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Foliar N/P ratio and nutrient limitation to vegetation growth on Keerqin sandy grassland of North-east China

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Abstract

To examine whether the critical leaf N/P ratios (of 14, 16) are valid to test nutrient limitation in the context of semi-arid sandy grasslands, an experiment was conducted on a Keerqin sandy grassland in North-east China to investigate the responses of plant biomass and nutrient concentrations to fertilization. Plant biomass production and leaf nutrient concentrations were measured after five consecutive years of fertilization with N (20 g N m^{-2} $year^{-1}$) and/or P (10 g P_2O_5 m^{-2} $year^{-1}$). Nitrogen fertilization increased the shoot biomass by twofold and consequently the shoot/root ratio, whereas P fertilization had little effect on either shoot biomass or shoot/root ratio. Leaf N/P ratio varied among species with an average of 5·6 in the control, while the mean leaf N/P ratio (7·5) under the N fertilization treatment remained below the threshold of 14. Our results suggest that the critical N/P ratio (14, 16) is not applicable as a test for nutrient limitations in the context of semi-arid, sandy grassland.

Keywords: biomass partitioning, Keerqin sandy lands, luxury consumption, N/P stoichiometry, nitrogen limitation

Introduction

Productivity in terrestrial ecosystems is constrained by soil fertility, especially soil nitrogen (N) and phosphorus (P) availability (Vitousek and Howarth, 1991; Lebauer and Treseder, 2008). An increase in net primary production in response to additions of limiting nutrients can indicate nutrient limitation of a community (Vitousek and Howarth, 1991). Therefore, whether plant growth is limited by N or P, or both, can be tested by a factorial fertilization experiment (Olde Venterink *et al.*, 2003; Rubio *et al.*, 2010). Much of our present understanding about N and/or P limitation of plant biomass production in grassland ecosystems has been determined from fertilization experiments (Craine *et al.*, 2008; Lebauer and Treseder, 2008).

In addition to field fertilization experiments, N/P stoichiometry may be an alternative tool to study nutrient limitation (Güsewell *et al.*, 2003; Zhang *et al.*, 2004). For conditions where either N or P controls plant growth, Koerselman and Meuleman (1996) proposed that the N/P ratio in plant tissues can be a good predictor of limitations of these nutrients in the soil, with a leaf N/P ratio of <14 indicating N limitation, a ratio of >16 indicating P limitation, and a ratio between 14 and 16 indicating limitation by N and P, or by neither. Their work was based largely on wetland ecosystems. Tessier and Raynal (2003) argued that N/P ratio can also be an effective predictor of N and/or P limitation in upland ecosystems. Güsewell (2004) also supported the use of N/P ratio to predict nutrient limitation and proposed a broader range of ratios, with N/P ratio <10 for N limitation and >20 for P limitation. However, there is still a debate on the applicability of the value of the N/P stoichiometry for nutrient limitation predictions or as a management tool in fertilizer planning (Drenovsky and Richards, 2004; Soudzilovskaia *et al.*, 2005; Craine *et al.*, 2008). Many other studies showed a considerable variability in the N/P ratio thresholds for N and P limitation among plant communities (Bennett and Adams, 2001; Tessier and Raynal,

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2003; Güsewell, 2004). Moreover, there are different N/P ratio thresholds for N and/or P limitation among species in a given area, i.e. even in the same community, coexisting species could be limited by different nutrient elements (Zhang *et al.*, 2004).

Sandy grassland in semi-arid areas is one of the important grassland types in China (Wang *et al.*, 2008). Located at the ecotone of agro-pastoral systems in northern China, the Keerqin Sandy Lands, covering an area of over 50 000 km^2, are the largest of China's four major sandy lands. As a main land cover, the sandy grassland is characterized by soils with low organic matter and nutrient content, supporting low vegetation cover and low levels of forage production (Brogaard and Prieler, 1998). In addition, economic development and human population growth in this region have resulted in increased demand for forage production. However, there is a lack of information on vegetation nutrient status and on the N/P ratios for this sandy grassland. An understanding of the nutrient status of semi-arid sandy grasslands may have significant implications for managing this ecosystem. In a previous study, Yu *et al.* (2009) investigated the responses of vegetation biomass to additions of water, N and P in Keerqin Sandy Lands and found that the limitation of the semi-arid sandy grassland was N, and not P and water. In the present study, we investigated the responses of plant biomass and leaf nutrient concentrations to N and/or P fertilization and further examined whether the critical N/P ratios (14, 16) are valid to test nutrient limitation in the context of semi-arid sandy grassland.

Materials and methods

Experimental site

The experimental site was located at the Daqinggou Ecological Station (42°58′N, 122°21′E; 260 m above sea level) of the Institute of Applied Ecology, Chinese Academy of Sciences, in the south-eastern Keerqin Sandy Lands, North-east China. The mean annual temperature is 6·4°C; the lowest mean monthly temperature (January) is −12·5°C; and the highest mean monthly temperature (July) is 23·8°C. The mean annual precipitation is 450 mm (60% occurring in June–August), with a high potential evaporation of 1300–1800 mm per year. The natural vegetation of the study site was grassland. It was converted to cropland in 1997 and farming ceased in 2000. The land was then naturally restored to grassland. Vegetation consists of grasses (*Pennisetum flaccidum* Griseb., *Phragmites communis* Trin. and *Cleistogenes chinensis* (Maxim.) Keng) and dicot species (*Chenopodium acuminatum* Willd., *Artemisia scoparia* Waldst. et Kit., *Cannabis sativa* L., *Lespedeza davurica* (Laxm.) Schindl. and *Lespedeza hedysaroides* (Pall.) Kitag.). The growing period starts in late April and ends in early September. The soil is Typic Ustipsamment, characterized by coarse texture and loose structure (Zeng *et al.*, 2009). The surface 15-cm soil layer has a pH value of 6·6 and the soil bulk density is $\sim$1·5 g cm^{-3}.

Experimental design

In 2003, an area of flat sandy grassland was fenced to exclude livestock grazing, and a factorial N $\times$ P fertilization experiment was established in a randomized block design with six replicates. Four treatments were installed: no fertilization (control), nitrogen fertilization (N), phosphorus fertilization (P) and combined fertilization of both N and P (N+P). The area of each plot was 4 $\times$ 4 m, and plots were separated by a 2-m buffer strip. Nitrogen was added at the rate of 20 g m^{-2} $year^{-1}$ of urea-N in the period 2004–2006, and as 20 g m^{-2} $year^{-1}$ of NH_4NO_3-N in 2007 and 2008. Phosphorus was added at the rate of 10 g P_2O_5 m^{-2} $year^{-1}$ in the form of NaH_2PO_4 in 2004–2008. Nitrogen and phosphorus (N+P) were added with the same forms and rates as N and P treatments. The amount of fertilizer applied in this study was similar to agricultural inputs typical in the region. The fertilizers were dissolved in 16 L water and applied to individual plots in late April–early May (30%) and mid-June (70%) each year. The control plots also received 16 L of water but without fertilizer. During the experimental periods (2003–2008), the whole trial plots were unmown except for biomass harvested from 20 $\times$ 50 cm quadrats.

Sampling and analytical methods

Plant samples including shoots (green leaves and stems), standing dead material, litter, and roots were collected separately from three randomly selected 20 $\times$ 50 cm quadrats in each plot in mid-August 2008, the time of peak biomass production. The aboveground biomass in these quadrats was clipped at ground level and separated into shoots and standing dead parts. Litter (including partially decomposed organic debris) was collected subsequently. Root biomass (0–30 cm) was collected using a soil corer (10 cm in diameter) in the same locations used for aboveground biomass clipping. Roots were separated from soil by hand and by washing with water. The three samples of each component were mixed into a composite sample, dried at 70°C for 48 h and weighed to determine biomass dry weight.

Additionally, fully expanded mature green leaves of six species (*C. sativa*, *Ph. communis*, *Ch. acuminatum*, *P. flaccidum*, *A. scoparia* and *L. davurica*) were randomly sampled from the plots for each treatment for chemical analyses. Three species (*C. sativa*, *Ph. communis* and *Ch. acuminatum*) were dominant in the plots in which

N and N+P had been added, and all six species were dominant in the control and P-added plots (the sum of their importance value was >60% respectively). For each plot and each species studied, a sufficient amount of leaf material was collected to enable chemical analyses. However, because the amount of *P. flaccidum*, *A. scoparia* and *L. davurica* leaves in the N- and N+P-added plots was not sufficient for sampling, only the other three species were selected in these plots.

The leaves were transported to the laboratory, oven-dried at 70°C and then stored in desiccators until further analysis. Leaves of each species for each plot were ground to pass through a 0·25-mm sieve; total N and total P concentrations were determined after the leaf samples were digested (340°C) with sulphuric acid using a mixture of potassium sulphate and copper sulphate as catalyst and analysed by using a continuous-flow autoanalyser (AutoAnalyser III, Bran+Luebbe GmbH, Norderstedt, Germany).

Data analyses

A general linear model (GLM) procedure with two-way ANOVA was used to examine the main and interactive effects of N and P fertilization treatments on biomass, shoot/root ratio, and leaf N/P ratios for each species individually, with N and P fertilization as main effects. All data were assessed for homogeneity of variance and normality. The data for standing dead and litter were log-transformed before the two-way ANOVA was performed. All analyses were performed using the SPSS 13.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

Results

Biomass production and partitioning

Nitrogen fertilization significantly increased biomass of shoots ($P < 0·001$), standing dead material ($P < 0·05$) and litter ($P < 0·001$), but it decreased root biomass ($P < 0·05$). No significant effects of P fertilization or its interaction with N fertilization were found on biomass of shoots, standing dead material or litter, whereas P fertilization significantly decreased root biomass (Figure 1). Nitrogen fertilization alone increased biomass of shoots, standing dead material and litter by 100, 90 and 120%, respectively, compared with the control (Figure 1). Similar to the effect of N fertilization alone, the combined fertilization of N and P increased the biomass of shoots, standing dead material and litter by 130, 70 and 170% respectively.

Owing to the significant increase in shoot biomass and the significant decrease in root biomass ($P < 0·05$) in treatments N and N+P, the biomass partitioning between shoots and roots in the two treatments was significantly different from the control (Figure 2). In

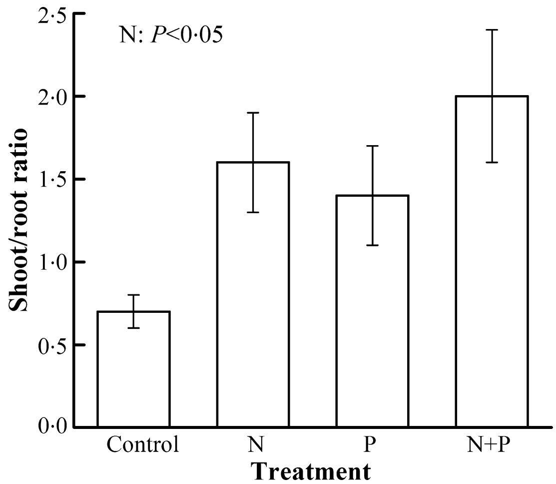

Figure 2 Biomass partitioning between shoots and roots under the four treatments (control, N, P and N+P fertilizations) in Keerqin sandy grassland in mid-August 2008. Bars indicate means and vertical lines are ±1 SE (n = 6). The superscript letter 'N' indicates a significant effect of N fertilization on shoot-to-root ratio, followed by the level of statistical significance.

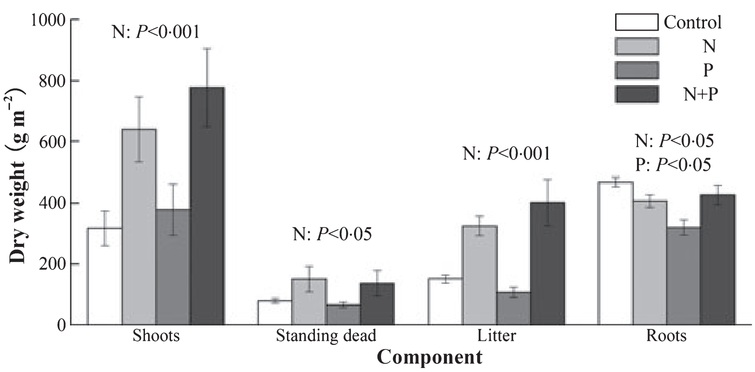

Figure 1 Plant biomass production in mid-August 2008 under the four treatments (control, N, P, and N+P fertilizations) in Keerqin sandy grassland. Bars indicate means, and vertical lines are ±1 SE (n = 6). Within a compartment, the superscript letters 'N' and 'P' indicate a significant effect of N or P fertilization, respectively, followed by the level of statistical significance. The data for standing dead and litter were log-transformed before two-way ANOVA was performed.

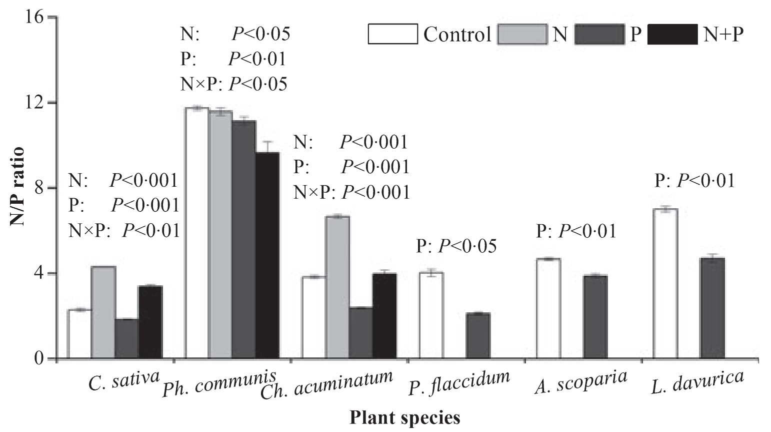

Figure 3 Leaf N/P ratios of plants under the four treatments (control, N, P and N+P fertilizations) in Keerqin sandy grassland in mid-August 2008. Bars indicate means, and vertical lines are ±1 SE (n = 6). Significance of N/P ratio responses to N- and P-additions and their interaction is indicated for *Cannabis sativa*, *Phragmites communis* and *Chenopodium acuminatum*. For *Pennisetum flaccidum*, *Artemisia scoparia* and *Lespedeza davurica*, significance of N/P ratio responses to P addition (based on one-way ANOVA) is indicated. Above the bars, the superscript letters 'N' and 'P' indicate a significant effect of N or P fertilization, respectively, and 'N × P' indicates an interactive effect, followed by the level of statistical significance.

addition, a negligible effect of P fertilization on biomass partitioning was observed. The shoot/root ratio increased from 0·7 in the control to 1·6 and 2·0 in treatments N and N+P respectively (Figure 2).

Leaf N/P ratio

Leaf N/P ratio varied among species in the control, ranging from 2·3 to 11·7 with an average of 5·6 (Figure 3). The N/P ratios in *C. sativa*, *Ph. communis* and *Ch. acuminatum* were significantly affected by N fertilization, P fertilization and their interactions (Figure 3). Phosphorus fertilization also significantly decreased the N/P ratios in *P. flaccidum* ($P < 0{\cdot}05$), *A. scoparia* ($P < 0{\cdot}01$) and *L. davurica* ($P < 0{\cdot}01$; Figure 3). In N-fertilized plots, leaf N/P ratios of different species ranged from 4·3 to 11·6, with an average of 7·5 (Figure 3).

Discussion

An increase in net primary production of a plant community under the effects of fertilization defines nutrient limitation (Vitousek and Howarth, 1991). Therefore, N or P limitation, or both, can be diagnosed by the improved plant growth under fertilization (Bedford *et al.*, 1999; LeBauer and Treseder, 2008). In this study, the fact that N fertilization led to a significant increase in net primary production (shoot biomass) (Figure 1) demonstrated that N was a limiting factor to plant growth in this sandy grassland. In contrast, P fertilization had negligible effects on the vegetation (Figure 1), suggesting that P was not a limiting factor to production in this sandy grassland. This pattern confirmed an earlier observation by Yu *et al.* (2009), who found that the semi-arid sandy grassland was limited by N, and not P and water, and that no interaction existed for biomass between water, N and P except that biomass was significantly increased by the interaction of water and N in July. Consistent with our findings, Bennett and Adams (2001) also demonstrated that plant growth of perennial grasslands in a semi-arid subtropical region in Australia was N-limiting and not P-limiting. On the basis of our results on biomass responses, the application of N fertilizer could be a management option for improving ecosystem productivity of the sandy grassland. However, the amount of fertilizer applied should be considered with caution, because of possible negative effects of fertilizer applications, such as species loss or great changes in species composition (Zeng *et al.*, 2010), and a decline in soil quality (Li *et al.*, 2010).

In this study, the leaf N/P ratio in the control varied substantially among species (Figure 3). The mean leaf N/P ratio of dominant plants in the control was 5·6, which was much lower than the mean value of 15·3 in Chinese grassland biomes (He *et al.*, 2008), and 13·8 for global flora (Reich and Oleksyn, 2004). Such a low leaf N/P ratio would indicate a strong N limitation according to the threshold fourteen proposed by Koerselman and Meuleman (1996) and even the lower threshold (N/P ratio <10) suggested by Güsewell (2004). Moreover, in this study, although the plant biomass production was N-limited, the mean leaf N/P ratio (7·5) under N fertilization was still much lower than the threshold of 14. This low leaf N/P ratio may represent an adaptive mechanism of species to the environment in the study region (Chen *et al.*, 2010). The low leaf N/P ratios, both in the control and in the N-fertilized plots, indicate that the critical N/P ratios (14, 16) are not valid for the semi-arid sandy grassland. Although these results were insufficient to reject the utility of leaf N/P stoichiometry in detecting nutrient limitation in the sandy grassland investigated in our study, there probably exist lower thresholds for nutrient limitation in this sandy grassland than currently suggested thresholds. To obtain accurate critical ratios for the semi-arid grassland, a multi-site and multi-species experiment should be designed.

The absence of clear responses of plant biomass to P treatment show that P is by no means limiting productivity in this sandy grassland, which is also indicated by the high value of leaf P concentration (with an average of 3·1 mg g^{-1}) in the control plots, compared with the mean value (1·9 mg g^{-1}) for Chinese grasslands (He *et al.*, 2008). In this study, the marked increases in leaf P concentration (data not shown) and the normal growth of plants (Figure 1) under P fertilization suggest that there exists luxury P consumption by plants in response to P fertilization (Oyarzabal and Oesterheld, 2009).

Although N has been shown to limit plant biomass production in our semi-arid grassland, water could temporally be growth-limiting because of large seasonal fluctuations in water availability (Cech *et al.*, 2008). However, we believe that the influence of water addition to each plot was diminished in the rainy season. Additionally, based on the potential evaporation rates (3·56–4·93 mm d^{-1}), the water added to dissolve the fertilizers (16 L per 16 m^2, equivalent to 1 mm) would evaporate within a day. Thus, this amount of water is not considered to be enough to lead to an interaction between water and fertilizer in the fertilizer-added plots. In addition, potassium (K) is another important growth-limiting element in grassland (Olde Venterink *et al.*, 2003), and it may become limiting to plant growth when N and P are supplied (Cech *et al.*, 2008). Without treatments with K additions, however, we cannot check whether K limitation exists or would occur in the N- and/or P-fertilized plots in the semi-arid grassland.

In conclusion, our experiment indicated that N fertilization increased shoot biomass significantly, while P fertilization had negligible effects on shoot biomass. The mean leaf N/P ratio in the N-added plots was 7·5, compared to 5·6 in the control. This result suggests that the critical N/P ratios (14, 16) for the shift between N- and P limitation (Koerselman and Meuleman, 1996) are not applicable for this sandy grassland.

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