Mowing alters nitrogen effects on the community-level plant stoichiometry through shifting plant functional groups in a semi-arid grassland

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Abstract
Land-use practices such as mowing and nitrogen (N) fertilization can have significant impacts on plant stoichiometry. However, the interactive effects of mowing and N fertilization on the community-level plant stoichiometry and the underlying processes are not well understood. We examined the impacts of mowing (once a year) and N fertilization (12 g N m⁻² yr⁻¹) on the community-level plant stoichiometry in a semi-arid grassland on the Loess Plateau. Results obtained showed that mowing alone had no effect on the community-level plant N or phosphorus (P) concentration. N fertilization alone significantly reduced the community-level plant P concentration, but did not affect the community-level plant N concentration, leading to an enhancement of plant N:P ratio. However, mowing altered the effects of N fertilization, leading to a higher plant N (and P) concentration than the fertilization-only plots. Also, mowing significantly reduced soil nitrate (NO₃⁻), but increased soil temperature, photosynthetic active radiation, plant diversity, richness and gross ecosystem productivity. In addition, mowing and N fertilization significantly affected plant community composition through shifting dominant plant functional groups (PFGs) (e.g. asteraceae, forbs and grass). Further, our structural equation modeling analysis showed that shifts in PFGs played an important role in regulating plant stoichiometry under mowing and N fertilization. Together, these results illustrate that effective management of mowing and N fertilization may induce changes in soil limiting nutrients and shifts in plant community composition, potentially altering plant N:P stoichiometry at the community level.

1. Introduction
The stoichiometric properties of plants, i.e. the relative concentrations of different nutrient elements in plants, critically affect ecosystem functions such as primary production and nutrient cycling (Aerts and Chapin 2000, Elser et al 2010). Different stoichiometric properties can influence the structure of plant community through conservative resource-use strategies in different functional groups (Xu et al 1994, Bai et al 2010). Land use practices such as mowing, grazing and fertilization can directly and/or indirectly affect nutrient availability, plant species composition and nutrient cycling, thereby altering nutrient stoichiometry in soil and plants (Xia et al 2009, Han et al 2014, Kotas and Choma 2017). Traditional regular mowing is a convenient management practice for forage removal and

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prevention of land degradation by enhancing seedling recuitment and species richness (Williams et al 2007; Zhou et al 2019). Also, mowing removes nutrients through plant biomass harvest, stimulating shoot regrowth and improving nutrient recycling efficiency (Oelmann et al 2009, Giese and Han 2013). Therefore, mowing may increase species-level nitrogen (N) and phosphorus (P) concentrations and alter N:P ratios in plants (Han et al 2014, Kotas and Choma 2017). In addition, mowing may reduce (Venterink et al 2009) or increase (Hamilton and Frank 2001) soil available N, thereby modifying potential N losses through N leaching or N-trace gas emissions (Kotas and Choma 2017). Further, mowing can partially offset the decline of plant species richness caused by N fertilization (Leps and Wan 2014).

Reactive N inputs due to N deposition and/or fertilization can also significantly affect plant stoichiometry. N inputs can alter the relative availability of soil nutrients and modify soil chemical properties (Bardgett and Mcalister 1999, Zhang et al 2018a). Effects of N enrichment on soil nutrient status and plant community composition have been extensively studied in grasslands (Collins 1998; Suding et al 2005). Increased N availability can alleviate plant N limitation and enhance net primary productivity of terrestrial ecosystems. However, excessive N inputs may reduce plant diversity through inducing competitive exclusion by nitrophilic species (Simkin et al 2016). Particularly, N inputs can indirectly affect plant stoichiometry through altering soil pH and soil cation availability (Clark et al 2007, Ye et al 2018). For example, N addition may alter soil P availability by influencing soil properties such as soil pH and phosphatase activities, potentially modifying the balance between soil available N and P (Peñuelas et al 2013, Deng et al 2017). Yet, N addition may still decrease plant P concentration through the dilution effect (Perring et al 2008, Liu et al 2013) and increase plant N concentration resulting in higher N:P ratios (Hou et al 2019). Many studies have shown that mowing, N fertilization and their interaction often affect soil nutrient availability (Guo et al 2010, Wang et al 2015) and plant stoichiometry at the species level (Han et al 2014, Liu et al 2014). Yet, less attention has been directed towards assessing their impacts on plant nutrient concentration and stoichiometry at the community level, particularly in fragile ecosystems such as semi-arid grasslands on the Loess Plateau, Northwestern China.

The Loess Plateau covers $6.2 \times 10^5$ km$^2$ in northwestern China, and is crucially important for Chinese arid agriculture (Ren et al 2016). In this region, increasing N fertilizers have been applied to agricultural fields, significantly modifying nutrient status and cycling (Yang et al 2011, Liu et al 2017). A significant proportion of loess farmlands have been abandoned in the last several decades as farmers moved to cities and the government encourages grassland reclamation (Li 2004, Gang et al 2018). Farmers remove grasses as hay and start to apply N fertilizers to maintain the productivity (Wang et al 2007, Cooney et al 2017). Mowing and/or N fertilization can affect plant composition and plant stoichiometry at both species and community levels in semi-arid grasslands (Collins 1998; Yang et al 2012, Han et al 2014, Hou et al 2019), but few have examined these effects and the underlying mechanisms in this region.

We initiated a field experiment in a semi-arid grassland on the Loess Plateau to understand how mowing, N fertilization and their interactions affect soil N and P dynamics, plant community composition and stoichiometry. Our objectives were to (1) determine the direct effects of mowing and N fertilization on plant community composition and plant stoichiometry at the community level, and (2) examine how mowing mediates the effects of N fertilization on plant stoichiometry at the community level. We hypothesized that (i) both mowing and N fertilization can significantly alter the plant community structure and the community-level plant stoichiometry, and (ii) mowing-induced changes in soil nutrients and plant community composition would cascade up to affect plant stoichiometry at the community level.

2. Materials and methods

2.1. Study site

Our study site was located at Yunwushan National Nature Reserve ($106^\circ 21'$–$106^\circ 27'$ E, $36^\circ 10'$–$36^\circ 17'$ N, altitude 1800–2000 m), a typical semi-arid grassland on the Loess Plateau, Ningxia Hui Autonomous Region, China. The study site has a typical semi-arid climate with a mean annual air temperature of 7.01 °C, ranging from the lowest in January (−14 °C) and the highest in July (23 °C). In this area, mean annual potential evaporation is 1330–1640 mm (Su et al 2019). Mean annual precipitation is about 450 mm with 60%–75% of annual precipitation occurring between July and September (Ren et al 2016). The soil is a montane grey cinnamon soil in the Chinese taxonomic system, equivalent to a Haplic Calcisol in the FAO/UNESCO system (Wei et al 2016), well drained with a pH of 8.0, and contained 39.6 g C and 5.3 g N kg$^{-1}$ soil when the experiment started (Tao et al 2018). The dominant plant species in the study area were Artemisia gmelinii, Saussurea altaica DC, Dendranthema lavandulifolium, Heteropappus altaicus, Stipa capillata. The study site has been fenced off since 2014 to prevent any disturbance by large animals.

2.2. Experimental design

The experiment was initialized in June 2016 on a mountaintop, where the topography is largely flat. This experiment was a $2 \times 2$ factorial design with four treatments assigned into five blocks (replicates). The four different treatments were a) no mowing and
no N addition control (CK), b) mowing with no N addition (M), c) N addition with no mowing (N), and d) mowing with N addition (MN). There were a total of 20 plots (2-m × 2-m each) with each plot being separated by a 1 m buffer strip. Reactive N (6.0 g N m$^{-2}$ yr$^{-1}$), aqueous urea (CO(NH$_2$)$_2$), was added into each N addition plot twice a year (one each in late April and early July). The annual N deposition rate in this area was 21.8 kg N ha$^{-1}$ yr$^{-1}$ (Liang et al. 2015). Our N inputs were high compared to the natural N deposition, but were within the range of which N fertilizers are applied to enhance grass productivity (Wei et al. 2018, Chen et al. 2020). Also, (Wei et al. 2018) reported that high N inputs (20.0 g N m$^{-2}$ yr$^{-1}$), but not at low N addition (10.0 g N m$^{-2}$ yr$^{-1}$), significantly enhanced aboveground plant biomass. Mowing was conducted once a year at the end of the plant growing period in late August, and the clippings were manually removed from the mowed plots. Additionally, a 0.5 m × 0.5 m mental frame was inserted into the soil to a depth of about 3 cm in the central part of each plot for measuring photosynthesis (Xia et al. 2009).

2.3. Plant sampling
In late August 2018 when plant community achieved peak biomass, aboveground vegetation was sampled in a 0.5 m × 0.5 m quadrat in each plot. Three randomly selected individuals of each species were measured by tape-line to determine the plant height. All plants in the quadrat were cut at ca. 2 cm above the soil surface. Live vascular plants were sorted by species, oven-dried at 60 °C for 48 h and weighed. The number of plant species occurred in the quadrats was used to represent the community species richness. In this study, the 13 dominant plant species were classified into three plant functional groups (PFGs): asteraceae (Artemisia gmelinni, Dendranthema lavandulifolium, Heteropappus altaicus (Willd) novopokr, Sausurea alta DC); grass (Stipa grandis, Stipa przewalskyi rshoev, Leymus, Saururus); forbs (Scutellaria linn, Carex spp, Thymus mongolicus ronn, Potentilla bifurca and Androsace erecta maximo). On average, these 13 species accounted for ca. 90% of the total plant biomass in our grassland. While asteraceae species were easy to form canopy by perennial standing litter, whereas forbs species were mostly shorter therophyte. Grass species usually have better soil nutrient absorption than forbs, leading to higher N:P ratios under N fertilization (Zhang et al. 2018b, Hou et al. 2019). Also, grasses and forbs usually have different responses in their stoichiometric and nutrient resorption efficiency to fertilization (Jiang et al. 2012, Zhang et al. 2016).

After the aboveground biomass was sampled, three soil cores (5 cm dia.) were taken to a 10 cm depth in each plot to harvest plant roots. Roots were washed with tap water, collected on a 1 mm screen, oven-dried at 60 °C for 48 h and weighed to determine their biomass.

The oven-dried plant parts of each species were then mixed and finely ground to powder. Total N concentrations in plant samples were determined by an elemental analyzer (Elementar Vario Micro Cube, Germany). Total plant P concentrations were determined by digesting ground samples in H$_2$SO$_4$-H$_2$O$_2$ and then measured colorimetrically at 880 nm after reaction with molybdenum blue (Carter 1993; SpectraMax i3x). Plant N:P ratio was reported as mass ratio.

2.4. Soil sampling and analyses
After three years of treatments, soil samples were collected in August 2018. Three soil cores (5 cm dia. and 0–10 cm depth) were randomly collected from each plot and then combined to form one composite sample. Fresh samples were sieved through a 2 mm screen to remove rocks, and the visible plant roots or debris. All soil samples were stored in an ice box (approximately 4 °C) during transportation to the laboratory for chemical and microbial analyses.

A 20 g subsample of moist soil was oven-dried at 105 °C for 24 h to determine water content. Soil pH was measured in a 1:2.5 (soil: water) suspension. Soil nitrate (NO$_3^-$) and ammonium (NH$_4^+$) were extracted with 50 ml of 0.5 mol L$^{-1}$ K$_2$SO$_4$ and then determined using a flow injection auto analyzer (SEAL-AA3, SEAL Analytical Inc. Germany). Soil P availability was determined following the extraction with NaHCO$_3$ according to the Olsen method (Olsen et al. 1954). Total soil carbon (TC) and soil nitrogen (TN) were determined by an elemental analyzer (Elementar Vario Micro Cube, Germany). Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined using the fumigation-extraction method (Vance et al. 1987). Briefly, a 12.5 g subsample (dry soil equivalent) was fumigated with ethanol-free chloroform for 48 h and then extracted with 50 ml of 0.5 M K$_2$SO$_4$ by shaking for 30 min. A non-fumigated subsample of soil (12.5 g) was immediately extracted with 0.5 M K$_2$SO$_4$ by shaking for 30 min and used as the control. Soil dissolved organic C (DOC) in the extracts was determined with a TOC analyzer (Elementar Vario Micro Cube, Germany). Inorganic N in the extracts was quantified on a flow injection auto analyzer (SEAL-AA3, SEAL Analytical Inc. Germany), after digestion with alkaline persulfate (Cabrera et al. 1993). The differences in extractable organic C and inorganic N between fumigated and nonfumigated soils were used to calculate MBC and MBN using a conversion factor of 0.33 (k$_{EC}$) and 0.45 (k$_{NS}$), respectively (Vance et al. 1987, Qiu et al. 2019).

2.5. Carbon flux and photosynthetic active radiation (PAR) measurements
We employed a static chamber method (Welker et al. 2004, Xia et al. 2009) to measure ecosystem C
fluxes, which were taken between 8:30 and 11:30 a.m weekly from April to August, 2018. Ecosystem C exchange was measured with a 0.5 × 0.5 × 0.5 m (length × width × height) transparent chamber connected to a Li-6400 CO₂ flux system (LI-COR Inc. Lincoln, NE, USA). During the measurement, the chamber was sealed to the surface of an iron frame, which was inserted into the soil to a depth of about 3 cm in the central part of each plot. Nine consecutive recordings of CO₂ concentrations were taken at 10 s intervals during a 70 s period after steady-state conditions were achieved inside the chamber. The CO₂ exchange was determined to calculate net ecosystem CO₂ exchange (NEE). Following NEE measurements, the chamber was vented for 30 s, put back on the mental frame, and covered with an opaque cloth. The CO₂ exchange was measured as ecosystem respiration (ER) rate as light was blocked and photosynthesis was eliminated. Gross ecosystem productivity (GEP) was estimated as NEE plus ER. Meanwhile, we employed the Tdr-100 (Spectrum) to measure the soil moisture (SM) and used portable temperature meter to determine the soil temperature (ST) (0–10 cm depth).

Transmitted PAR was estimated by AccuPAR LP-80 (Meter Group, Inc. USA). Averages of eight measurements of the above and below PARs were taken from the triplicates of each treatment by placing the probe perpendicularly to the rows above and below plant canopy between 8:30 a.m and 11:30 a.m. The method to calculate the PAR is as follows (1):

\[
PAR(\%) = 100 \times \frac{PAR_b}{PAR_t}
\]

where PAR_t: the average PAR at the top of the canopy (μmol m⁻² s⁻¹); PAR_b: the average PAR at the bottom of the canopy (μmol m⁻² s⁻¹)

2.6. Data analyses

Repeated measures ANOVA was employed to test the effects of mowing and N fertilization on ST, SM and GEP. Two-way ANOVA was used to evaluate the effects of mowing and N fertilization on soil and plant variables. Pearson correlation coefficients were used to evaluate relationships between the relative abundance of plant species and community-level plant N or P concentration. Also, α-diversity (Shannon Wiener diversity) of plant communities in different treatments was determined based on the relative abundances of different plant species. In addition, the total number of plant species occurred in the quadrats was used to represent the community species richness. Furthermore, in order to visualize differences in plant communities across experimental treatments, we used non-metric multidimensional scaling (NMDS) ordination with the ‘vegan’ package in R software with 1000 permutations. Bray-Curtis similarity matrices were created using the relative abundance of plant species biomass (Desai et al 2016). To test for the effects of the experimental treatments on plant community composition, we performed two-way permutational multivariate analyses of variance (PERMANOVA) using the default settings. All analyses were performed with R version 3.4.3 (R Development Core Team, 2016).

Finally, structural equation modeling (SEM) was conducted to quantify direct and indirect effects of mowing and N fertilization on plant N:P ratio at the community level. AMOS 24.0 (IBM, SPSS) was used to create a conceptual model of hypothetical relationships based on a prior and theoretical knowledge and the data was fitted into the model by using the maximum-likelihood estimation method, with a criteria of \( P > 0.05 \) to accept the fitted model. Several tests were used to assess model fit: the \( \chi^2 \)-test, comparative fit index (CFI) and root square mean error of approximation (RMSEM).

3. Results

3.1. Responses of soil variables to mowing and N fertilization

Mowing significantly increased soil temperature (ST) at the 10 cm depth during plant growing season (figure 1(a); table S3 (available online at: https://stacks.iop.org/ERL/15/074031/mmedia)) and enhanced soil C/N ratio (\( P < 0.05 \); table S1), but decreased soil nitrate (NO₃⁻) and soil N/P ratio (\( P < 0.05 \); figure S4; table S1). Compared to the control, N fertilization significantly increased soil NO₃⁻ by 38% (figure S4; table S1), but slightly decreased soil pH from 8.0 to 7.8 on average (\( P < 0.01 \); table S1). Also, N fertilization alleviated the effect of mowing on increasing ST (figure 1(a)). Neither mowing nor N fertilization, however, had any significant effects on SM, soil ammonium (NH₄⁺), TC, TN, MBC and MBN (table S1).

3.2. Response of PAR and ecosystem C flux to mowing and N fertilization treatments

Neither mowing nor N fertilization had any significant effect on GEP from April to June, whereas mowing significantly increased GEP from June to August in 2018 (figure 1(b); table S3). Compared to the control, mowing significantly enhanced PAR by 80% (table S2). Also, mowing significantly decreased total aboveground biomass by 53% (figure 2(a), table S2), whereas N fertilization had no significant impact.

3.3. Response of plant community composition to mowing and N fertilization treatments

Mowing significantly increased the Shannon-Wiener diversity (figure S2(a); table S2) and species richness of plant community (figure S2(c); table S2). However, N fertilization had no effect on either of
them (figure S2). NMDS ordination and PERMANOVA analysis showed that both mowing (Pseudo-F = 3.68, \(P < 0.05\)) and N fertilization (Pseudo-F = 3.72, \(P < 0.01\)) significantly altered overall plant community composition (figure 4; table 2).

Compared with the control, mowing and N fertilization significantly reduced the relative biomass of asteraceae by 28% and 30%, respectively (figure 2(b); table S2). However, N fertilization significantly increased the relative biomass of grass by 31% compared with the unfertilized plots. Mowing significantly increased the relative biomass of forbs by 30% (figure 2(b); table S2), whereas N fertilization had no significant effect on that.

### 3.4 Interactive effects of mowing and N fertilization on plant N and P concentrations, and N:P ratio at the community level

Mowing and N fertilization interacted to affect the plant N and P concentrations at the community level (figure 3). Mowing alone had no effect on plant N or P concentrations. Compared with the control, N fertilization significantly reduced plant P concentration by 16% (table S2) at the community level. Under the combination of mowing and N fertilization, both plant N and P concentrations were significantly higher than those under the fertilization-only treatment (figures 3(a) and (b)). N fertilization, but not mowing or the interaction of mowing and

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**Figure 1.** Response of soil temperature (ST) (a) and gross ecosystem productivity (GEP) (b) to mowing and N fertilization treatments. CK, control; N, N fertilization with no mowing; M, mowing with no N fertilization; MN, mowing with N fertilization; T, time. Data shown are the means of five replicate plots.

**Figure 2.** Effects of mowing and N fertilization on total (a) and PFG (b) biomass of plant community. Asteraceae: Artemisia gmelinii, Dendranthema lavandulifolius, Heteropappus alticus (Willd) novoproko, Saussurea alata DC; Grass: Stipa grandis, Stipa przewalskii rochev, Leymus, Saururus; Forbs: Scutellaria linn, Carex spp, Thymus mongolicus ronn, Potentilla bifurca and Androsace erecta maxim. CK, control; N, N fertilization with no mowing; M, mowing with no N fertilization; MN, mowing with N fertilization. Data shown are mean ± SE (n = 5).
N fertilization, significantly increased plant N:P ratio (figure 3(c); table S2).

3.5. Relationships between the community-level plant N and P concentrations and the relative biomass of different species

Linear regressions showed that the relative biomass of *Dendranthema lavandulifolium* and *Saussurea alata* DC were positively related to the community-level plant P concentration ($P < 0.05$; table 1). The relative biomass of *Stipa grandis* had a significant positive relationship with the community-level plant N concentration, whereas the relative biomass of *Stipa przewalskyi roshev* was significantly negatively related with the community-level plant P concentration (table 1). However, the relative biomass of other species had no significant relationship with the community-level plant P or N concentration (table 1).

3.6. The main drivers of the community-level plant N and P responses to mowing and N fertilization

We used abiotic variables (i.e. PAR and soil NO$_3^-$) and biotic variables (i.e. the relative biomass of forbs, asteraceae and grass) to conduct the SEM analysis. To evaluate the effect of PFGs shifts via light competitiveness and soil N availability on plant N:P ratio, our SEM adequately fitted our data into two models (a: asteraceae-forbs model with $\chi^2 = 12.51$, df = 6, $P = 0.09$; b: grass-forbs model with $\chi^2 = 9.26$, df = 5, $P = 0.10$). Both models indicated that mowing and N fertilization had significant impacts on soil N availability and the shifts in PFGs. With respect to asteraceae-forbs model, the significant increase in relative biomass of grasses induced by soil available N mostly explained the higher plant N:P ratio through two major pathways: 1) the decreased relative biomass of asteraceae by both mowing and N fertilization and 2) the increase in forb biomass induced by direct effect from mowing and indirect effect through altered PAR (figure 5(a)). Different from asteraceae-forbs model, the significant increase in relative biomass of grasses induced by soil available N mostly explained the higher plant N:P ratio.
Light competition is critical for plant survival growth and reproduction in a dense stand (Demalach et al 2017). Plant height has been considered as a critical functional trait for a plant to effectively compete for light (Dickson et al 2014, Demalach et al 2017). One might expect taller plants to capture more light than lower ones in a dense stand where light availability decreases from the top downwards (Anten and Hirose 2001, Li et al 2017, Demalach et al 2017). The average height of asteraceae (24.1 cm) and grass (27.4 cm) was significantly higher than that of forbs (7.4 cm) in our field plots (figure S5). Also, mowing significantly reduced the relative biomass of asteraceae, but increased that of forbs (figures 2(b) and 5). This shift in the relative dominance in plant species often leads to higher light energy utilization efficiency by forbs (Flanagan et al 2015, Li et al 2017). (Demalach et al 2017) also showed that species in the lower canopy received lower amounts of light per unit size and experienced more intense light competition than taller plants, increasing the probability of losses of shorter plants. Our asteraceae-forbs model also confirmed that the PFG shift was significantly modified by dominant light competition (figure 5(a)).

Soil N availability is another factor that can significantly affect the relative dominance of different plant species (Suding et al 2005, Hillebrand et al 2008). N addition has often been shown to promote the dominance of grasses over forbs in grasslands (Chapin et al 1995, You et al 2017), likely due to their different capacity for N uptake. Our results showed that mowing reduced, but N addition increased soil NO$_3^-$ (figure S4; table S1). The significant effects of mowing and N inputs on soil NO$_3^-$ were expected as mowing removes N from field (Yang et al 2011, Carey et al 2015, Liu et al 2017) and N addition directly adds N nutrients. Most grasses have highly branched fibrous root systems and may have a better ability to absorb more nutrients from the soil surface.

### Table 1. Correlations (R) between species relative biomass and the community-level plant N or P concentration.

| Groups | Species                  | Plant N concentration | Plant P concentration |
|--------|--------------------------|-----------------------|-----------------------|
| Asteraceae | A. gmelinii            | 0.18                  | 0.05                  |
|         | D. lavandulifolium      | 0.08                  | 0.45$^*$              |
|         | H. altaicus. (Wild) novopokr | 0.19                  | 0.03                  |
|         | S. alata DC            | 0.35                  | 0.46$^*$              |
| Grass   | S. grandis             | 0.64$^{**}$           | 0.15                  |
|         | S. przewalskyi roshev  | 0.3                   | 0.72$^{**}$           |
|         | Leymus                  | 0.3                   | 0.08                  |
|         | Saururus                | 0.18                  | 0.16                  |
| Forbs   | S. Linn                 | 0.06                  | 0.07                  |
|         | T. mongolicus ronn     | 0.21                  | 0.16                  |
|         | A. erecta maxim         | 0.38                  | 0.24                  |
|         | P. bifurca             | 0.03                  | 0.07                  |
|         | C. spp                  | 0.08                  | 0.23                  |

Significant effects: $^*$ 0.01 < P < 0.05, $^{**}$0.001 < P < 0.01. A. gmelinii, Artemisia gmelinii; D. lavandulifolium, Dendranthema lavandulifolium; H. altaicus (Wild) novopokr, Heteropappus altaicus (Wild) novopokr; S. alata, DC, Sesussurea alata DC; S. grandis, Stipa grandis; S. przewalskyi roshev, Stipa przewalskyi roshev, S. Linn, Scutellaria Linn; T. mongolicus ronn, Thymus mongolicus ronn; A. erecta. maxim, Androsace erecta maxima; P. bifurca, Potentilla bifurca; C. spp, Carex spp.

### Table 2. Effects of mowing and N fertilization treatments on the structure of plant communities assessed by PERMANOVA analysis.

| Factor                  | F    | R$^2$ |
|-------------------------|------|-------|
| CK vs. M                | 3.34$^*$ | 0.29  |
| CK vs. N                | 9.51$^{**}$ | 0.54  |
| CK vs. MN               | 3.85$^*$ | 0.32  |
| M vs. N                 | 3.53$^*$ | 0.31  |
| M vs. MN                | 0.47  | 0.06  |
| MN vs. N               | 2.65  | 0.25  |

Significant effects: $^*$ 0.01 < P < 0.05, $^{**}$ 0.001 < P < 0.01. CK, control; N, N fertilization with no mowing; M, mowing with no N fertilization; MN, mowing with N fertilization.

4. Discussion

Our results showed that mowing and N fertilization significantly altered the plant community structure (figures 2 and 4) and the community-level plant stoichiometry (figure 3), which supports our first hypothesis. Plant N and P stoichiometry is indicative of the relative nutrient limitation to plant growth (Güsewell 2005) and sensitive to mowing and N fertilization (Han et al 2014, Hou et al 2019). Mowing and/or N fertilization can significantly alter the structure and functioning of grassland ecosystems by altering nutrient inputs, dominant species and plant community composition (Elser et al 2010, Shi et al 2015, Li et al 2017). Yet, their interactive effects on plant stoichiometry are less known, particularly in ecologically sensitive systems such as semi-arid grasslands on the Loess Plateau, Northwestern China. Our results showed that mowing, N fertilization and their interactions significantly shifted PFGs, which were induced by light permeation and/or soil N availability (figures 3 and 5).
and use these nutrients more effectively than forbs (Chapin et al. 1995; Yu et al. 2011, You et al. 2017). Our SEM results further confirmed that the shift in grasses and forbs was largely due to alterations of N availability under mowing and N fertilization (figure 5(b)). Our study also documented that the PFG shift (i.e. the decrease of asteraceae species and the increase of grass species induced by N fertilization) (figure 2(b)), rather than nutrient dilution (Perring et al. 2008), mostly explained the lower community-level plant P concentration and the higher N:P ratios in N-fertilized plots (figure 3(b)). The enhancement of N:P ratios under the interactive treatment likely stems from either the lower plant P concentration under N fertilization treatment or the higher plant N concentration (figure 3). Our SEM analysis also showed that the community-level plant N:P ratio was largely modified by the different PFG-shift mechanisms (figure 5). Together, these results indicated that mowing-induced changes in soil nutrient availability (e.g. soil NO$_3^-$) and plant community composition can scale up to affect plant stoichiometry at the community level, supporting our second hypothesis.

The N:P ratio represents the relative availability of N to P (Aerts and Chapin 2000) and is a valuable indicator of the nutrient status for plant growth.
(Elser et al. 2010). It is usually accepted that a N:P ratio less than 14 suggests N limitation, while a ratio greater than 16 indicates P limitation (Koerselman and Meuleman 1996, Aerts and Chapin 2000). (Lü et al. 2013) reported that the positive effects of N addition on plant N concentration could result in the higher N:P ratios. In contrast, our results showed that the higher N:P ratios following N addition resulted from the higher sensitivity of plant P concentration than that of plant N concentration. The N:P ratios (at 17.4 and 17.2 under N and MN treatments, respectively) observed in this study were higher than other observations (Elser et al. 2000, Reich and Oleksyn 2004), suggesting that plant nutrient limitation shifted from N in the CK and M treatments (with N:P ratios at 13.7 and 13.1, respectively) to P under N fertilization.

A shift in the dominant species in a plant community can not only affect the community-level stoichiometry but also may significantly affect functioning of the ecosystem (Bai et al. 2010), such as GEP. Usually, GEP positively relates with the aboveground biomass (Goudriaan et al. 1985, Peng et al. 2017). Interestingly, the dominant community with forbs and grass under our interactive treatment with lower aboveground biomass still had higher GEP than that in un-mowed plots (figure 1(b)), except for the trophophase when the regrowing shoots did not germinate well from April to June in 2018. This occurred probably due to the increased recourses of light to the whole plant communities. Also, accompanying with more fixed carbon via higher photosynthesis induced by mowing, the new-born forbs and grasses would take up more N due to carbon-nitrogen coupling (Langley and Megonigal 2010). In addition, mowing altered PFG composition with an enhancement of forbs, leading to a higher Shannon Wiener diversity and richness of plant communities (Bobbink et al. 1987; Fynn 2004). A recent study adjacent to our field plots showed that both climate warming and rainfall changes tended to increase the relative contribution of the dominant species to the total biomass (Su et al. 2019). The temperature at our site has increased by 1.92 °C from 1960 to 2013 (Guo et al. 2011, Sun et al. 2016), while the global average temperature has increased by ca. 0.85 °C from 1880 to 2012 (IPCC 2013). Finally, rainfall has become more variable on the Loess Plateau (Wan et al. 2013, Sun et al. 2016, Wang et al. 2019). Together, these changes in temperature and rainfall, combined with the increasing N deposition in this area, suggest that local plant diversity may be at risk under future climate change scenarios. Mowing may provide a tool to moderate the ecological effects of climate change factors under the frequent occurrences of extreme climate events.

5. Conclusions

Our results showed that mowing significantly changed the plant community structure through altering the light competitiveness between forbs and asteraceae, whereas N fertilization significantly changed the plant community composition through altering soil available N. Mowing mediated the effects of N fertilization on the community-level plant N:P ratio through the multiple PFG shifts. The enhancement of plant N:P ratio with both mowing and N fertilization is of significance in understanding the shifts of relative N or P limitation within soil and plants. Our results may provide guidance for designing management practices that prevent the grassland degradation, while promoting the functioning of the ecosystem through increasing species richness and primary production in arid and semi-arid Loess Plateau. Future studies should examine how climate change factors mediate the effect of mowing and N fertilization on plant-soil-microbial interactions and potential long-term feedbacks.

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Data availability statements

Any data that support the findings of this study are included within the article.

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