Biogeochemistry (2017) 133:295–305
DOI 10.1007/s10533-017-0333-x

Temporal variability of foliar nutrients: responses to nitrogen deposition and prescribed fire in a temperate steppe

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Received: 22 November 2016 / Accepted: 10 April 2017 / Published online: 18 April 2017

Abstract Plant nutrient concentrations and stoichiometry drive fundamental ecosystem processes, with important implications for primary production, diversity, and ecosystem sustainability. While a range of evidence exists regarding how plant nutrients vary across spatial scales, our understanding of their temporal variation remains less well understood. Nevertheless, we know nutrients regulate plant function across time, and that important temporal controls could strongly interact with environmental change. Here, we report results from a 3-year assessment of inter-annual changes of foliar nitrogen (N) and phosphorus (P) concentrations and stoichiometry in three dominant grasses in response to N deposition and prescribed fire in a temperate steppe of northern China. Foliar N and P concentrations and their ratios varied greatly among years, with this temporal variation strongly related to inter-annual variation in precipitation. Nitrogen deposition significantly increased foliar N concentrations and N:P ratios in all species, while fire significantly altered foliar N and P concentrations but had no significant impacts on N:P ratios. Generally, N addition enhanced the temporal stability of foliar N and decreased that of foliar P and of N:P ratios. Our results indicate that plant nutrient status and response to environmental change are temporally dynamic and that there are differential effects on the interactions between environmental change drivers and timing for different nutrients. These responses have important implications for consideration of global change effects on plant community structure and function, management strategies, and the modeling of biogeochemical cycles under global change scenarios.

Keywords Ecological stoichiometry · Ecosystem stability · Inter-annual variation · N:P ratio · Nutrient limitation · Primary productivity · Semiarid grassland

Introduction

Plant nutrient concentrations and stoichiometry play a critical role in driving myriad fundamental ecological

Responsible Editor: John Harrison.

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processes, including photosynthesis, plant growth and competition, primary production, herbivory, pathogen infection, decomposition, and coupled biogeochemical cycling (e.g., Aerts and Chapin 2000; Agren 2008; Elser et al. 2010; Schlesinger et al. 2011). Accordingly, changes to plant nutrient concentrations and stoichiometric ratios have important ecosystem consequences. A variety of research lends rich insight into the variation of plant nutrients and stoichiometry at different spatial scales, ranging from local to global (e.g., Elser et al. 2010; Han et al. 2011; Reed et al. 2012; Reich and Oleksyn 2004). In contrast, less attention has been paid to the temporal dynamics of plant nutrients, with fewer studies examining the seasonal, intra-annual, and inter-annual dynamics of plant nutrients and stoichiometry (Treydte et al. 2008; Van de Vijver et al. 1999). Moreover, inter-annual patterns in absolute and relative nutrient changes in the face of global change remain poorly known, although manipulation experiments suggest the strong potential for effects (e.g., Sardans et al. 2012). Inter- and intra-annual changes in plant nutrients and stoichiometry have significant implications for the temporal stability of primary productivity, due to the close relationship between foliar nutrient status and plant carbon (C) fixation (Wright et al. 2004).

Climate change is expected to enhance inter-annual variability of precipitation through increasing the frequency and intensity of extreme precipitation events and years (IPCC 2013). Nutrient transformation processes in semiarid grasslands—the productivity of which are believed to be limited by water and nitrogen (N) availability (Hooper and Johnson 1999)—are highly sensitive to spatial and temporal variation in precipitation (Dijkstra et al. 2012; Wang et al. 2014). Indeed, the grasslands of northern China have been shown to be sensitive to inter-annual changes of precipitation (John et al. 2013). For instance, cumulative soil net N mineralization rates are 133% higher in the growing season of a wet year than that of a dry year in the semiarid grasslands of northern China (Zhou et al. 2009). In the same region, water additions have been shown to increase soil phosphorus (P) availability, likely due to the stimulation of P-mineralizing enzyme activities (Wang et al. 2016). Given the strong climate-dependence of soil nutrient transformations and the robust relationships between nutrient availability and plant community structure and function (e.g., Elser et al. 2000), plant nutrient status and stoichiometry are also likely to vary in functionally relevant ways across years with substantial variation of precipitation.

Beyond variation in environmental controls, such as precipitation, plant nutrients and stoichiometry are also directly sensitive to changes resulting from anthropogenic activities, such as N deposition and prescribed burning. Given the widespread increase in atmospheric N deposition in both developed and developing countries (Galloway et al. 2008), there is growing concern about the imbalance of nutrients in ecosystems (Peñuelas et al. 2013; Vitousek et al. 2010). From a stoichiometric perspective, N deposition generally increases plant N:P ratios and can result in P limitation to plant growth (Peñuelas et al. 2013). Previous studies have shown that increases in foliar N concentrations following simulated N deposition are much higher than N-induced changes to foliar P (Lü et al. 2013), although increased N can stimulate the activities of soil P-mineralizing enzymes (Marklein and Houlton 2012). However, while we know that the effects of N deposition on plant nutrients and stoichiometry can vary across years due to the interactive effects of soil N and water availability (Lü et al. 2012), our understanding of how multiple interactions will regulate foliar chemistry among years in the face of environmental change remains poor. For example, N deposition is not the only anthropogenic driver facing ecosystems, and perturbations such as fire have strong potential to additionally and differentially affect foliar nutrient concentrations and stoichiometry.

Prescribed fire is a common and important management strategy in grassland ecosystems (Blair 1997; Heisler et al. 2004; Pellegrini et al. 2015). Given that prescribed fire alters soil temperature and water availability (Henry et al. 2006), as well as directly affects the availability and chemical forms of nutrients, it is no surprise that fire has direct consequences for soil nutrient cycling (Wan et al. 2001). Data suggest that, over time, prescribed fire would inhibit soil N cycling and reduce N availability (Soong and Cotrufo 2015; Wan et al. 2001), but would have positive impacts on soil P availability (Henry et al. 2006; Rau et al. 2007; Schaller et al. 2015). However, the impacts of fire on plant stoichiometry can vary significantly among species and ecosystems. For example, fire resulted in lower shoot N:P ratios in a California annual grassland (Henry et al. 2006), while plant stoichiometry in a tropical savanna was quite resistant to prescribed fire (Pellegrini et al. 2015). In a previous study in a semiarid grassland in China, the responses of plant stoichiometry to fire were species-specific (Cui et al. 2010). Nevertheless, to our knowledge no study has focused on the temporal stability (inter-annual) of plant nutrients and stoichiometry and their responses to N deposition and annual burning, which significantly constrains our capacity to consider the importance of these effects both now and into the future.

Here, we report results from a field experiment exploring the main and interactive effects of N deposition and prescribed annual burning on foliar nutrient status and its temporal stability across three years (2006–2008) for three dominant grasses in a semiarid temperate steppe in northern China (together these species make up >70% of the aboveground biomass). We hypothesized: (1) plant nutrient status would vary among years due to inter-annual variation in precipitation and its effects on soil nutrient availability in this water-limited semiarid ecosystem (e.g., Lü et al. 2010), (2) the responses of foliar nutrients and stoichiometry to N deposition and prescribed fire would vary among years because their impacts on soil nutrient availability would be dependent on annual precipitation (Zhou et al. 2009), and (3) N addition would increase the stability of foliar N due to a higher N availability following additional N supply, but would decrease foliar P stability due to the unmatched meeting of plant demand for P (Vitousek et al. 2010). Due to these varied responses, changes in individual plant nutrients would translate into a decreased stability of foliar N:P ratios. We focused on grasslands because these ecosystems are experiencing significant increases in N inputs worldwide (Galloway et al. 2008), because fire is a common management technique in grasslands (Blair 1997; Heisler et al. 2004; Pellegrini et al. 2015), and because such semiarid ecosystems may dominate the interannual variability (and the trajectory) of the global carbon sink (Ahlstrom et al. 2015; Poulter et al. 2014).

Methods and materials

The experiment was carried out in a semiarid temperate steppe in Inner Mongolia of northern China, near the Inner Mongolia Grassland Ecosystem Research Station (IMGERS, 43°38′N, 116 °42′E, 1250 m a.s.l). Long-term mean annual precipitation is 334 mm (1980–2008), ranging from 182 mm in 1980 to 507 mm in 1998, with 90% of precipitation distributed from May to October. The mean annual temperature is 0.92 °C with a monthly mean temperature ranging from −20.7 °C in January to 16.5 °C in July. The soil is a calcic-orthic Aridisol in the US Department of Agriculture soil taxonomy classification system, and is chestnut according to Chinese classification. Mean bulk density of the topsoil is ~ 1.3 g cm^{-3}, and soil pH is ~ 7.2. The ecosystem has experienced heavy grazing and encroachment by the legume shrub *Caragana microphylla*. In 1983, the ecosystem was fenced to exclude all livestock grazing. The plant community is dominated by a perennial rhizomatous grass *Leymus chinensis* (*L. chinensis*), several perennial bunchgrasses including *Stipa grandis* (*S. grandis*), *Cleistogenes squarrosa* (*C. squarrosa*), and *Agropyron cristatum*, and the legume shrub, *C. microphylla*. Together, the three focal species (*L. chinensis*, *S. grandis*, and *C. squarrosa*) make up >70% of the ecosystem's aboveground biomass. Other common species in the ecosystem include perennial forbs, *Potentilla acaulis*, *Potentilla bifurca*, *Allium tenuissimum*, *Allium bidentavum*, *Thalictrum squarrosum*; annual and biannual forbs, *Axyris amaranthoides*, *Chenopodium aristatum*, *Salsola collina*, *Chenopodium glaucum*; Subshrb species, *Artemisia frigida*, *Kochia prostrata*; and sedge species, *Carex korshinskii*. There were about 15–25 species per square meter in the pre-treatment plots and the abundance of the focal species did not vary prior to treatment application. The annual precipitation of 2006, 2007, and 2008 was 304, 240, and 362 mm, respectively. The mean annual temperature was 1.5, 2.0, and 1.7 °C for those three years, respectively. The year 2007 was the 4th driest year and the 3rd warmest year in the past four decades (since 1971). Precipitation in 2006 and 2008 was a bit lower and higher than the long-term mean annual precipitation (334 mm), respectively. This wide range in precipitation across the three years allowed us to explore a relatively large span of potential precipitation effects. In this region, present annual ambient atmosphere N deposition is estimated to be <1.0 g N m^{-2} (Zhu et al. 2015), and is expected to increase in the future (Galloway et al. 2008).

This research is a part of a larger experiment that has multiple fire and N addition treatments. For the larger experiment, in 2005, after more than 20-year of fencing, an experiment applying prescribed fire at five frequencies (unburned, burned every 1, 2, 4, 6 year), N addition at four different levels (0, 5.25, 17.25, 28 g N m^{-2} $year^{-1}$), and mowing at four frequencies (unmown, mowing every year, mowing every other year, and mowing annually for three years followed by one year of no mowing) was established. In total, there were 80 different treatments, with each replicated nine times (nine blocks) for a total of 720 plots. Each block was divided into 80 plots with the area of 10 m × 10 m, which were separated by 1 m buffers. Plots under the burning treatments were burned either at the end of April or in early May, depending on the timing of snow melt. Nitrogen was supplied as NH_4NO_3 in dry form on rainy days in early July of each year. We fertilized during a wet month and on rainy days in order to promote fertilizer dissolution and responses of forage production. In this study, we focused on the impacts of annual burning and N addition treatments of 5.25 g N m^{-2} $year^{-1}$, which is a rate of N addition similar to that used in many studies focusing on N deposition (usually 5 g N m^{-2} $year^{-1}$). Thus, there were four treatments: control (C), N addition (N), annual burning (F), and combined N addition and burning (NF) for a total of 28 10 m × 10 m plots, with seven replications for each treatment.

In the second week of August in 2006–2008, we sampled leaves of *L. chinensis*, *S. grandis*, and *C. squarrosa* in plots receiving each of the four treatments. The leaf samples were taken from leaves at the same developmental stage across the three years, and the date ranges were quite limited within each year. Plant samples were collected from a 2 m × 2 m subplot in the center of each plot. For *L. chinensis*, and *S. grandis*, about 15–20 fully expanded mature leaves were sampled from 8 to 10 individuals in each subplot. Due to the smaller leaf area of *C. squarrosa*, 30–40 leaves from 15 to 20 individuals were sampled from each subplot. We collected two fully expanded leaves of almost the same size from each individual, usually the third and fourth visible leaves from the top of each shoot. Leaf samples were dried at 70 °C for 48 h, and then ground with a ball mill (Retsch MM 400, Retsch GmbH and Co KG, Haan, Germany). Subsamples ($\sim$0.1 g) were digested in H_2SO_4–H_2O_2 (Bennett et al. 2002). Total N concentrations were analyzed with an Alpkem autoanalyzer (Kjektec System 1026 Distilling Unit, Sweden) and total P concentrations were determined colorimetrically at 880 nm after reaction with molybdenum blue. The N:P ratio was reported as a mass ratio, calculated from the N and P concentrations for each sample.

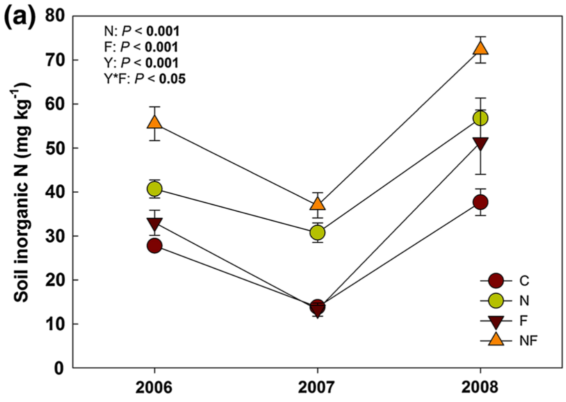
At the same time as leaf sampling each year, samples of the top soils (0–10 cm depth) were collected for each plot. Three soil cores from each plot were collected using a 3 cm diameter auger and were then mixed into one composite sample. All samples were sieved through a 2 mm mesh size to remove roots and stones. Soil moisture was gravimetrically determined by drying soil subsamples at 105 °C for 48 h. Fresh soil samples were extracted with 50 ml of 2 M KCl and the filtered soil extract was used to determine ammonium and nitrate concentrations with a continuous flow spectrophotometer (FIAstar 5000, Foss Tecator, Denmark). Plant available soil P was estimated by extracting soil with 0.5 M $NaHCO_3$ (pH 8.5) and analyzed using the molybdenum blue-ascorbic acid method (Olsen et al. 1954).

We focused on the temporal stability of plant N and P concentrations and stoichiometric ratios, which measures the degree of constancy in each nutrient variable relative to its mean across the three years examined in this study. The stability is defined as the ratio of the mean value of a variable (N, P, or N:P) for the three years relative to its standard deviation over the same interval (Tilman et al. 2006), which equals the inverse of the coefficient of variation.

Data were tested for normality using the Kolmogorov–Smirnov test and for equality of error variance using Levene's test. As all the data met model assumptions, thus we used untransformed data for statistical analysis. Repeated measures ANOVAs were used to examine the effects of N and fire on soil nutrient concentrations, foliar N and P concentrations, and N:P ratios. Three-way ANOVAs were used to test the main and interactive effects of species identity, N addition, and fire on the stability (1/CV) of N and P concentrations and N:P ratios. The between-subject effects evaluated were species identity, N addition, and prescribed fire, and the within-subject effect was year. As there were significant interactions between species identity and the treatments, we performed repeated measures ANOVA analysis for each species separately. All analyses were conducted using SPSS 13.0 (SPSS Inc., Chicago, USA) and significance was determined at $\alpha = 0.05$.

Results

Soil inorganic N concentrations differed significantly across the three years, with the lowest values occurring in the dry year of 2007 (F = 7.85, d.f. = 2; Fig. 1a). Nitrogen addition significantly enhanced soil inorganic N concentrations (F = 144.22, d.f. = 1; Fig. 1a). In contrast, the impacts of prescribed burning varied across years, as indicated by a significant interaction between fire treatment and year (F = 3.95, d.f. = 2). The positive effects of prescribed burning on soil inorganic N concentrations were highly significant in the wetter years (2006 and 2008), but much less so in the dry year (2007). The concentrations of plant available P in soils also showed a year-dependent variation, with much higher values in the wet years than in the dry year (F = 24.23, d.f. = 2; Fig. 1b). Nitrogen addition and prescribed burning interacted to affect plant available soil P (F = 7.73, d.f. = 1), such that prescribed burning tended to increase available P only under conditions of N enrichment and only in the wetter years.



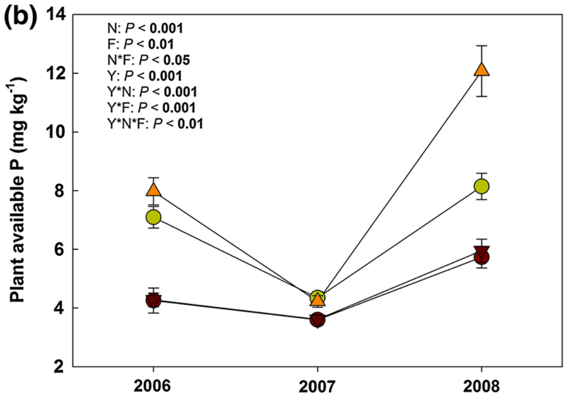


Fig. 1 The concentrations of extractable inorganic N (**a**) and plant available P (**b**) in soils in response to N deposition and prescribed burning treatments in a temperate steppe (C, control, N, N addition with a rate of 5.25 g N m^{-2} year^{-1}, F, annual burning, NF, N addition and annual burning). Data shown are means for each year (Y) and *error bars* represent 1 SE. ANOVA P-values are reported when $p < 0.05$ (*in bold*)

Plant nutrient concentrations and stoichiometric ratios significantly varied among different species (Table 1). Across all treatments and years, the mean foliar N in *L. chinensis* was highest (23.24 ± 0.34 mg g^{-1}) and that of *S. grandis* was lowest (19.17 ± 0.35 mg g^{-1}) (Fig. 2a–c). The mean foliar P was highest in *C. squarrosa* (1.82 ± 0.05 mg g^{-1}) and lowest in *S. grandis* (1.3 ± 0.05 mg g^{-1}) (Fig. 2d–f). The foliar N:P ratios in *C. squarrosa* (12.83 ± 0.56) were much lower than those found for *L. chinensis* (16.9 ± 0.60) and *S. grandis* (16.55 ± 0.72) (Fig. 2g-i).

All nutrient variables for all species showed significant inter-annual variation (Table 1, Fig. 2), except for foliar N of *L. chinensis* (Fig. 2a). For example, across all treatments, foliar P of *L. chinensis* in the hot and dry year 2007 was 40% lower than that in 2006 and 2008 (Fig. 2d). Similarly, foliar P in 2007 was lower than in 2006 and 2008 for the other two

Table 1 Results (*F*-value) of repeated measures ANOVAs on the main and interactive effects of species identity (S), nitrogen addition (N), and prescribed burning (F) on the concentrations of foliar nitrogen (foliar N) and phosphorus (foliar P) and the mass-based ratio of the elements (foliar N:P) across different years (Y)

	d.f.	Foliar N	Foliar P	Foliar N:P
Block	1	0.14	0.39	0.95
S	2	68.99***	73.10***	53.73***
Y	2	41.77***	174.62***	351.28***
F	1	23.27***	10.29**	0.39
N	1	88.03***	1.19	55.22***
S*Y	4	20.54***	3.44**	4.18**
S*F	2	3.54*	1.54	0.97
S*N	2	0.49	1.64	0.40
Y*F	2	2.85^	1.26	4.09*
Y*N	2	5.79**	4.33**	4.16**
F*N	1	13.44***	6.31*	0.001
S*F*N	4	2.02^	0.49	1.03
S*Y*F*N	4	3.13**	1.56	6.29***

Statistical significance is depicted as: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ^ $0.05 < p < 0.1$

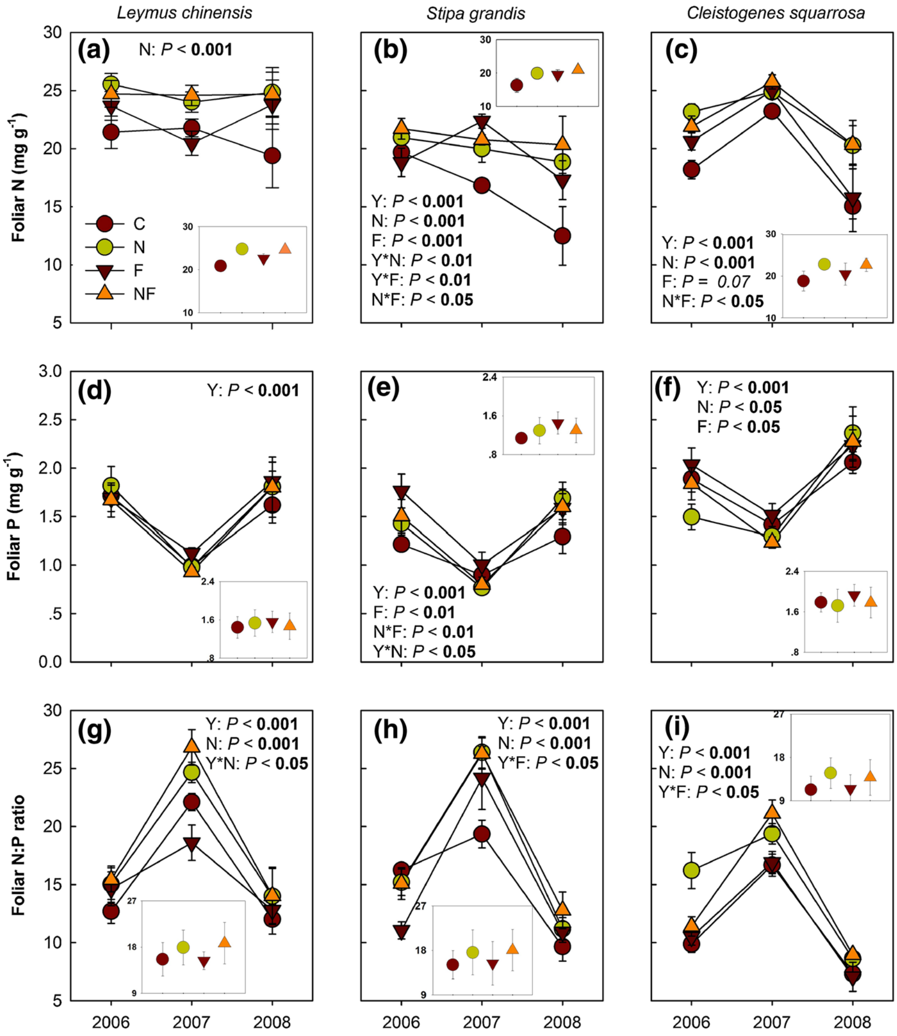


Fig. 2 Foliar N (**a–c**), P (**d–f**), and N:P ratios (**g–i**) of three grasses in response to N deposition and prescribed burning treatments (C, control, N, N addition, F, annual burning, NF, N addition and annual burning). Data shown are means for each year and *error bars* represent 1 SE. ANOVA P-values are reported when $p < 0.05$ (*in bold*) or $p < 0.1$ (*in italics*). Embedded *small boxes* show the mean values across the three years (Y)

species (Fig. 2e, f). For all species across the treatments, foliar N:P ratios in 2007 were much higher than the N:P ratios for 2006 and 2008 (Fig. 2g–i).

Nitrogen additions significantly increased foliar N concentrations in all species (Table 1, Fig. 2a–c), had no significant effect on foliar P in *L. chinensis* and *S. grandis* (Fig. 2d, e), but significantly decreased P concentrations of *C. squarrosa* (Fig. 2f). Nitrogen additions significantly increased foliar N:P of all species (Fig. 2g–i). Across all three species, the impacts of N addition on foliar N, P, and N:P varied among different years, as indicated by significant interactions between N addition and year (Table 1).

The impacts of prescribed burning on foliar N were dependent on species identity, in that fire had no impacts on foliar N in *L. chinensis* (Fig. 2a), but significantly increased foliar N for *S. grandis* and *C. squarrosa* (Fig. 2b, c). Annual burning increased foliar P in *S. grandis* and *C. squarrosa*, but had no effect on foliar P for *L. chinensis* (Fig. 2d–f) or on N:P ratios for any species (Table 1, Fig. 2g–i).

We found highly interactive effects of N deposition and fire on foliar N and P concentrations (Table 1). For instance, averaged across the three years, foliar N of *S. grandis* showed a 22% increase in response to N addition in the unburned plots, whereas the increase was only 7.4% in burned plots (Fig. 2b). Similarly, increases in foliar N for *C. squarrosa* were 21 and 10.8% in response to N addition under unburned and burned conditions, respectively (Fig. 2c). However, there were no interactive effects of N addition and prescribed fire on foliar N:P ratios in any species (Table 1).

Nitrogen addition significantly enhanced the inter-annual stability (1/CV) of foliar N (F = 21.92, d.f. = 1; Fig. 3a), decreased that of foliar P (F = 6.97, d.f. = 1; Fig. 3b), and (marginally significantly) reduced the stability of foliar N:P (F = 3.16; d.f. = 1; Fig. 3c). Prescribed fire showed no effects on the temporal stability of nutrient concentrations and stoichiometric ratios. Furthermore, in control plots, the stability of foliar N and N:P ratios varied among species, with *L. chinensis* showing the highest stability (Fig. 3a, c).

Discussion

With data from three years, we found that soil nutrient concentrations, foliar N and P concentrations, and N:P stoichiometric ratios of the three dominant grasses in the Chinese temperate steppe showed significant inter-annual variation. Annual precipitation also varied greatly among these years, with precipitation in 2007 (240 mm) being 25 and 30% lower than that in 2006 (304 mm) and 2008 (362 mm), respectively. Such temporal variation in ambient precipitation is likely to have important consequences for soil nutrient transformations and plant available soil nutrient stocks. Indeed, we found significant inter-annual variability in soil nutrient pools. Previous results showed that growing season cumulative net N mineralization in unburned and burned plots was 133 and 183% higher, respectively in the wetter year (2006) relative to the dry year (2007) in this same ecosystem (Zhou et al. 2009). Similarly, Wang et al. (2016) reported that increased water availability enhanced the activities of P-transforming enzymes and increased soil available P in aggregate fractions by up to 85% in a nearby site. The temporal variation in plant available soil nutrients observed in this study and others has the potential to strongly influence plant nutrition. In fact, the inter-annual variation in foliar N was higher than that between control plots and N addition plots for *S. grandis* and *C. squarrosa*, highlighting the importance of climate factors in driving the inter-annual variation of plant nutrition. Together, the results show that nutrients in soil and plants varied markedly among years, and present new evidence related to the impacts of inter-annual climate dynamics on fundamental ecological processes.

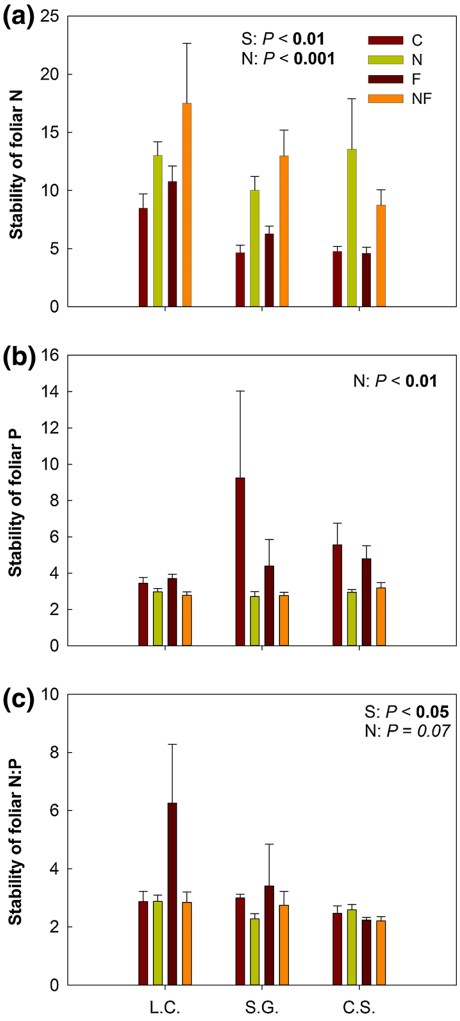


Fig. 3 The stability of foliar N (**a**), P (**b**), and N:P ratios (**c**) of three dominant grasses in response to N deposition and prescribed burning treatments (C, control, N, N addition with a rate of 5.25 g N m^{-2} $year^{-1}$, F, annual burning, NF, N addition and annual burning). The stability was calculated as the ratio of the mean value of a variable (N, P, or N:P) to its standard deviation over the three years, with larger numbers signifying higher stability. Data shown are means for each species and *error bars* represent 1 SE. ANOVA P-values are reported when $p < 0.05$ (*in bold*) or $p < 0.1$ (*in italics*). S, species identity

For all three species, foliar P was much lower and N:P ratios much higher in the dry year relative to the two years with average precipitation. This gives support for our first hypothesis that patterns would vary among years in relation to precipitation. A significantly longer time course would be needed to refine the understanding of how foliar nutrients vary with inter-annual variability in climate, but these data point to the potential for large variability among years. In light of known, tight linkages between foliar nutrient concentrations and fundamental processes such as photosynthesis and respiration (Reich et al. 1998), such large inter-annual variability in foliar nutrients could represent a strong indirect control of climate over ecosystem functions like primary productivity. It is also notable that N and P variability among years did not vary in a synchronized manner, suggesting important differences in how different nutrients are cycling and are being accessed and allocated by the plant. In particular, if plant growth patterns alone were driving foliar differences among years, the expectation would be for N and P concentrations to vary in concert.

Foliar stoichiometry provides insight into nutrient limitation of primary production (e.g., Koerselman and Meuleman 1996; Tessier and Raynal 2003). Our results suggest that the nature of nutrient limitation for plants may depend upon precipitation patterns. In particular, while N:P ratios among different species and across ecosystem types may not be consistent indicators of nutrient limitation (e.g., Alvarez-Clare and Mack 2015), the variability seen here within species and in concert with precipitation patterns suggest the potential for notable changes in nutrient controls over plant productivity. For example, using the commonly cited cutoff of 14 and 16 (Koerselman and Meuleman 1996), we see that each grass studied here would be transitioning from N limitation to P limitation among wetter and drier years, respectively. This pattern is notable, as it suggests a climate-induced switch in nutrient status from one year to another. For example, *L. chinensis* in the control treatment showed a foliar N:P ratio of 12.7 and 12.0 in 2006 and 2008 (years with normal precipitation), which using N:P ratios as indicators of nutrient limitation would suggest N limitation to growth in both years (Koerselman and Meuleman 1996). In contrast, N:P ratios for *L. chinensis* in the dry year of 2007 was nearly double (22.1), implying P limitation to plant growth. Inter-annual variation of N:P in *S. grandis* and *C. squarrosa* shared the same pattern with *L. chinensis*. If N:P ratios are indicators of nutrient limitation in this system, our results suggest the complete interchange of nutrient limitation to plant growth across three years with different precipitation. Considering that more frequent extreme climate events are predicted in the coming decades, especially in the form of drought in semiarid grasslands (Smith 2011), our data raise important questions about the impacts of extreme climate on plant nutrient status and limitation.

Moreover, while overall patterns in N:P ratios varied in concert among years for each species, our results also showed significant interactions between species identity and years in absolute effects on foliar N, P, and N:P ratios. These data suggest that inter-annual variation of plant foliar nutrients and stoichiometry would be strongly dependent on species identity. This makes predictions for long-term patterns in plant-mediated biogeochemical cycling under projected global change scenarios more complicated, and also highlights the importance of considering species within a community as well as the community as a whole (Austin and Zanne 2015; Hobbie 2015). In this way, climate change may not only have important effects on grasslands due to higher sensitivity of community composition to global change factors (Henry et al. 2006), but also due to differential responses of different species across years.

In contrast to our second hypothesis, we did not see large variation in N addition effects on foliar N among years. While it is often observed that N addition results in lower foliar P concentrations due to dilution effects (Perring et al. 2008), we found no significant impacts of N addition on foliar P across the three species over the three years. It has been proposed that the effects of fertilization on plant nutrient status might be dependent on the nutrient limitation conditions of each ecosystem (Ostertag 2010), but these interactions remain unknown for this system. Moreover, such divergent responses may also be related to the N addition rates in different studies. Together, the higher N:P ratios under N enriched conditions suggest that N deposition may result in P limitation for plant growth in this ecosystem through unbalanced effects on foliar N and P, but not through negative effects on the absolute change of foliar P.

The responses of plant nutrients to fire were species-specific. Fire had no detectable influence on foliar N or P in *L. chinensis*, but significantly increased N and P in the other two species. While the divergent responses of plant nutrients to fire have been reported among different plant life forms (Pellegrini et al. 2015), results here present evidence for the importance of species identity in driving the responses of plant nutrients to prescribed fire, even within three grasses of the same ecosystem. Further, prescribed fire showed no significant effects on foliar N:P ratios in any of the three species. Consistent with these results, a recent study reported stoichiometric ratios in plants of the tropical South African savanna did not change with fire (Pellegrini et al. 2015). In an annual grassland of California, however, Henry et al. (2006) found that burning significantly decreased the N:P ratio in the shoots of annual grasses.

Overall, our second hypothesis that the responses of plant foliar nutrient concentrations and stoichiometry to N addition and fire would vary among years was partially supported for the three species. Across all species, we found that N addition and year interacted to affect foliar N, P, and N:P. At the same time, fire and year interacted to affect foliar N:P ratios rather than foliar N and P. Recently, Guiz et al. (2016) reported the importance of species turnover in constraining the temporal changes of plant community stoichiometry in two long-term diversity experiments. Our results indicate that the temporal changes of plant stoichiometry at the intra-specific level, for example, the three species examined here, would also be an important driver for changes to plant stoichiometry at the community-scale. These results also raise important questions about the insight provided from a single year study relative to assessment across multiple years. For instance, N addition led to 11 and 13% enhancement of N:P in *L. chinensis* in 2006 and 2008, whereas the enhancement was doubled (26%) in the dry year 2007.

While there is increasing evidence for the potential for N enrichment to drive P limitation of plant growth in diverse ecosystems (Peñuelas et al. 2013; Perring et al. 2008; Vitousek et al. 2010, but also see Finzi 2009), which is also supported by N:P ratios in the present study, our findings showed that N deposition could also alter such patterns through negative impacts on the temporal stability of plant P status. Foliar P and N:P ratios became less stable following N enrichment at the inter-annual scale. Although we are uncertain about the mechanisms underlying changes of foliar P in response to N enrichment, we suspect that such a change has significant implications for N deposition threats to the temporal stability of primary productivity and trophic networks (Binzer et al. 2016; Hautier et al. 2015).

Fertilization experiments suggest that primary productivity in many terrestrial ecosystems is co-limited by N and P (Elser et al. 2007; Ågren et al. 2012; Harpole et al. 2011). Further, N and P concentrations in leaves determine the rates of a wide variety of processes and interact with ecosystem function in important ways. For example, the specific correlation between foliar N and photosynthetic processes is dependent upon P concentrations (Reich et al. 2009; Walker et al. 2014). Thus, it is reasonable to suspect that the inter-annual variation in foliar P and N:P ratios may lead to consequent variation in primary productivity, even though foliar N was notably stable under N-enriched conditions. Further, beyond discussions of limitation to primary production, foliar N and P concentrations can have significant implications for a host of ecosystem processes, including herbivory and cross-trophic level interactions (Bardgett et al. 1998; Mattson 1980). As grasses are the food source of herbivores ranging from grasshoppers to livestock, nutrient quality changes could have a strong bottom-up effect on herbivore performance (Cease et al. 2016). More and more evidence highlights the important role of P in regulating herbivore behavior and the food web dynamics (Declerck et al. 2015). Taken together, the results described here shed a temporal light on the potential for plant and soil nutrients, and their responses to global change factors, to vary significantly among years with variation being strongly driven by climate patterns.

Acknowledgements We thank the Inner Mongolia Grassland Ecosystem Research Station for logistical support. This work was supported by National Natural Science Foundation of China (31470505), National Key Research and Development Program (2016YFC0500601 and 2015CB150802), Strategic Priority Research Program of the Chinese Academy of Sciences (XDB15010403), Youth Innovation Promotion Association CAS (2014174), and the Key Research Program from CAS (QYZDB-SSW-DQC006 and KFZD-SW-305-002). Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government.

Author Contributions XTL and XGH designed this research, XTL, SLH, YYH, HWW, FML, QC carried out the experiment and collected data, XTL and SCR analyzed and interpreted the data, all authors contributed to drafting the paper.

Compliance with ethical standards

Conflict of interest Authors declared no conflict of interest.

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