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REGULAR ARTICLE

Hierarchical responses of plant stoichiometry to nitrogen deposition and mowing in a temperate steppe

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Abstract

Background and aims Stoichiometric relations drive powerful constraints on many ecosystem processes. However, our understanding of the hierarchical responses of plant C:N:P stoichiometry at different levels of biological organization to global change factors remains limited.

Methods we examined the plant C:N:P stoichiometric responses to N deposition and mowing (hay making) at both species- and community-level by carrying out a 4-year field experiment in the temperate steppe of northern China.

Results Our results showed that N addition and mowing resulted in higher plant N concentrations, lower C:N, and higher N:P at both species- and community-level. Mowing had a limited negative influence on the effects of N addition. We observed divergent responses of both plant P concentrations and C:P to N addition at species-level and community-level: N addition led to higher plant P and lower C:P at species-level, but this effect was not observed at the community-level.

Conclusions Our results indicate that stoichiometric responses at community-level to N addition and mowing diverge from more traditionally examined species-specific responses. Our results suggest that the hierarchical responses of plant stoichiometry to anthropogenic disturbance deserves more attention when we model the interactions of terrestrial ecosystem C, N, and P cycling under scenarios of increasing N availability concomitantly occurring with active land management.

Keywords C:N:P · Mowing · Hay-making · Ecological stoichiometry · Ecosystem functioning · Litter quality · Nitrogen deposition · Semiarid grassland

Introduction

Anthropogenically driven increases in reactive nitrogen (N) availability greatly influence ecosystem structure and function (Galloway et al. 2008; Peñuelas et al. 2012). Because the majority of terrestrial ecosystems are N-limited (LeBauer and Treseder 2008), N deposition is expected to enhance plant growth and thus primary productivity (Elser et al. 2007; Harpole et al. 2011). The positive effects of N deposition on primary

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productivity, however, are stoichiometrically governed by the relative availability of other elements, such as phosphorus (P) (Peñuelas et al. 2012; Vitousek et al. 2010), as well as ecosystem management practices, such as defoliation due to mowing (for hay production) or grazing in the grasslands (Gruner et al. 2008; Ziter and MacDougall 2012). Plant C:N:P stoichiometry plays an important role in mediating many ecological processes through its effects on plant growth and substrate quality for organisms at other trophic levels (Koerselman and Meuleman 1996; Mooshammer et al. 2011). Consequently, it is critical to understand the responses of both individual plant species and whole communities to increasing N availability in managed landscapes under an ecological stoichiometry framework.

While N deposition typically results in higher foliar N concentrations and lower C:N ratios in plant tissues (Henry et al. 2005; Lü et al. 2012a; Sardans and Peñuelas 2012), large uncertainties remain about the effects of N deposition on foliar P concentrations and N:P ratios. Positive (Menge and Field 2007), neutral (Lü et al. 2012a), even negative (Rowe et al. 2008) effects of N deposition on plant tissue N:P have been reported, partly due to variation in both species-specific response to fertilization and in the amount of N being added in different studies. This variable response reflects that N deposition may promote conservative P use and thus higher N:P due to growth dilution effect (Perring et al. 2008), while under P-limiting conditions, N deposition may increase soil P availability and plant P uptake (Fujita et al. 2010) by increasing P-mineralizing phosphatase activity (Marklein and Houlton 2012). The balance between those two processes would lead to variable response patterns of plant N:P to nitrogen addition. Plant N:P status is considered an indicator for nutrient limitation of plant growth and primary productivity (Güsewell 2004; Koerselman and Meuleman 1996). However, whether increasing N deposition would result in consistent changes of N or P limitation status at both species and ecosystem levels remains an open question.

The impacts of increasing N availability on stoichiometrically regulated ecosystem functions will be further modulated by ecosystem management strategies. Mowing, which removes the majority of current-year aboveground biomass (and thus the nutrients contained within those plant tissues from the system), is a widely practiced grassland management strategy across the world (Giese et al. 2013; Klumpp et al. 2011). Mowing is associated with both positive and negative effects on plant nutrient status, depending on management intensity, history, and species identity (Hiernaux and Turner 1996; Mikola et al. 2009). Mowing may enhance plant nutrient status by stimulating the growth of newly produced leaves; the impacts of mowing on tissue chemistry can be much stronger than that of nutrient addition (Ziter and MacDougall 2012). Persistent mowing can also reduce plant N contents by negatively affecting soil nutrient availability through the chronic removal of litter (Turner et al. 1993). Further, the effects of mowing on plant nutrients and element stoichiometry can vary with site productivity (Robson et al. 2007).

Both N deposition and mowing can significantly influence grassland community composition through their impacts on nutrient availability (Collins et al. 1998; Isbell et al. 2013). Differential responses of individual plant species due to their inherent variation in nutrient use strategies may result in alteration of competitive interactions and dominance patterns, and subsequently changes of plant community structure and composition (Klanderud and Totland 2005; Yang et al. 2011). Consequently, the plant-stoichiometry-mediated effects of N deposition and mowing on ecosystem processes will depend not only on element ratio changes at the species-level, but also at the community-level (e.g. biomass effects, altered species composition). Most natural ecosystems are species diverse and species-specific stoichiometric responses to disturbances such as N deposition and mowing are widely reported (Lü et al. 2012b; Sardans et al. 2012). While it is hypothesized that the transition from species-level stoichiometric responses to the community-level is a key threshold when considering the effects of global change factors on ecosystem function (Sistla and Schimel 2012), the empirical evidence is rather scarce. Instead, research has historically focused solely on species-level responses of plant stoichiometry to global change factors, with few studies identifying the connections between species- and community-level stoichiometric responses to change (Sardans and Peñuelas 2012).

Interactions between greater N availability and mowing may cause variable effects on plant stoichiometry at both the species and community scales, complicating our understanding of the interactive effects of global change factors and ecosystem management strategies on plant-soil systems. This study was designed to identify species- and community-level C:N:P stoichiometric responses to N addition and mowing in a temperate steppe of Inner Mongolia, northern China. The perennial C3-dominated grassland ecosystems in northern China are experiencing increasing N deposition (Liu et al. 2011) and have been traditionally used for haymaking, with consequences for community composition and ecosystem functioning (Bai et al. 2010). The main aims of this study were: (1) to examine whether mowing would impact the responses of plant C:N:P ratios to N deposition, and (2) to investigate whether community-level C:N:P responses to N addition and mowing parallel that of species-level stoichiometric responses to these disturbances.

Materials and methods

Study site and experimental setup

This study was conducted near the Inner Mongolia Grassland Ecosystem Research Station (IMGERS, 116°14’E, 43°13’N), which is located in Xilin River Basin, Inner Mongolia, northern China. The topography of this experimental area is flat, with elevations ranging from 1,255–1,260 m. The mean annual temperature is 0.9 °C, with mean monthly temperatures ranging from −21.4 °C in January to 19.7 °C in July for 1983–2012. Long-term mean annual precipitation is 355 mm, with 60–80 % falling during the growing season from May to August. The soil is classified as Calcic-Orthic Aridisol (USDA soil classification). The plant community is co-dominated (>60 % aboveground biomass) by *Leymus chinensis* (Trin.) Tzvel. and *Stipa grandis* P. Smirn.

This study was conducted in a subsection of an N deposition experiment, which has been maintained since 2008. There are four treatments (Control, N addition, Mowing, N addition + Mowing) with ten replicates for each treatment, for a total of 40 8 m×8 m plots. The study is arranged in a completely randomized block design, with each plot separated by a 1 m buffer. Nitrogen was added as purified NH_4NO_3 (>99 %), with a rate of 10 g N m^{-2} $years^{-1}$. The ambient atmosphere N deposition is estimated at 1–2 g N m^{-2} $years^{-1}$ in this region (Liu et al. 2011). To mirror the seasonal pattern of natural N deposition, half of the N was mixed with water (9.0 L), and then was sprinkled evenly using a sprayer to plot in June (wet deposition), the other half was mixed with sand (the sand was sieved to 1 mm in size, hydrochloric acid dipped, purified water washed and then heated at 120 °C for 24 h in an oven), and then spread by hand in November (dry deposition) each year. We estimated that less than 1 mm of water was added to the N addition plots. Plots under the mowing treatments were mowed with a mower about 10 cm above the soil surface at the end of September each year. All the litter was removed to the edge of each plot after mowing.

Field sampling and chemical analysis

Aboveground plants were sampled in mid August 2011 by clipping all plants above the soil surface using a 0.5 m×2 m quadrat, which was randomly placed in each plot (at least 50 cm inside each plot to avoid edge effects). All living vascular plants were sorted to species, oven-dried at 65 °C for 48 h, weighed and then finely ground in a ball mill. Nutrient analyses were completed on the seven dominant plant species in the system: one perennial rhizome grass, *Leymus chinensis* (L.c.), five perennial bunchgrasses, *Stipa grandis* (S.g.), *Agropyron cristatum* (A.c.), *Achnatherum sibiricum* (A.s.), *Cleistogenes squarrosa* (C.s.), *Koeleria cristata* (K.c.), and one sedge *Carex korshinskii* (C.k.) (Bai et al. 2004). Together, these species accounted for 65–100 % of the total aboveground biomass in all plots. Total C content was determined using a H_2SO_4-$K_2Cr_2O_7$ oxidation method (Lü et al. 2012b). Total N concentrations were determined colorimetrically by the Kjeldahl acid-digestion method with an Alpkem auto-analyzer (Kjektec System 1026 distilling unit, Sweden) after extraction with sulfuric acid. Total P concentration was measured by persulfate oxidation followed by colorimetric analysis (Kuo 1996). Mass ratios of C:N, C:P and N:P are used here to facilitate comparisons with previous studies (Güsewell 2004; Sardans et al. 2012).

Data calculation and statistical analysis

To calculate the community-level nutrient concentrations for each plot, the sum of total nutrient contents of the seven dominant species in each plot were divided by the sum of their total aboveground biomass. Consequently, nutrient concentration at community-level in this study is defined as the biomass-weight concentrations (instead of an average of nutrient concentrations) of all dominant species in the community. Community C, N, and P pools were calculated for each plot, based on the biomass and element concentration of each species presented in the plot.

Data were tested for normality using the Kolmogorov-Smirnov test and for equality of error variance using Levene's test. When necessary, data were natural log transformed to meet the assumption of normal distribution of data and homogeneity of variances. Four-way ANOVAs with species identity, N addition, mowing as the fixed factors and block as random factor were performed to examine the main and interactive effects on foliar nutrient concentrations, C:N:P ratios, and aboveground biomass at species-level. For those parameters at community-level, three-way ANOVA with N addition and mowing as the fixed factors and block as random factor were performed. Non-significant interactions were removed from the analysis. A Tukey HSD post hoc test was used to test for significant differences between treatments following the ANOVA ($p<0.05$). All the analyses were conducted with SPSS v13.0 (SPSS, Chicago, Illinois, USA).

Table 1 Results (*F*-value) of four-way ANOVAs on the effects of block (B), species identity (S), nitrogen addition (N), mowing (M) and their interactions on plant nutrient variables and stoichiometric ratios at species-level

	%C	%N	%P	C:N	C:P	N:P
B	15.9***	30.1***	2.5	10.6***	0.4	7.2**
S	9.2***	5.1***	9.0***	5.1***	11.6***	12.4***
N	0.4	334.0***	45.0***	236.3***	38.6***	46.2***
M	4.3*	45.4***	2.6	42.2***	2.1	47.0***
N × M	1.7	16.9***	0.6	22.9***	0.1	7.2**
N × S	1.2	1.9	2.7*	1.9	2.3*	3.8***
M × S	0.5	1.0	1.5	1.3	1.1	1.7
N × M × S	0.5	0.7	1.8	0.9	1.5	1.0

*$p<0.05$; **$p<0.01$; ***$p<0.001$

Results

Species-level stoichiometric responses

There were significant differences of nutrient concentrations and stoichiometric ratios among the seven species (Table 1; Figs. 1 and 2). N addition did not affect plant C concentration in any species, but increased average plant N concentrations by 35 % and thus decreased C:N by 28 % for all species (Table 1; Figs. 1a, b and 2a). Nitrogen addition and species identity interacted to affect plant P concentrations and C:P (Table 1). Nitrogen addition significantly increased plant P and decreased C:P in A.s., A.c., and C.k. (Figs. 1c and 2b). Across all species, N addition enhanced plant N:P by 12.2 % (Table 1, Fig. 2c). Nitrogen addition increased plant N:P in L.c., C.s., K.c., and S.g. (Fig. 2c), as reflected by the significant N addition and species identity interaction on N:P (Table 1).

Across all the species, mowing significantly increased plant C and N concentrations (by 1.2 % and 13.2 %, respectively) and decreased C:N (by 13.3 %) (Table 1). Mowing and N addition interacted to affect plant N concentrations, in that the effects of mowing were much stronger under N enriched conditions than ambient N conditions (Fig. 1b). There was no effect of mowing on plant P concentrations. Nitrogen addition and mowing significantly enhanced plant N:P for all species except K.c. (Table 1; Fig. 2c). Nitrogen addition and mowing interacted to affect plant N concentration, C:N, and N:P (Table 1). Mowing alone increased plant N:P in A.c., S.g., and C.k. (Fig. 2c).

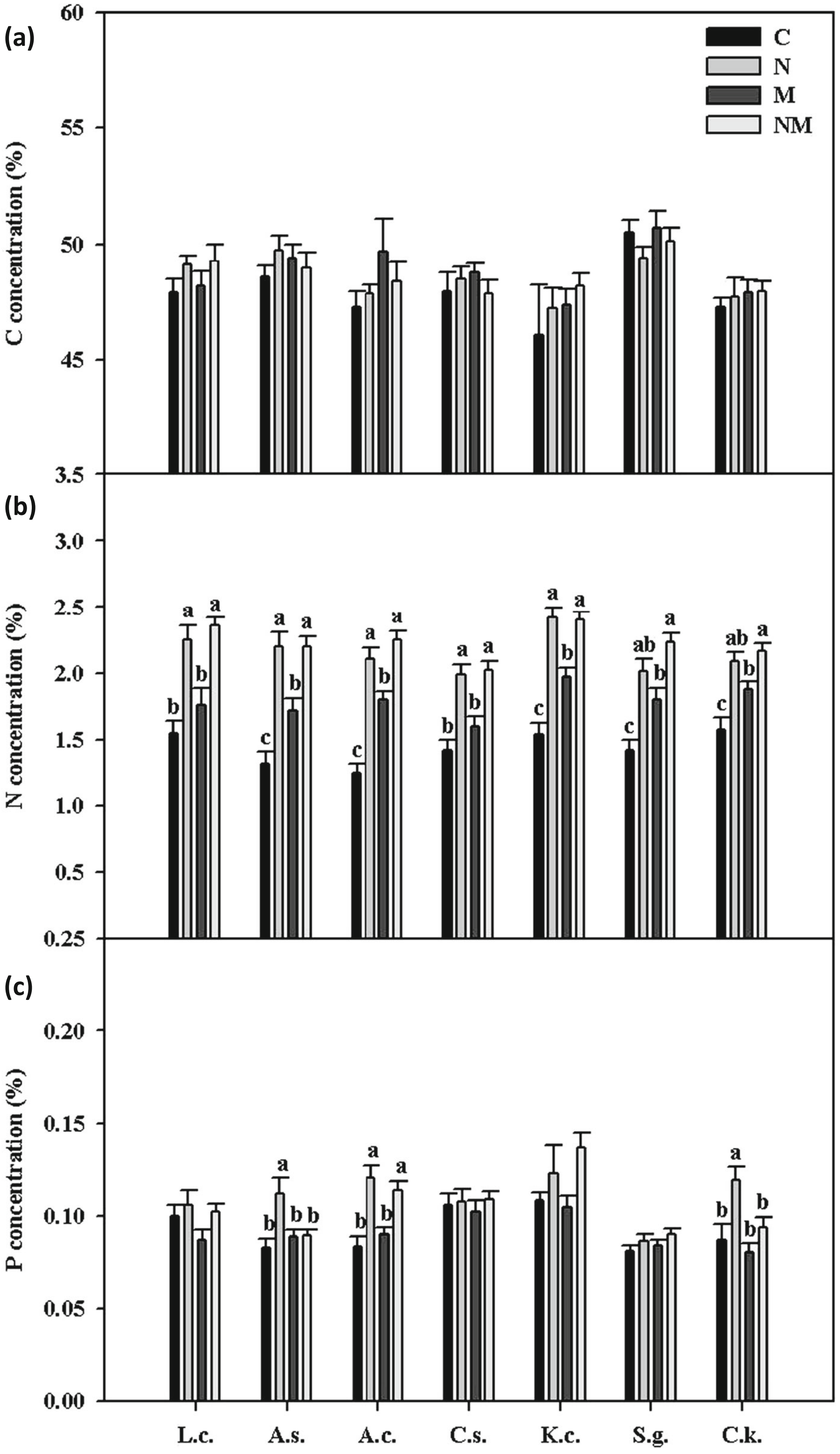
Species- and community-level biomass responses to treatments

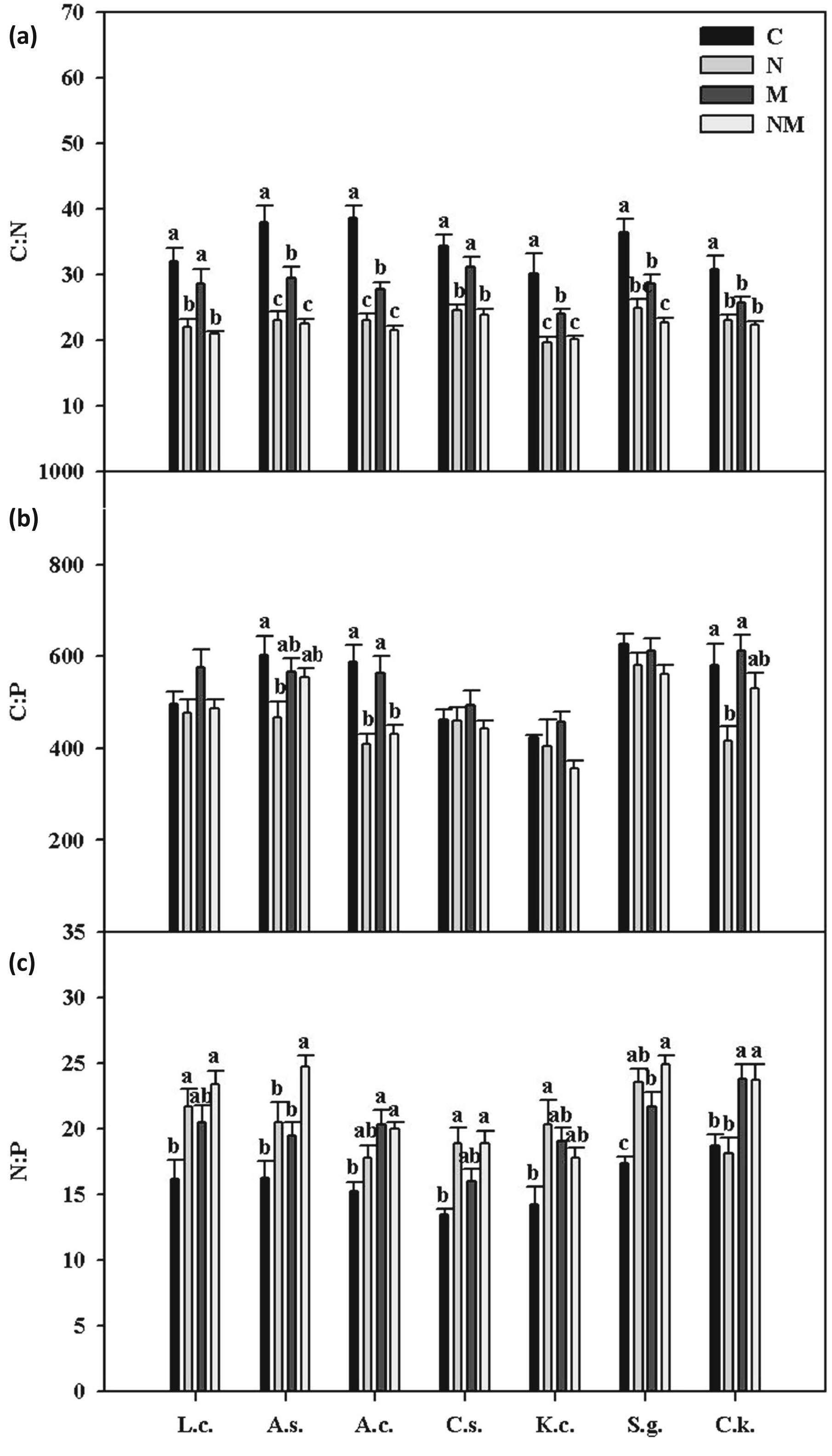
Nitrogen addition significantly increased total plant biomass C, N, and P pools by 27.5 %, 76.2 %, and 44.4 %, respectively, whereas mowing did not impact plant C, N, or P pool at community-level (Table 2, Fig. 3). The growth responses of main individual species to N addition and mowing were idiosyncratic (Fig. 4). N addition enhanced the biomass of S.g. by 59.7 %. Mowing doubled C.s. biomass and increased the biomass of C.k. by 80 % (Fig. 4). No other treatment effects on species-specific aboveground biomass were observed, with the combination of N addition and mowing mitigating the species-specific growth responses observed in the individual treatments.

Community-level stoichiometric responses

Neither N addition nor mowing significantly affected plant C concentrations, P concentrations, or C:P at

Fig. 1 The effects of nitrogen addition and mowing (*C*, control; *N*, nitrogen addition; *M*, mowing; NM, both *N* addition and mowing) on plant carbon **a**, nitrogen **b**, phosphorus **c** concentrations of seven dominant species. Data are shown as means +1 SE. *Letters* indicate results of Tukey's HSD posthoc test of significant difference between treatments; *bars that share the same letter* are not significantly different ($p<0.05$) ▶


(a)
C concentration (%)
60
55
50
45
3.5
C
N
M
NM
(b)
N concentration (%)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.25
b
a
b
a
c
a
b
a
c
a
b
a
b
a
b
a
c
a
b
a
c
ab
b
a
c
ab
b
a
(c)
P concentration (%)
0.20
0.15
0.10
0.05
0.00
b
a
b
b
b
a
b
a
b
a
b
b
L.c.
A.s.
A.c.
C.s.
K.c.
S.g.
C.k.


(a)
(b)
(c)
C
N
M
NM
C:N
C:P
N:P
70
60
50
40
30
20
10
1000
800
600
400
200
35
30
25
20
15
10
5
0
a b a b
a c b c
a c b c
a b a b
a c b c
a bc b c
a b b b
a b ab ab
a b a b
a b a ab
b a ab a
b b b a
b ab a a
b a ab a
b a ab ab
c ab b a
b b a a
L.c.
A.s.
A.c.
C.s.
K.c.
S.g.
C.k.

◀ **Fig. 2** The effects of nitrogen addition and mowing (*C*, control; *N*, nitrogen addition; *M*, mowing; NM, both *N* addition and mowing) on plant stoichiometric ratios, C:N **a**, C:P **b**, N:P **c** of *seven dominant species*. Data are shown as means +1 SE. *Letters* indicate results of Tukey's HSD posthoc test of significant difference between treatments; *bars that share the same letter* are not significantly different ($p<0.05$)

community-level. N addition, mowing, and their combination all increased N concentration at the community-level (by up to 50 % in the N addition treatment) and decreased community-level C:N (by up to 33 % in the N addition treatment), with no significant difference observed between the N addition treatments (Table 2, Fig. 5a,b). All treatments significantly enhanced community N:P from 27.1 % (mowing only) to 36.8 % (N addition treatments) (Table 2; Fig. 5c).

Discussion

Characterizing the effects of anthropogenic perturbation on plant stoichiometric relationships is critical to accurately projecting the consequences of these disturbances on ecosystem functions. Moving beyond the traditional focus on species-level stoichiometric responses to anthropogenic disturbance, this study also identified stoichiometric changes at the community-level. We found that C:N:P ratios at species- and community-level showed both convergent and divergent responses to N addition and mowing. For plant N concentrations, C:N, and N:P, the effects of N addition and mowing were similar at both the species- and community-level. This convergence reflects the largely consistent responses of plant N and C:N in different species to N addition and mowing. In contrast, the effects of N addition on plant P concentrations and C:P varied across different organization levels. When analyzed at species-level, N addition increased plant P concentrations and decreased C:P ratios. In contrast, N addition did not affect those two variables at community-level, suggesting that while P-limitation may be alleviated with increasing N availability for certain species, P-limitation may be exacerbated at the community level.

The asynchronous responses of P concentrations and biomass to N addition across individual species appear to be a primary driver of the divergent responses of plant P stoichiometry to N addition at species- and community-level. The positive effects of N addition on plant P concentrations and the negative effects on plant C:P only occurred in three out of the seven species (A.s., A.c., C.k.; Figs. 1c and 2b); however, N addition did not impact the growth of those species. In contrast, N addition stimulated the growth of S.g., while having no affect on its P stoichiometry. Therefore, N deposition would alter plant stoichiometry at community-level both directly through physiological changes (i.e. changes of nutrient concentrations in plant tissues), and indirectly through alteration of community composition. As such, changes in community composition with increasing active N availability may have significant implications for community-level plant stoichiometry. Furthermore, our observation that N addition altered N-related stoichiometric ratios at both species- and community-level, while changing only species-level P-related ratios, gives further evidence for the hypothesis of Ågren and Weih (2012) that different elements respond differently at the different scales. This study demonstrates robust evidence for the varied responses of plant stoichiometry between the species- and community-scales to environmental variation.

The seven dominant grassland species studied varied greatly with respect to nutrient concentrations and C:N:P stoichiometric ratios. We found that N addition significantly stimulated the growth of a bunchgrass, S.g., and increased total plant C and N pools,

Table 2 Results (*F*-value) of three-way ANOVAs on the effects of block (B), nitrogen addition (N), mowing (M) and their interactions on biomass-weighed carbon, nitrogen, and phosphorus concentrations (%C, %N, P%), their pools in plant biomass ($g\ m^{-2}$) and stoichiometric ratios at community-level

	%C	%N	%P	C pool	N pool	P pool	C:N	C:P	N:P
B	3.4	9.7^{**}	0.1	0.9	4.9^{*}	0.5	2.3	0.0	3.3
N	0.0	96.6^{***}	4.0	17.8^{***}	74.9^{***}	20.5^{***}	71.8^{***}	3.5	29.4^{***}
M	2.2	19.4^{***}	0.1	0.4	1.3	0.9	16.5^{***}	0.3	13.3^{***}
N × M	3.4	4.2^{*}	0.3	0.9	2.2	0.8	6.1^{*}	0.0	1.5

*$p<0.05$; **$p<0.01$; ***$p<0.001$

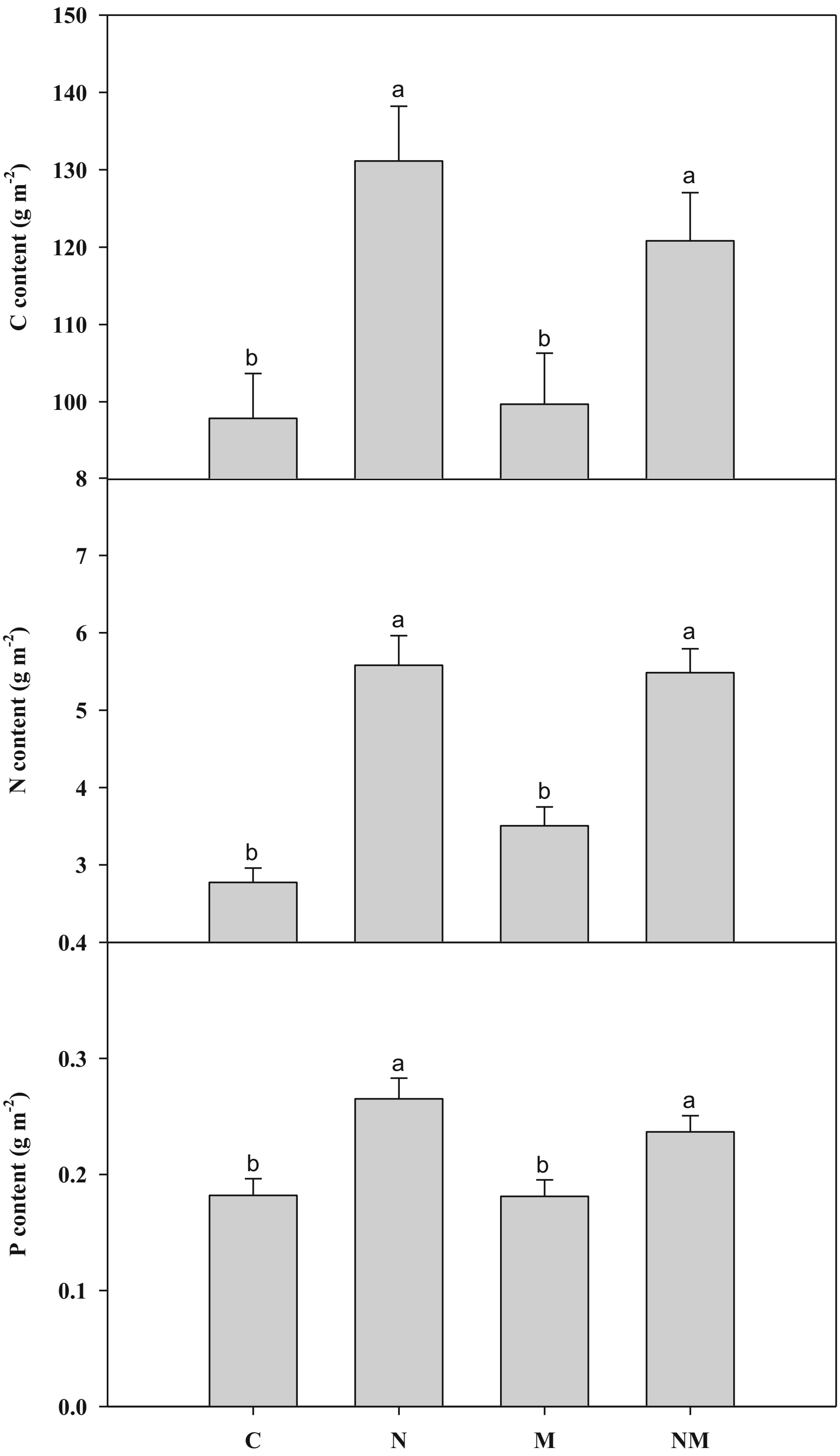
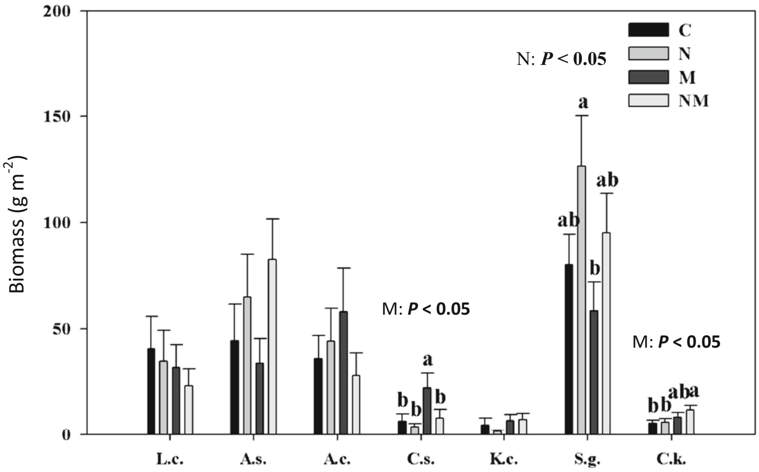


Fig. 3 The effects of nitrogen addition and mowing (*C*, control; *N*, nitrogen addition; *M*, mowing; NM, both *N* addition and mowing) on aboveground community carbon, nitrogen, and phosphorus content. Data are shown as means +1 SE. *Letters* indicate results of Tukey's HSD posthoc test of significant difference between treatments; *bars that share the same letter* are not significantly different (p<0.05)

Fig. 4 The effects of nitrogen addition and mowing (*C*, control; *N*, nitrogen addition; *M*, mowing; NM, both *N* addition and mowing) on aboveground biomass of *seven dominant species*. Data are shown as means +1 SE. *Letters* indicate results of Tukey's HSD posthoc test of significant difference between treatments; *bars that share the same letter* are not significantly different ($p<0.05$)



corresponding to a decline in community-level C:N and increase in N:P. Supporting previous studies suggesting that mowing is a strong driver for community composition in grassland ecosystems (Collins et al. 1998), we found that mowing facilitated the growth of the low-stature species, C.k. and C.s., which decreased community-level C:N and increased N:P (to a lesser extent than the N addition treatments). Intriguingly, the combination of mowing and N addition did not significantly alter community composition, although it did reduce community-level C:N status. Given the large interspecific variation in C:N:P ratios as found in this study, N deposition and mowing exert non-linear effects on community stoichiometric ratios by changing community composition as well as species-specific C:N:P ratios.

Nitrogen addition increased plant N concentrations for all the seven species. It is well documented that increasing N availability leads to higher plant N status (Kozovits et al. 2007; Lü et al. 2012a; Rowe et al. 2008). A recent meta-analysis (Xia and Wan 2008) showed that plant N concentrations were increased on average by 28.5 % across 456 terrestrial plant species following N-fertilization. Moreover, our results indicate that more N will be immobilized in plant biomass under N enrichment in this ecosystem, as N addition significantly enhanced community biomass and plant N storage in this ecosystem. This phenomenon, however, was mitigated when N addition was combined with mowing. The enhancement of community-level plant N pool in response to N addition indicates that the quality of herbage will increase with increased N availability in the temperate steppe. From a grassland management perspective, N fertilization at an appropriate rate would facilitate the animal husbandry in this area.

Our observation that N addition enhanced plant P status at species-level is consistent with previous findings (Lü et al. 2013). Notably, the positive effects of N addition on plant P concentrations were only significant in three (A.s., A.c., and C.k.) out of seven species in this study, indicating a species-specific responses of plant P to increased N availability. There are several pathways through which N addition would lead to increased P concentrations in plants. First, increased N availability could stimulate the activity of P mineralizing enzymes in soils and on plant roots (Marklein and Houlton 2012). Phosphatase enzymes play an important role in mobilizing P from ester-bonded forms (Fujita et al. 2010). The enhancement of phosphatase activities would provide more phosphate ions for plant uptake and assimilation. N addition may also increase the root colonization of P-acquiring arbuscular mycorrhizal fungi in grassland ecosystems, especially systems characterized by low soil P availability (Blanke et al. 2012; Egerton-Warburton et al. 2007; Johnson et al. 2003). Plants can also up-regulate the production of root-localized P-carrier enzymes to fulfill increased P-demand under N enrichment (Treseder and Vitousek 2001).

Achnatherum sibiricum is heavily infected by fungal endophytes, which could significantly improve acid phosphatase activity under N enriched conditions (Li et al. 2012). However, none of those three species showed positive responses of N addition with respect to aboveground biomass, indicating the possibility of luxury P use (Menge and Field 2007) for those species following N enrichment. The divergent responses of P

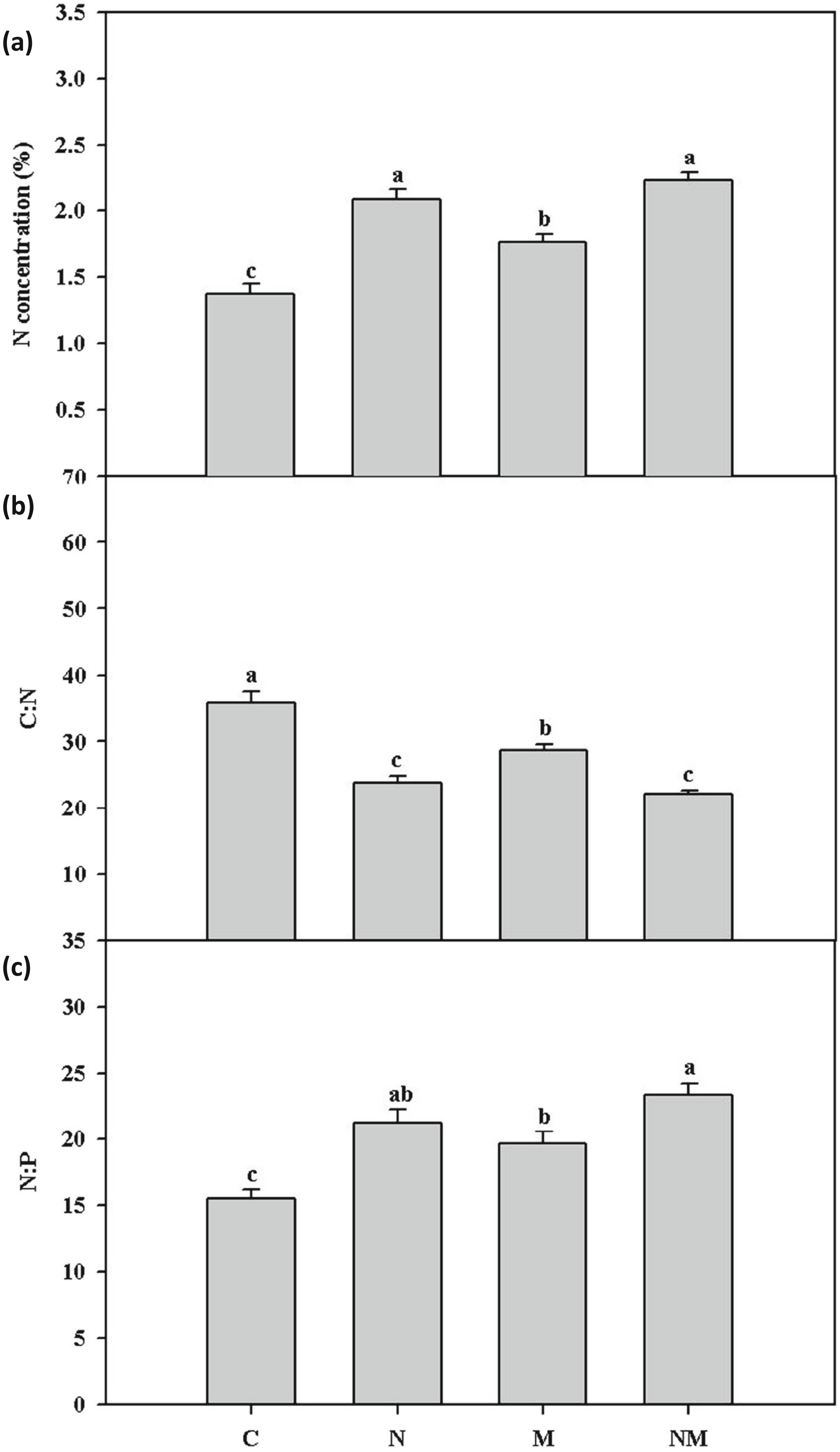


Fig. 5 The effects of nitrogen addition and mowing (*C*, control; *N*, nitrogen addition; *M*, mowing; NM, both *N* addition and mowing) on plant nitrogen concentrations **a**, C:N **b**, N:P **c** at community-level. Data are shown as means +1 SE. *Letters* indicate results of Tukey's HSD posthoc test of significant difference between treatments; *bars that share the same letter* are not significantly different ($p<0.05$)

concentrations among different plant species to N addition also imply a plant-mediated mechanism through which the micro-scale heterogeneity of soil nutrient status may be maintained under scenarios of increased soil N availability. Nitrogen addition also enhanced plant P uptake as indicated by the significant increase of plant biomass P pool at community-level after N enrichment. Increases of P requirement following increased N availability may alter nutrient limitation status for plant growth from N- to P-limitation (Vitousek et al. 2010).

Although both N and P concentrations in plant biomass were enhanced by N addition, plants increased N to a much greater degree than P as reflected by the increasing N:P ratios following N addition. Our results suggest that greater N availability has the potential to shift the nutrient limitation status towards greater P-limitation, though plant P status positively responded to N addition. Furthermore, the community-level biomass-weighed N:P ratios were significantly enhanced by N addition, indicating a robust and positive effect of N addition on community-level N:P in this temperate steppe. Together, our results suggest that the plant community of this temperate steppe ecosystem would tend to be limited by P availability with increased soil N availability resulting from both atmospheric N deposition and global warming (stimulating soil N turnover) (Ma et al. 2011).

We found that plant N concentrations were significantly enhanced after being mown annually for four years. In contrast, plant P status remained constant irrespective mowing or not. Consequently, mowing drove higher plant N:P ratios across all the seven species, indicating the potential for increasing P-limitation under this land management strategy. Positive effects of mowing on shoot N status have been reported in other studies (Green and Detling 2000; Mikola et al. 2009). Several mechanisms have been suggested to account for the elevated N concentrations under mowed conditions; plants would allocate more N to their regrowing shoots after defoliation (Mikola et al. 2009) or mowing may also enhance soil N availability by facilitating soil microbial N-mineralization (Hamilton and Frank 2001). While the underlying mechanism remains unclear, our results suggest that N and P in plant tissues would show divergent responses to mowing in this semiarid grassland.

We hypothesize that this divergence may be caused by differences in the N and P cycling patterns. Soil N transformation is a soil microbe mediated processes that is affected by abiotic factors such as water availability (Aerts and Chapin 1999). In contrast, P mineralization is largely a chemical processes (Aerts and Chapin 1999). The observed neutral effects of mowing on community-level biomass-weighed P and positive effects on community-level N:P indicate that mowing may promote plant P limitation in this ecosystem, which would be expected to have negative consequences for sustained haymaking. The potential P-limitation following continuously annual mowing as indicated by the changes of N:P ratios at both species- and community-level would be a particularly important consideration for forage production in the temperate steppe of China, where plant communities already tend to be P-limited (Han et al. 2005).

One of our main aims was to examine whether mowing would impact the responses of plant stoichiometry to N deposition. Our results indicate that N addition and mowing significantly interact to affect shoot N status, C:N, and N:P. These observations highlight the importance of identifying how concurrently occurring global change factors and land management strategies affect nutrient biogeochemical cycles in grasslands and other ecosystems. The effects of mowing were generally much stronger in ambient conditions than in N enriched conditions, indicating that the role of mowing is limited in mediating the effects of N addition on plant C:N ratios. Similarly, A.s. and C.k. expressed the highest N:P ratios in the treatment with both N addition and mowing. Long-term annual mowing and biomass removal may decrease soil N availability (Turner et al. 1993). Consequently, it is reasonable to expect that mowing would partly counteract the effects of N addition on N-related stoichiometric ratios in plant tissues. Our results, however, suggest that the effects of N addition on grassland plant C:N:P stoichiometric ratios are only marginally affected by 4 years of mowing. This study highlights that the effects of N deposition on nutrient concentrations and stoichiometric ratios in plant tissues is of concern, even in the grasslands under continuous biomass removal and consequently nutrient losses. Given the expected globally increasing atmospheric N deposition in the coming decades (Galloway et al. 2008), its potentially negative effects on biogeochemical cycling and community composition deserve more attention, even in heavily-used ecosystems, such as the temperate steppe of northern China.

In conclusion, the divergent responses of species- and community-level nutrient concentrations and stoichiometric ratios highlight a hierarchical response of plant C:N:P stoichiometry to environmental changes. Our results suggest that the community-level stoichiometric responses to anthropogenic disturbance would not be simply mirrored by what is happening at species-level. Rather, plants at both the species- and community-level are stoichiometrically flexible in their response to N addition at different scales. The hierarchical responses of plant P-related variables to N addition should be given full consideration in modeling the interactions of terrestrial C, N, and P cycles under the scenarios of global change. Moreover, we found limited effects of mowing in mediating the effects of N on plant stoichiometry, indicating the effects of increased N availability would be still of concern even in continuously harvested ecosystems.

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