

## Long-term mowing did not alter the impacts of nitrogen deposition on litter quality in a temperate steppe



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### ABSTRACT

Litter quality plays an important role in determining litter decomposition and nutrient cycling in terrestrial ecosystems. We investigated the main and interactive effects of nitrogen (N) addition and mowing on litter quality (%N, lignin, cellulose, and hemicellulose concentrations and lignin:N ratio) of four dominant species in a temperate steppe in northern China. In addition to species-specific impacts, both N addition and mowing can alter community composition by changing relative dominance of individual species, with consequences on litter decomposition. Nitrogen addition significantly increased N concentration of shoot litters at species- and community-level, while mowing did not affect N concentration. Nitrogen addition decreased concentrations of lignin, cellulose, hemicellulose, and lignin:N ratio at both species- and community-level. Mowing decreased lignin and cellulose concentrations, increased hemicellulose concentrations, and did not affect lignin:N ratio at species-level. Mowing decreased cellulose concentrations but did not alter lignin and hemicellulose concentrations, and lignin:N ratio at the community level. Furthermore, mowing did not alter the impacts of N addition on litter quality at both organization levels. Our results suggested that both N addition and mowing can influence litter quality by affecting individual species litter quality and community structure, and thus regulate the processes of litter decomposition and nutrient cycling in grassland ecosystems through a plant-mediated pathway. Importantly, our results showed that the role of annual mowing as a widely-used ecosystem management strategy globally appears to be limited in mediating the impacts of N deposition on plant-mediated nutrient cycling.

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## 1. Introduction

Plant litter quality plays an important role in mediating the impacts of plants on nutrient cycling in terrestrial ecosystems (Vitousek, 1982; Hobbie, 1992; Knops et al., 2002). Lignin, cellulose, and hemicellulose are the major structural carbon (C) compounds in plant litters that retard decomposition (Hättenschwiler and Jørgensen, 2010; Henry et al., 2005). The inhibitory effects of cellulose and hemicellulose on litter decomposition mainly occur in the early stage, while lignin degradation often occurs in the late stage. Further, the ratio of initial nitrogen (N) to lignin contents

often closely correlates with litter decomposition rates (Melillo et al., 1982). Litter quality—defined by both structural C components and N content—is expected to be sensitive to abiotic global change factors such as N enrichment (Liu et al., 2016; Zhang et al., 2016a,b) and ecosystem management strategies such as haymaking by mowing (Mikola et al., 2009). However, the interactive impacts of those factors on litter quality and its implications for decomposition dynamics remain largely unknown in grassland systems.

Nitrogen deposition has rapidly increased due to intensive anthropogenic activities, such as fossil fuel combustion, fertilizer use, and the cultivation of N-fixing crops (Galloway et al., 1995; Gruber and Galloway, 2008; Vitousek et al., 1997). Liu et al. (2013) found that the average annual N deposition increased by about 8 kg N ha<sup>-1</sup> between 1980 and 2010 in China. Plant foliar N concentration in natural ecosystems and crop N uptake from long-term

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unfertilized croplands were significantly increased by N deposition (Liu et al., 2013). Changes in the soil environment following N deposition can influence litter chemistry, for example, by decreasing lignin concentration and thus accelerating decomposition, particularly for low-lignin litter (Norby, 1998; Hobbie, 2000). The effects of N deposition on nutrient status (Lü et al., 2012a; Wang et al., 2014) and community composition (Collins et al., 1998; Isbell et al., 2013) have been studied extensively in grassland ecosystems; however, the response of their C chemistry to N deposition is less clear at both species- and community-level. Furthermore, the effects of N deposition on phytochemistry characteristics are expected to be simultaneously modulated by other factors, such as ecosystem management strategy.

Grassland management strategies can also affect nutrient availability through their impacts on nutrient cycling and community structure in semi-arid ecosystems (Han et al., 2014; Niu et al., 2009; Xia et al., 2009). Mowing, a widely-used grassland management strategy for haymaking across northern China and other parts of the world, removes a majority of current year's aboveground biomass from the ecosystem, and thus has significant consequences on ecosystem composition (Collins et al., 1998; Isbell et al., 2013) and nutrient cycling (Giese et al., 2013; Klumpp et al., 2011). One of the dominant pathway through which mowing can alter nutrient cycling is by reducing plant C supply to soil and decreasing plant nutrient availability by the chronic removal of litter and thus nutrients within litter (Turner et al., 1993). However, short-term annual mowing can also increase nutrient concentrations as a result of increasing soil temperature and moisture values (Bardgett et al., 1998; Tix et al., 2006), changing relative allocation of nutrient, or increasing plant nutrient uptake after defoliation (Green and Detling, 2000; Mikola et al., 2009).

In contrast to our understanding of the impacts of mowing on plant nutrient status, whether mowing affects litter chemistry is less well understood at both species and community levels. Positive (Hiernaux and Turner, 1996; Mikola et al., 2009; Han et al., 2014), neutral (Lü et al., 2012b), and negative (Mikola et al., 2009) effects of mowing on plant N concentration has been reported, which were highly dependent on species identity and ecosystem types. Nutrient availability may also regulate ecosystem responses to mowing (Lü et al., 2012b), but it is not clear whether mowing interacts with N deposition to impact litter quality.

Here, we examined the effects of seven years of N deposition and mowing on litter quality, including N, lignin, cellulose, and hemicellulose concentrations of four dominant grasses at species and community levels in a temperate steppe in northern China. These grasslands are generally mown once annually at the end of the growing season for the forage harvest. We hypothesized that: (1) N addition would increase litter N concentration and decrease the concentrations of lignin, cellulose, and hemicellulose in litters and thus increase litter quality; (2) mowing would decrease N concentration but increase concentrations of lignin, cellulose and hemicellulose, and consequently decrease litter quality through the chronic removal of nutrients with plant biomass. Furthermore, given the divergent impacts of long-term N addition and mowing on nutrients in both soil and plants previously observed (Giese et al., 2009; Han et al., 2014), we hypothesized (3) mowing would decrease the effects of N addition on litter quality, and thus have an interactive effect of mowing and N addition.

## 2. Materials and methods

### 2.1. Study site

This study was carried out in a temperate steppe that located in Xilin River Basin, Inner Mongolia in northern China, which is dom-

inated by *Leymus chinensis* and *Stipa grandis* (Zhang et al., 2014). Mean annual precipitation in this area is approximately 355 mm, with 80% falling during the growing season from May to September, and mean annual temperature is 0.9 °C. The soil is classified as Calcic-Orthic Aridisols (Zhang et al., 2016a,b). Plant primary productivity in this semi-arid grassland is N-limited (Bai et al., 2010; Zhang et al., 2015).

### 2.2. Experimental design

In September 2008, 24 plots measuring 8 m × 8 m were established with four treatments (control, N addition, mowing, and both N addition and mowing), and each treatment was replicated six times separated by 1 m buffer. Treatments were randomly assigned to plots within each block. Purified NH<sub>4</sub>NO<sub>3</sub> (>99%) was added two times each year for N addition treatment, with a rate of 10 g N m<sup>-2</sup> yr<sup>-1</sup>. Each year in June, fertilizer was mixed with purified water, and then was sprinkled evenly to each plot to simulate wet N deposition. In November, fertilizer was mixed with sand (which was sieved to 1 mm size, dipped by hydrochloric acid, washed by purified water, and then heated by an oven at 120 °C for 24 h), and then spread evenly by hand to each plot to simulate dry N deposition. Less than 1 mm of water was added to the N addition plots. The ambient atmosphere N deposition rate is estimated as 1–2 g N m<sup>-2</sup> yr<sup>-1</sup> (Jia et al., 2014), and no additional fertilizer was added in this area before our experiment began. Plots were mown with a mower about 10 cm above the soil surface after the growing season each year, then mowing litter was removed to the edge of each plot.

### 2.3. Sampling and chemical analysis

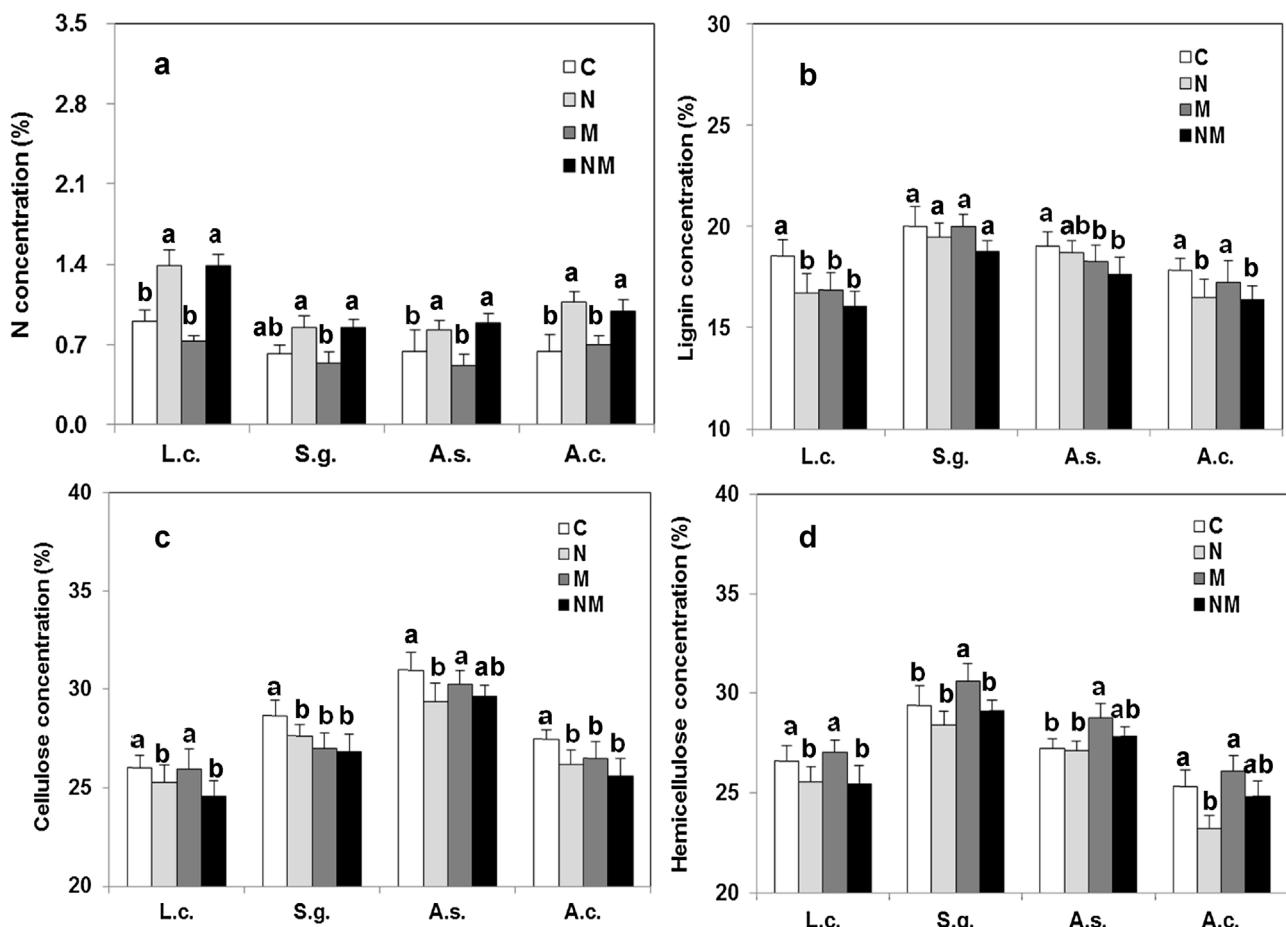
Plant aboveground biomass was sampled in mid-August 2015 using a 1 m × 1 m quadrat, which was randomly placed in each plot at least 50 cm inside each plot to avoid edge effects. All green aboveground plants were classified to species, and then weighed after oven-drying at 70 °C for 48 h.

Representative senesced aboveground tissues of the four perennial grasses, *L. chinensis* (L.c.), *S. grandis* (S.g.), *Achnatherum sibiricum* (A.s.) and *Agropyron cristatum* (A.c.) of the current year, were sampled in the plots in October 2015 (seven years after the start of the experimental treatments), when all the grasses were senesced and before the mowing treatment was carried out in that year. We considered the tissues ready to abscise when they were completely dry and yellow without signs of deterioration (Wright and Westoby, 2003). Together, these four species contributed >70% of the total aboveground biomass in this ecosystem.

The senesced shoots were transported to the laboratory, oven dried for 48 h at 70 °C, and then finely ground with a ball mill (Retsch MM 400, Retsch GmbH & Co KG, Haan, Germany). Total N concentration were analyzed by an Alpkem autoanalyzer (Kjektec System 1026 Distilling Unit, Sweden). Lignin was determined directly as the acid-insoluble residue after samples (50 mg) were extracted with phenol: acetic acid: water (1.1:1.0:0.9) and 72% dilute H<sub>2</sub>SO<sub>4</sub> to remove confounding low molecular weight phenolics, followed by digestion in concentrated H<sub>2</sub>SO<sub>4</sub> (Booker et al., 1996). Cellulose and hemicellulose were measured as glucose equivalents (Taylor, 1995) following a two-stage hydrolysis procedure consisting of a treatment with concentrated H<sub>2</sub>SO<sub>4</sub> followed by a second hydrolysis step with 72% dilute H<sub>2</sub>SO<sub>4</sub> at elevated temperature to release the glucose monomers.

### 2.4. Statistical analysis

Levene's test was used to test for the equality of error variance and the Kolmogorov-Smirnov test was used to test data normality. The effects of species identity, N addition, mowing and their



**Fig. 1.** Effects of nitrogen addition and mowing (C, control; N, nitrogen addition; M, mowing; NM, both N addition and mowing) on N, lignin, cellulose and hemicellulose concentrations in litter tissues of four dominant grasses at species-level. Data are shown as means  $\pm 1$  SE. Different letters indicate significant differences ( $P < 0.05$ ) among treatments for each species.

possible interactions on N, lignin, cellulose, and hemicellulose concentrations and lignin:N ratio were determined using three-way ANOVAs. The impacts of N addition, mowing and their possible interactions on the litter chemistry and aboveground biomass of each species were analyzed using two-way ANOVAs with block as a random factor. The litter chemistry at the community level in each plot was calculated based on the litter chemistry at species level and the biomass of each species in the plot. Two-way ANOVAs were used to examine the impacts of N addition and mowing on litter chemistry at the community level with block as a random factor. Significant differences among treatment means at species- and community-level were analyzed using Tukey's multiple comparison post hoc test. All data analyses were conducted with SPSS version 13.0 (SPSS, Chicago, IL, USA).

### 3. Results

#### 3.1. Responses of litter chemistry at the species level

Litter C and N chemistry varied among the four species ( $P < 0.001$ ; Table 1). Nitrogen concentration was highest in *L. chinensis* and lowest in *S. grandis* (Fig. 1a). Averaged across the four species, N addition significantly increased N concentration by 35.9% (Table 1; Fig. 1a) and its effects were dependent on species identity (Table 1; Fig. 1a). *S. grandis* had the highest lignin concentration while *A. cristatum* had the lowest lignin concentration (Fig. 1b). Averaged at the species level, N addition decreased lignin, cellu-

**Table 1**  
Results ( $F$ -value) of three-way ANOVAs on the effects of species identity (S), mowing (M), nitrogen addition (N) and their interactions on concentrations of nitrogen ([N]), lignin, cellulose, and hemicellulose, and lignin:N ratio in litter tissues.

[N]	lignin	cellulose	hemicellulose	Lignin:N
S	23.8***	31.0***	37.2***	16.4***
M	1.2	9.5**	5.1*	8.0**
N	101.4***	17.7***	10.4**	19.5***
S × M	0.2	0.9	0.5	0.6
S × N	5.3*	0.6	0.3	0.7
M × N	1.0	0.1	0.1	0.1
S × M × N	1.0	0.8	0.6	0.4

\*\*  $P < 0.01$ .

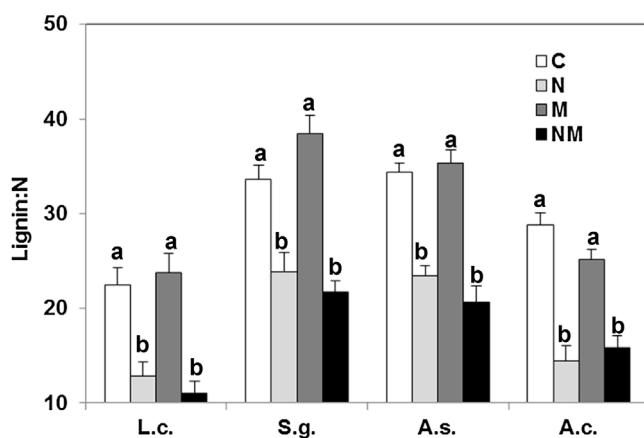
\*\*\*  $P < 0.001$ .

lose, hemicellulose, and lignin:N ratio by 5.2%, 3.9%, 4.6% and 40.6%, respectively (Figs. 1b-d; 2).

Mowing did not affect N concentration and lignin:N ratio at the species level (Table 1; Figs. 1a; 2), however, mowing decreased lignin concentration by 3.8% (Fig. 1b) and cellulose concentrations by 2.7% (Fig. 1c), but increased hemicellulose by 2.9% (Fig. 1d). Furthermore, there was no significant interaction between mowing and N addition on litter C and N chemistry across the four species (Table 1; Figs. 1a-d; 2).

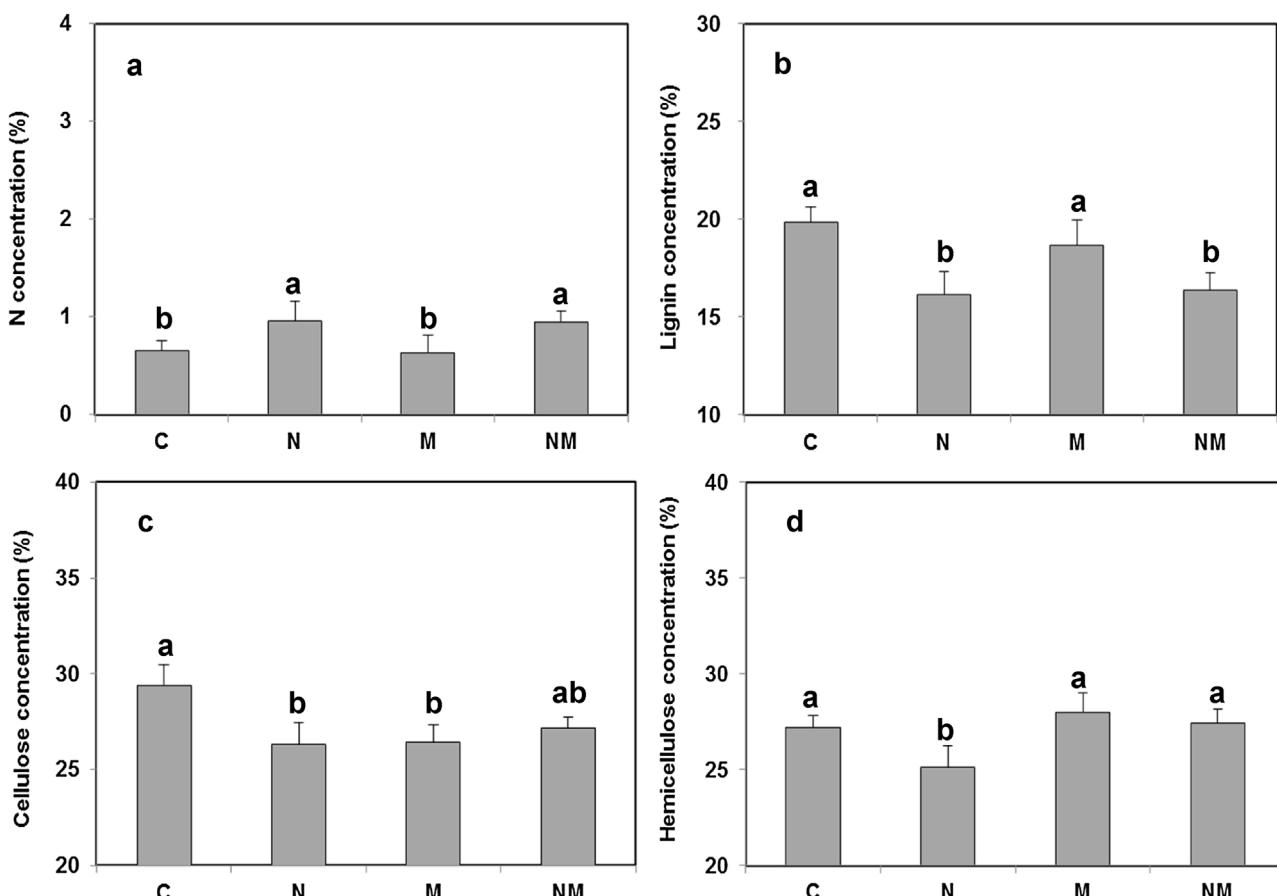
#### 3.2. Responses of litter chemistry at the community level

Nitrogen addition increased litter N by 32.1%, and reduced lignin, cellulose, hemicellulose and lignin:N ratio by 18.7%, 10.5%,



**Fig. 2.** Effects of nitrogen addition and mowing (C, control; N, nitrogen addition; M, mowing; NM, both N addition and mowing) on lignin:N ratio in litter tissues of four dominant grasses at the species level. Data are shown as means + 1 SE. Different letters indicate significant differences ( $P < 0.05$ ) among treatments for each species.

7.6% and 35.28%, respectively at the community level (Table 2; Figs. 3 a-d; 4). Mowing decreased litter cellulose by 10.1% (Fig. 3c), but showed no significant impact on litter N, lignin, hemicellulose and lignin:N ratio at the community level (Table 2; Figs. 2a,b,d; 4). Furthermore, no significant interactions between N addition and mowing were detected at the community level in the semi-arid grassland (Table 2; Figs. 3a-d; 4).



**Fig. 3.** Effects of nitrogen addition and mowing (C, control; N, nitrogen addition; M, mowing; NM, both N addition and mowing) on N, lignin, cellulose and hemicellulose concentrations in litter tissues of four dominant species at the community level. Data are shown as means + 1 SE. Different letters indicate significant differences ( $P < 0.05$ ) among treatments at the community level.

**Table 2**

Results ( $F$ -value) of two-way ANOVAs on the effects of mowing (M), nitrogen addition (N) and their interactions on concentrations of nitrogen ([N]), lignin, cellulose, and hemicellulose, and lignin:N ratio in litter tissues at the community level.

	[N]	lignin	cellulose	hemicellulose	Lignin:N
M	0.5	1.0	9.2**	0.9	1.1
N	43.9***	24.8***	9.4**	8.7**	16.6***
M × N	0.8	0.3	0.4	0.1	0.6

\*\*  $P < 0.01$ .

\*\*\*  $P < 0.001$ .

**Table 3**

Results ( $F$ -value) of two-way ANOVAs on the effects of nitrogen addition (N), mowing (M) and their interactions on aboveground biomass of the four species.

Factors	<i>L. chinensis</i>	<i>S. grandis</i>	<i>A. sibiricum</i>	<i>A. cristatum</i>
N	3.1	7.1*	37.6***	2.5
M	40.3***	5.3*	12.6**	1.7
N × M	1.6	2.0	2.8	0.4

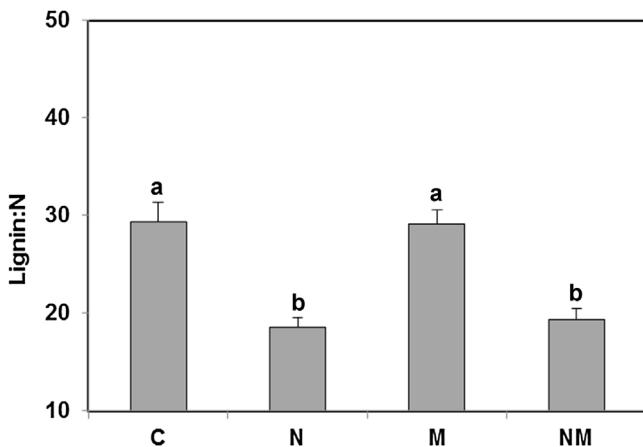
\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

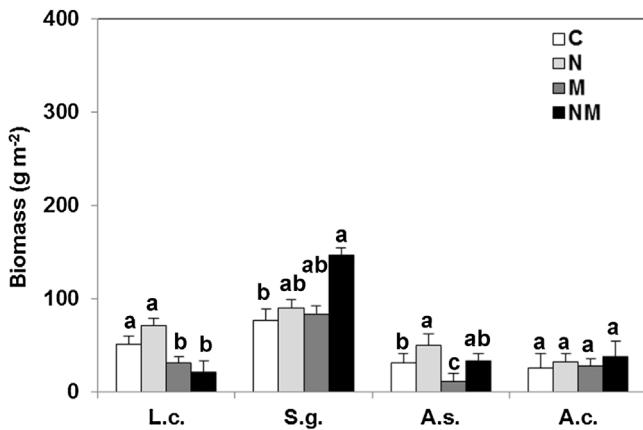
\*\*\*  $P < 0.001$ .

### 3.3. Responses of plant biomass to mowing and fertilizer treatments

The biomass responses of the four species to N addition and mowing varied (Table 3; Fig. 5). Nitrogen addition increased the biomass of *S. grandis* by 32.5% and *A. sibiricum* by 49.9%, but had no effects on the biomass of *L. chinensis* (Fig. 5). Mowing increased the



**Fig. 4.** Effects of nitrogen addition and mowing (C, control; N, nitrogen addition; M, mowing; NM, both N addition and mowing) on lignin:N ratio in litter tissues of four dominant species at the community level. Data are shown as means + 1 SE. Different letters indicate significant differences ( $P < 0.05$ ) among treatments at the community level.



**Fig. 5.** Effects of nitrogen addition and mowing (C, control; N, nitrogen addition; M, mowing; NM, both N addition and mowing) on aboveground biomass of the four species. Data are shown as means + 1 SE. Different letters indicate significant differences ( $P < 0.05$ ) among treatments for each species.

biomass of *S. grandis* by 27.4%, but decreased the biomass of *L. chinensis* by 57.7% and *A. sibiricum* by 45% (Fig. 5). Neither N addition nor mowing affected the biomass of *A. cristatum* (Table 3; Fig. 5), and there was no significant interaction between N addition and mowing on plant aboveground biomass for any of the species sampled (Table 3).

#### 4. Discussion

We examined the main and interactive effects of N addition and mowing on the litter C and N chemistry in the shoots of four dominant grasses in a temperate steppe. Long-term annual mowing did not alter the effects of N addition on litter quality in this system. Nitrogen addition, but not mowing, significantly increased litter N concentration at species- and community-level. Nitrogen addition also decreased the concentrations of lignin, cellulose, and hemicellulose and lignin:N ratio at species and community levels, while the impacts of mowing on C chemistry was inconsistent. At the species level, mowing decreased lignin and cellulose concentrations and lignin:N ratio, but increased hemicellulose concentrations. At the community level, mowing decreased cellulose concentrations, but had no detectable effects on other litter quality variables. We found no significant interaction of N addition and mowing on concentra-

tions of N, lignin, cellulose, and hemicellulose and lignin:N ratio in the shoot litter at both species and community levels in this temperate steppe system. Similarly, Han et al. (2014) also reported that the effects of N addition on nutrient status of mature plant tissues was only marginally affected by mowing in this area. The four dominant grasses in this system showed similar responses to both N addition and mowing. As litter quality significantly varied among the four grasses sampled, both N addition and mowing also indirectly affected litter quality at the community level by changing community structure in this semi-arid grassland.

Consistent with our first hypothesis, N addition significantly increased litter N concentration at both species and community levels in this temperate steppe, which is also consistent with our previous results of the responses of green leaves of dominant species in this ecosystem (Han et al., 2014). Although plant N uptake and use varies among species (Lü et al., 2011, 2014), results from this study and that from Han et al. (2014) indicate that interspecific variation of N concentration appears to be consistent in both mature and senesced tissues. Furthermore, our results demonstrated lignin, cellulose, and hemicellulose concentrations and lignin:N ratio varied greatly among different species, with implications for the differences of initial litter quality among those species. As plants generally maintain characteristic stoichiometric relationships under natural conditions (Sistla and Schimel, 2012), the increased N concentration in senesced tissues potentially accelerate litter decomposition. Hobbie (2015) concluded that plant litter traits differ predictably along fertility gradients, and such differences reinforce soil fertility gradients through effects on litter decomposition and N release. In light of the increased N deposition in this ecosystem (Galloway et al., 2008; Gruber and Galloway, 2008; Peñuelas et al., 2012), it is reasonable to expect that this ongoing fertilization will accelerate litter decomposition and nutrient release through its positive impacts on the N status of plant litters.

Beyond the litter N concentration, litter C chemistry of the four grasses was also sensitive to N addition. Nitrogen addition reduced lignin, cellulose, and hemicellulose concentrations and lignin:N ratio in the litters of all the four species. We considered that N addition may increase the content of other compounds, such as soluble sugar, starch and amino acids, and thus decrease the proportion of lignin, cellulose and hemicellulose in litters. Lignin:N ratio are negatively correlated with net N mineralization rate of soil (Scott and Binkley, 1997), and thus feed back on soil N availability. Our results suggest that the lower cellulose, hemicellulose and lignin concentrations, and lignin:N ratio in the steppe dominant grass species may be promoted by increased N deposition over time. Coupled with increased primary productivity and a shift in community composition, this change in litter quality is expected to accelerate decomposition and thus further increase nutrient availability in this N-limited temperate steppe system (Bai et al., 2010; Zhang et al., 2015).

We found no significant effects of seven years of mowing on litter N concentration at both species and community levels. Mowing decreased lignin and cellulose concentrations, increased hemicellulose concentrations, but showed no significant impact on lignin:N ratio at species-level. At the community level, however, mowing only decreased litter cellulose concentrations. These results may reflect that mowing promoted the synthesis of some more easily decomposed compounds, such as soluble sugar, starch and hemicellulose, and thus decreased lignin and cellulose concentrations in litter tissues. Ziter and MacDougall (2013) also showed that mowing could reduce the C complexity of plant leaves, in particular by reducing lignin concentration. Our results thus suggest that mowing could increase initial litter quality by reducing C complexity, and would potentially accelerate litter decomposition. Overall, our results suggested that the impacts of mowing on litter quality

mainly occurred through the alteration of C chemistry instead of N chemistry.

Both N addition and mowing affected community composition by changing aboveground biomass of individual species in this semi-arid grassland. Across the individual species, N fertilization increased the biomass of *S. grandis* and *A. sibiricum*, but had no effects on the biomass of *L. chinensis* and *A. cristatum*. Mowing increased the biomass of *S. grandis*, decreased the biomass of *L. chinensis* and *A. sibiricum*, with no effects on that of *A. cristatum*. The different responses of individual species likely reflects inherent variation in growth strategy, which may result in changes of competitive interactions and dominance patterns, and subsequently alterations of community composition (Klanderud and Totland, 2005; Yang et al., 2011). Importantly, both N addition and mowing increased the biomass and thus dominance of *S. grandis* in this ecosystem, which has the lowest litter quality among the examined four species (Figs. 1 and 2). Similarly, Giese et al. (2009) also found that mowing could affect litter chemistry at the community level by changing species composition in a nearby ecosystems.

Given the interspecific variation of nutrient status and C quality among different species and their divergent responses in biomass to both N addition and mowing, our results indicated that both N addition and mowing would affect litter quality by changing community composition, and consequently influence litter decomposition and nutrient cycling in the semi-arid grassland. Consequently, the observed variation in litter quality among the dominant grass species in the steppe system indicates that changes in community composition with N deposition and mowing could potentially influence nutrient cycling via a litter feedback.

## 5. Conclusion

Our results demonstrated that both N addition and mowing can influence litter quality at both the species and community levels in semi-arid grassland systems. Both N addition and mowing increased litter quality, and long-term annual mowing did not alter the impacts of N fertilization on litter quality. Furthermore, both N addition and mowing affected community composition, which can indirectly affect litter quality in this temperate steppe. Because litter quality is coupled to decomposition dynamics and primary productivity, the enhanced litter quality following N addition and mowing would potentially accelerate nutrient cycling and affect community structure in ecosystems. Our study highlights the importance for documenting the consequences of simultaneously occurring of global climate change phenomena such as N deposition and ecosystem management strategies, such as haymaking, on ecosystem properties and functions. Results from this study thus contribute to grassland management decision-making strategies in this rapidly changing system.

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