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Short communication

Nitrogen, not phosphorus, enrichment controls biomass production in alpine wetlands on the Tibetan Plateau, China

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ABSTRACT

Little is known about the effect of nitrogen (N) enrichment on alpine wetland plant N and phosphorus (P) limitations and N:P stoichiometry. We assessed the effects of N and P additions on plant nutrient limitations and N:P ratio in an alpine wetland complex on the Tibetan Plateau, China using a 3-year long factorial experiment. N and P were applied twice per year at an annual rate of 10 $g\,m^{-2}\,yr^{-1}$ and 5 $g\,m^{-2}\,yr^{-1}$, respectively. N addition significantly increased aboveground biomass, plant leaf N, plant leaf N:P ratio and soil available N:P ratio, but had no effect on plant leaf P and soil available P. Although N addition increased the plant N:P ratio it was still < 14. P addition increased plant and soil available P, decreased plant and soil N:P ratio, but had no effect on plant biomass. N + P additions had no additive effect on plant biomass over N additions alone, plant N or P, or soil available N or P. These results indicate that N, rather than P, is likely the limiting nutrient for biomass production in alpine wetlands in this region of Tibet and nutrient limitation does not shift from N to P with increasing N deposition. Thus, N deposition could have a significant influence on vegetation growth and biomass production.

1. Introduction

Nitrogen (N) and phosphorus (P) are essential nutrients for plant growth (Elser et al. 2007). In many ecosystems N limits plant growth (Lebauer and Treseder, 2008), while P is also important or complementary to N in some ecosystems (Naples and Fisk, 2010; Rejmánková et al., 2008). However, whether N or P is the most important nutrient limitation in wetland ecosystems is unclear. Some studies have identified N (Mao et al., 2016; Manninen et al., 2016), while others have identified P (Cusell et al., 2014; Seastedt and Vaccaro, 2001; Rejmánková et al., 2008) or N + P (Fritz et al., 2011) as limiting biomass production. These variable results could be due to differences in climate, soil character, or the vegetation of the studied wetlands (Rejmánková 2001; Soudzilovskaia et al., 2005). Little is known about nutrient limitation in high mountain wetlands, and research is needed to draw more general conclusions.

The Tibetan Plateau is the highest and largest plateau in the world, and alpine wetlands are common in many areas (Ren et al., 2013). Due to an increase in regional economic development as well as long-range transport of atmospheric reactive N, atmospheric N deposition is very high in this region, ranging from 8.0 to 13.8 $kg\,N\,ha^{-1}\,yr^{-1}$, and an increasing trend in N deposition could continue for many decades (Zhao et al., 2017). In addition, the Tibetan Plateau is experiencing striking climate warming (Luo et al., 2010; Wu et al., 2015) that is expected to increase soil organic matter decomposition rates, which could increase soil N and P availability (Gao et al., 2016). There has been little research on the effects of N and P loading on plant biomass production and nutrient limitation in high mountain wetlands. Therefore, the purpose of this study was to determine; (1) the effect of N and P additions on (a) plant biomass production, (b) plant and soil N and P concentrations, and (c) N:P ratios, and (2) whether nutrient limitation shifts from N to P with increasing N additions, simulating continued N deposition?

2. Materials and methods

This study was conducted in Hongyuan County at 3500 m above sea level, on the eastern Tibetan Plateau, China (33° 03′ N, 102° 36′ E). The region has a harsh continental climate with a mean annual temperature of 1.1 °C. Annual precipitation averages 752 mm, with approximately 86% received from May through September (Gao et al., 2016). The soil is classified as peat, and the soil organic C, total N, and total P

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concentrations in the upper 10 cm are 253.9, 21.3 and 0.9 g kg^{-1} (Gao et al., 2015). The vegetation is dominated by *Carex muliensis* Hand-Mazz., *Chamaesium paradoxum* Wolff and *Sanguisorba filiformis* Hand.-Mazz. (Gao et al., 2015).

In early May 2012, sixteen 2 m × 2 m plots were established in a wetland area with homogenous environmental characteristics and vegetation and was sufficiently large to allow the placement of four replicates each of the control and three treatments; (1) addition of 10.0 g m^{-2} yr^{-1}N, (2) addition of 5.0 g m^{-2} yr^{-1}P, (3) addition of 10.0 g N plus 5.0 g P m^{-2} yr^{-1}, and (4) no additions control. The plots were arranged in a complete randomized block design and each plot was separated by a 2-m buffer zone to minimize nutrient exchange between plots. The applied nutrient rates are 2–3 times higher than the annual N and P requirement of vascular plants on the Tibetan Plateau and corresponded to levels reported to overcome plant N and P limitations (Zhou, 2001). N and P were applied as a solution of NH_4NO_3 and NaH_2PO_4 dissolved in 2 L of water. Solutes were added evenly across the plots by hand watering in May and July 2012, 2013 and 2014. The ground water level of all plots was permanently below the soil surface and nutrient additions remained within the plot. The same volume of water was applied to control plots during each treatment event.

Samples of plant and soil were collected in mid-August of each study year. Aboveground biomass was clipped at ground level in three random 0.25 m^2 quadrats within each plot, and was collected in each quadrat only once. Plant material was oven-dried at 70 °C to constant weight, and then weighed. All soil analyses were performed on five soil cores, each 5 cm in diameter and 10 cm deep, collected from random locations in each plot and mixed into one composite sample.

Soil and plant N was measured using the micro-Kjeldahl method (Lu, 1999). Soil and plant P was measured by spectrophotometer (Perkin Elmer Lambda 35, USA) after wet digestion with H_2SO_4 and $HClO_4$ (Lu, 1999). Available soil N was determined by the alkaline diffusion method (Lu, 1999). Available soil P was determined using the Olsen method by extracting samples with 0.5 M $NaHCO_3$, and determining P colorimetrically using molybdate (Olsen and Sommers, 1982).

Repeated measures ANOVA was used to examine the effects of N and P additions over time on plant biomass and plant and soil nutrient concentrations. The least significant difference (LSD) was used to determine differences among treatments. Linear regression was used to assess the relationships between plant N:P ratio and soil N:P ratio across all treatments. Significance was accepted at $P < 0.05$. All statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago).

3. Results and discussion

A significant increase in aboveground biomass occurred in N addition plots, compared to the control plots. P additions had no effect on aboveground biomass in any year (Table 1 and Fig. 1). This suggests that N, not P, limits biomass production in wetlands in this region of the Tibetan Plateau. Similar results have been observed in other temperate (Cusell et al., 2014; Mao et al., 2016) and boreal (Iversen et al., 2010) freshwater wetlands. Alpine plant N-limitation could be caused by low N mineralization rates in cold high mountain soils (Körner 2003). Our N + P additions increased plant biomass as compared with control and P added plots, but the N + P plots were not statistically different from the N only treatment, indicating that N and P additions had no additive effect on aboveground biomass production. Thus, when N input increased, nutrient limitation did not shift from N to P. Studies in several temperate freshwater wetlands have suggested that N enrichment increased potassium (K) limitation on wetland plant growth (Olde venterink et al., 2003; Hoosbeek et al., 2002). Thus, nutrient limitation may change from N to other elements, such as K, under N enrichment.

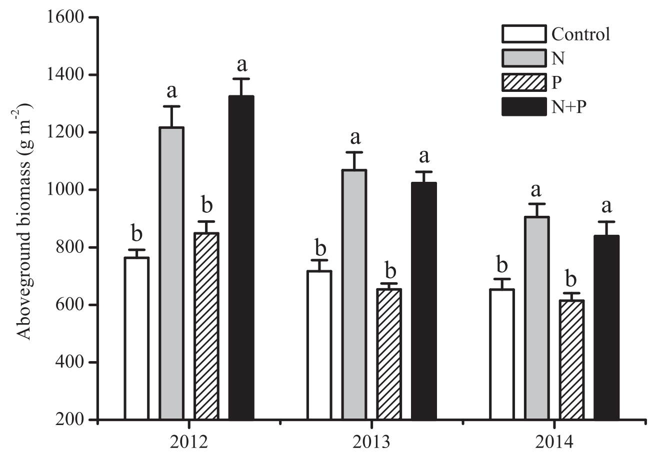

Fig. 1. Aboveground biomass as affected by N, P and N + P additions compared to the control for 2012, 2013 and 2014. Error bars show standard error (n = 4). Means with the same letter are not significantly different ($P > 0.05$) between treatments in the same year.

Significant differences in aboveground biomass occurred between 2012 and 2014. For example, biomass in control plots in 2012 was 16.9% higher than in 2014 (Fig. 1). This may be due to differences in precipitation. During the growing season from May to August, the total precipitation in 2012 was 20% higher than in 2014, but the average air temperature was similar (Zhen et al., 2016). This suggests that even wetlands can be water limited in this region. For every study year, N addition significantly increased plant biomass, but P has no effect, indicating the effects of N and P on plant biomass production do not appear strongly affected by climate factors and the plant biomass production due to the addition of N or P may not depend on climate.

The additions of N increased N concentration in plant biomass in plots not receiving P additions (Table 1 and Fig. 2a). These findings are consistent with studies in freshwater marshes where N additions stimulated primary production (Güsewell et al., 2003; Manninen et al., 2016). P addition did not significantly affect plant N concentrations (Fig. 2b). The lack of N concentration response in plants receiving P additions has also been observed in temperate freshwater (Van Duren et al., 1997) and boreal peatlands (Iversen et al., 2010). In plots receiving N additions, the reduction in aboveground biomass P concentration was likely due to a dilution effect caused by the significant

Table 1
F-values and probability levels from repeated measures ANOVA on the effect of N and P addition and sampling year on plant and soil parameters in the Tibetan wetlands.

Effect	Aboveground biomass	Plant N	Plant P	Plant N:P	Soil available N	Soil available P	Soil available N:P
N	176.446***	42.117***	17.264***	65.973***	1.901	2.169	14.435**
P	0.017	5.496*	276.231***	205.432***	0.628	326.614***	235.593***
Year	38.758***	7.843**	28.091***	5.377**	28.954***	16.692***	11.302***
N × P	0.008	0.580	0.494	7.662**	0.458	3.913	11.535**
N × Year	6.017**	1.000	0.602	0.016	0.001	1.483	0.961
P × Year	3.487*	0.038	0.889	1.058	0.064	11.066***	8.040**
N × P × Year	0.089	0.238	0.018	0.074	0.374	0.746	0.035

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.