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Contrasting responses of nitrogen: Phosphorus stoichiometry in plants and soils under grazing: A global meta-analysis

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Abstract

1. Grazing by ungulate herbivores can greatly alter nitrogen ([N]) and phosphorus ([P]) concentrations in plants and soils. It is not clear, however, how grazing might affect N:P co-limitation in grasslands depending on soil N and P availability.

2. Here we selected 173 peer-reviewed studies, which measured 12 key variables associated with changes in [N], [P] and N:P ratios (i.e. N:P stoichiometry) in soils and plants in the presence or absence of herbivore grazing. Subsequently, we addressed the magnitude and direction of grazing effects on these variables using a meta-analysis approach.

3. Grazing increased leaf [N] and [P] but decreased total and available soil [N] and [P]. Grazing also increased leaf N:P ratios while decreasing root and total soil N:P ratios.

4. The response ratio (RR) of leaf N:P was negatively correlated with RR of plant-available soil [P] and positively correlated with RR of available soil N:P ratio (rather than with RR of total soil N:P).

5. Intensive grazing (e.g. heavy grazing or long-term grazing) had in general more positive effects on plant N:P stoichiometry and negative effects on soil N:P stoichiometry than light grazing. Responses of plant-soil N:P stoichiometry to grazing greatly varied depending on plant functional group identity, plant organizational level (i.e. species and community) and grassland type.

6. *Synthesis and applications*. Our study suggests that understanding changes in available soil N:P stoichiometry (rather than total soil N:P) in response to grazing is crucial to predict nutrient co-limitation in grassland biomes. Our findings show that P is more important for plant growth than generally thought due to greater reduction of plant-available soil [P] under grazing. A better mechanistic understanding of the relationships between plant and available soil N:P stoichiometry under grazing will greatly help improve the sustainability of natural and semi-natural grassland ecosystems.

KEYWORDS
grassland type, grazing duration, grazing intensity, grazing regime, herbivore assemblage, nitrogen: phosphorus stoichiometry, plant functional group, plant-available soil phosphorus

Rui-Peng Yu and Wei-Ping Zhang contributed equally to this work.

1 | INTRODUCTION

Grazing animals can profoundly affect the ecology and functioning of grassland ecosystems world-wide through direct effects on plant community structure and species composition (Augustine et al., 2019; Milchunas & Lauenroth, 1993). The modification of grassland habitats by grazers often also leads to significant indirect effects on cycling and storage of key elements such as carbon (C), nitrogen (N) and phosphorus (P) (Bai et al., 2012; Ritchie et al., 1998). These potential ecosystem-level impacts of animal grazers could be revealed by changes in the relative abundance of C, N and P (i.e. C:N:P element stoichiometry) in plants and soils (Güsewell, 2004; He et al., 2020; Sterner & Elser, 2002). Previous studies show, for example, significant changes in C:N stoichiometry and ecosystem C and N cycling in response to grazing (Wang et al., 2016; Zhou et al., 2017). However, less attention has been paid to changes in N:P stoichiometry in plants and soils under different grazing intensities and regimes. In particular, it is not clear whether and how shifts in N:P co-limitation may occur in grazed grasslands depending on the prevailing levels of soil N and P availability.

Both negative and positive changes of plant and soil [N] and [P] in grassland ecosystems in response to grazing have been reported in previous studies (e.g. Anderson et al., 2007; Bai et al., 2012; Wang et al., 2016). Similar studies have also showed either positive (Frank, 2008), neutral (Yang et al., 2019) or negative (Chaneton et al., 1996) effects of grazing on plant or total soil N:P ratios. We argue, however, that plant growth is regulated to a large extent by the availability of key soil nutrients (Marschner, 2011). Therefore, changes in available soil nutrient concentrations and/or changes in the ratio of bioavailable soil N and P forms (rather than total soil N and P) could be more important for plant growth with far-reaching implications for ecosystem-level impacts of grazing. Limited studies have reported the effect of available soil N: P ratio on plant growth (Ågren et al., 2012), while no study has been conducted on the relationship between plant and available soil N:P ratios under grazing. Therefore, integrating and linking N:P ratios of plant and soil can help better understanding N:P co-limitation and thus nutrient cycling in grazed grassland ecosystems.

Contradictory responses of plant and soil N:P stoichiometry to grazing may be explained by differences in grazing intensity and duration, herbivore assemblage and grassland type. For example, heavy grazing tends to have more negative effects on total and available soil [N] than moderate grazing does (Fuhlendorf et al., 2002). The effects of a given grazing pattern on plant and soil N:P stoichiometry also vary across grassland types, for instance, heavy grazing tends to decrease total soil N:P in the typical steppe but increase N:P in the meadow steppe (Bai et al., 2012). Exploring the potential responses of N:P ratios to a given grazing pattern across different grassland types is crucial to assess and understand contradictory results among individual studies and to suggest better grazing management strategies.

Grazing by livestock and wildlife can also have different effects on plant and soil N:P stoichiometry (Frank, 2008; Liu et al., 2016; Ngatia et al., 2015). However, previous meta-analysis studies have not examined potential differences in the response of N:P stoichiometry to livestock and wildlife grazing. Other key factors that could affect N:P stoichiometry include differences in grazing duration (Wang et al., 2014), grazing regimes (i.e. continuous grazing and rotational grazing; Mathews et al., 1994), plant functional group identity (Chaneton et al., 1996), plant organizational levels (i.e. species vs. community; Zheng et al., 2012) and other environmental factors including soil depth (Zhou et al., 2017).

The overall responses of plant and soil N:P stoichiometry to animal grazing addressed in previous individual studies are associated with a great deal of uncertainty. There is thus the need to study potential general patterns of N:P stoichiometry across different grazing systems and environmental conditions through a meta-analysis approach (Hedges et al., 1999).

In this study, a meta-analysis was conducted on 3,482 paired comparisons (i.e. grazing vs. grazing exclusion) from 173 experimental studies globally to evaluate the response of plant and soil N:P stoichiometry to animal grazing. Specifically, the key objectives of this study were to (a) examine the response of twelve variables associated with changes in plant and soil N:P stoichiometry to grazing, (b) explore the correlation among the response ratios of plant and soil N:P stoichiometry as affected by different environmental factors, (c) investigate whether and how grazing intensity, duration, regime, herbivore assemblage, grassland type and other environmental factors might affect the responses of plant and soil N:P stoichiometry to grazing.

2 | MATERIALS AND METHODS

2.1 | Data compilation

We collected data from peer-reviewed studies that quantified changes in plant and soil [N], [P] or N:P ratios in response to ungulate grazing. We searched Web of Science (http://apps.webofknowledge.com), Google Scholar and China National Knowledge Infrastructure (CNKI) using the following keywords: 'grazing or grazer or grazed or ungulate or herbivore or degraded or degradation' and 'nitrogen or phosphorus'. We supplemented this database by searching more general keywords, for example, 'stocking rate', 'defoliation', 'grassland' and 'plant and soil nutrient'.

Five criteria were further used to select peer-reviewed publications including: (a) plant species must be vascular plants; (b) ungulate herbivore species must belong to Artiodactyla and Perissodactyla taxa; (c) studies were carried out in rangeland ecosystems (including grasslands and pastures) that directly compared plant and/or soil nutrient in response to grazing and grazing exclusion without other experimental treatments (e.g. warming, sowing, fertilizer, mowing or fire); (d) mean value, standard error (*SE*) or standard deviation (*SD*) and the sample size were given in the original studies; (e) plant and soil were sampled at the peak period of grass biomass production, as sampling time can affect plant and soil nutrient status (Kleinebecker et al., 2011). One hundred and seventy-three peer-reviewed publications in 142
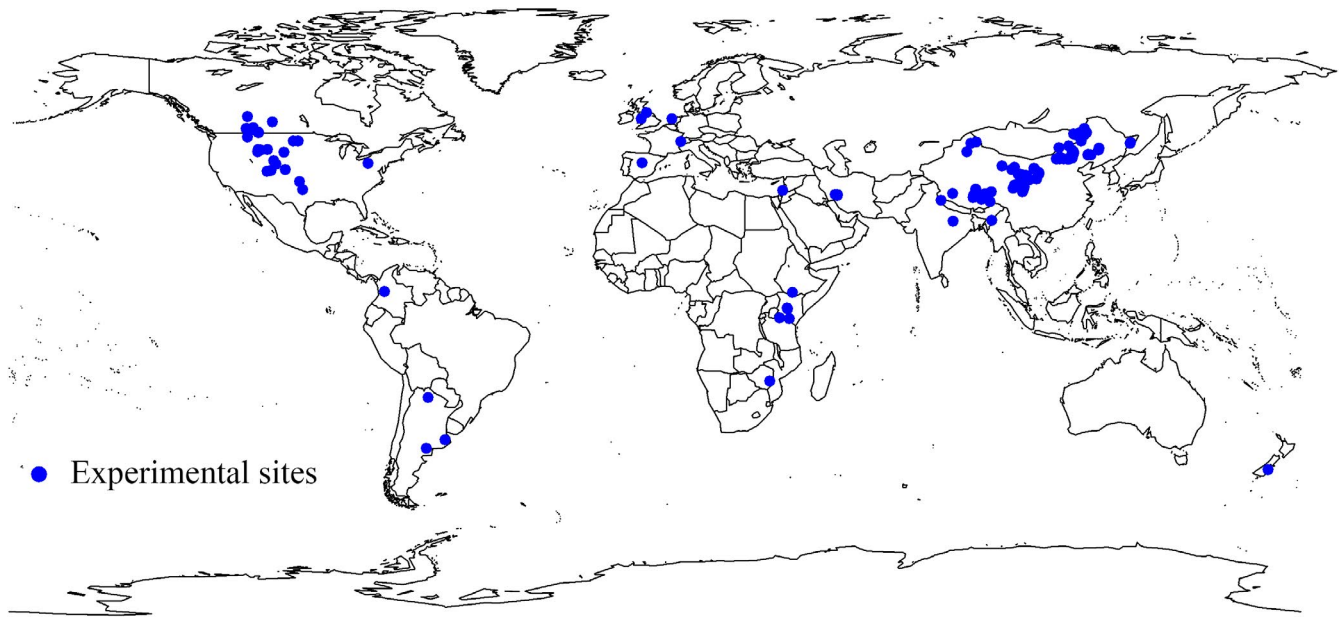
FIGURE 1 Global distribution of 173 studies and 142 experiments sites included in the meta-analysis

experimental sites finally complied with our selection criteria (Figure 1; Appendix S1).

Twelve variables related to plant and soil N and P nutrient concentrations and ratios were included in our database, namely (1) leaf [N], (2) leaf [P], (3) leaf N:P ratio, (4) root [N], (5) root [P], (6) root N:P ratio, (7) total soil [N], (8) available soil [N], (9) total soil [P], (10) available soil [P], (11) total soil N:P ratio, (12) available soil N:P ratio. Plant and total soil N:P ratios were calculated as [N] divided by [P] if N:P ratios were not reported in the original literature. Available soil N:P ratio was calculated as available [N] divided by available [P] since no study included in this meta-analysis has reported the response of available soil N:P ratio to grazing. If the original literature provided nitrate and ammonium concentration, available soil [N] was defined as the sum of these two inorganic N forms; available soil [P] was defined as soil Olsen P or resin P concentration in the original literature. Soil nutrient concentration was calculated using data on nutrient stocks, soil bulk density and sampling depth given in the primary literature (Lyu et al., 2015). For each variable, the mean value, *SE* or *SD* and sample size were extracted in both grazing and no grazing treatments. All data used in this study were extracted from tables or figures from the published papers using the software GetData Graph Digitizer 2.24 software (http://getdata-graph-digitizer.com). Environmental variables including elevation, mean annual precipitation (MAP), mean annual temperature (MAT) were also recorded directly from published papers.

We examined the effect of grazing intensity, duration and regime (i.e. continuous grazing and rotational grazing), herbivore assemblage, functional group, plant organizational level (i.e. plant species and community levels), grassland type and soil depth on plant and soil N:P stoichiometry. Grazing intensities were grouped into light grazing (LG), moderate grazing (MG), heavy grazing (HG) and overgrazing (OG) according to the original study. Grazing duration was

partitioned into short-term grazing (1–10 years) and long-term grazing (>10 years). Herbivore assemblage included grazing by sheep or goat, cattle or yak, mixed grazing (including sheep, cattle, or horse) and wild ungulates. Plant functional groups were classified as perennial bunchgrass (PB), perennial rhizome grass (PR), perennial forb (PF), shrub and semi shrub (SS), annuals and biennial (AB) according to life forms (Bai et al., 2012). Soil depth was extracted from the primary paper and divided into topsoil (0–20 cm), sub-topsoil (20–60 cm) and deep soil (>60 cm).

Grassland types were classified as temperate grassland (including steppe, prairie and pampas), savanna and alpine grassland based on climatic factors and grassland biome (Whittaker, 1975). The Eurasian steppe, a subgroup of temperate grassland, is a widespread and productive grassland type with great ecological importance and historical grazing custom (Bai et al., 2012). Therefore, the steppe was further classified into the desert steppe, typical steppe and meadow steppe according to climatic factors and dominant plant species. To better understand the inconsistent results among individual studies, we further examined the effect of a given grazing pattern (i.e. grazing intensity, duration, regime and herbivore assemblage) on plant and soil N:P stoichiometry across different grassland types.

2.2 | Statistical analysis

A weighted response ratio approach was used to conduct the meta-analysis (Hedges et al., 1999; Luo et al., 2006). Animal grazing was regarded as treatment and grazing exclusion as control. For each variable, the mean value, *SE* or *SD* and sample size (*n*) in both grazing and no grazing treatment were used for calculation. Response ratio (RR) is an index of the effect of grazing, which was calculated as the logarithm of the ratio between the mean value of grazing treatment

(X_t) and exclosure control (X_c) for each variable (Equation 1). The variance of RR (v), the weighted response ratio (RR_{++}), the weighting factor of each RR ($w = 1/v$), standard error of RR_{++} ($s_{RR_{++}}$), 95% CI of RR_{++} and the percentage change in each variable was calculated as shown in Equations 2–6.

$$RR = \ln \frac{\overline{X_t}}{\overline{X_c}}, \tag{1}$$

$$v = \frac{SD_t^2}{n_t \overline{X_t}^2} + \frac{SD_c^2}{n_c \overline{X_c}^2}, \tag{2}$$

$$RR_{++} = \frac{\sum_{i=1}^{m} \sum_{j=1}^{k_i} w_{ij} RR_{ij}}{\sum_{i=1}^{m} \sum_{j=1}^{k_i} w_{ij}}, \tag{3}$$

$$s_{RR_{++}} = \sqrt{\frac{1}{\sum_{i=1}^{m} \sum_{j=1}^{k_i} w_{ij}}}, \tag{4}$$

$$95\% \, CI = RR_{++} \pm 1.96 \, s_{RR_{++}}, \tag{5}$$

$$Percentage \, change = \left(e^{RR_{++}} - 1\right) \times 100\%. \tag{6}$$

In cases where no SE, SD or confidence intervals (CI) were reported, we assigned SD as 1/10 of the mean value (Luo et al., 2006). If the 95% CI of the RR_{++} of a variable overlapped with zero, the effect of grazing on the variable was not considered significant between two treatments (Luo et al., 2006). Otherwise, grazing was considered causing a significant difference in the value of that specific variable. Z-tests were performed to examine potential differences among levels of a given explanatory variable (e.g. grazing intensity) in BSDA package. We estimated the potential publication bias using funnel plots and Kendall rank correlation coefficient (Kendall's Tau, Duval & Tweedie, 2000). Pearson correlation analyses were performed to test potential relationships among environmental factors and the RR of twelve variables associated with plant and soil N:P

stoichiometry. All the statistical analyses were performed in R version 3.6.0 (R Development Core Team, 2019).

3 | RESULTS

3.1 | Mean effect size of plant and soil N:P stoichiometry in response to grazing

We did not find any publication bias across all the twelve N and P variables included in our meta-analysis (Figure S1). The mean RR_{++} of eleven variables were significantly different from zero, except for available soil N:P ratio (Figure 2). Grazing increased RR_{++} of leaf [N], [P], N:P ratio by 16%, 15% and 6% respectively. RR_{++} values of the root [N] and [P] increased each by 4% in response to grazing, whereas root N:P ratio decreased by 3% in response to grazing. Mean effect sizes of soil total [N], total [P], available [N], available [P] and total N:P ratio were significantly lower than zero and decreased by 7%, 5%, 2%, 5% and 2% respectively.

The RR_{++} of soil N and P stoichiometry was dependent on soil depth (Figure S2). Grazing disturbance had greater negative effects on total [N], available [N] and total N:P ratios of deep soils compared to the top and sub-topsoil. In contrast, total soil [P] decreased in the topsoil. However, grazing had a positive effect on available soil [N] in topsoil and available soil [P] exhibited less negative effect in response to grazing in the topsoil.

3.2 | Correlations among response ratio of plant and soil N:P stoichiometry and with environmental factors

The response ratio (RR) of most plant and soil variables to grazing was significantly correlated with environmental factors (Figure 3). Elevation and MAT were negatively related to the RR of leaf [N],

FIGURE 2 The weighted response ratio (RR_{++}) of twelve variables related to changes in N and P within plant tissues and soils in response to grazing. Bars represent 95% confidence intervals of RR_{++}. The vertical line is drawn at $\log_e RR = 0$. The sample size for each indicator is shown next to the bar
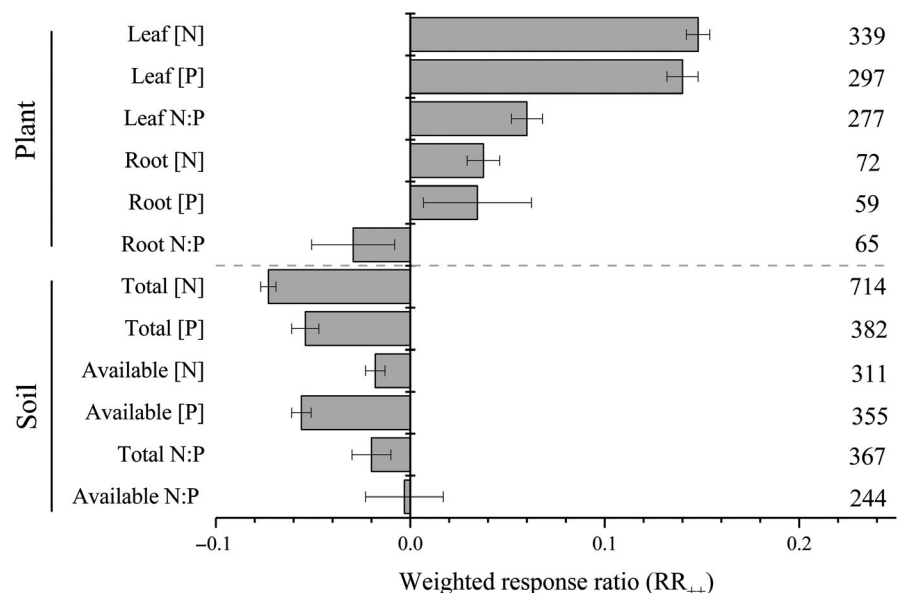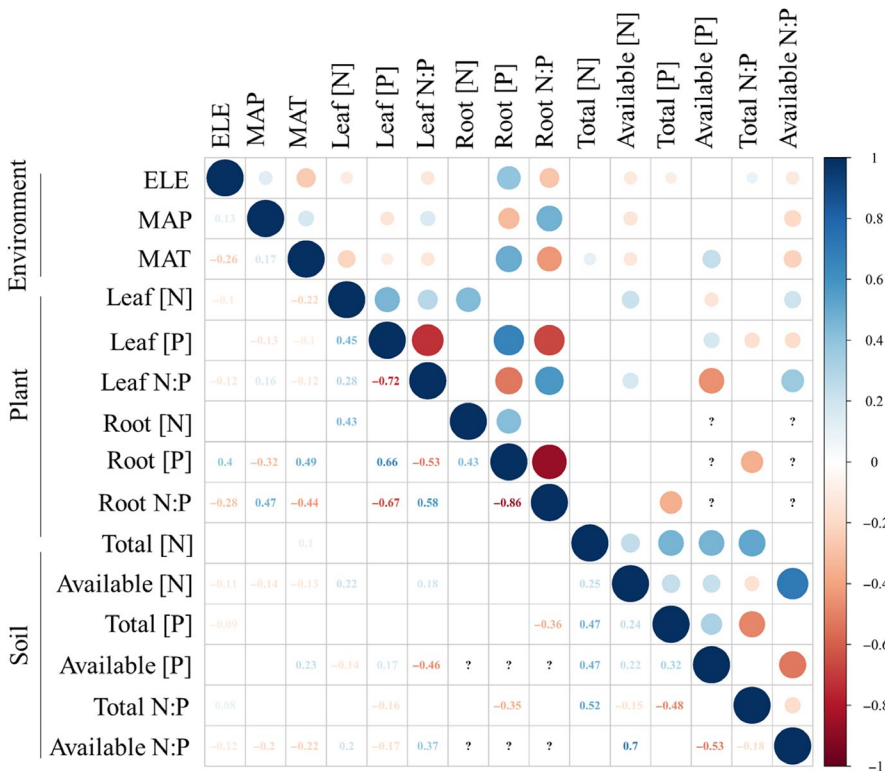
FIGURE 3 Pearson correlation matrix of potential relationships between key environmental factors and the response ratio of twelve variables related to changes in N and P in plant tissues and soils. Significant correlation values considered at $p < 0.05$. Circles and values in blue and red represent positive and negative correlations respectively. '?' means no enough data for correlation. ELE, elevation; MAP, mean annual precipitation; MAT, mean annual temperature

leaf and root N:P ratio but positively correlated with root [P]. Environmental factors exhibited a negative correlation with RR of available soil [N] and available soil N:P ratio. MAP was positively correlated with RR of leaf and root N:P ratio, and negatively correlated with the RR of leaf and root [P]. MAT was positively related to total soil [N] and available [P], while being negatively correlated with leaf [P].

We also examined the relationships among RR of response variables (Figure 3). RR of leaf N:P ratio, total soil N:P ratio and available N:P ratio covaried with changes in [N] and [P] in response to grazing; while RR of root N:P ratio covaried with RR of root [P]. RR of leaf [N] and [P] were positively correlated with RR of available soil [N] and [P] but not with RR of total soil [N] and [P]. RR of [N] and [P] in each compartment of plant and soil covaried in response to grazing. Specifically, the RR of leaf N:P ratio positively correlated with RR of root N:P ratio, available soil [N] and N:P ratio, while being negatively correlated with RR of available soil [P] in response to grazing.

3.3 | Effect of grazing pattern on plant and soil N:P stoichiometry

Leaf [N], [P] and root [N] exhibited a greater increase under intensive grazing than under light grazing (Figure 4a). All grazing intensities enhanced leaf N:P ratios except for light grazing. Root N:P ratio increased under light grazing but decreased under moderate and heavy grazing treatments. The RR_{++} of soil [N] and [P] decreased under all grazing intensities, except for total soil [N],

available soil [N] and [P] under light grazing. Total soil [N] and available soil [P] decreased with increasing grazing intensities. Overgrazing induced the greatest negative effects on soil total [P], total N:P ratio and available N:P ratio compared with other grazing intensities.

Grazing duration significantly affected most variables with the exception of the root [P] and root N:P ratio, which neither revealed significant differences from zero nor between the grazing durations evaluated (Figure 4b). Long-term grazing generally had a greater effect on leaf and soil N:P stoichiometry than short-term grazing, except for available soil [P]. Mixed livestock grazing had greater positive effects on leaf and soil N:P ratios and negative effect on soil nutrient concentration than cattle or sheep grazing alone and wildlife (Figure 4c). Wildlife grazing increased total soil [P], available [N] and [P] and decreased total soil N:P ratio compared to livestock grazing.

Compared to continuous grazing, rotational grazing did not significantly affect leaf [N], root [P], leaf and root N:P ratio (Figure 4d). For total soil [N], available soil [N], [P] and total soil N:P ratio, there were both significant differences from zero and between continuous and rotational grazing.

3.4 | Differential responses of plant N:P stoichiometry under grazing across functional groups and plant organizational levels

Grazing increased leaf [N], [P] and N:P ratio of most functional groups, with the exception of annuals and biennials (leaf [N]) and

FIGURE 4 The weighted response ratio of twelve variables related to changes in N and P within plant tissues and soils in response to (A) grazing intensity, (B) grazing duration, (C) herbivore assemblage, and (D) grazing regime. Bars represent 95% confidence intervals of RR_{++}. The vertical line is drawn at $\log_e RR = 0$. The sample size for each indicator is shown next to the bar. The same letter means there is no significant difference among explanatory variables for a given response variable (Z-test)
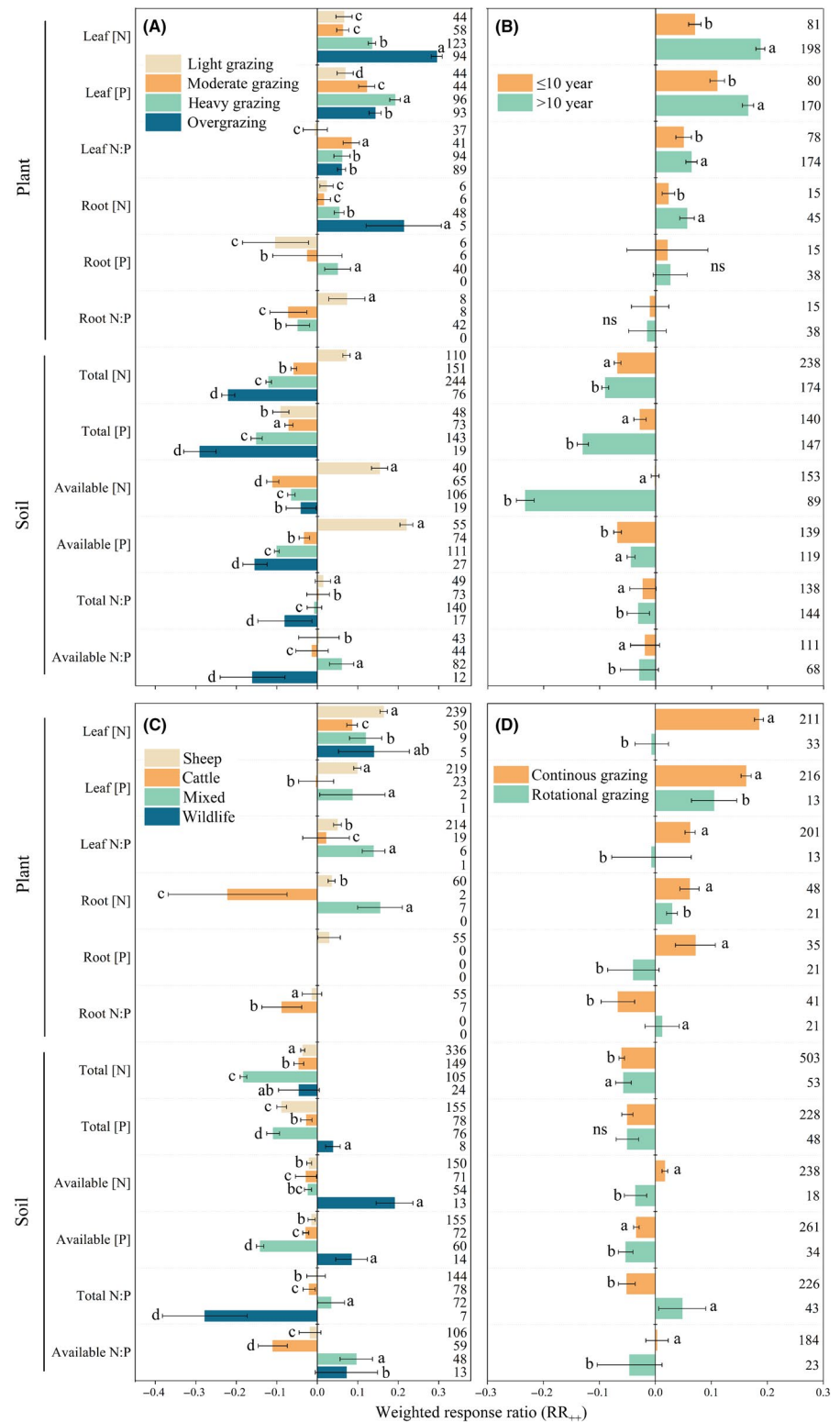
perennial rhizome grass (leaf N:P ratio; Figure 5a). Perennial rhizome grass exhibited greater leaf and root [N], root [P] and lower root N:P ratios in response to grazing when compared with other functional groups. Root [N] of perennial forb increased under grazing, while root N:P ratio of perennial bunchgrass and perennial rhizome grass decreased under grazing.

Plant N:P stoichiometry at species and community levels showed different responses to grazing (Figure 5b). Grazing increased leaf and root [N] at species and community levels; leaf [P] at the species level and root [P] at the community level. Grazing decreased leaf [P] at the community level, which thus increased community-level leaf N:P ratio by 19%.
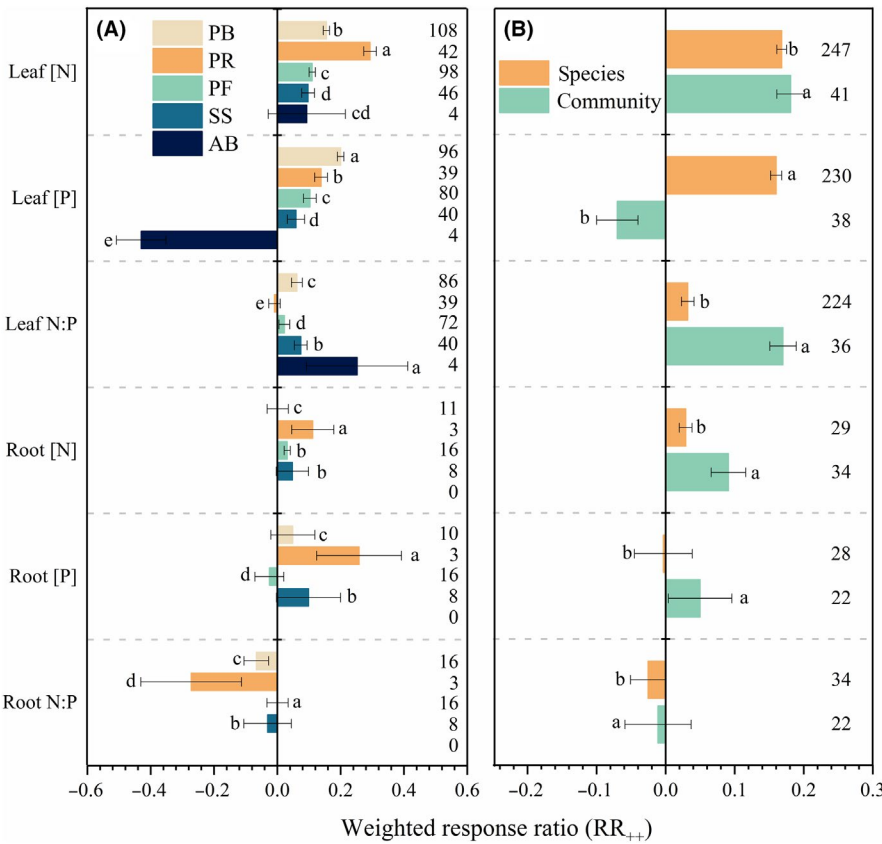
FIGURE 5 The weighted response ratio of six variable-related changes in N and P in plant tissues in response to (A) functional group and (B) organizational level (i.e. species vs. community level). PB, perennial bunchgrass; PR, perennial rhizome grass; PF, perennial forb; SS, shrub and semi shrub; AB, annuals and biennials. See Figure 4 for the definition of numbers and letters
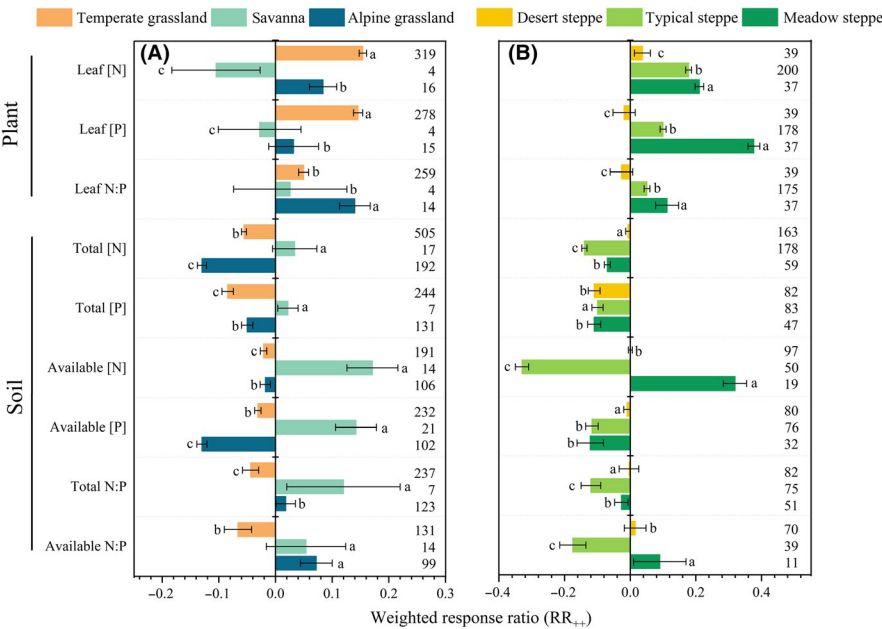

FIGURE 6 The weighted response ratio of nine variables related changes in N and P in leaf and soil variables among (A) temperate grassland, savanna, and alpine grassland based on climatic factors and grassland biome (Whittaker, 1975) and (B) desert steppe, typical steppe, and meadow steppe according to climatic factors and dominant plant species in the Eurasian steppe (Bai et al., 2012). See Figure 4 for the definition of numbers and letters

3.5 | Effect of grazing on plant and soil N:P stoichiometry is dependent on grassland types

Grazing increased leaf [N], [P] and N:P ratio and simultaneously decreased soil [N] and [P] concentrations in temperate (including steppe, prairie and pampas as a subgroup) and alpine grasslands (Figure 6a). However, leaf and soil [N] and [P] in savanna exhibited different trends compared to temperate and alpine grasslands. Steppe ecosystem was further classified into the desert steppe, typical steppe and meadow steppe. Leaf and soil stoichiometry in typical steppe and meadow steppe exhibited similar patterns except for available soil [N] and N:P ratio (Figure 6b). Most variables in the desert steppe were less affected by grazing than those in the typical steppe and meadow steppe.

Total soil N:P ratio decreased in temperate grassland, typical steppe and meadow steppe, while it increased in savanna and alpine grassland under grazing. Grazing increased available soil N:P ratio in alpine grassland and meadow steppe but decreased that in temperate grassland and typical steppe.

To suggest better grazing management strategies across different grassland types, we further examined the effect of different grazing patterns (i.e. grazing intensity, duration, regime and herbivore assemblage) on leaf and soil N:P stoichiometry in the desert steppe, typical steppe, meadow steppe and alpine grassland where sufficient data were available (Table S1). Responses of leaf and soil N:P ratios to a given grazing pattern varied in different grassland types, for example, long-term grazing increased leaf and available soil N:P ratio in the meadow steppe, but these variables did not change in the desert steppe.

4 | DISCUSSION

4.1 | Grazing-induced changes in plant N:P co-limitation

We found that ungulate grazing significantly increased leaf and root [N], [P] as well as leaf N:P ratios, and that the magnitude of these effects is dependent on grazing intensity and duration, grazing regime (i.e. continuous vs. rotational) and herbivore assemblage (Figures 2 and 4). Increases in leaf nutrient concentrations could be related to plant compensatory growth responses to defoliation by grazers (McNaughton, 1983; Milchunas & Lauenroth, 1993). Improved plant nutrient concentrations may also result from increased root-shoot ratios. For example, increased C allocation to roots improves plant water status and stomatal conductance thus supporting photosynthetic rates and maintaining relatively high nutrient concentration in leaves and roots (Chaneton et al., 1996; McNaughton, 1983).

The magnitude of grazing effect on leaf N:P stoichiometry is greater at the plant community level compared to the species level (Figure 5b). Shifts in dominant species and functional groups composition could be a major mechanism driving plant community-level stoichiometric responses to grazing (Figure 7; Bai et al., 2012; Frank, 2008). For example, grazing can decrease the dominance of perennial bunchgrasses and rhizome grasses, and increase the dominance of annuals, biennials and perennial forbs (Bai et al., 2012). Findings from a recent study show that the increasing presence of grazing-tolerant forbs contributes to enhance community-level leaf N:P ratios from 11.8 in exclosures to 16.2 in overgrazed communities (Yu, Zhang, Yu, et al., 2020), which become more P limited (Koerselman & Meuleman, 1996). Our evidence is that grazing increased leaf N:P ratios of most functional groups suggesting that P
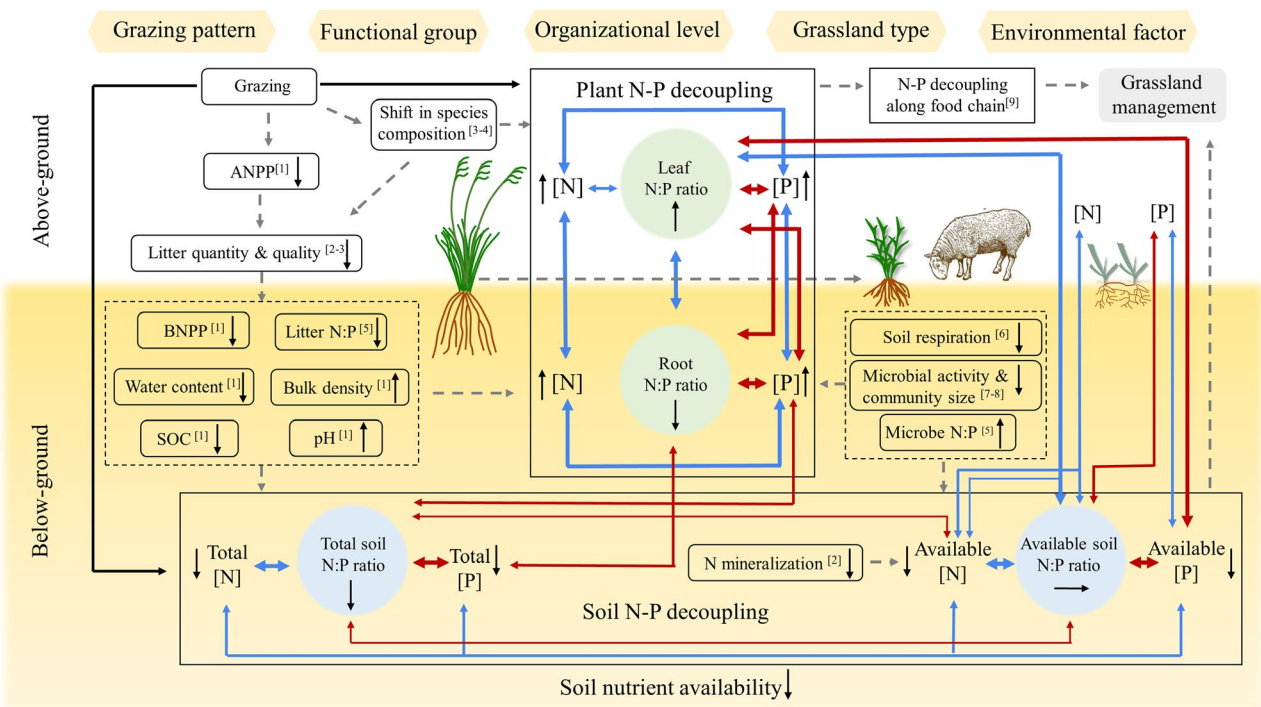

FIGURE 7 Framework of grazing effects on ecosystem processes controlling plant and soil nitrogen (N)-phosphorus (P) stoichiometry. Upward and downward black arrows indicate the positive and the negative effect of grazing on a given variable respectively. Blue and red arrows indicate positive and negative correlations between the response ratio of each variable based on Figure 3 ($p < 0.05$) respectively. Dashed arrows denote the potential underlying mechanisms based on previous literature studies. ANPP, above-ground net primary productivity; BNPP, below-ground net primary productivity; SOC, soil organic carbon. [1]Hao and He (2019), [2]Zhou et al. (2017), [3]Garibaldi et al. (2007), [4]Bai et al. (2012), [5]He et al. (2020), [6]Zhou et al. (2019), [7]Raiesi and Asadi (2006), [8]Zhao et al. (2017), [9]Sterner and Elser (2002)

could become more important for plant productivity at the community level (average leaf N:P ratio increased from 13.7 in exclosures to 15.6 under grazing, data not presented). These results agree with the view that community-level nutrient stoichiometric responses to grazing may be largely dependent on inherent nutrient use strategies of species or functional groups, which adapt to soil nutrient losses induced by grazing (Figure 5; Güsewell, 2004; Lyu et al., 2015; Niu et al., 2016).

4.2 | Grazing-induced changes in soil N:P stoichiometry

The results show that both total and available soil [N] and [P] were reduced under ungulate grazing (Figure 2). The potential mechanisms responsible for shifts in soil N:P stoichiometry in response to grazing are shown in Figure 7. First, because N cycling is closely coupled to C cycling, it could be that a decrease in total soil [N] results from reduced plant litter inputs (i.e. reduced C inputs to soil) following above-ground biomass removal by grazers (Figure 7; Zhou et al., 2017). Second, trampling by herbivores could increase soil compaction thus inhibiting microbial activity and soil respiration, which decreases the amount of available nutrients released into the soil and leads to soil nutrient losses (Neff et al., 2005; Raiesi & Asadi, 2006; Zhao et al., 2017; Zhou et al., 2019). In addition, grazing-induced shifts in species composition can alter litter decomposition and further reduce nutrient release into the soil (Garibaldi et al., 2007). The decrease in total soil [N] and [P] under grazing is consistent with the findings of a previous meta-analysis study (Hao & He, 2019). Our result shows that grazing decreases available soil [N] and [P], whereas Hao and He (2019) found that grazing increases available soil [N] and maintains available soil [P]. The differences in available soil nutrient responses to grazing between our meta-analysis and Hao and He (2019) could be possibly due to (a) variation in grazing patterns, (b) differences in the data source and sample size, and (c) nutrient responses to grazing at different soil depths.

The results show that available soil [N] increased in topsoils, but decreased in the sub- and deep soil under grazing (Figure S2). This suggests that the return of faeces from animal grazers may support soil biological processes in the rhizosphere in topsoils (Frank & Groffman, 1998), could lead to an increase in available soil [N] under grazing (Hao & He, 2019). The fact that RR_{++} of soil [N] and total N:P ratio dropped sharply with soil depth in response to grazing (Figure S2) may be related to grazing-induced effects on soil compaction and soil physical structure, which may limit nutrient flow to deeper soils, a potential mechanism that merits future research effort.

Grazing increased leaf N:P ratios while decreasing root and total soil N:P ratios (Figure 2), suggesting that plant nutrients may not covary with total soil nutrients in response to grazing (Yang et al., 2017). This is probably because different regulatory mechanisms in plants (Güsewell, 2004) may contribute to detach plant

and soil N-P stoichiometry. Also, plant nutrient status may be more closely associated with available soil [N] and [P] than with total soil [N] and [P] (Marschner, 2011), implying that N:P ratios between plant-available soil N and P forms (rather than total soil N:P ratios) may better predict changes in plant N:P co-limitation under grazing.

4.3 | Increases in leaf N:P ratio are related to losses of available soil [P] under grazing

Our study is the first to show that the response ratio (RR) of leaf N:P ratio is negatively correlated with the RR of available soil [P] and positively correlated with the RR of available soil [N] and available soil N:P ratio in response to grazing (Figure 3). This suggests that under grazing, plant species tend to experience greater P limitation because of the decrease in soil P availability. Reduced soil P availability may result from grazer-induced effects on soil compaction and plant root functioning. For example, plants acquire inorganic phosphate (Pi) through diffusion processes within the soil matrix (Marschner, 2011), where P mobility remains poor because most P is sorbed in soils (Hinsinger, 2001). Pi mobility can be reduced when trampling by grazers increases soil compaction and decreases soil water content (Hao & He, 2019) resulting in lower P availability to plant roots (Figure 7). Also, grazers can reduce root P acquisition by negatively affecting root morphological traits (e.g. specific root length) and rhizosphere processes (e.g. root-released phosphatase; Yu, Zhang, Yu, et al., 2020). Thus by inhibiting plant root functioning and increasing soil compaction, mammalian grazers can decrease soil P availability, which in turn can limit plant growth in grazed communities (Figure 7).

Our results support the findings of a previous meta-analysis study which shows positive relationships between response ratio of plant above-ground biomass and that of available soil [P], rather than available soil [N] (Hao & He, 2019). These findings suggest that P could be more important for plant N-P balance and productivity following changes of plant-available soil P under grazing. Previous studies mainly focused on the effect of grazing on total soil [N], [P] and N:P ratio, rather than on plant-available soil N:P stoichiometry (Hao & He, 2019; He et al., 2020). Our findings indicate that by addressing changes in available soil N:P stoichiometry, we could better understand potential plant N:P co-limitation in grazed ecosystems.

4.4 | Differential responses of leaf and soil N:P stoichiometry to grazing across grassland types

Leaf and soil N:P stoichiometry responses to grazing are different in magnitude and direction among the grassland types included in our meta-analysis (Figure 6). Herbivore–plant–soil microbial feedbacks can be responsible for changes in plant and soil nutrient stoichiometry under grazing. For example, leaf [N], [P] and available soil [N] increased in more productive meadow steppe ecosystems. This is because the

predominant plant functional groups (e.g. perennial rhizome grasses) are well adapted to grazing, and grazing stimulates positive feedbacks between C-rich root exudates and microbial activities accelerating N cycling (Bai et al., 2012; Bardgett & Wardle, 2003).

Environmental factors correlated with most of the variables, and this explains the observed differential responses of grassland types to grazing (Anderson et al., 2007; Bai et al., 2012; Rui et al., 2012). For example, we found negative relationships between MAT and RR of leaf and root N:P stoichiometry, while the RR of leaf and root N:P ratio positively correlated with MAP under grazing (Figure 3). MAT of alpine grassland in this meta-analysis was −0.1°C, which is lower than the MAT of other grasslands; consistently, plant species in alpine grassland ecosystems (with lower MAT and higher RR of leaf N:P ratio) tend to suffer from grazing-induced P limitation (average leaf N:P ratio was 19.9 under grazing, data not presented). This could be attributed to increased inhibition of soil microbial enzyme activities (e.g. phosphatase) under low temperatures (Rui et al., 2012). In addition, the RR of available soil [N] and N:P ratio decreased with increasing MAP, suggesting potential greater available soil N limitation in wetter regions due to N leaching. More studies are needed to address how climate change could mediate grazing impact on N:P stoichiometry and ecosystem functioning (Zhou et al., 2019; Figure 7).

4.5 | Implications for future grassland management

In terms of changes in grazing intensity, our results show that total soil [N], available soil [N] and [P] all increased under light grazing but significantly decreased under intensive grazing (Figure 4a). Light grazing contributes to increasing root exudates, which may further enhance N and P accumulation in soils. In contrast, heavy grazing decreases above-ground plant production, leading to reduced litter quantity and nutrient inputs to soils, which in turn negatively affect microbial biomass and microbial activities (Zhou et al., 2017). In addition, the effect of grazing intensity on leaf and soil N:P ratio is dependent on grassland types, for example, heavy grazing increased leaf N:P ratio and available soil N:P ratio in the meadow steppe, but no change was observed in the desert steppe (Table S1). These findings suggest that the management of grazing intensity based on N-P balance should be considered in different grassland types.

Long-term grazing had a greater effect on all plant and soil nutrient variables when compared to short-term grazing in most of the grassland types (Figure 4b; Table S1). This is possibly related to soil buffer ability and recovery capacity to maintain ecosystem functioning under consistent grazing disturbance (Hao & He, 2019; Raiesi & Asadi, 2006). The overall effects of mixed grazing on leaf and soil variables are generally more pronounced than those of individual sheep, cattle and wildlife grazing (Figure 4c; Table S1). This finding partly agrees with evidence from a previous meta-analysis (Hao & He, 2019) and could be explained by different diet selection among the grazing mammals (Frank, 2008; Liu et al., 2016). For example,

cattle are less selective on above-ground biomass removal, which may not be too detrimental to plant growth (Liu et al., 2016) or may not affect N:P ratios (Yang et al., 2019). However, mixed grazing by sheep and cattle increases utilization intensity of grassland biomass (Hao & He, 2019), with consequent grazing-induced negative effects on plant and soil nutrient cycling. In addition, wildlife grazing usually facilitates soil nutrient accumulation (i.e. positive feedback), while livestock grazing usually decreases nutrient availability (i.e. negative feedback, Ritchie et al., 1998), indicating that sustainable grazing systems should be managed based on availability of nutrient resources in space and time and according to specific feeding strategies within animal assemblages.

In general, P-limited grassland ecosystems are associated with higher plant species diversity and the presence of endangered plant species (Fujita et al., 2014). Animal grazing is likely to intensify P limitation as shown in our study. Therefore, sustainable grassland management has to balance animal production, N:P stoichiometry and the conservation of endangered plant species based on grassland type and soil P availability.

5 | CONCLUSIONS

Our meta-analysis shows how plant and soil N-P stoichiometry vary globally with predictable patterns, which are determined by grazing patterns, plant functional groups, plant organizational levels, grassland types and key environmental factors. Our findings provide evidence that grazing is responsible for decoupling plant and soil N:P stoichiometry in grassland ecosystems. Grazing increased leaf N:P ratio but reduced root and total soil N:P ratio. Response ratio (RR) of leaf N:P was strongly negatively correlated with RR of plant-available soil [P], suggesting that P can significantly affect plant growth when available soil [P] decreases under the effect of ungulate grazing. We suggest that understanding the unbalanced response of plant N:P and available soil N:P stoichiometry to grazing is crucial to improve the long-term sustainability of grazed ecosystems and inform future grassland management policies across different climatic regions world-wide.

AUTHORS' CONTRIBUTIONS

L.L., W.-P.Z. and R.-P.Y. designed the research; R.-P.Y. collected and analysed the data, R.-P.Y. and W.-P.Z. drafted the paper and all authors contributed substaintily to the revisions.

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