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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

Lan Li¹  | Mengyuan Wang¹ | Xiong Zhao He² | Zhen Wang¹  |
Xiumin Zhang¹ | Junqi Hu¹ | Ling Huang¹ | Fujiang Hou¹ 

¹State Key Laboratory of Herbage Improvement and Grassland Agro-ecosystems, Key Laboratory of Grassland Livestock Industry Innovation, Ministry of Agriculture and Rural Affairs, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, PR China

²School of Agriculture and Environment, Massey University, Palmerston North, New Zealand

Correspondence

Fujiang Hou, State Key Laboratory of Herbage Improvement and Grassland Agro-ecosystems, Key Laboratory of Grassland Livestock Industry Innovation, Ministry of Agriculture and Rural Affairs, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730020, PR China.
Email: cyhoufj@zu.edu.cn

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Abstract

Despite their significance, how interactions of plant diversity at multiple spatial scales and soil microbial stoichiometry alter a series of ecosystem functions (multifunctionality, EMF) in response to anthropogenic nitrogen (N) input and herbivores are poorly known. We conducted a 17-year sheep grazing experiment with 6-year N addition to explore the impacts of grazing (0, 2.7, 5.3 and 8.7 sheep ha⁻¹) and N addition (N0, N5, N10 and N20, i.e., 0, 5, 10 and 20 g N m⁻² yr⁻¹, respectively) on grassland functions and EMF via changes in plant α - and β -diversity, and carbon to nitrogen ratio (C:N) of soil microbes in a typical steppe. The results show that grazing or N addition alone significantly affected EMF with a treatment order of 2.7 and 8.7 sheep ha⁻¹ > 0 and 5.3 sheep ha⁻¹ for grazing intensity or N5 > N10 and N20 > N0 for N addition, which resulted in a significant higher EMF in the combination treatment of 2.7 sheep ha⁻¹ and 5 g N m⁻² yr⁻¹. Plant α - and β -diversity, and soil microbial C:N were the predominant drivers of changes in EMF. Grazing reduced EMF indirectly by decreasing the plant β -diversity. N addition promoted EMF indirectly by decreasing plant α -diversity. In addition, lower plant α -diversity enhanced EMF indirectly by increasing soil microbial C:N. Our results suggest that the negative effects of herbivore on EMF were stronger at larger spatial scales compared to the smaller local communities, while N addition could maintain a higher level of EMF at smaller scales rather than at the larger ones. Our results highlight that multiple spatial scales should be considered to fully unravel the effects of herbivore and eutrophication on ecosystem functions. Our results also demonstrate the important role of soil microbe in maintaining higher grassland multifunctionality, thus we should include the soil microbial functions (i.e., C and N transformation) in further studies. Our results suggest that grazing at a low grazing intensity of 2.7 sheep ha⁻¹ with a low N supplementation of 5 g N m⁻² yr⁻¹ could maintain the most important ecosystem functions. Our work provides important insight into grassland conservation and management, aiming to maintain the capacity of grasslands to sustainably supply ecological and productive functions.

KEYWORDS

biodiversity-multifunctionality, cross-scale, eutrophication, grassland management, soil carbon and nitrogen transformation, stocking rate

1 | INTRODUCTION

Grazing is the most intensive land-use activity in grasslands, accounting for more than 30% of Earth's terrestrial land surface (Herrero et al., 2013). Grazing livestock plays a profound role in shaping vegetation, soil nutrient dynamics, and global material and energy cycle (Breidenbach et al., 2022; Filazzola et al., 2020). Herbivore affects grassland ecosystem functioning through its direct effects including hoof action, defoliation, and excreta fertilization, and also the indirect effects mediated by changes in plant community composition and soil microbial activity (Tuomi et al., 2021; Xun et al., 2018). Since the early 20th century, more than doubled anthropogenic nitrogen (N) deposition has been attributed to fossil-fuel combustion and fertilizer application (Stevens, 2019). A substantial body of research demonstrates that increasing N loading largely affects plant communities and soil microbes, and a range of ecosystem functions that depend on them (Liu et al., 2021; Valencia et al., 2018). Yet, estimates of multiple ecosystem functions in response to N loading are commonly performed in ecosystems without considering the effects of herbivores (e.g., Hautier et al., 2020; Lekberg et al., 2021). Further studies need to combine grazing and N fertilizer and explore their interaction effects on multiple ecosystem functions (i.e., multifunctionality [EMF], hereafter).

Many observational and experimental studies have provided a wide range of evidence for the positive effect of diversity at small scales (i.e., the number and abundance of species within local communities, α -diversity; Loreau, 2000) on ecosystem function, through the selection and complementarity effects (Hautier et al., 2015; Tilman et al., 2014). Previous studies have documented that grazing or N addition may reduce plant α -diversity (e.g., Liang et al., 2021; Liu et al., 2021; Midolo et al., 2019; Wang et al., 2020), resulting in the negative effects on ecosystem functions. For example, the increasing the stocking rate may reduce biodiversity by 10% and ecosystem functions by 20%–40% in Australia (Eldridge & Delgado-Baquerizo, 2017), and increasing N inputs could decrease the number of plant species by 30% and parallel ecosystem stability by 8% (Hautier et al., 2015). However, grazing and N deposition occur simultaneously and are difficult to disentangle experimentally in the real world. Furthermore, grazing could probably affect the directions and magnitudes of N enrichment functioning on plant α -diversity and ecosystem functions, because the influences of N addition on plant community composition and soil biotic and abiotic properties differ in grazed and non-grazed areas (Wang, Wu, et al., 2021; Wang, Zhang, et al., 2021). Thus, it remains unknown whether the multifunctionality is related to α -diversity in real-world ecosystems under complicated anthropogenic environmental changes and grazing disturbance.

Beyond α -diversity, the spatial variation in abundances or identities of species among local communities (β -diversity) also affects ecosystem functions (Hautier et al., 2018; Mori et al., 2018; Yan et al., 2020). The threat of biotic homogenization could decrease EMF via two main mechanisms. First, the same functional species in different locations could not maintain the functions at higher levels in the landscape (Grman et al., 2018; Le Provost et al., 2021). Second, homogenization in species composition among local communities could weaken ecological interactions including the movement of resources that is important for ecosystem functioning (Hautier et al., 2018). Increasing the stocking rate may increase dominant species abundance and decrease β -diversity, and then substantially decrease local community stability (Liang et al., 2021). A long-term nitrogen input could decrease β -diversity (Liu et al., 2021) and increase the risk of pest damage (Dainese et al., 2019). Many studies have shown that plant β -diversity contributes to ecosystem multifunctionality (EMF) (Jing et al., 2021; Yan et al., 2020). However, it remains unclear whether the variation in communities observed across landscapes (β -diversity) also contributes to the functioning of real-world ecosystems based on herbivore and eutrophication control in a grassland.

Soil microbes are the most abundant and diverse taxa on the Earth, and they are responsible for the key ecosystem processes, including decomposition and nutrient cycling (Delgado-Baquerizo et al., 2017; Roy & Bagchi, 2021). Many of these functions are also critical to ecosystem services, such as primary production and global carbon (C) cycle (Delgado-Baquerizo et al., 2017). Changes in soil microbial stoichiometry could also regulate EMF by altering soil organic matter quality. For instance, soil microbial C:N ratio can reflect the capacity of microbes to regulate the mineralization and immobilization of soil C and N (Chen et al., 2019). High available N in excreta returning by grazing animals and N addition may decrease soil microbial C:N ratio (Li et al., 2021), resulting in higher soil process rates and multifunctionality (Lucas-Borja & Delgado-Baquerizo, 2019; Yang et al., 2022). So far, few studies have determined the thresholds for the interaction of stocking rate and N input on the stoichiometric characteristic of soil microbes in controlling EMF, despite the current surge of interest in soil microbial stoichiometry responses to elevated N input or grazing.

Numerous research studies have explored the impacts of grazing or N input alone on single or several functions (Liu et al., 2021; Pulido et al., 2018). Multiple ecosystem functions need to be considered simultaneously to achieve an integrative understanding of the changes in multifunctionality, especially nutrient cycling and ecosystem production under interplay between herbivore grazing and eutrophication, which is critical for grassland management (Cui et al., 2020; Ma et al., 2021; Wang et al., 2019; Zhang et al., 2021). In this study, we

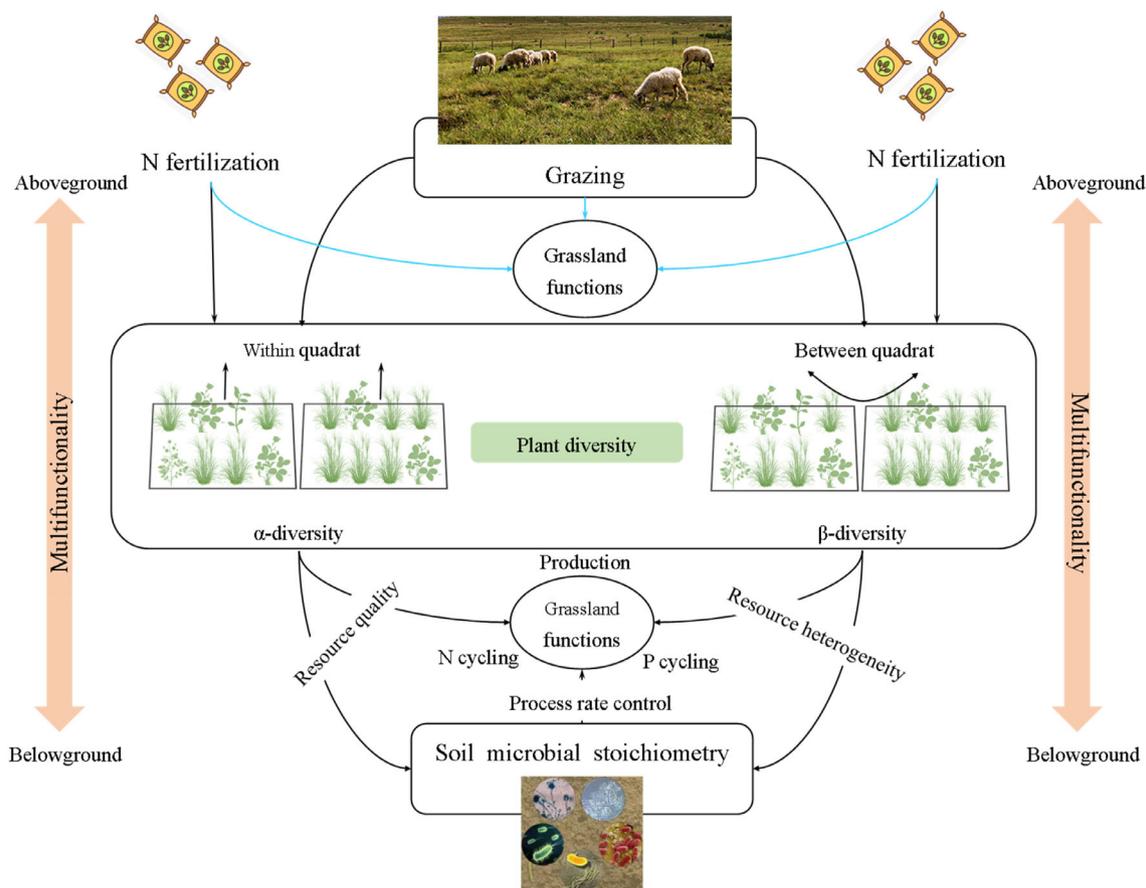


FIGURE 1 A conceptual framework showing how plant diversity (α -diversity and β -diversity) and soil microbial stoichiometry mediate multifunctionality in response to grazing and N addition. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/lid.4652)]

focused on the respective and integrated influences of stocking rate and N addition rate on EMF in a typical steppe. EMF is a comprehensive index derived from multiple ecosystem functions, such as (a) aboveground biomass, (b) plant nitrogen concentration, (c) plant phosphorus (P) concentration, (d) soil available nitrogen, (e) soil total nitrogen, and (f) soil total phosphorus. We postulated three hypotheses: (1) light grazing could maintain higher levels of EMF, and EMF is dramatically reduced by any subsequent stocking rate increases (Ren et al., 2018); (2) N addition will increase EMF at the low N levels ($\leq 10 \text{ g N m}^{-2} \text{ yr}^{-1}$, Bai et al., 2010; Cui et al., 2022), and relative low N fertilization rates can weaken negative influences of higher stocking rate on EMF; (3) Changes in EMF will be associated with variations in the aboveground resource quantity and heterogeneity. It is well known that changes in plant α - and β -diversity could regulate multiple ecosystem functions by generating aboveground resource heterogeneity (Figure 1) via litter and resources for soil microbes (Liu et al., 2021; Zhang et al., 2021). Grazing or N addition will decrease soil microbial C:N ratio resulting in higher soil process rates and multifunctionality (Chen et al., 2019; Lucas-Borja & Delgado-Baquerizo, 2019; Yang et al., 2022). Our objectives were: (a) to determine how the respective and integrated influences of stocking rate and N addition rate on affect EMF; and (b) to understand how plant α - and β -diversity, and soil microbial stoichiometry co-regulate the

alterations in EMF in response to grazing and N addition in this typical steppe.

2 | MATERIALS AND METHODS

2.1 | Study site

This study was conducted in the Huanxian Grassland Agriculture Trial Station of Lanzhou University, which is located in Huan County, Gansu Province, China (37.14°N, 106.84°E, 1650 m asl). The grassland type is a typical temperate steppe. The mean annual temperature is 7.8°C. The mean annual rainfall is 289.8 mm, mostly occurring from April to September. The typical soil type is classified as loessal soils with sand textures (Hu et al., 2021). The vegetation is dominated by *Artemisia capillaris*, *Heteropappus altaicus*, *Lespedeza davurica*, *Potentilla bifurca* and *Stipa bungeana*.

2.2 | Design of experiment

A manipulative rotation of Tan-sheep (*Ovis aries*) grazing experiment was initiated in 2001. We established 12 paddocks (100 × 50 m for each

paddock), in which four stocking rates (i.e., 0, 4, 8, and 13 sheep corresponding to 0, 2.7, 5.3, and 8.7 sheep ha⁻¹, respectively) with three replicates were randomly set up. The long-term grazing systems included 30-day rotational grazing with three 10-day grazing periods in each paddock from June to September (Li et al., 2021). Tan sheep of about 20 kg in body weight were purchased all in a similar condition at the start of each experimental period in spring. The standard stocking rate was 0, 1.6, 3.2 and 5.2 sheep units ha⁻¹, corresponding to 0, 2.7, 5.3, and 8.7 sheep ha⁻¹, respectively. A fenced experimental cell (11 × 2 m) was randomly set up in all grazing paddocks. In each fenced cell, 4 subplots (2 × 2 m) were built and separated by 1-m walkways. Since 2012, each subplot was randomly allocated by one of the four N addition rates (i.e., CK, 0 g N m⁻² yr⁻¹; N5, 5 g N m⁻² yr⁻¹; N10, 10 g N m⁻² yr⁻¹; N20, 20 g N m⁻² yr⁻¹). The N supplement was NH₄NO₃ dissolved in water, and it was uniformly sprayed on a plot twice a year in May and July. The amount of water is equivalent to 1 mm of rainfall.

2.3 | Vegetation survey

In mid-August 2017, we randomly conducted plant community surveys in a quadrat (1 × 1 m) of each subplot to quantify plant above-ground (AGB) biomass and determine the species richness. All plant materials were oven-dried at 65°C for 48 hr to constant weight before they were weighed to determine the AGB. We calculated species richness as the total number of species and quantified plant species diversity in terms of the Shannon index:

$$\text{Shannon index} = -\sum p_i \ln(p_i),$$

Where: p_i is species i 's relative abundance, the AGB of each species accounting for the total AGB of community in each subplot.

Plant N concentration was determined following Kjeldahl digestion by an auto-Kjeldahl's apparatus (UDK149, VELP, Usmate Velate, Italy), while measurement of P concentration was used molybdenum blue colourimetric approach (Sparks et al. 1996). The plant community weighted mean (CWM; Garnier et al., 2004) of N and P (CWM_N and CWM_P, respectively) was measured by the weighted relative abundance in each subplot.

2.4 | Soil abiotic and biotic property

After plant biomass was harvested, two soil cores were sampled in the same subplot at a depth of 0–10 cm using a 7-cm-diameter bucket auger and mixed into one sample. Soil composite samples were sieved through 0.25-mm mesh to remove the roots and stones and then shipped to the laboratory. Subsamples were kept at –80°C to preserve soil microorganisms and at 4°C for soil abiotic properties' analysis.

The measurement of soil moisture content (SM) was determined according to the weight loss after being oven-dried at 105°C for 48 hr.

Soil available nitrogen (SAN) was the sum of NH₄⁺-N and NO₃⁻-N, which were extracted using 2 M KCl and estimated by colorimetric analysis (Spectrophotometer Cary 60UV-Vis from Agilent, Alto, America). The measurements of total N and P of soil (STN and STP) were determined as the same as those of plants. The C and N of soil microbes were measured via chloroform fumigation extraction methods (Joergensen & Mueller, 1996).

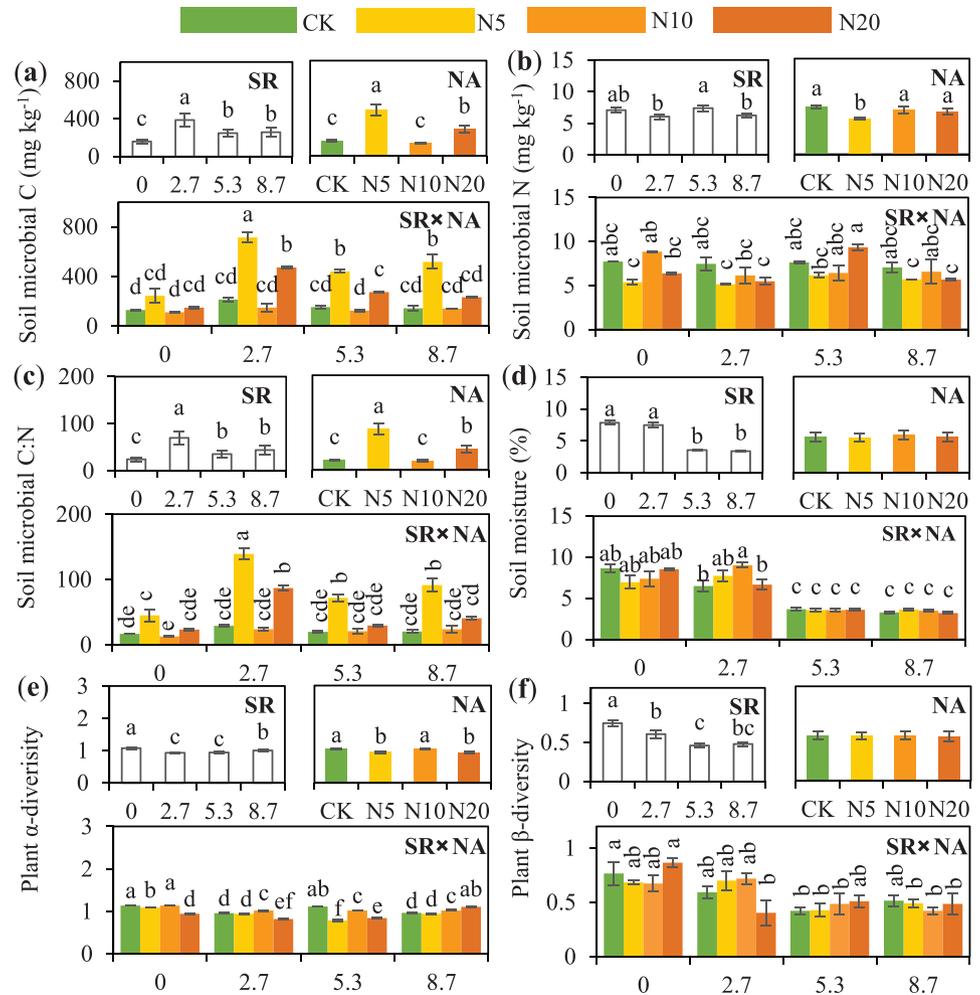
2.5 | Evaluation of ecosystem multifunctionality index

We quantified six grassland functions related to nitrogen (CWM_N, STN, and SAN), phosphorus (CWM_P and STP), and biological productivity (AGB). These parameters serve as good proxies for the processes underlying nutrient cycling, primary production, and the formation of nutrient pools (Giling et al., 2019; Manning et al., 2018). To investigate the potential trade-offs between individual functions, we calculated Pearson's correlation coefficients between each pair of individual functions (Figure S3). Of the 15 combinations of pairs of functions, we found significant positive correlations between two pairs, significant negative correlations between three pairs (Figure S3), and a strong negative correlation between AGB and STP (–0.54). We kept AGB and STP in our analyses because a negative correlation meant that choosing one function or the other would favour either a positive or negative impact of diversity on average multifunctionality. In contrast, retaining these two variables demonstrated a trade-off between them. We standardized each function using z-scores and calculated the averaged EMF via an averaging method (Maestre et al., 2012). Then, we used principal component analysis (PCA) to reduce the dimensions, and visualize the direction and strength of the ecosystem's individual functions or multifunctionality relative to the overall distribution (Figure S2).

2.6 | Associations between plant diversity, environmental predictors, and ecosystem functioning (multifunctionality)

We examined the relationships between plant richness, Shannon diversity and most of the individual functions measured, assessing whether plant diversity contributed to EMF. We found positive relationships between Shannon diversity and individual functions and EMF, thus plant Shannon diversity was used as the metric of plant α-diversity to predict EMF (Figure S3). Except the plant diversity, we also assessed the relationships between environmental predictors, ecosystem functioning and EMF (Figure S3). We then used a linear regression to identify the relative importance of plant diversity and environmental predictors on EMF, and selected plant α- and β-diversity and soil microbial C:N as the main predictors in the following structural equation model (SEM) analyses. Due to the multicollinearity between soil microbial C, N and C:N, we used soil microbial C:N in our analyses.

FIGURE 2 Effects of stocking rate (SR, sheep ha⁻¹), nitrogen addition (NA), and their interaction (SR × NA) on ecosystem attributes: (a) Soil microbial C, (b) Soil microbial N, (c) Soil microbial C:N, (d) Soil moisture, (e) Plant α-diversity, and (f) Plant β-diversity. Nitrogen addition rates: CK, 0 g N m⁻² yr⁻¹; N5, 5 g N m⁻² yr⁻¹; N10, 10 g N m⁻² yr⁻¹; and N20, 20 g N m⁻² yr⁻¹. Significant differences between means are indicated by different upper-case letters based on the Tukey–Kramer test ($p < 0.05$). [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]



2.7 | Statistical analysis

We used plant Shannon diversity as the metric of plant α-diversity. Plant β-diversity was calculated by the biomass-based Bray–Curtis dissimilarity to assess the dissimilarity in community composition between repetitive subplots of the same treatment (Oksanen et al., 2013). Before conducting statistical analyses, the separated and integrated effects of stocking rate and N addition rate on plant β-diversity were assessed by a permutational multivariate analysis of variance (PERMANOVA) using the R package's Adonis function.

R version 4.1.0 (R Development Core Team 2021) was used for all analyses of statistics, with the significance of levels at $p < 0.05$. We assessed a generalized linear mixed model (Proc GLIMMIX), with stocking rate, N addition rate, and their interaction as fixed factors, and subplots as the random effect to assess plant diversity, soil biotic and abiotic properties, individual ecosystem functions, and EMF (Figures 2–4, S1; Table 1). Before conducting the Tukey–Kramer adjustment test for multiple comparisons, the distribution's normality and the variance's homogeneity were verified by the Shapiro–Wilk and Levene's tests.

PCA was used to display the directions and magnitudes of individual functions by using the “factoextra” package (Figure S2). The sampling adequacy of individual and set variables by the Kaiser–Meyer–Olkin

measure (KMO value > 0.50) and Bartlett's test of sphericity ($p < 0.05$) should be taken into account. We estimated Pearson's correlations between plant diversity indices, environmental predictors, ecosystem functioning and EMF (“corplot” package, Figure S3). We quantified the contributions of selected main predictors to EMF by using the “relaimpo” package based on the dominance analysis in linear regression (Groemping, 2006), and further filtered SM and plant richness (Figure 5). We presumed that stocking rate, N addition and their interaction directly affect EMF, and changes in EMF would be associated with plant α- and β-diversity, and soil microbial C:N ratio. For example, changes in plant α- and β-diversity could regulate EMF by generating resource heterogeneity (Liu et al., 2021; Zhang et al., 2021). Soil microbial C:N ratio was associated with a range of soil processes and microbial community composition, thus mediating multifunctionality (Yang et al., 2022) (Figure S4). We also considered the causal relationship between α and β diversity in this conceptual model (see Liang et al., 2021). Furthermore, we promoted the hypothetical model fitted by progressively removing insignificant pathways and simplifying the hypothetical modelling (Figure S5). We removed the pathways about interactive effects of grazing and N addition on EMF, and the final model was fitted by d-separation tests and examined by Fisher's C statistics obtained lower Akaike information criterion (AIC) scores (Figure 6; Shipley, 2013).

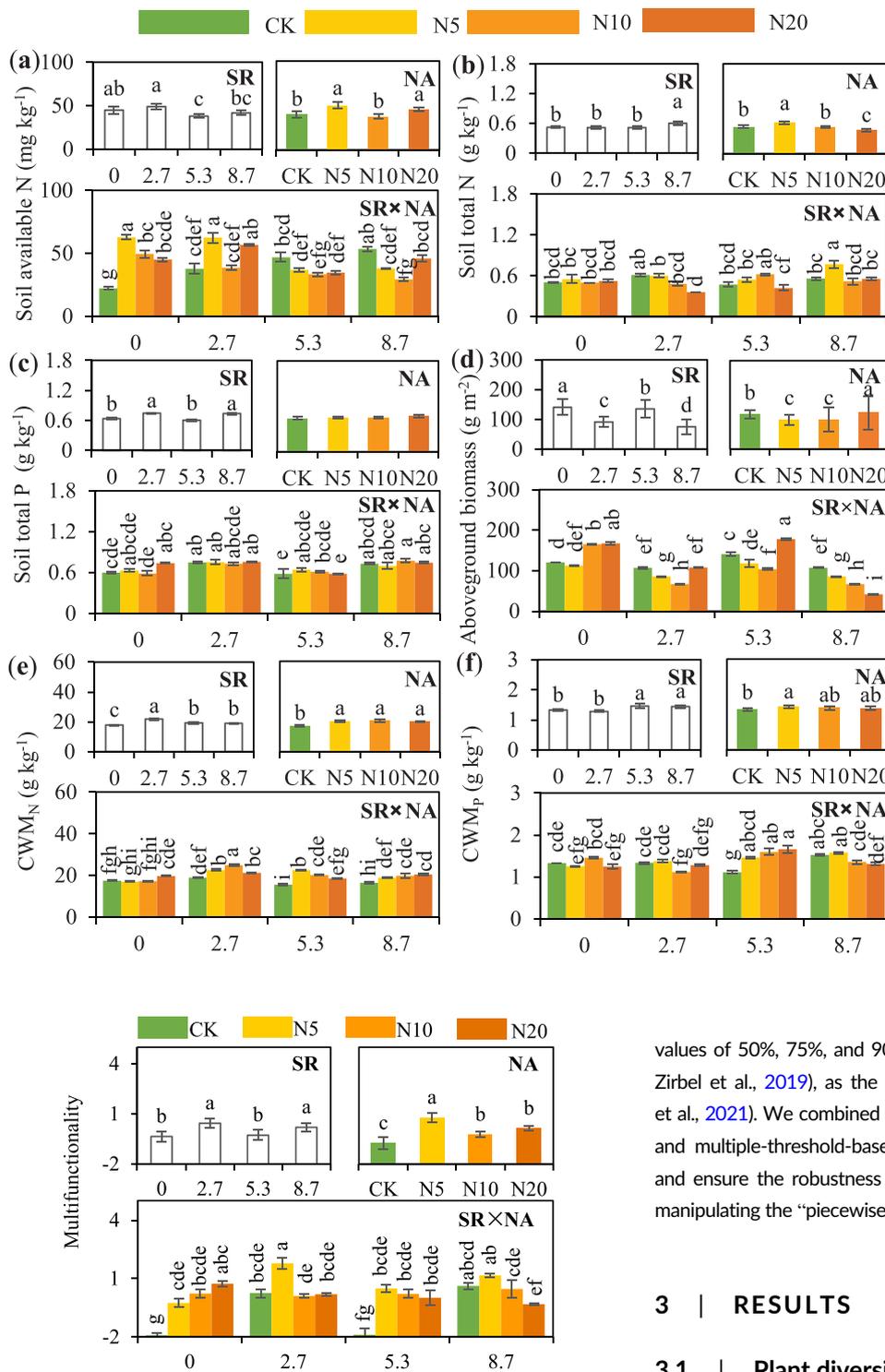


FIGURE 4 Effects of stocking rate (SR, sheep ha⁻¹), nitrogen addition (NA), and their interaction (SR × NA) on ecosystem multifunctionality. Nitrogen addition rates: CK, 0 g N m⁻² yr⁻¹; N5, 5 g N m⁻² yr⁻¹; N10, 10 g N m⁻² yr⁻¹; and N20, 20 g N m⁻² yr⁻¹. Significant differences between means are indicated by the different upper-case letters based on the Tukey–Kramer test ($p < 0.05$). [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/lid.4652)]

Meanwhile, we assessed the direct effects of stocking rate, N addition and their interaction, and indirect effects of stocking rate and N addition on individual functions (Figure S6), and estimated the threshold

FIGURE 3 Effects of stocking rate (SR, sheep ha⁻¹), nitrogen addition (NA), and their interaction (SR × NA) on ecosystem functions: (a) soil available N, (b) Soil total N, (c) Soil total P, (d) Aboveground biomass, (e) Community weighted mean of plant N (CWM_N), and (f) Community weighted mean of plant P (CWM_P). Nitrogen addition rates: CK, 0 g N m⁻² yr⁻¹; N5, 5 g N m⁻² yr⁻¹; N10, 10 g N m⁻² yr⁻¹; and N20, 20 g N m⁻² yr⁻¹. Significant differences between means are indicated by different upper-case letters based on the Tukey–Kramer test ($p < 0.05$). [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/lid.4652)]

values of 50%, 75%, and 90% EMF (Figure S6, S7; Byrnes et al., 2014; Zirbel et al., 2019), as the previous studies (Wang et al., 2019; Zhang et al., 2021). We combined single-function, averaging-based approaches, and multiple-threshold-based approaches to overcome their limitation and ensure the robustness of analytical methods. The SEM was fit by manipulating the “piecewiseSEM” package (Lefcheck, 2016).

3 | RESULTS

3.1 | Plant diversity, environmental factor, ecosystem function, and multifunctionality

Grazing significantly increased soil microbial C and C:N ratio, while N addition significantly increased soil microbial C and C:N ratio in N5 and N20 (Figure 2a, c). The interplay between grazing and N addition induced significantly higher soil microbial C and C:N ratio in N5 with a stocking rate of 2.7 sheep ha⁻¹ (Figure 2a, c). Compared to the control, grazing had no significant effect on soil microbial N, N addition significantly decreased soil microbial N in N5 (Figure 2b). N addition alone had no significant effect on SM, whilst SM significantly declined

TABLE 1 Model summaries describing the responses of soil biotic and abiotic properties, plant diversity, ecosystem function, and multifunctionality (EMF) to stocking rate (SR), N addition (NA), and their interaction (SR × NA).

	SR		NA		SR × NA	
	F	P	F	P	F	P
Soil biotic and abiotic properties						
Soil moisture	272.97	<0.001	0.91	0.449	3.94	0.002
Microbial C	56.29	<0.001	182.05	<0.001	5.82	<0.001
Microbial N	7.06	0.001	12.49	<0.001	4.20	0.001
Microbial C:N ratio	64.16	<0.001	194.76	<0.001	4.66	<0.001
Plant diversity						
Species richness	1.84	0.161	0.27	0.846	0.40	0.923
α-diversity	279.93	<0.001	286.41	<0.001	151.07	<0.001
β-diversity	17.64	<0.001	0.11	0.952	3.02	0.011
Ecosystem functions						
SAN	20.14	<0.001	34.55	<0.001	49.88	<0.001
STN	10.45	<0.001	24.11	<0.001	10.14	<0.001
STP	34.47	<0.001	2.00	0.136	3.17	0.008
AGB	1976.62	<0.001	191.26	<0.001	438.45	<0.001
CWM _N	136.83	<0.001	128.55	<0.001	36.98	<0.001
CWM _P	36.09	<0.001	7.65	<0.001	39.20	<0.001
EMF	60.276	<0.001	20.452	<0.001	49.937	<0.001

Abbreviations: AGB, aboveground biomass; CWM_N, community weighted mean of plant N; CWM_P, community weighted mean of plant P; SAN, soil available N; STN, soil total N; STP, soil total P.

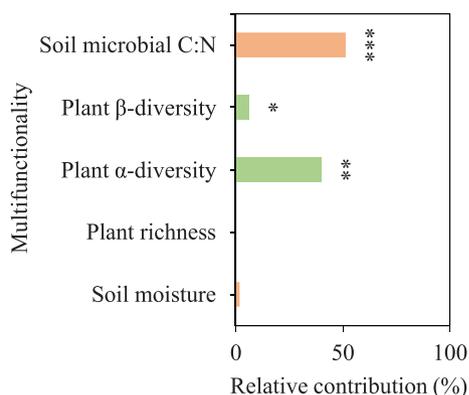


FIGURE 5 Relative contribution of soil biotic and abiotic properties and plant diversity to ecosystem multifunctionality. Significance levels: * $p < 0.05$ ** $p < 0.01$ and *** $p < 0.001$ [Colour figure can be viewed at wileyonlinelibrary.com]

at stocking rates of 5.3 and 8.7 sheep ha^{-1} (Figure 2d). Grazing, N addition, and their interaction did not significantly affect plant species richness (Figure S1). Increasing the stocking rate decreased plant α -diversity, while it was significantly lower in N5 and N20 (Figure 2e). The interaction effect of grazing and N addition induced lower plant α -diversity except that it was significantly higher in N20 with the highest stocking rate of 8.7 sheep ha^{-1} (Figure 2e). N addition had no significant impact on plant β -diversity, while grazing significantly decreased plant β -diversity (Figure 2f).

SAN was significantly lower at a stocking rate of 5.3 sheep ha^{-1} , while it was significantly higher in N5 and N20 compared to control, and

the interaction effect of grazing and N addition induced significantly lower SAN at three N addition rates with a stocking rate of 5.3 or 8.7 sheep ha^{-1} (Figure 3a). The highest stocking rate of 8.7 sheep ha^{-1} significantly increased STN, and STN was significantly higher in N5 but lower in N20 compared to control and N10 (Figure 3b). Low and high stocking rates of 2.7 and 8.7 sheep ha^{-1} significantly increased STP, but STP was not significantly affected by N addition. The significant interaction of grazing and N addition led to the highest STN in N5 and STP in N10 with a stocking rate of 8.7 sheep ha^{-1} (Figure 3b, c). Grazing significantly decreased AGB, while compared to control, AGB was significantly lower in N5 and N10 but higher in N20 (Figure 3d). The interaction between grazing and N addition induced lower AGB at three N addition rates with stocking rates of 2.7 and 8.7 sheep ha^{-1} (Figure 3d). Grazing, N addition and their interaction significantly increased CWM_N and CWM_P (Figure 3e, f). Grazing and N addition significantly increased EMF (Figure 4). Interaction of grazing and N addition led to significantly higher EMF in N5 with a stocking rate of 2.7 sheep ha^{-1} .

3.2 | Plant diversity and soil microbial C:N ratio mediating EMF under grazing and N addition conditions

Plant α -diversity was significantly and negatively related to C:N ratio of soil microbes, CWM_N, CWM_P, and EMF ($p < 0.05$; Figure S3). Soil microbial C:N ratio had a significant negative effect on AGB, while it was significantly and positively related to SAN, CWM_N, and EMF ($p < 0.05$; Figure S3). Although plant β -diversity was not significantly

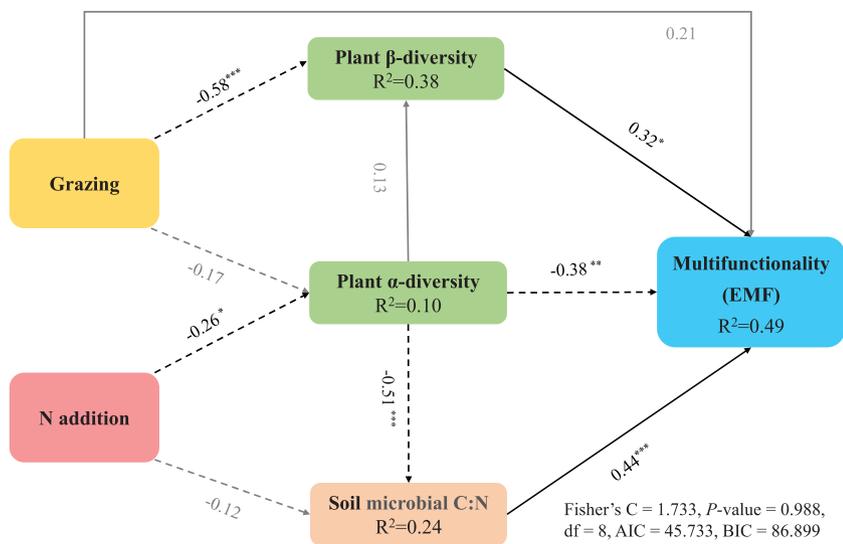


FIGURE 6 Final structural equation model of effects of stocking rate and N addition on ecosystem multifunctionality through plant α -diversity and β -diversity, and soil microbial C:N ratio. Solid and dashed arrows indicate significant positive and negative pathways, respectively. Black and grey arrows indicate significant and non-significant pathways, respectively. Numbers at arrows are standardized path coefficients. The proportion of variance explained (R^2) appears alongside the response variable in the model. Significance levels: * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/lid.4652)]

correlated with ecosystem functions ($p > 0.05$; Figure S3), it was one of main predictors of changes in EMF (Figure 5). Soil microbial C:N, plant α - and β -diversity were the main predictors of EMF (Figure 5). We also show that SM was significantly and positively correlated with plant β -diversity and negatively correlated with CWN_p and plant richness ($p < 0.05$; Figure S3).

The direct and indirect effects of grazing and N addition contributed to 49% of the variance in EMF (Figure 6). Grazing and N addition had no significant direct effect on EMF ($p > 0.05$). Grazing and N addition had a range of indirect influences on EMF mediated by the C:N ratio of soil microbes and plant diversity. For instance, grazing significantly decreased EMF by reducing plant β -diversity. N addition promoted EMF indirectly by decreasing plant α -diversity. In addition, lower plant α -diversity enhanced EMF indirectly by increasing soil microbial C:N. Similar impacts of grazing and N addition were observed on other functions (e.g., STN, STP, AGB, CWM_N and CWM_p), or EMF over the 75% thresholds (Figures S6a-f, S7b-c).

4 | DISCUSSION

4.1 | A range of functions are essential to assessing ecosystem functioning in response to herbivore grazing and N enrichment

Increasing the stocking rate could enhance EMF directly, which is inconsistent with findings from studies conducted in other arid and semi-arid grasslands (Ren et al., 2018; Zhang et al., 2021). A previous study reveals that high stocking rates reduce EMF, and 4.5 sheep ha^{-1} or less intensive grazing may be a threshold for maintaining >50% EMF in semi-arid grasslands (Ren et al., 2018). In our study, the low stocking rate of 2.7 sheep ha^{-1} maintained the highest levels of EMF, which was mostly explained by the notable improvement in ecosystem functions that are integrated with EMF under light grazing

(Figure 2). For example, grazing animals usually increase SAN, STP, and CWM_N through stimulating N availability by returning urine and excrement, compensatory growth by livestock feeding, and decreasing decomposition due to herbivores treading-induced anaerobic conditions (Li et al., 2021; Yu et al., 2021). Contrary to our hypothesis, the highest stocking rate of 8.7 sheep ha^{-1} significantly increased EMF. This is mainly due to STN, STP, CWM_N , and CWM_p significantly increased at 8.7 sheep ha^{-1} (Figure 3) caused by greater nutrient return via urine and faeces (Li et al., 2021), which nearly compensates for the decreased EMF by AGB loss (Figure 3b, c). Meanwhile, under the rotational grazing system, the “real” moderate and heavy stocking in the current study may be less intensive than the threshold (~ 4.5 sheep ha^{-1}) in Ren et al. (2018).

Partly consistent with our second hypothesis, EMF increased under all N addition conditions, with the highest EMF detected at a low N supplement level (~ 5 g N m^{-2} yr^{-1}). In N-limited systems, N fertilization has direct positive effects on EMF by enhancing SAN (Figure 3a), which can be readily acquired and utilized by plants. Our threshold of N addition rate was lower than that in the previous studies (5–10 g N m^{-2} yr^{-1} ; Bai et al., 2010; Cui et al., 2022), probably because N return by livestock urine and excrement and atmospheric N deposition is not accounted for analysis (Wang, Wu, et al., 2021; Wang, Zhang, et al., 2021). Meanwhile, the increased magnitude of multifunctionality was lower at the higher N levels (Figure 4). The reason is likely that the constant input of N may shift N to P limitation, which is often observed in many temperate biomes (Cui et al., 2022). Supporting this idea, we find that N addition had strong positive effects on CWM_N than on CWM_p (Figure 3e, f), suggesting that the reduced increase rates of EMF may be attributed to a result of inadequate P under excessive N fertilization.

Global grasslands may experience various degrees of ecosystem function deterioration due to intensive livestock grazing and nitrogen deposition (Herrero-Jáuregui & Oesterheld, 2018). It has been shown that grazing affects the response of soil carbon processes to nitrogen addition (Li et al., 2018). We show that grazing increased the EMF at a

low level of 5 g N m⁻² yr⁻¹ or less, but did not change or even decrease EMF at the higher N rates (Figure 4). These results partly objected to our second hypothesis, that higher N fertilization rates can weaken positive influences of grazing on EMF. In the non-grazed areas, EMF increased with increasing N addition rate, while the optimum N supplement level related to the highest EMF was detected at a low N supplement level (~5 g N m⁻² yr⁻¹) with a light stocking rate of 2.7 sheep ha⁻¹, indicating that less N may retain the higher capacity of the ecosystem to provide multiple functions under grazing (Figure 4). Inconsistent with results of previous studies that grazing and nitrogen addition have antagonistic effects on ecosystem functions (Li et al., 2018; Shi et al., 2019), we reveal that low N addition rate and stocking rate had synergetic effects on ecosystem functions and integrated EMF, but an antagonistic effect was detected as N addition rate and stocking rate increased. This is evidenced by the decline in certain ecological parameters (e.g., SAN, STN, and AGB) integrated with EMF under intensive livestock grazing and nitrogen addition (Figure 3a, b, d, f). These are mainly because excess N inputs and overgrazing may result in the homogenization of soil nutrients (Eldridge et al., 2020) and inadequate P, which could further affect soil processes, microbial community structure and enzymatic activities (Wang, Wu, et al., 2021; Wang, Zhang, et al., 2021) and result in decreasing EMF. In addition, both grazing and N addition increased CWM_N, which further promotes food palatability for grazing animals and increases the removal of plant biomass, resulting in lower EMF. The low N addition rate could be compensated for the negative effects of removal of biomass by animals by increasing N availability, and it may not change nutrient-limitation status and exacerbate competition for light compared to high N addition levels. Taken together, excessive N input may strengthen grazing-associated negative effects on EMF. Hence, the lower stocking rate (~2.7 sheep ha⁻¹) with a lower N supplement level (~5 g N m⁻² yr⁻¹) is optimum for grassland management to maintain multiple ecosystem functions in the study region.

4.2 | Plant α - and β -diversity dominating multifunctionality in response to grazing and N addition

The predominance of plant α -diversity in sustaining multifunctionality has been well documented (Gamfeldt & Roger, 2017; Isbell et al., 2011). Many studies report that plant α -diversity plays positive roles in regulating EMF (e.g., Byrnes et al., 2014; Meyer et al., 2018). In contrast, we show that plant α -diversity had significant negative relationships with EMF under grazing and N addition (Figure 6). This is probably because grazing and N addition may decrease plant α -diversity, and increase the overwhelming effect of dominant species on ecosystem functioning (Garnier et al., 2004; Le Bagousse-Pinguet et al., 2019). On the one hand, small herbivores like sheep are capable of reducing plant α -diversity (Kleynhans et al., 2011; Olofsson et al., 2004) because their predation on rare species (Zhang et al., 2022) would increase the dominant species throughout the

landscapes. Supporting the notion that in dry and less productive grasslands, there is a negative link between diversity and disturbance (Olff & Ritchie, 1998). On the other hand, N addition alleviates N-limitation of plant, and benefits dominant *Gramineae* (i.e., *Stipa bungeana* in our study) (Midolo et al., 2019), resulting in low plant α -diversity but higher EMF. Supporting this idea, Le Bagousse-Pinguet et al. (2019) also report that mass ratio effects (i.e., the effect of plant species on ecosystem functioning is directly proportional to their biomass) induced by dominant species contribute more to the changes in plant aboveground functions (i.e., AGB, CWM_N and CWM_P in our study) and better explained C, N, and P cycling separately. We show that increasing N addition at the highest stocking rate of 8.7 sheep ha⁻¹ induced higher plant α -diversity (Figure 2e), indicating that higher N fertilization rates can weaken negative influences of grazing on plant α -diversity. However, the decrease in dominant species may result in lower EMF (Figure S5).

Plant β -diversity is important for maintaining a single ecosystem function, and even more important in the context of multifunctionality (Hautier et al., 2018; Mori et al., 2016; Pasari et al., 2013). The positive effect of β -diversity on multifunctionality was likely due to the fact that a higher β -diversity can enhance ecological interactions and exchanges among local communities, such as the movement of organisms and resources, which are important for ecosystem functioning (Hautier et al., 2018). Our results show that grazing significantly decreased plant β -diversity and thus reduced EMF (Figure 6). Because grazing may reduce spatial asynchrony and lead to the homogenization of plants (Liang et al., 2021; Wang & Loreau, 2014), which results in the ecosystem functions loss. Similarly, functionally important species cannot maintain functioning across landscapes (Hautier et al., 2018). Moreover, grazing-induced aggravating soil resource limitation and competition for belowground resources may reduce spatial heterogeneity of plant community (Molina et al., 2021). Supporting this idea, we also detected significant relationships between SM, STN and plant β -diversity under grazing (Figures S3, S6b). Liu et al. (2021) report the N addition increases the stochasticity of community assembly and elevates plant β -diversity. In contrast, O'Connor et al. (2015) demonstrate that nutrient enrichment reduces plant β -diversity due to an increase in biotic homogenization. In our study, the relatively stable plant β -diversity under N addition may be attributed to the balance of stochastic and deterministic processes in community succession.

The analyses of SEM show that the indirect effects of herbivores on the response of EMF to plant β -diversity is less important than the direct grazing effects. This is probably because herbivores are most likely to have a significant influence on plant quality by rapidly returning easily N accessibility from urine and excrement and altering the soil microenvironment (Li et al., 2021; Yang et al., 2019). Compared with grazing, N addition mainly affected EMF indirectly through plant α -diversity-mediated effects. Our results indicate that N addition induced plant-diversity-mediated EMF mainly at small scales (α scales), while herbivore-regulated EMF at the larger spatial scales (β scales).

4.3 | Soil microbial stoichiometry mediating changes in multifunctionality under grazing and N addition

Soil microbial C:N:P ratios play a critical role in shaping microbial community structure and soil nutrient cycling processes (Chen et al., 2019; Yang et al., 2022). We show that soil microbial C:N functioned as an important factor regulating the changes in EMF (Figures 5, 6). Previous studies have shown that the higher microbial C:N ratio can result in greater input of C-rich structural cellular materials and higher C decomposition capacity, while lower microbial C:N ratio could inhibit soil N accumulation and boost N mineralization (Delgado-Baquerizo et al., 2017). In our study, the positive relationship between soil microbial C:N ratio and EMF indicates that higher soil microbial C:N ratio may increase C inputs to soil, stimulate microbial community, and further improve soil biochemistry processes and EMF in grassland ecosystems. An N addition-induced decrease in plant α -diversity significantly increased soil microbial C:N ratio and thus increased EMF (Figure 6), which was contrary to our hypothesis. This may be because N addition may increase above-ground biomass of dominant species and modify the diversity and composition of soil microbial communities (i.e., increasing fungi to bacteria ratio), consequently promoting C cycling rate and increasing EMF (Chen et al., 2019). For example, N addition may decrease the relative biomass of *Leguminosae* but increase that of *Gramineae*, increasing fungi to bacteria ratio and soil microbial C:N, because fungi tend to have a higher C:N ratio than bacteria (Dassen et al., 2017; Zhong et al., 2020). We also observed the highest soil microbial C and C:N ratio in N5 with a stocking rate of 2.7 sheep ha⁻¹, which are critical to maintain multiple ecosystem functions in grasslands. Our results highlight the role of soil microbe in maintaining higher grassland multifunctionality, further work should include soil microbial functions (i.e., C and N transformation) in ecosystems.

5 | CONCLUSIONS

This study provides important insight into EMF maintenance through grazing management and N enrichment in a semiarid steppe. We show that (1) both grazing and N enrichment increase EMF, while an increase of both stocking rate and N addition rate directly decreases EMF in a typical steppe. Our findings suggest that N supplementation at an N addition rate of 5 g N m⁻² yr⁻¹ under a grazing intensity of 2.7 sheep ha⁻¹ could maintain the most important ecosystem functions and services; and (2) plant α - and β -diversity, and soil microbial C:N ratio are the predominant drivers of EMF changes. Grazing decreases EMF indirectly via reducing plant β -diversity, while N addition increases EMF indirectly via decreasing plant α -diversity and its negative effects on soil microbial C:N ratio. Our results show that the negative effects of herbivore on EMF are stronger at the larger spatial scales than at the smaller local ones, while nutrient addition could maintain a high level of EMF at the smaller local scales than at the larger ones. Our results suggest that multiple spatial scales should be

considered to fully unravel the effects of eutrophication and herbivore on ecosystem functions.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Lan Li  <https://orcid.org/0000-0003-0577-8922>

Zhen Wang  <https://orcid.org/0000-0002-9523-3303>

Fujiang Hou  <https://orcid.org/0000-0002-5368-7147>

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SUPPORTING INFORMATION

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

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