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Response of *Kobresia pygmaea* and *Stipa purpurea* Grassland Communities in Northern Tibet to Nitrogen and Phosphate Addition

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The Tibetan Plateau is of fundamental ecological significance to China, Asia, and the world. In recent years, Tibetan grasslands have suffered from severe degradation due to climate change and anthropogenic disturbance. In this study, nitrogen (N) and phosphate were applied to a moderately degraded Kobresia pygmaea *meadow and* Stipa purpurea *steppe in the arid alpine northern Tibetan Plateau. The results showed that with increasing nitrogenous fertilizer, the height, coverage, biomass, and importance value of the* K. pygmaea *population decreased whereas the population of* S. purpurea *exhibited the opposite trend. Application of a mixed fertilizer with the same amount of N and phosphorus (P) (5 g each per* m^2*) doubled the biomass of the* K. pygmaea *meadow and increased the aboveground biomass of the* S. purpurea *steppe by 72.3%. The nitrogenous fertilizer increased the total biomass and belowground biomass of the* S. purpurea *steppe, whereas the mixed fertilizer was beneficial to aboveground grass recovery. Application of* $10\ g\ N + 5\ g\ P\ m^{-2}$ *fertilizer increased aboveground biomass by 164.8%, whereas the belowground biomass was less than the control by 4.7%. The N and P fertilizer did not affect soil pH, except for some changes in soil N and P contents.*

Keywords: *Fertilization; nitrogen and phosphorus addition; alpine grassland; soil productivity; Tibetan Plateau.*

Introduction

The Tibetan Plateau, known as the world's "third pole," is of fundamental ecological significance for China, Asia, and even the world (Qiu 2008). In recent years, the vegetation on the Tibetan Plateau has undergone severe degradation due to intensified grazing, urbanization, population growth, burgeoning tourism, and climate change (Zhang et al 2008; Harris 2010). Degradation of ecosystems has already led to obvious negative consequences such as lower grassland carrying capacity, an increased number of endangered species, shrunken water sources, and landslide disasters (Zhao et al 2006; Cui and Graf 2009). Recognizing the trend of ecological disasters caused by ecosystem degradation, state and local governments are implementing a series of measures to restore the local environment (Akiyama and Kawamura 2007).

Fencing and fertilization are currently the main measures applied to rehabilitate the degraded grassland ecosystem (Smith et al 2000). Fertilization can provide the necessary nutrition for plants and increase grass yield. Previous studies have shown that fertilization, especially nitrogen (N) fertilization (William et al 1995), is one of the most important and cost-effective measures to improve grass productivity in various climatic zones and soils. Indeed, N has been found to be the predominant factor limiting the primary productivity of most land ecosystems (Vitousek and Howarth 1991; LeBauer and Treseder 2008).

However, the effects of N fertilization vary with ecosystem types and local soil conditions. In recent decades, with an increasing input of N to natural ecosystems due to N deposition and fertilization, N cycling, natural ecosystem productivity, ecosystem structure and function, and species composition have all been greatly influenced (Al-Mufti et al 1977; Willems et al 1993; Sala et al 2000). Degradation of an ecosystem has been found to be due to a disruption of the balance between energy flows and material cycling (Haileslassie et al 2005). Intensive studies of the effects and efficiency of fertilization have been conducted in the Mongolian grasslands (Bai et al 2010; Pan et al 2011), but very few similar studies have been reported for the northern Tibetan Plateau, a key area of the plateau, which is very sensitive to climatic change. The northern Tibetan

FIGURE 1 Location of study sites. (Map by Baoxiong Chen)
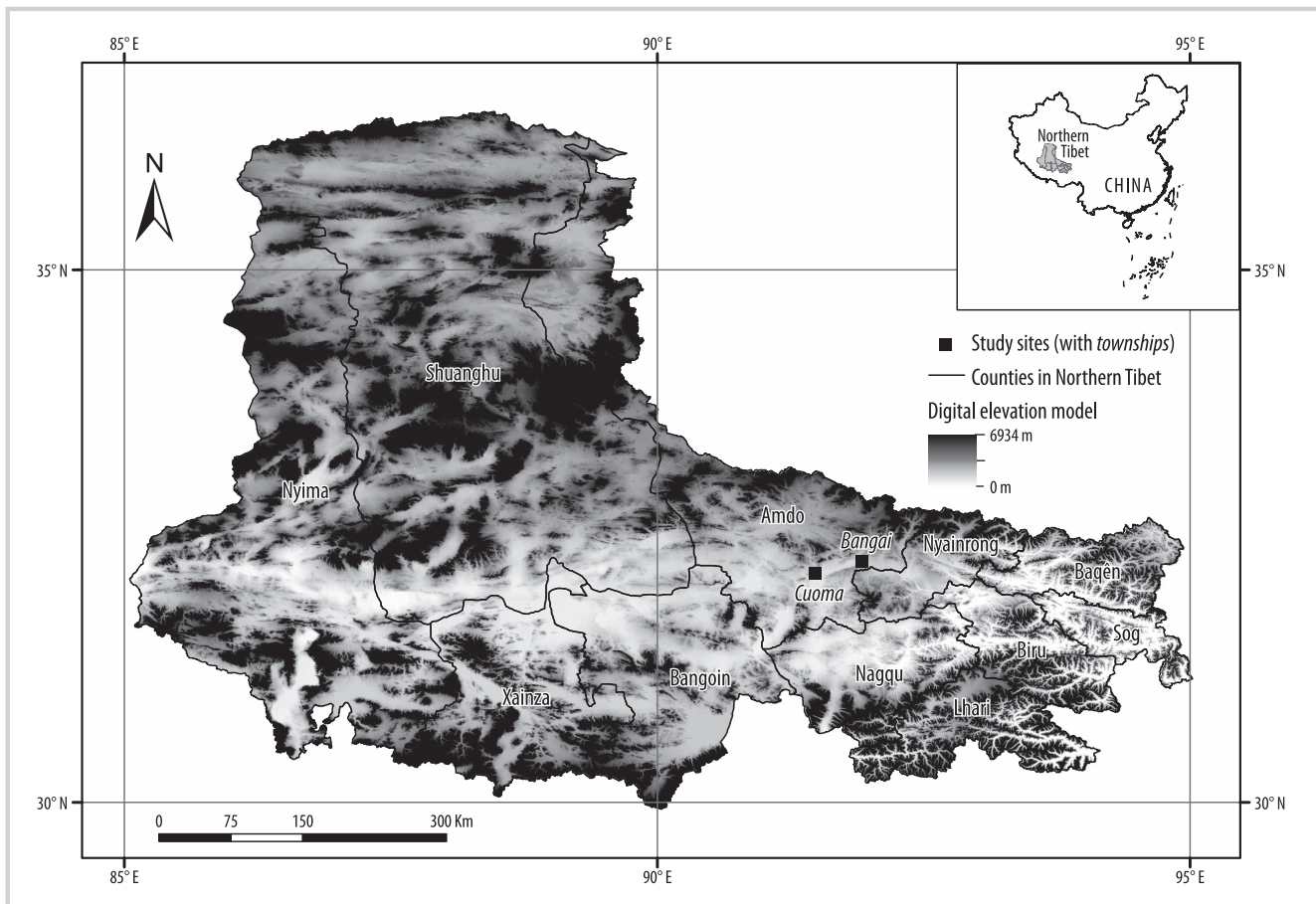
Plateau covers an area of 0.7 million km^2 and has an average altitude of over 4000 m. It would be inappropriate to apply research findings obtained from other ecosystems to this region with its unique climate, including long and cold winters, strong winds, strong radiation, and low oxygen pressure.

With a view to restoring the degraded grass ecosystem of the northern Tibetan Plateau, the "Returning Grazing Land to Grass" policy has been implemented since 2003. A series of measures such as fencing, grazing prohibition, replanting, grass plantation, fertilization, and sprinkler irrigation have been implemented. However, scientists are still debating whether fertilization is suitable to restore the degraded ecosystems in this arid alpine northern region (Wei et al 2010; Zong et al 2013), and little has been reported on how N and phosphorus (P) inputs influence ecosystem structure and function and the stability of grasslands there. Moreover, there are numerous issues related to the appropriate amount and mix of fertilizers in northern Tibetan grasslands.

Thus, the objective of this study was to examine the response of arid alpine grasslands with *Kobresia pygmaea* and *Stipa purpurea* as dominant species to N fertilization and mixed N and P fertilization. The study findings should help to shed light on the efficiency of fertilization for recovering Tibetan grasslands.

Material and methods

Study site

Unique climate conditions on the northern Tibetan Plateau have generated several ecosystems (Gao et al 2010), including *K. pygmaea* meadow and *S. purpurea* steppe, which are widely distributed in the Nagqu and Ngari regions of Tibet, an alpine zone in the upper reaches of the Brahmaputra river and the lake basin region in the northern Tibetan Plateau (Figure 1). The altitude where these grasslands are distributed ranges between 4500 and 4800 m. The vegetation is semiarid alpine cold meadow or alpine steppe. Mean annual temperature is about $-1.5°$C. The accumulated temperature above $0°$C per year is less than $1500°$C, whereas that above $10°$C is less than $650°$C, with 9–50 frost-free days per year. The average annual precipitation is 150–300 mm.

Two sites that represent the 2 grassland and soil types in the northern Tibetan Plateau were selected for this

FIGURE 2 Arrangement of experimental plots.
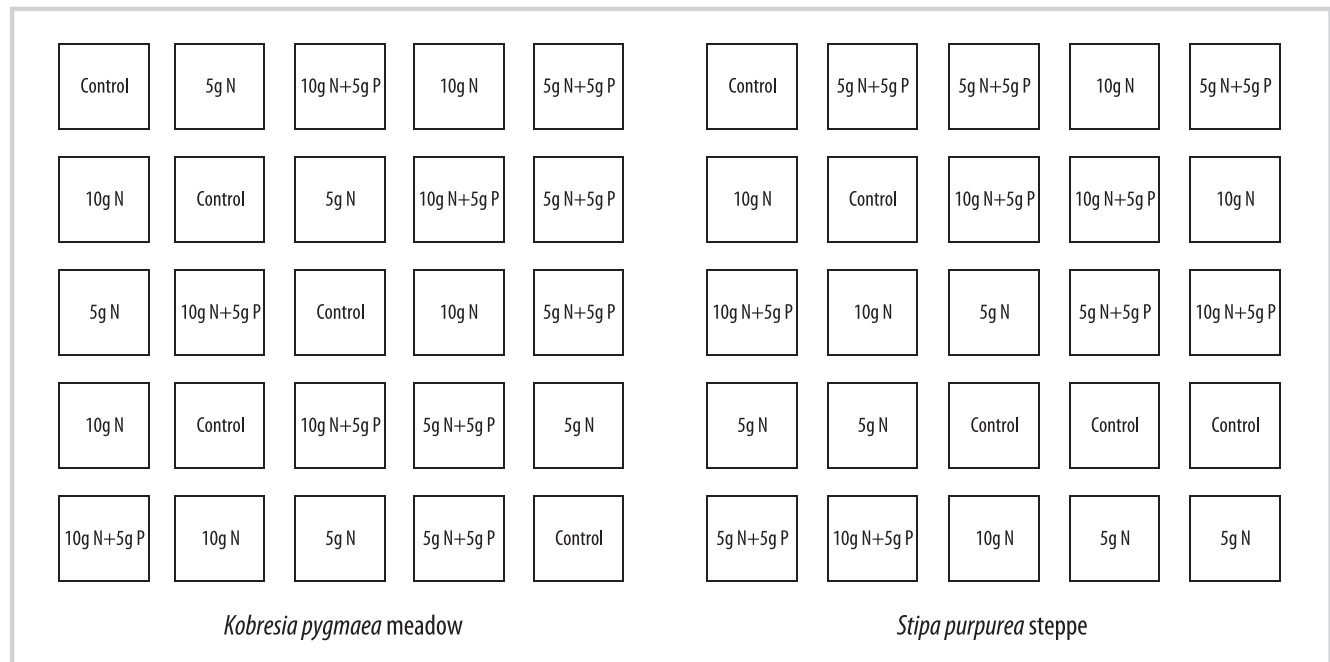
study. The *K. pygmaea* meadow (moderately degraded) is located in the Four Villages of Bangai township (32°16.225′N, 91°54.948′E), Amdo county, in the Nagqu region in Tibet. The elevation of the experimental site is 4678 m. The accompanying species include *Stipa purpurea* Griseb., *Festuca wallichania* E.B. Alexeev, *Arenaria serpyllifolia* L., and *Potentilla parvifolia* Fisch. ex Lehm. In surface soil (0–10 cm), organic matter accounts for 0.344% of total soil matter, and N accounts for 0.048% of the total. The ammoniacal and nitrate N contents are 99.90 and 94.28 mg kg^{-1}, respectively. The sample plot of *S. purpurea* steppe was established at the First Village of Cuoma township (32°10.009′N, 91°28.882′E), at 4613 m above sea level. Accompanying species include *Roegneria thoroldiana* (Oliv.) Keng, *Potentilla bifurca* L., and *Viola philippica* Cav. Organic matter and total N in surface soil make up 0.347% and 0.038% of the total mass, respectively. Ammoniacal N content is 74.6 mg kg^{-1}, and nitrate N content is 75.0 mg kg^{-1}.

Experimental design

The experiment was based on experimental design theories and conditions in the alpine grassland of northern Tibet (Niu et al 2008; Ren et al 2010). It was set up in a randomized block design (5-m^2 plots separated by 2-m buffers) in both sample areas in mid-June 2008 (Figure 2). The control plots received no treatment; there were 4 treatments with fertilizer: 5 g N m^{-2}, 10 g N m^{-2}, 5 g N + 5 g P m^{-2}, and 10 g N + 5 g P m^{-2}. The fertilizers used were urea, for which the effective component is

CO(NH$_2$)$_2$, containing 46.4% N, and superphosphate, for which the effective component is Ca(H$_2$PO$_4$)$_2$, containing 8.5% P. Each treatment was applied 5 times in mid to late June 2008 (Zhang et al 2010).

We surveyed the plant community and collected aboveground biomass, belowground biomass, and soil samples from 20 to 22 August 2008. To minimize the disturbance to vegetation and soil, 0.5-m square sampling plots were used to investigate aboveground biomass. All aboveground plants were clipped to the surface, sorted into species, and saved in separate marked paper bags. In the plots, 0.25-m^2 quadrats were randomly selected to assess belowground biomass. Soil in the quadrats was excavated to a depth of 30 cm. Soil cores were loaded in cloth bags and washed with fresh water repeatedly to separate the roots. All herbaceous samples were oven dried at 65°C for 48 hours and weighed on an electronic scale. Root-free soil cores were sent to laboratories to obtain the pH value and the N, P, and potassium content.

Coverage and height were also measured. The importance value (IV) was calculated using the following formula:

$$IV = \frac{\text{relative height} + \text{relative coverage}}{2} \qquad (1)$$

The aboveground biomass was determined by adding up the aboveground biomass of each species in every plot. The belowground biomass is the total root biomass. The total biomass is the sum of aboveground and belowground biomass.

FIGURE 3 Effects of fertilization on *K. pygmaea* population characteristics. Bars indicate double standard deviations.
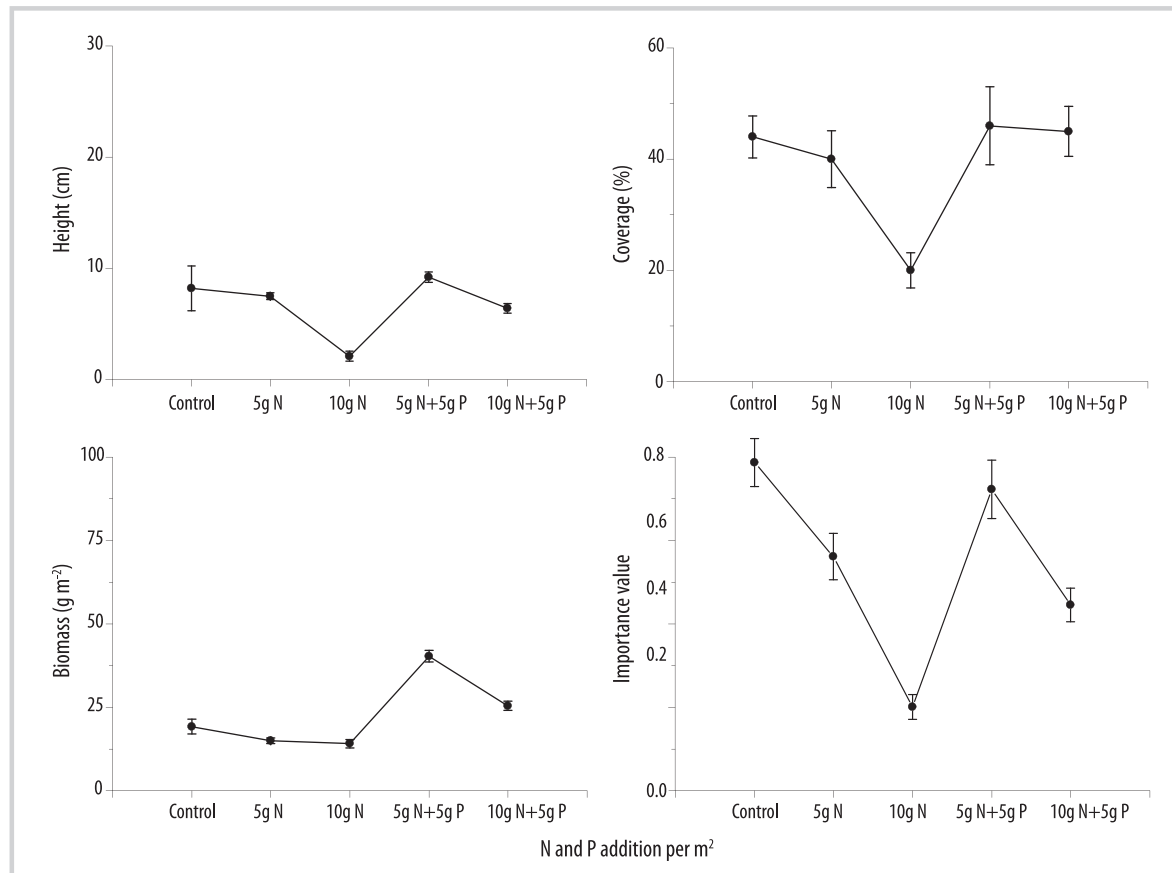
$$\text{Increment of biomass } (\%) = \frac{m_{treatment} - m_{control}}{m_{control}} \qquad (2)$$

$$20\text{Shoot-root ratio} = \frac{\text{aboveground biomass}}{\text{belowground biomass}} \qquad (3)$$

where $m_{treatment}$ is the biomass (aboveground, belowground, total) of the treatment plots and $m_{control}$ is the corresponding biomass of the control plots.

Statistical analysis
Significant differences between the means of results from the treatment and control plots were determined by paired *t*-test, with a significance level of $P < 0.05$. For different fertilizer applications, 1-way analysis of variance (ANOVA) and Fisher's least significant difference test with a significance level of $P < 0.05$ were used to test the effect of fertilizer. All statistical analyses were performed using SPSS version 13.0 (SPSS Inc, IBM, Armonk, NY, USA).

Results

Changes in the community structures, biomass, and soil conditions of the 2 grassland communities due to

fertilizer applications were analyzed. All application amounts given below are per square meter.

Community structure
On the *K. pygmaea* meadow, with N additions, the height, coverage, biomass, and importance value of the *K. pygmaea* population all decreased (Figure 3). When the fertilizer amount was 10 g N, the height, coverage, and importance value decreased to 2.1 cm, 20%, and 0.2, respectively. When the amount was 5 g N + 5 g P, the height, coverage, biomass, and importance value of the *K. pygmaea* population reached their peak. When a mixture with more N (10 g N + 5 g P) was applied, all the corresponding index values were reduced.

On the *S. purpurea* steppe, compared with the control plot, all fertilization treatments increased the height, coverage, biomass, and importance value of the *S. purpurea* population (Figure 4).

Biomass
In the *K. pygmaea* meadow, when N alone was added, total, aboveground, and belowground biomass all declined (Figure 5). With treatment with 5 g N, aboveground, belowground, and total biomass decreased by 35.4%,

FIGURE 4 Effects of fertilization on *S. purpurea* population characteristics. Bars indicate double standard deviations.
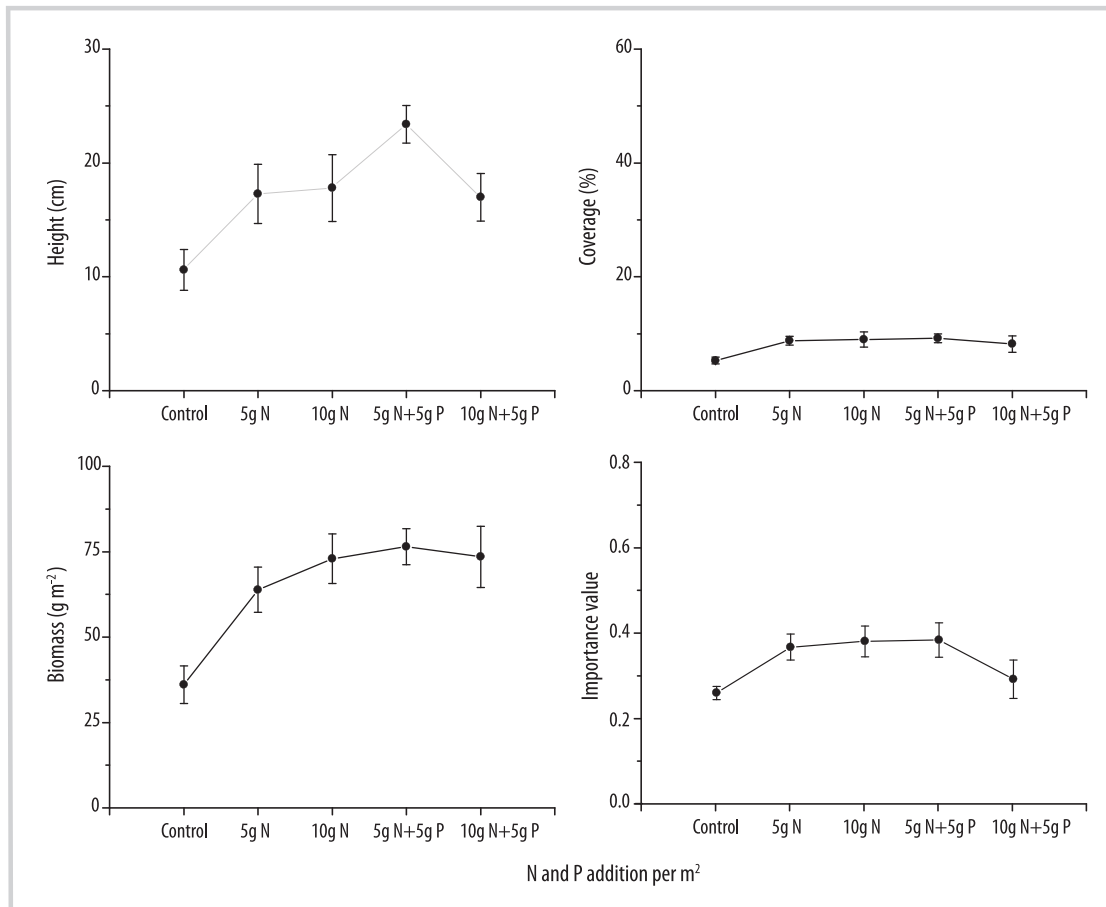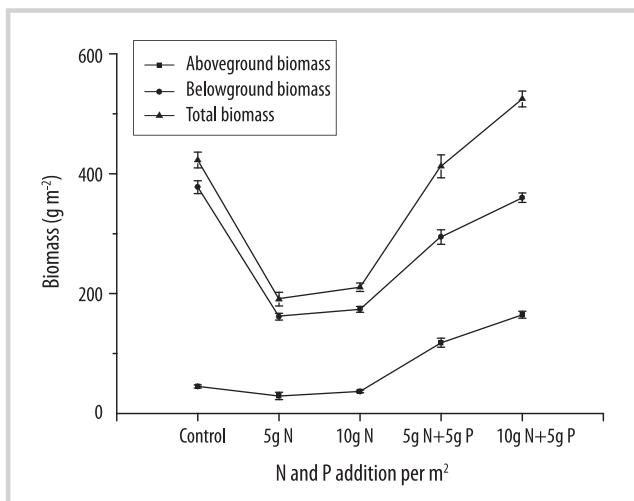

FIGURE 5 Changes in biomass with different fertilization treatments on a *K. pygmaea* meadow. Bars indicate double standard deviations.

57.2%, and 54.9%, respectively (Table 1). After adding a higher amount of N (10 g), we observed a slight but not significant rise in biomass (paired *t*-test, $P = 0.254$), but it was also much lower than that in the control plots. Compared with application of N alone, the combined application of N and P significantly boosted biomass in the *K. pygmaea* meadow (paired *t*-test, both $P < 0.01$). There was a positive correlation between biomass and fertilizer amount (1-way ANOVA, $P = 0.042$). Treatment with 10 g N + 5 g P performed better than treatment with 5 g N + 5 g P. Under the former, aboveground biomass increased by 164.8%, whereas belowground biomass declined by 4.7% compared with the control. Total biomass increased by 24.1%, suggesting that mixed application of N and P is more efficient in increasing aboveground than belowground biomass on a (moderately degraded) *K. pygmaea* meadow.

In the *S. purpurea* steppe, compared with the control, applications of N and mixed N and P both significantly increased aboveground, belowground, and total biomass (Figure 6)—especially for treatment with 5 g N + 5 g P, for which aboveground biomass (261.2 g) increased by 72.3%, compared with the control (151.6 g). However,

TABLE 1 Average biomass and shoot–root ratio after 5 applications of N and P, *K. pygmaea* meadow.

| Treatment | Aboveground biomass | | Belowground biomass | | Total biomass | | Shoot–root ratio (%) |
	Amount (g m^{-2})	Increase[a] (%)	Amount (g m^{-2})	Increase[a] (%)	Amount (g m^{-2})	Ratio (%)	
Control	45.2 ± 2.7	0	377.6 ± 10.6	0	422.8 ± 13.3	0	12.0
5 g N	29.2 ± 6.0	−35.4	161.6 ± 5.7	−57.2	190.8 ± 11.3	−54.9	18.1
10 g N	36.8 ± 2.0	−18.6	172.8 ± 4.9	−54.2	209.6 ± 6.9	−50.4	21.3
5 g N + 5 g P	118.0 ± 7.5	161.1	294.4 ± 12.2	−22.0	412.4 ± 19.0	−2.5	40.1
10 g N + 5 g P	164.8 ± 5.8	264.6	360.0 ± 7.9	−4.7	524.8 ± 13.2	24.1	45.8

[a]Increase by comparison with values in the control plot.

belowground biomass under the mixed application of N and P increased less than under N applications. A possible reason for this might be that too much P restrained the root growth or accelerated the root turnover of some species. The 10 g N treatment resulted in the maximum biomass in all treatments, with total and belowground biomass increasing by 56.8% and 59.1% compared with the control, respectively (Table 2).

Compared with N alone, the effects of 5 g N + 5 g P were more obvious in enhancing aboveground biomass (Figure 6), with a maximum increase of 72.3% (Table 2). Although mixed N and P application failed to increase belowground biomass significantly, the boost in aboveground biomass improved the area's carrying capacity.

N and mixed N and P both increased the shoot–root ratio on the *K. pygmaea* meadow, with a greater increase caused by mixed N and P (Figure 7). N application alone decreased shoot–root ratio on the *S. purpurea* steppe, and mixed application of N and P increased the ratio (Figure 7).

Soil

The soil pH values of the *K. pygmaea* meadow and *S. purpurea* steppe were 8.50 and 8.38, respectively. As is typical of weak alkaline soils, the pH value did not change markedly after fertilization (paired *t*-test, all $P > 0.05$).

For the *K. pygmaea* meadow, the soil N level remained constant after N addition, possibly being utilized promptly by plants or washed away by rainwater. For the treatment of 5 g N + 5 g P, the amount of available P increased by 1.2 times, indicating that P was not entirely used by plants and was not affected by rainwater. Thus, the increased soil P would contribute to the next growing season or over a longer term. For the *S. purpurea* steppe, adding a small amount of N (5 g) failed to change soil N, whereas adding 10 g N increased soil N by 30%. Most of the P was sequestered in the soil. Mixed N and P application significantly improved the stock of P in the soil (paired *t*-test, all $P < 0.05$). Comparing the treatments 5 g N + 5 g P and 10 g N + 5 g P, though both had the same amount of P, the amount of available P in the soil doubled from 17.0 to 33.8 mg kg^{-1} (Table 3).

Discussion

Based on our data, we conclude that fertilization can boost aboveground biomass, decrease belowground biomass on *K. pygmaea* meadow (Figure 5), and increase the shoot–root ratio (Figure 7). Fertilization increased both aboveground and belowground biomass of the *S. purpurea* steppe, but the increase in aboveground biomass under both N and mixed N and P application was greater

TABLE 2 Average biomass and shoot–root ratio after 5 applications of N and P, *S. purpurea* steppe.

| Treatment | Aboveground biomass | | Belowground biomass | | Total biomass | | Shoot–root ratio (%) |
	Amount (g m^{-2})	Increase[a] (%)	Amount (g m^{-2})	Increase[a] (%)	Amount (g m^{-2})	Ratio (%)	
Control	151.6 ± 10.1	0	926.4 ± 18.5	0	1078.0 ± 27.8	0	16.4
5 g N	161.6 ± 10.7	6.6	1329.6 ± 50.9	43.5	1491.2 ± 42.6	38.3	12.2
10 g N	216.8 ± 11.4	43.0	1473.6 ± 96.2	59.1	1690.4 ± 105.3	56.8	14.7
5 g N + 5 g P	261.2 ± 9.8	72.3	1211.2 ± 61.9	30.7	1472.4 ± 70.4	36.6	21.6
10 g N + 5 g P	238.4 ± 13.8	57.3	1390.4 ± 101.3	50.1	1628.8 ± 114.7	51.1	17.2

[a]Increase by comparison with values in the control plot.

FIGURE 6 Changes in biomass with different fertilization treatments on *S. purpurea* steppe. Bars indicate double standard deviations.

FIGURE 7 Effects of fertilization on the shoot–root ratio on *K. pygmaea* meadow and *S. purpurea* steppe.
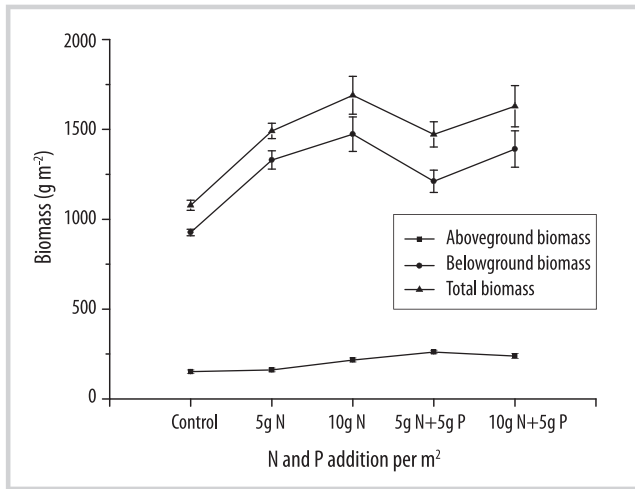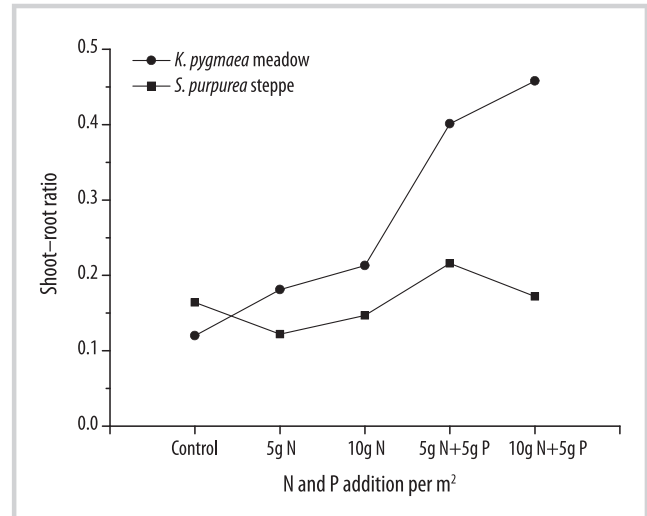

than that belowground (Figure 6). Fertilization also resulted in a decreased shoot–root ratio under the N applications and an increased shoot–root ratio under the mixed applications (Figure 7).

Responses to N and P application vary greatly among different grassland types. Some studies have shown that N addition can promote grassland productivity (He et al 2003; Fargione et al 2007). Other studies have shown that competition for light, fertilizer, and water among different species under N application can decrease the productivity of some functional groups and even cause some species to disappear (Rajaniemi 2002; Ives and Carpenter 2007; Clark and Tilman 2008; Hautier et al 2009). The present study suggests 2 possible causes for the decreased dominance and productivity of *K. pygmaea* after fertilization:

1. Competition: *K. pygmaea* is not sensitive to N addition (Ma et al 2003), whereas species of Gramineae, Legumi-nosae, Asteraceae (Zhang et al 2010) and weeds grow rapidly and occupy more resources under N fertilization.
2. Negative effects: N addition in alpine and arid conditions decreases the productivity of Cyperaceae (Du et al 2003).

The mixed N and P application significantly increased the aboveground biomass of *K. pygmaea*, from which we can conclude that (1) the soil in the *K. pygmaea* meadow had a P deficiency and (2) interaction between N and P added in equal amounts may facilitate the growth of *K. pygmaea* (Ji 2002).

In addition, our results showed that the application of N alone and the mixed N and P application both significantly increased the productivity of the *S. purpurea* steppe, which has been shown in other grassland types as well (Ji 2002; Du et al 2003; He et al 2003; Fargione et al 2007). However, the effects of the mixture of equal amounts of N and P (5 g N + 5 g P) were more obvious than those of 10 g N and 10 g N + 5 g P. This might be caused by (1) the interactions between N and P being generally more effective (Bernhardt-Römermann et al 2011) or (2) the different species compositions and soil conditions in the 2 study areas—the equivalent N and P application best promoted the growth of *S. purpurea*, resulting in the different responses of the 2 grassland types to fertilizer applications.

TABLE 3 Average pH and soil nutrient content after 3 applications of N and P, *K. pygmaea* meadow and *S. purpurea* steppe.

Treatment	*K. pygmaea* meadow				*S. purpurea* steppe			
	pH	Potassium	Nitrogen	Phosphorus	pH	Potassium	Nitrogen	Phosphorus
Control	8.50 ± 0.13	69.5 ± 5.1	67.7 ± 6.5	4.2 ± 0.2	8.38 ± 0.14	61.5 ± 6.2	60.9 ± 3.2	5.3 ± 1.1
5 g N	8.48 ± 0.08	67.7 ± 6.2	62.1 ± 7.3	4.1 ± 0.6	8.42 ± 0.23	67.3 ± 7.3	62.7 ± 4.3	5.0 ± 0.7
10 g N	8.55 ± 0.15	65.0 ± 4.7	71.1 ± 9.1	6.7 ± 0.9	8.35 ± 0.11	69.5 ± 4.8	79.5 ± 3.9	8.9 ± 1.6
5 g N + 5 g P	8.42 ± 0.12	61.6 ± 7.6	67.9 ± 6.1	9.3 ± 1.4	8.39 ± 0.09	60.4 ± 7.6	60.9 ± 5.6	17.0 ± 2.9
10 g N + 5 g P	8.48 ± 0.20	58.9 ± 6.8	61.9 ± 5.6	9.2 ± 1.8	8.34 ± 0.17	69.6 ± 3.6	76.6 ± 6.3	33.8 ± 3.4

Two government measures currently underway to restore degraded grasslands in the northern Tibetan Plateau—fencing and grass plantation—have made progress (Wang et al 2010), but they still have not fully achieved the desired objectives. If a mixture of equal parts of N and P (3–5 g m^{-2} y^{-1}) could be applied, the productivity of grasslands in northern Tibet would greatly increase, and grassland restoration would accelerate accordingly.

Conclusions

In restoring degraded northern Tibetan grasslands through fertilization, the mixture of equal parts of N and P turned out to be the most effective—increasing the aboveground biomass of the *K. pygmaea* population by 100% and that of the *K. pygmaea* meadow as a whole by over 160% (while decreasing total biomass by 2.5%), and increasing the aboveground biomass of the *S. purpurea* population by 112% and that of the steppe as a whole by 72% (while increasing total biomass by 36%).

A 1-year fertilization period may not have changed pH values in the 2 study areas. For the *K. pygmaea* meadow, soil N content was not changed either, whereas mixed N and P additions resulted in an increased amount of P remaining in the soil. For the *S. purpurea* steppe, 10 g N and 10 g N + 5 g P increased soil N content very obviously, and mixed N and P addition increased P content in the soil.

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