization, data curation and supervision. Bin Zhang: Data curation. Xiaoxia Yang: Methodology, supervision and visualization. Quanmin Dong: Conceptualization, methodology, supervision, visualization, and writing—review and editing. Jinlin Zhang: Conceptualization, supervision, visualization, and writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.dr7sqvbcr (Zhang et al., 2026).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Variation in C:N, C:P and N:P ratios of *M. ruthenica* and *K. humilis* over three consecutive years under different grazing patterns. All data are presented as the mean ± SE, $n = 9$, and bars with different letters indicate significant differences at $p < 0.05$. CK, no grazing; YG, single yak grazing; SG, single sheep grazing; MG (1:2), yak to sheep as 1:2; MG (1:4), yak to sheep as 1:4; MG (1:6), yak to sheep as 1:6.

Figure S2. Stoichiometric ratios of C, N and P in the rhizosphere soil of *M. ruthenica* and *K. humilis* for three consecutive years under different grazing patterns. All data are presented as the mean ± SE, $n = 9$, and bars with different letters indicate significant differences at $p < 0.05$. CK, no grazing; YG, single yak grazing; SG, single sheep grazing; MG(1:2), yak to sheep as 1:2; MG(1:4), yak to sheep as 1:4; MG(1:6), yak to sheep as 1:6.

Figure S3. Stoichiometric ratios of C, N and P in the rhizosphere soil microbial biomass of *M. ruthenica* and *K. humilis* for three consecutive years under different grazing patterns. All data are presented as the mean ± SE, $n = 9$, and bars with different letters indicate significant differences at $p < 0.05$. CK, no grazing; YG, single yak grazing; SG, single sheep grazing; MG(1:2), yak to sheep as 1:2; MG(1:4), yak to sheep as 1:4; MG(1:6) and yak to sheep as 1:6.

Figure S4. Relationships between stoichiometric ratios of C, N and P among plant, soil and rhizosphere soil microbial biomass of *M. ruthenica* and *K. humilis* under different grazing patterns. For each panel, the colour is proportional to the Pearson's correlation coefficients (Pearson ρ) (dark blue, $r = -1$, dark red, $r = 1$). MBC, microbial biomass carbon; PCN, plant total carbon: plant total nitrogen; PCP, plant total carbon: plant total phosphorus; PNP, plant total nitrogen: plant total phosphorus; SCN, soil total carbon: plant total nitrogen; SCP, soil total carbon: plant total phosphorus; SNP, soil total nitrogen: plant total phosphorus; MCN, microbial biomass carbon: microbial biomass nitrogen; MCP, microbial biomass carbon: microbial biomass phosphorus; MNP, microbial biomass nitrogen: microbial biomass phosphorus; CK, no grazing; YG, single yak grazing; SG, single sheep grazing; MG(1:2), yak to sheep as 1:2; MG(1:4), yak to sheep as 1:4; MG(1:6), yak to sheep as 1:6.

Figure S5. Pearson's correlation coefficient analysis between rhizosphere soil microbial biomass and stoichiometry C, N, and P of plants and soil in *M. ruthenica* and *K. humilis* under different grazing patterns. Rhizosphere soil microbial biomass was related to each nutrient factor using the Mantel test. The edge colour denotes significance. Blue and red show negative and positive relationships, respectively, between the two variables. For each panel, the colour is proportional to the Pearson's correlation coefficients (Pearson ρ) (dark blue, $r = -1$, dark red, $r = 1$). PC, plant total carbon; PN, plant total nitrogen; PP, plant total phosphorus; SC, soil total carbon; SN, soil total nitrogen; SP, soil total phosphorus; SM, soil water content; SBD, soil bulk density; SOC, soil organic carbon; CK, no grazing; YG, single yak grazing; SG, single sheep grazing; MG(1:2), yak to sheep as 1:2; MG(1:4), yak to sheep as 1:4; MG(1:6), yak to sheep as 1:6.

Figure S6. Canonical correlation analysis of soil properties under different grazing patterns. SM, soil water content; SBD, soil bulk density; SOC, soil organic carbon; CK, no grazing; YG, single yak grazing; SG, single sheep grazing; MG(1:2), yak to sheep as 1:2; MG(1:4), yak to sheep as 1:4; MG(1:6), yak to sheep as 1:6.

Table S1. Summary of the grazing experiment design.

Table S2. Soil properties of rhizosphere soil under different grazing patterns.

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