Photosynthesis and nutrient-use efficiency in response to N and P addition in three dominant grassland species on the semiarid Loess Plateau

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Abstract

Understanding the ecophysiological and nutrient-use strategies of dominant species is important for clarifying plant growth and an ecological process in their community under unbalanced N and P inputs. This study investigated effects of N and P addition [main plot: 0, 25, 50, and 100 kg(N) ha^{-1} $year^{-1}$; subplot: 0, 20, 40, and 80 kg(P) ha^{-1} $year^{-1}$] on leaf N and P contents (LNC_a and LPC_a), photosynthetic capacity (P_{Nmax}) and photosynthetic N- and P-use efficiencies (PNUE and PPUE) in three species (*Stipa bungeana*, *Bothriochloa ischaemum*, and *Lespedeza davurica*) in a semiarid grassland in China. At the assessing time, N addition alone significantly increased LNC_a and LNC_a/LPC_a ratio of *Stipa bungeana* (C_3 grass) only, while P_{Nmax} increased significantly in all three species. Under N addition, P addition caused significantly lower LNC_a, but higher PNUE and P_{Nmax} in *Bothriochloa ischaemum* (C_4 grass) and *Stipa bungeana*. The LNC_a, P_{Nmax}, and PNUE of *Lespedeza davurica* (C_3 legume) increased significantly after P addition regardless of N application. The LNC_a/LPC_a for optimum PNUE changed with species. The P_{Nmax} and PNUE of *Bothriochloa ischaemum* and *Stipa bungeana* had a peak when LNC_a/LPC_a attained ~ 11 and ~ 20. *Lespedeza davurica* tended to retain greater P_{Nmax} and PNUE at lower LNC_a/LPC_a. Our results indicated that the C_3 grass was more sensitive to N and P addition than the C_4 grass and the C_3 legume in terms of leaf N and P contents and P_{Nmax}. Such interspecific variations of nutrient use in response to N and/or P addition are favorable for maintaining the diversity and the stability of the grassland community.

Additional key words: ecological adaptation; function groups; leaf traits; nutrient addition.

Introduction

In the past 150 years, the N deposition has more than doubled globally and N deposition rate is still increasing (Peñuelas *et al.* 2013, Regus *et al.* 2017). Enhanced N deposition has dramatically altered terrestrial ecosystem properties and processes, such as plant nutrient cycling, photosynthetic carbon assimilation, and species diversity (Li *et al.* 2016, Mao *et al.* 2018). In N-limited ecosystem, N deposition can satisfy plant N demand and stimulate photosynthetic capacity and plant growth (Peñuelas *et al.* 2013, Mao *et al.* 2018). Excessive N deposition would break the stoichiometric balance of N and P in plant and soil (Peñuelas *et al.* 2013, Li *et al.* 2016). Plant photosynthesis in response to N deposition is suppressed by P limitation on Rubisco activity and the synthesis of triose phosphate, ATP and NADPH productions (Hikosaka 2004, Reich *et al.* 2009).

The species-specific response to increased N deposition depends largely on their physiological adaptations in natural grassland community (Chen *et al.* 2005, Bubier *et al.* 2011). Different functional groups exhibit divergent photosynthetic capacity and nutrient cycling in response to altered N and P availability (Hikosaka 2004, Bubier *et al.* 2011). Photosynthetic N or P use efficiency (PNUE or PPUE, defined as photosynthetic capacity per unit leaf N or P) have been considered as important species-specific functional traits in characterizing leaf physiology (Hikosaka 2004, Feng *et al.* 2008, Liu *et al.* 2015). Generally, there are four physiological factors that cause interspecific variations in PNUE and PPUE: (*1*) CO_2 concentration at the carboxylation site, (*2*) N and P allocation between photosynthetic and nonphotosynthetic nitrogenous compounds, (*3*) N and P partitioning among photosynthetic components, and (*4*) specific activity of photosynthetic enzymes (Evans and Poorter 2001, Feng

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Abbreviations: AQE – apparent quantum efficiency; C_i – intercellular CO_2 concentration; g_s – leaf stomatal conductance; LCP – light-compensation point; LNC_a – area-based leaf nitrogen content; LPC_a – area-based leaf phosphorus content; LSP – light-saturation point; P_N – net photosynthetic rate; P_{Nmax} – light-saturated net photosynthetic rate; PNUE – photosynthetic nitrogen-use efficiency; PPUE – photosynthetic phosphorus-use efficiency; WUE – water-use efficiency.

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et al. 2008). Besides, the differences in PNUE and PPUE are also associated with the leaf light respiration and light absorption (Hikosaka 2004).

Interspecific variations of PNUE and PPUE are related to species functional attributes, such as photosynthetic pathway, N fixation, plant relative height, and specific leaf area (SLA) (Hikosaka 2004, Kattge *et al.* 2009, Mao *et al.* 2018). Firstly, photosynthetic pathway affects PNUE and PPUE. For example, C_4 plants perform better than C_3 plants under nutrient-limited environment due to its superior PNUE and PPUE (Hikosaka 2004, Ghannoum *et al.* 2005). This is because Rubisco of C_4 plants has carboxylation rates up to double of those of C_3 plants (Ghannoum *et al.* 2005, Ma *et al.* 2017). In addition, Rubisco operates at or close to substrate-saturated enzyme activity (V_{max}) in C_4 plants due to CO_2 concentrating mechanism, while Rubisco in C_3 plants only operates at about 25% of V_{max} (Sage and Pearcy 1987, Ghannoum *et al.* 2005). C_4 plants need relatively less N allocation to photosynthetic enzymes to achieve high photosynthetic capacity (Ghannoum *et al.* 2005, Ma *et al.* 2017). Secondly, PNUE and PPUE in response to N and P addition are also associated with N fixation. Legume plants require extra amount of P in the course of nodulation and N_2-fixation. P addition would improve their PNUE and photosynthetic capacity by stimulating nodulation and nitrogenase activity (Naeem *et al.* 2010, Augusto *et al.* 2013). Thirdly, plant height relative to the canopy has been suggested as an important functional trait to affect the PNUE and PPUE. Taller species generally have higher PNUE and PPUE than shorter species. Taller species need to allocate relatively more N to Rubisco to maintain higher carboxylation capacity under sufficient irradiance, while shorter species grown in shade allocate relatively more N into thylakoids and chlorophyll to trap more light (Evans and Poorter 2001, Ma *et al.* 2017). Plant species with high specific leaf area (SLA) usually allocate more N to photosynthetic compounds. Species with low SLA have higher N allocation to structural tissue (*e.g.*, cell walls) to improve leaf toughness and plant resistance (Hikosaka 2004, Feng *et al.* 2008).

Changes in plant community can be justified from individual species before ecosystem processes are greatly affected (Regus *et al.* 2017, Mao *et al.* 2018). Ecophysiological response of dominant species could directionally predict underlying changes in plant growth and guide grassland restoration under N and P addition (Chen *et al.* 2005, Liu *et al.* 2015). Grassland degradation caused by long-term overgrazing and intensive cultivation has lowered vegetation productivity and quality on the semiarid Loess Plateau (Gang *et al.* 2018, Cui *et al.* 2019). Plant growth in the region is normally limited by the availability of both N and P due to high nutrient loss through soil erosion (Cui *et al.* 2019). Exogenous N and P fertilization is an efficient measure to improve soil nutrition and grassland productivity (Rawnsley *et al.* 2019, Sandral *et al.* 2019). The 'Grain for Green Project (GGP)', defined as replacing cropland and grazing land with trees and grass, has been proposed by Chinese authority since 1999 across the Loess Plateau. After the GGP implementation for ~ 20 years, the vegetation coverage nearly doubled. Grassland is the main vegetation type, covering about 42.9% of the total land area in the region (Gang *et al.* 2018, Chen *et al.* 2020). *Bothriochloa ischaemum* (C_4 grass), *Stipa bungeana* (C_3 grass), and *Lespedeza davurica* (C_3 legume) are three most common dominant species in the local grassland communities. The three species had different photosynthetic capacity and nutrient-use strategy. N and P capture and absorption in *B. ischaemum* were greatly improved when mixed with *L. davurica* (Xu *et al.* 2018). P addition alone or combined with N improved the biomass production and N and P accumulation of *L. davurica*. N addition (either alone or with P) could improve the photosynthetic capacity of *B. ischaemum* under water stress, while P addition alone exhibited no significant effect (Xu *et al.* 2013). Niu *et al.* (2016) and Xiong *et al.* (2017) found that *B. ischaemum* possessed much higher net photosynthetic rate than that of *L. davurica* in natural community on the semiarid Loess Plateau. We hypothesized that the interspecific variations of the three species are functionally interrelated (*e.g.*, photosynthetic pathway, N fixation) under N and P addition. Here, we examined leaf N and P contents, maximum photosynthetic rate, photosynthetic N- and P-use efficiencies and their relationships of the three species under different N and P addition rates. The aims of our study were to: (*1*) compare differences in leaf N and P contents, P_{Nmax}, PNUE, and PPUE in responses to different N and P addition rates between the three species; (*2*) reveal the relationship between leaf N and leaf P concentration, N/P ratio, and P_{Nmax} of the three species based on nutrient-use efficiency following N and P addition; and (*3*) explore the ecophysiological adaptive strategies employed by the three species in response to different N and P addition rates.

Materials and methods

Study site: The study was conducted at the Zhifanggou watershed in Ansai County, Yan'an City, Shaanxi Province, China (109°13'46"–109°16'03"E, 36°42'42"–36°46'28"N, and elevation range from 1,010–1,431 m a.s.l.), which is a typical loess hilly-gully watershed on the Loess Plateau (Zhao *et al.* 2016). It has temperate and semiarid climate with mean annual temperature of 8.8°C (mean minimum of 6.9°C in January and maximum of 22.6°C in July). The frost-free period is ~ 160 d in each year. Mean annual precipitation during 1951–2016 is 528.8 mm, of which about 82.2% (434.8 mm) falls in the growing season (from May to September). The annual precipitation in 2017 and 2018 were 485.6 and 464.4 mm, respectively. The growing season precipitation in 2018 (399.8 mm) was higher than that in 2017 (328.8 mm), and both was lower than the 65-year mean. Precipitation in July in 2017 (74.8 mm) was obviously lower than the 65-year mean (120.1 mm), but it was obviously higher in 2018 (149.4 mm) (Fig. 1).

The soil type is classified as Calcaric Cambisol according to FAO-UNESCO Soil Map of the World (FAO and ISRIC, 1988). The soil total N content is ~ 6.1%, and total P content is ~ 5.1%. Soil pH (H_2O) is from 8.4 to 8.6 and soil gravimetric moisture content at field capacity is ~ 20.0% (Zhao *et al.* 2016). The vegetation belongs to

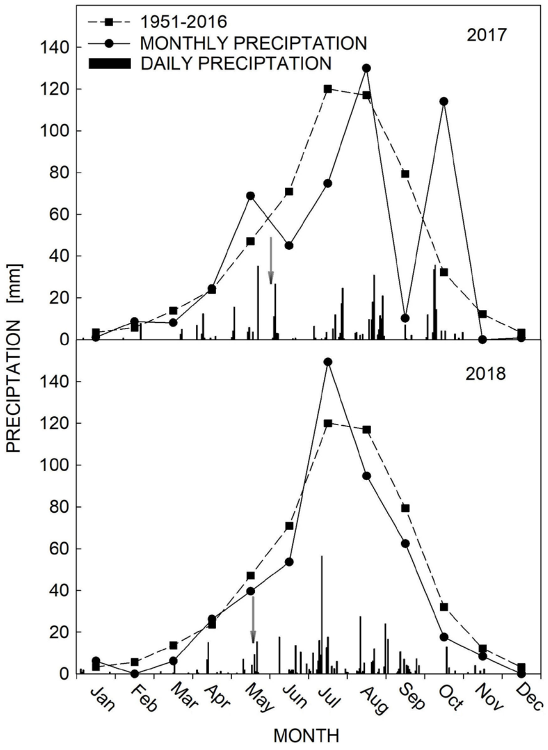

Fig. 1. Daily and monthly precipitation in 2017 and 2018, and monthly mean precipitation during 1951–2016 at the experimental site. *Arrows* indicate dates for N and P additions.

the warm-temperate forest steppe area, which is located in the ecotone of forest and grassland. In natural vegetation, common herb species include *B. ischaemum*, *L. davurica*, *S. bungeana*, *Artemisia gmelinii*, and *Artemisia giraldii*. Common shrubs include *Caragana korshinskii*, *Hippophae rhamnoides*, and *Sophora viciifolia*. Common trees include *Robinia pseudoacacia* and *Pinus tabuliformis*.

Experimental design: In May 2017, a common farming-withdrawn grassland community dominated by *B. ischaemum*, *S. bungeana*, and *L. davurica* in the catchment was selected. The experimental area of 20 × 30 m was fenced to exclude grazing disturbance by animals. A randomized split-plot design comprising four N addition treatments (main plot) and four P addition treatments (subplot) was carried out. Three blocks were set up and arranged in three rows along the slope. The blocks were separated by a 2.0-m buffer strip. Four 4.0 × 4.0 m main plots were situated in each block for N fertilizer treatments. N fertilizers were applied at rates of 0, 25, 50, and 100 kg(N) ha^{-1} $year^{-1}$ (hereafter denoted as N0, N25, N50, and N100, respectively) as calcium ammonium nitrate [$5\ Ca(NO_3)_2 \cdot NH_4NO_3 \cdot 10\ H_2O$]. The main plots within each block were separated by a 1.0-m buffer strip. Each main plot was divided into four 2.0 × 2.0 m subplots. Four P fertilizer treatments [0, 20, 40, and 80 kg(P) ha^{-1} $year^{-1}$; hereafter denoted as P0, P20, P40, and P80, respectively] were implemented in four subplots randomly as triple superphosphate [$Ca(H_2PO_4)_2 \cdot H_2O$]. The subplots without addition of both N and P were considered as control treatment. Each treatment was replicated three times, and totally there are 12 main plots and 48 subplots. N25 treatment was applied according to ambient annual N deposition level [21.76 kg(N) ha^{-1} $year^{-1}$] in the region of Loess Plateau (Liang *et al.* 2016). N50 and N100 treatments represented two-fold and four-fold annual N deposition levels, respectively, to estimate further N deposition effects. Granular calcium ammonium nitrate and triple superphosphate were weighed and broadcasted manually to each plot uniformly before the rain seasons on 4 June in 2017 and 21 May in 2018, respectively.

P_N-PAR response curves: Photosynthesis measurements of the three dominant species in the community, *i.e.*, *B. ischaemum*, *S. bungeana*, and *L. davurica*, were undertaken between 8:00 and 11:30 h from 20 July to 5 August in 2018. The most recent fully expanded healthy sun-exposed leaves were used for the measurements on the same six sunny days using the portable photosynthesis system with a red-blue LED light source (*CIRAS-2*, *PP Systems*, USA). The light-response curves were determined for PAR values of 2,000; 1,600; 1,200; 1,000; 800, 600, 400, 300, 200, 100, and 0 μmol(photon) m^{-2} s^{-1} with the leaf chamber at C_a of 360–370 (365 ± 3.1) μmol(CO_2) mol^{-1} (mean ± SE), leaf temperature of 25 ± 3°C, and relative humidity between 50 and 65%. Measurements of photosynthetic gas exchange were conducted after P_N-PAR response curves measurement on the same leaves. Net photosynthetic rate (P_N [μmol(CO_2) m^{-2} s^{-1}]), transpiration rate (E [mmol(H_2O) m^{-2} s^{-1}]), stomatal conductance (g_s [mmol(H_2O) m^{-2} s^{-1}]), and intercellular CO_2 concentration (C_i [μmol(CO_2) mol^{-1}]) of the three species were measured at a saturating irradiance [1,600 μmol(photon) m^{-2} s^{-1}]. Instantaneous water-use efficiency (WUE [μmol(CO_2) mmol(H_2O)$^{-1}$]) was calculated by determining P_N/E.

Leaf N and P concentration: Just after gas-exchange measurement, 30 healthy leaves were randomly collected from five plants with a similar height under each treatment. For *B. ischaemum* and *S. bungeana*, only the blade (without the sheath) was collected, and for *L. davurica*, both the blade and the petiole were sampled. Leaf area was calculated for the adaxial side of each leaf by *ImageJ* software (*National Institutes of Health*, USA) after being photographed by a digital camera (*Powershot G7X*, *Canon*, Japan). The SLA [m^2 g^{-1}] was calculated as leaf area divided by oven-dried mass. Leaf samples were oven-dried at 75°C for 48 h to obtain leaf dry mass, which were ground to a fine powder using a ball mill (*MM-400*, *Retsch*, Germany) to determine leaf N and P concentration and content. Mass-based leaf N concentration (N_{mass}) was determined using the auto-Kjeldahl method (*Kjektec System 2300 Distilling Unit*, *Foss*, Sweden). Mass-based leaf P concentration (P_{mass}) was analyzed using a molybdenum–antimony colorimetric method after H_2SO_4-H_2O_2 digestion quantified by reference to a national standard material with a known P concentration (*UV-2600* spectrophotometer, Japan). Area-based leaf N concentration (LNC_a [g(N) m^{-2}]) and leaf P concentration (LPC_a [g(P) m^{-2}]) were calculated as N_{mass} and P_{mass} divided by SLA, respectively. The LNC_a/LPC_a

ratio was calculated as the LNC_a divided by the LPC_a. PNUE and PPUE [μmol(CO_2) g(nutrient)$^{-1}$ s^{-1}] were calculated as maximum photosynthetic rate (P_{Nmax}) divided by LNC_a and LPC_a, respectively.

Statistical analysis: Rectangular and nonrectangular hyperbolas have been used widely to describe the irradiance-response curves of P_N (Yu *et al.* 2004, Messinger *et al.* 2006). However, these models do not deal with photoinhibition for plants, thus P_{Nmax} calculated by these hyperbolas is much higher than the actual measured data (Yu *et al.* 2004, Messinger *et al.* 2006). Thus, the parameter estimation was accomplished by using the modified rectangular hyperbolic model which can accurately describe the irradiance-response curve of photosynthesis, including irradiance below compensation (PAR_c) and above PAR_{max} (Ye 2007). The nonlinear regression module was conducted in the *SPSS* statistical package (*version 20.0 for Windows*, *SPSS*, Chicago, IL, USA). The regression equation is expressed as:

$$P_N = \alpha\frac{1-\beta\text{PAR}}{1+\gamma\text{PAR}}(\text{PAR}-\text{LCP}) \tag{1}$$

where PAR is irradiance, LCP is light-compensation point, α is the initial slope of P_N-PAR curves when irradiance approaches zero, β and γ are coefficients which are independent of PAR. Apparent quantum efficiency (AQE) was estimated from the initial slope of P_N-PAR curves when irradiance approaches zero (Xu *et al.* 2013).

Light saturation point (LSP) and P_{Nmax} were given by following formulae:

$$\text{LSP} = \frac{\sqrt{(\beta+\gamma)(1+\gamma\text{LCP})/\beta}-1}{\gamma} \tag{2}$$

$$P_{\text{Nmax}} = \alpha\frac{1-\beta\text{LSP}}{1+\gamma\text{LSP}}(\text{LSP}-\text{LCP}) \tag{3}$$

The main and interaction effects of N and P addition on P_{Nmax}, AQE, LSP, LCP, LNC_a, LPC_a, PNUE, and PPUE were analyzed using analysis of variance (*ANOVA*) in randomized blocks of the split-plot design. Effects for all tests were assessed for significance at $p \le 0.05$, $p \le 0.01$, and $p \le 0.001$. Significant differences between treatments means were identified using the least significant difference (LSD) test only when the effect of N, P or N × P interaction was significant ($p \le 0.05$). Statistical analyses were performed with *GenStat 18th edition* (*VSN International Ltd.*, Rothamsted, UK). Relationships between P_{Nmax} and LNC_a, LPC_a were fitted with linear or polynomial functions by curves estimation regression analysis using *SPSS Statistics 20.0* (*SPSS Inc.*, Chicago, IL, USA). These functions were chosen because they provided a simple and good description of the phenomenon. The determination coefficient (r^2) and the significance of the regression (p) were also computed. The graphs were plotted using *SigmaPlot 12.5* (*Systat Software Inc.*, San Jose, CA, USA).

The P_{Nmax}, LNC_a, LPC_a, PNUE, and PPUE of the three species were processed by redundancy analysis (RDA) in *CANOCO 5.0* program (ter Braak and Smilauer 2012). Monte Carlo permutation test with 999 permutations was used to reveal whether the LNC_a, LPC_a, $\text{LNC}_a/\text{LPC}_a$, and g_s significantly affected the P_{Nmax}, PNUE, and PPUE. The results of RDA analyses were visualized in the form of ordination diagrams constructed by *CANOCO 5.0* program. The percentage of P_{Nmax}, PNUE, and PPUE explained by LNC_a, LPC_a, $\text{LNC}_a/\text{LPC}_a$, and g_s was used as a measure of their explanatory power, p value represented corresponding probability obtained by the Monte Carlo permutation test.

Results

P_N-PAR curves and photosynthetic gas-exchange parameters: The P_N values increased with the increase of PAR showing obvious light-saturation phenomenon across all treatments in the three species (Fig. 2). In N0P0 treatment, *B. ischaemum* had the highest P_{Nmax} [14.4 μmol(CO_2) m^{-2} s^{-1}] and *S. bungeana* had the lowest P_{Nmax} (9.1 μmol m^{-2} s^{-1}), while *L. davurica* possessed intermediate P_{Nmax} [13.5 μmol(CO_2) m^{-2} s^{-1}]. P_{Nmax} and AQE of *B. ischaemum* and *S. bungeana* showed an increasing trend as N addition increased regardless of P addition, while those of *L. davurica* showed significant decreases under N100 compared to those under N25 and N50 addition. P_{Nmax} and AQE increased significantly only in *L. davurica* after P addition alone. Under combined N and P addition, P_{Nmax} and AQE of all three species significantly increased. The highest P_{Nmax} value of *B. ischaemum* [32.7 μmol(CO_2) m^{-2} s^{-1}], *S. bungeana* [26.6 μmol(CO_2) m^{-2} s^{-1}], and *L. davurica* [29.7 μmol(CO_2) m^{-2} s^{-1}] appeared at N50P80, N100P80, and N25P80 treatments, respectively. N and P significantly affected the P_{Nmax} of all three species, while N × P interactions significantly affected P_{Nmax} of *B. ischaemum* and *S. bungeana* only (Table 1). The g_s of *S. bungeana* and *L. davurica* improved significantly after P addition with or without N addition, while P addition had no effects on that of *B. ischaemum*. The g_s of *S. bungeana* and *L. davurica* increased significantly under all N addition treatments, whereas those of *B. ischaemum* exhibited significant increases only under N50 and N100 (Table 2).

The LSP of *B. ischaemum* showed a significant increase only under N50 and N100, while those of *S. bungeana* and *L. davurica* increased significantly under all N addition treatments. LSP increased significantly only in *L. davurica* after P addition regardless of N addition (Fig. 2; Table 1S, *supplement*). The LSP of *B. ischaemum* and *L. davurica* increased to ~ 1,750 μmol(photon) m^{-2} s^{-1} under N100 combined with P addition, while that of *S. bungeana* was only ~ 1,650 μmol(photon) m^{-2} s^{-1}. The LCP of *S. bungeana* decreased significantly after N addition with or without P addition, while the LCP of *L. davurica* decreased significantly only under N addition alone (Fig. 2, Table 1S). N, P, and their interaction showed significant effects on the LSP of all three species. N, P, and their interaction significantly affected the LCP of *S. bungeana* and *L. davurica* (Table 1S).

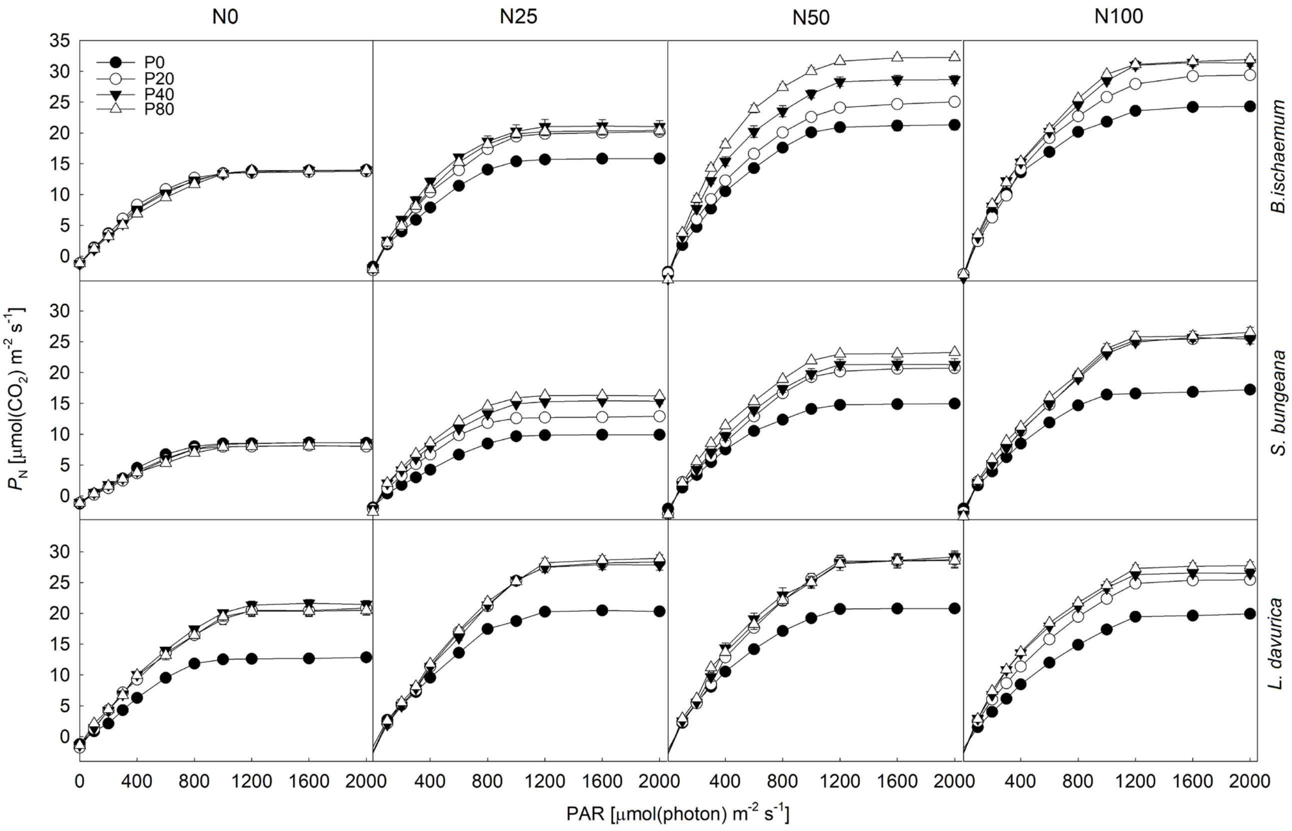

Fig. 2. Leaf net photosynthetic rate (P_N)–photosynthetically active radiation (PAR) response curves for *Bothriochloa ischaemum*, *Stipa bungeana*, and *Lespedeza davurica* under different N and P additions.

Leaf N_a and P_a concentration (LNC_a and LPC_a): In N0P0 treatments, *B. ischaemum* (0.64 and 0.062 g m^{-2}) had lower LNC_a and LPC_a than that of *S. bungeana* (1.21 and 0.076 g m^{-2}) and *L. davurica* (1.72 and 0.077 g m^{-2}), respectively. Only LNC_a of *S. bungeana* showed an increasing trend as N addition increased. LNC_a increased significantly under P addition alone only in *L. davurica*. Under combined N and P addition, LNC_a of *B. ischaemum* and *S. bungeana* decreased significantly, while it increased significantly in *L. davurica* (Fig. 3). N addition alone had no effects on LPC_a of all three species. Under P addition, LPC_a of *B. ischaemum* and *S. bungeana* decreased gradually with increasing N addition, while no significant difference was found between N addition treatments in *L. davurica* (Fig. 3). LNC_a/LPC_a ratio of *B. ischaemum* and *S. bungeana* increased significantly with N addition level, while N addition had no effect on those of *L. davurica*. LNC_a/LPC_a ratio of all three species showed a decreasing tendency as P addition level increased (Fig. 3). In *B. ischaemum*, N and P showed significant effects on LPC_a and LNC_a/LPC_a and only P significantly affected LNC_a. N, P, and their interaction had significant effects on LNC_a, LPC_a, and their ratio of *S. bungeana*. In *L. davurica*, only P significantly affected LNC_a, LPC_a, and their ratio (Fig. 3).

Relationship between P_{Nmax} and LNC_a, LPC_a, LNC_a/LPC_a: The P_{Nmax} of *B. ischaemum* had no functional relationship with LNC_a, while showed parabolic relation with LNC_a/LPC_a ratio ($r^2 = 0.19$, $p \leq 0.001$; Fig. 4*C*), and the P_{Nmax} reached the highest value when the LNC_a/LPC_a ratio was ~ 11 (Fig. 4*C*). The P_{Nmax} of *S. bungeana* increased initially with increasing LNC_a and LPC_a and peaked at 1.98 g(N) m^{-2} and 0.13 g(P) m^{-2} under N100 combined with P addition, while the subsequent decreases of P_{Nmax} appeared at greater LNC_a and LPC_a under N or P addition alone ($r^2 = 0.32$, $p \leq 0.001$ for LNC_a; $r^2 = 0.18$, $p \leq 0.05$ for LPC_a; Fig. 4*A*,*B*). P_{Nmax} peaked at 20 and showed as a function of LNC_a/LPC_a ratio in *S. bungeana* (Fig. 4*C*). P_{Nmax} of *L. davurica* increased significantly with increasing LNC_a and LPC_a until it reached the highest LNC_a and LPC_a under N25 and N50 combined with P addition ($r^2 = 0.26$, $p \leq 0.01$ for LNC_a; $r^2 = 0.41$, $p \leq 0.001$ for LPC_a; Fig. 4*A*,*B*). P_{Nmax} showed a negative relationship with LNC_a/LPC_a ratio in *L. davurica* ($r^2 = 0.26$, $p \leq 0.01$; Fig. 4*C*).

Photosynthetic water-, nitrogen-, and phosphorus-use efficiency (WUE, PNUE, and PPUE): In N0P0 treatment, *B. ischaemum* possessed obviously higher WUE, PNUE, and PPUE than those of *S. bungeana* and *L. davurica* (Fig. 5). PNUE of *B. ischaemum* and *L. davurica* increased significantly while that of *S. bungeana* showed decreasing tendency after N addition alone. Under P addition, PNUE

Table 1. Leaf light-saturated net photosynthetic rate (P_{Nmax}) and apparent quantum efficiency (AQE) of *Bothriochloa ischaemum*, *Stipa bungeana*, and *Lespedeza davurica* under different N and P additions (mean ± SE, $n = 3$). Data in brackets are LSD values among different N and P addition treatments ($p \leq 0.05$). *, **, and *** indicate statistically significant difference at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. ^{ns} means no significant difference.

Species	Treatment	P_{Nmax} [µmol(CO$_2$) m^{-2} s^{-1}]				AQE [µmol(CO$_2$) µmol(photon)$^{-1}$]			
		P0	P20	P40	P80	P0	P20	P40	P80
B. ischaemum	N0	14.41 ± 0.44	14.21 ± 0.24	14.38 ± 0.41	14.42 ± 0.08	0.03 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
	N25	16.42 ± 0.41	20.70 ± 0.25	21.64 ± 1.09	21.11 ± 0.67	0.03 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
	N50	21.81 ± 0.77	25.22 ± 0.24	28.96 ± 0.71	32.74 ± 0.45	0.04 ± 0.00	0.05 ± 0.00	0.07 ± 0.00	0.08 ± 0.00
	N100	24.51 ± 0.52	29.63 ± 0.19	31.84 ± 0.57	32.39 ± 0.37	0.06 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.06 ± 0.00
S. bungeana	N0	9.10 ± 0.64	8.61 ± 0.34	9.00 ± 0.46	8.47 ± 0.12	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
	N25	10.40 ± 0.59	13.32 ± 0.67	15.84 ± 0.24	16.76 ± 0.19	0.02 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
	N50	15.30 ± 0.29	21.34 ± 0.51	22.05 ± 0.91	23.78 ± 0.20	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.00
	N100	17.55 ± 0.25	26.56 ± 0.67	26.33 ± 0.68	26.87 ± 0.93	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.00
L. davurica	N0	13.48 ± 0.27	21.30 ± 1.10	22.39 ± 0.64	21.22 ± 0.79	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00
	N25	21.03 ± 0.27	29.21 ± 0.26	29.03 ± 0.84	29.67 ± 0.72	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
	N50	21.20 ± 0.52	29.52 ± 1.21	29.35 ± 0.90	28.91 ± 1.18	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
	N100	20.27 ± 0.36	25.83 ± 0.34	27.33 ± 0.25	28.01 ± 0.64	0.03 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.06 ± 0.00
B. ischaemum		N*** (0.52)	P*** (0.85)	N × P*** (1.52)		N*** (0.0033)	P*** (0.0026)	N × P*** (0.0052)	
S. bungeana		N*** (0.77)	P*** (0.82)	N × P*** (1.55)		N*** (0.0030)	P*** (0.0019)	N × P*** (0.0041)	
L. davurica		N*** (1.53)	P*** (1.01)	N × P^{ns}		N*** (0.0031)	P*** (0.0021)	N × P*** (0.0044)	

Table 2. Leaf stomatal conductance (g_s) of *Bothriochloa ischaemum*, *Stipa bungeana* and *Lespedeza davurica* under different N and P additions (mean ± SE, $n = 3$). Data in brackets are LSD values among different N and P addition treatments ($p \leq 0.05$). *, **, and *** indicate statistically significant difference at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. ^{ns} means no significant difference.

Species	Treatment	g_s [mmol(H_2O) m^{-2} s^{-1}]			
		P0	P20	P40	P80
B. ischaemum	N0	205.24 ± 2.78	200.46 ± 9.19	192.94 ± 17.42	196.43 ± 10.18
	N25	196.64 ± 8.30	195.94 ± 9.62	193.97 ± 4.87	217.97 ± 5.77
	N50	234.20 ± 12.72	237.09 ± 30.32	248.67 ± 13.29	233.88 ± 14.74
	N100	267.42 ± 25.69	236.86 ± 20.79	281.77 ± 7.26	287.01 ± 18.29
S. bungeana	N0	279.73 ± 4.31	374.36 ± 16.19	383.45 ± 26.12	358.85 ± 18.37
	N25	364.90 ± 27.43	425.89 ± 23.29	464.71 ± 18.71	426.45 ± 21.01
	N50	418.54 ± 16.62	434.23 ± 40.96	463.16 ± 19.53	498.47 ± 16.13
	N100	401.57 ± 7.26	465.18 ± 8.28	437.58 ± 13.03	470.56 ± 20.05
L. davurica	N0	280.93 ± 11.67	291.48 ± 4.22	290.95 ± 9.53	353.43 ± 15.85
	N25	387.58 ± 17.41	466.03 ± 25.23	464.75 ± 9.97	462.47 ± 10.48
	N50	351.44 ± 32.99	481.32 ± 19.12	487.09 ± 8.16	459.19 ± 23.26
	N100	343.18 ± 30.34	462.19 ± 22.36	446.87 ± 11.55	471.44 ± 12.20
B. ischaemum		N^{***} (20.40)	P^{ns}	N × P^{ns}	
S. bungeana		N^{***} (31.47)	P^{***} (30.34)	N × P^{ns}	
L. davurica		N^{***} (41.55)	P^{***} (23.88)	N × P^{*} (54.32)	

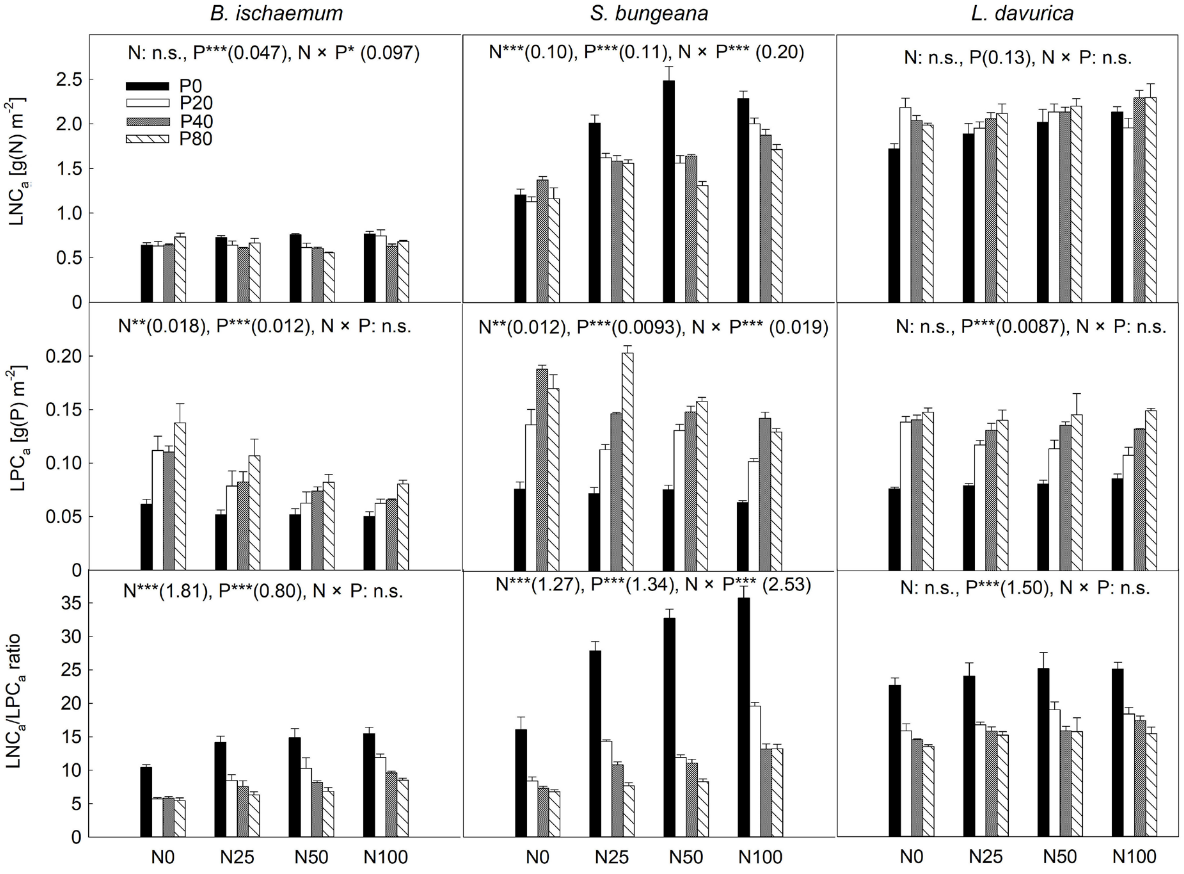

Fig. 3. Leaf N concentration per unit area (LNC_a), leaf P concentration per unit area (LPC_a), and LNC_a/LPC_a ratio of *Bothriochloa ischaemum*, *Stipa bungeana*, and *Lespedeza davurica* in response to N and P additions. Data in brackets are LSD values among different N and P addition treatments ($p \leq 0.05$). *, **, and *** indicate statistically significant difference at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. ^{ns} means no significant difference.

of *B. ischaemum* and *S. bungeana* significantly increased with N addition level, while it exhibited a decreasing tendency under N100 relative to N25 and N50 in *L. davurica*. PNUE significantly increased in *L. davurica* after P addition alone, while PNUE showed the significant increase in all three species under combined N and P addition (Fig. 5). PPUE of *B. ischaemum* and *S. bungeana* increased significantly under N addition regardless of P addition, while that of *L. davurica* had a decreasing tendency to under N100 compared to N25 and N50. N and

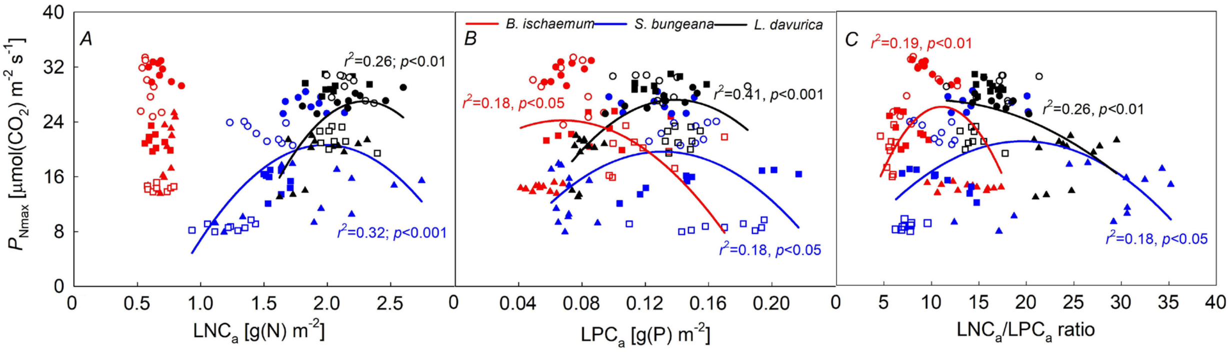

Fig. 4. Relationships between leaf light-saturated net photosynthetic rate (P_{Nmax}) and leaf N concentration per unit area ($\mathrm{LNC_a}$), leaf P concentration per unit area ($\mathrm{LPC_a}$), $\mathrm{LNC_a/LPC_a}$ ratio in *Bothriochloa ischaemum*, *Stipa bungeana*, and *Lespedeza davurica* grown under different N and P additions. ▲ represented N0P0, N25P0, N50P0, and N100P0 treatments. □ represented N0P20, N0P40, and N0P80 treatments. ■ represented N25P20, N25P40, and N25P80 treatments. ○ represented N50P20, N50P40, and N50P80 treatments. ● represented N100P20, N100P40, and N100P80 treatments.

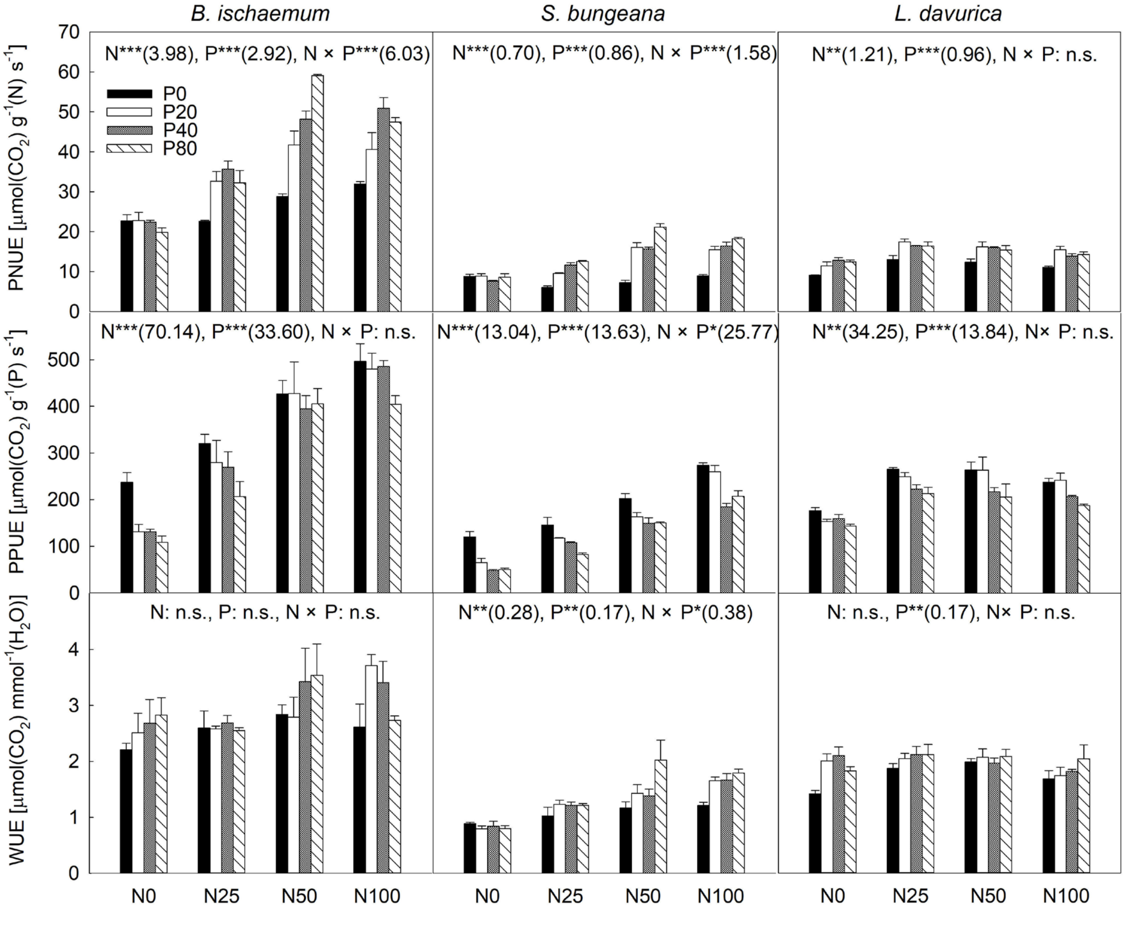

Fig. 5. Photosynthetic nitrogen-use efficiency (PNUE) and photosynthetic phosphorus-use efficiency (PPUE) of *Bothriochloa ischaemum*, *Stipa bungeana*, and *Lespedeza davurica* in response to N and P additions. *, **, and *** indicate statistically significant difference at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. $^{\mathrm{ns}}$ means no significant difference.

P addition significantly affected PNUE and PPUE of all three species, while N and P interaction only significantly affected PNUE of *B. ischaemum* and *S. bungeana*. WUE of *S. bungeana* increased significantly after P addition under N addition. N addition resulted in significant increase in WUE irrespective of P addition in *S. bungeana*. Only P had significant effects on WUE of *L. davurica*. In *B. ischaemum*, N, P, and their interaction had no significant effect on WUE (Fig. 5).

Dependence of P_{Nmax}, PNUE, and PPUE upon $\mathrm{LNC_a}$, $\mathrm{LPC_a}$, and g_s: The RDA analysis illustrates the divergent responses of P_{Nmax}, PNUE, and PPUE of the three species to N and P addition in relation to changes in $\mathrm{LNC_a}$, $\mathrm{LPC_a}$, $\mathrm{LNC_a/LPC_a}$, and g_s. The first two axes explained 71.7% of the total variability in *B. ischaemum* (F = 28.5, p=0.002), 68.0% in *S. bungeana* (F = 24.6, p=0.002), and 73.7% in *L. davurica* (F = 35.3; p=0.002) (Fig. 6). The highest variability in P_{Nmax}, PNUE, and PPUE of *B. ischaemum* was explained by $\mathrm{LPC_a}$ (48.2%), followed by g_s and $\mathrm{LNC_a/LPC_a}$. g_s, $\mathrm{LNC_a}$, and $\mathrm{LPC_a}$ explained 30.6, 30.3, and 14.1% of the variability in P_{Nmax}, PNUE, and PPUE of *S. bungeana*, respectively. g_s and $\mathrm{LPC_a}$ explained 46.5 and 26.5% of the variability in P_{Nmax}, PNUE, and PPUE of *L. davurica*, respectively, while $\mathrm{LNC_a}$ explained only 3.3% of the variability (Fig. 6).

Discussion

Effects of N and P addition on photosynthetic capacity and leaf nutrient content: Interspecific differences between functional groups in response to N and P addition are highly related to plant leaf traits, such as SLA, leaf N and P contents, and nutrient-use efficiency (Hikosaka 2004, Kattge *et al.* 2009, Mao *et al.* 2018). Elevated N availability greatly increased leaf N in *S. bungeana*, but not in *B. ischaemum* and *L. davurica* (Fig. 3). *B. ischaemum* and *L. davurica* have high stoichiometric homeostasis and can maintain relatively stable leaf N under increased N availability (Xu *et al.* 2013, 2016). The insensitive leaf N response of *B. ischaemum* may be associated with low N requirements and high N-use efficiency (Xu *et al.* 2016). The insensitive response of *L. davurica* is attributed to its relatively high N concentration because of intrinsic N fixation (Naeem *et al.* 2010, Regus *et al.* 2017). However, the photosynthetic capacity increased significantly under increased N availability in all three species (Table 1). Generally, the improved photosynthetic capacity can result from the increase in stomatal conductance and carboxylation capability (Chen *et al.* 2005, Messinger *et al.* 2006). Species grown in semiarid regions generally have xerophyte characteristics (*e.g.*, small cell size and thick cell wall) and small stomatal conductance to reduce water loss. Increased N availability could enlarge cell size and thin the cell wall to improve stomatal conductance (Xiong *et al.* 2015). Enhanced g_s of the three species under increased N availability improved the photosynthetic capacity by increasing CO_2 supply to the intercellular spaces (Messinger *et al.* 2006). In addition, elevated leaf N in *S. bungeana* could improve photosynthetic enzymes activity and pigments content, and further increase photosynthetic capacity (Chen *et al.* 2005). Despite lower leaf N in *B. ischaemum*, it had higher photosynthetic capacity than *S. bungeana* (Table 1, Fig. 3). The greater photosynthetic capacity and N-use efficiency in *B. ischaemum* were associated to its C_4 photosynthetic property. C_4 plants generally allocate relatively less N to photosynthetic enzymes to achieve high photosynthetic capacity because of high internal CO_2 concentrations and Rubisco activity (Sage and Pearcy 1987, Ghannoum *et al.* 2005). Generally, more N and P were invested to nonphotosynthetic component (*i.e.*, epidermis, cuticle, and sclerenchyma) in plant leaf to resist drought stress (Hikosaka 2004, Feng *et al.* 2008). Increased N availability could increase the infection rate of arbuscular mycorrhizal fungi and improve drought tolerance of the host plants by increasing water absorbing surface area, leading to smaller nonphotosynthetic N but higher photosynthetic N partition at a given leaf N content (Alkaraki *et al.* 2004, Reynolds *et al.* 2005).

The photosynthetic capacity of *B. ischaemum* and *S. bungeana* had no significant response to increased P availability (Table 1). This suggested that photosynthetic capacity of *B. ischaemum* and *S. bungeana* was primarily limited by N availability in natural grassland on the Loess Plateau (Xu *et al.* 2013, 2016). Legume is more responsive to P addition than the two grasses since legume plants require massive P amounts during nodulation and N_2-fixation. The stimulated nodulation and improved nitrogenase activity of nodule would increase leaf N and photosynthetic capacity (Table 1; Figs. 3, 4), indicating that the photosynthetic capacity of *L. davurica* was primarily limited by P availability in this region (Chaudhary *et al.* 2008, Naeem *et al.* 2010, Augusto *et al.* 2013).

The leaf N of *B. ischaemum* and *S. bungeana* under N and P combined addition decreased significantly relative to N addition alone due to dilution effect induced by leaf expansion (Xu *et al.* 2018). However, leaf P, photosynthetic capacity, and N-use efficiency exhibited significant increase under N and P combined addition. Increased arbuscular mycorrhizal fungi after N and P combined addition could promote poorly mobile nutrients (*e.g.*, P) uptake and further increase leaf P (Reynolds *et al.* 2005). Contrary to the two grasses, the leaf N of *L. davurica* increased significantly after combined addition of N and P. The dilution effects of plant growth on leaf N might be offset by the simulating effects of P addition through benefiting symbiotic N_2-fixation (Chaudhary *et al.* 2008, Naeem *et al.* 2010). There were two physiological factors that cause the increase of photosynthetic capacity under N and P combined addition. First, elevated leaf P can directly improve leaf photosynthetic capacity by promoting the synthesis of ATP and NADPH production, triose-phosphate exchange rate, and regeneration of Rubisco (dos Santos *et al.* 2004, Liu *et al.* 2015). In addition, elevated leaf P can also indirectly affect P_{Nmax}–N relationship and increase PNUE by influencing Rubisco activity and N partition (Hikosaka *et al.* 2004, Reich *et al.* 2009). The P_{Nmax}–N relationship and N-use efficiency was constrained by low P in P-limited ecosystems and the slope

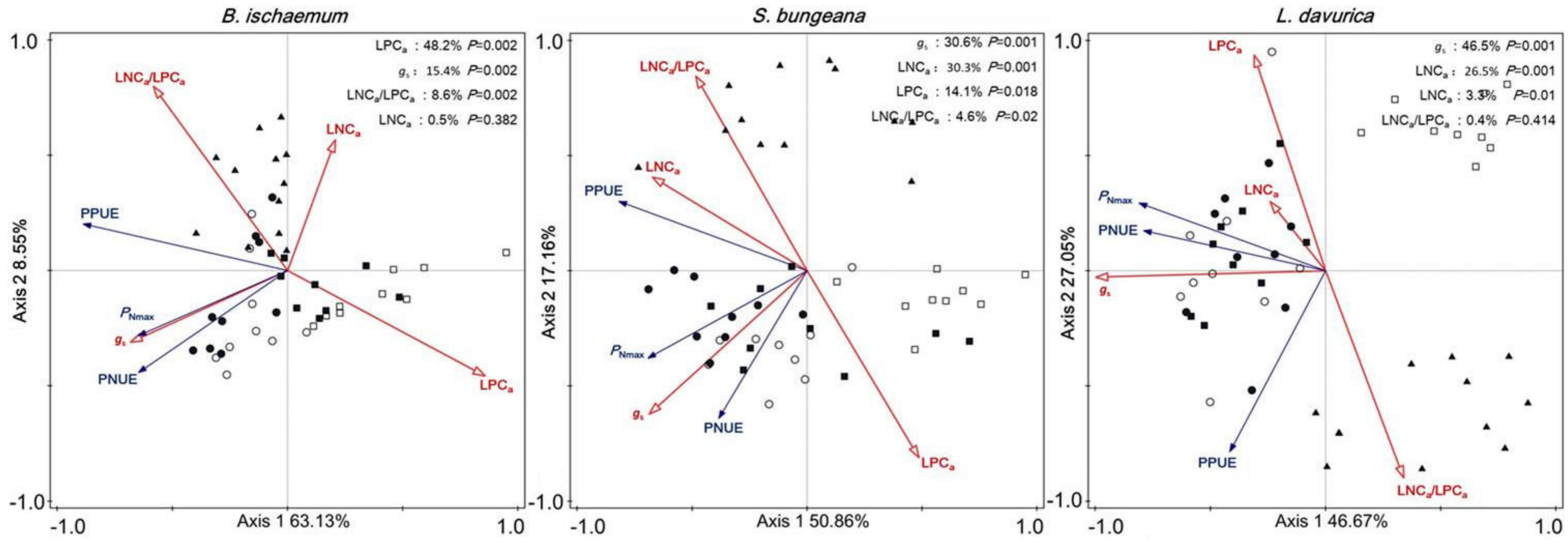

Fig. 6. Ordination diagram showing the result of RDA analysis of leaf light-saturated net photosynthetic rate (P_{Nmax}), photosynthetic nitrogen-use efficiency (PNUE) and photosynthetic phosphorus-use efficiency (PPUE) in relation to leaf stomatal conductance (g_s), leaf N concentration per unit area ($\mathrm{LNC_a}$), leaf P concentration per unit area ($\mathrm{LPC_a}$), and $\mathrm{LNC_a/LPC_a}$. ▲ represented N0P0, N25P0, N50P0, and N100P0 treatments. □ represented N0P20, N0P40, and N0P80 treatments. ■ represented N25P20, N25P40, and N25P80 treatments. ○ represented N50P20, N50P40, and N50P80 treatments. ● represented N100P20, N100P40, and N100P80 treatments.

of P_{Nmax}–N increased with the increase of leaf P content (Reich *et al.* 2009). Second, N and P combined addition further improved g_s in *S. bungeana* and *L. davurica* with better photosynthetic capacity (dos Santos *et al.* 2004, Naeem *et al.* 2010). The increased photosynthetic capacity with increasing N and P addition rates was primarily attributed to the increases in Rubisco activity rather than stomatal conductance (Shangguan *et al.* 2000, Chen *et al.* 2005).

Interspecific photosynthetic N- and P-use strategies and its ecological significance: The three different functional species exhibited contrasting photosynthetic N and P use strategies in response to N and P addition. *B. ischaemum* had the lowest leaf N and P and varied marginally in response to N and P addition (Fig. 5). The photosynthetic capacity of *B. ischaemum* had no significant relationship with leaf N but showed highly positive relationship with N- and P-use efficiency (Figs. 5, 6). This indicated that the enhanced photosynthetic capacity of *B. ischaemum* primarily depended on the improvement of PNUE and PPUE instead of leaf N and P content. *S. bungeana* exhibited large variation in both leaf N and P content and N- and P-use efficiency. The increased photosynthetic capacity in *S. bungeana* might be explained by simultaneous increase of g_s, leaf nutrient, and nutrient-use efficiency. In *L. davurica*, the relationship of P_{Nmax}–P was stronger than that of P_{Nmax}–N, and P_{Nmax} exhibited negative relationships with $\text{LNC}_a/\text{LPC}_a$, indicating that *L. davurica* tended to achieve higher PNUE and P_{Nmax} at higher LPC_a than at higher LNC_a. These indicated that leaf N/P ratios for optimum photosynthetic capacity and nutrient-use efficiency varied among the species (Hikosaka *et al.* 2004, Reich *et al.* 2009). *S. bungeana* was more sensitive to N deposition than the other two species in terms of leaf nutrient and photosynthetic capacity. *B. ischaemum* and *L. davurica* may not be able to take the advantage of elevated N availability under long-term N deposition, while anthropogenic P addition can improve their N-use efficiency and photosynthetic capacity.

Understanding how different functional groups respond to N and P addition is important for both management and restoration of grasslands (Chen *et al.* 2005). *B. ischaemum* and *L. davurica* achieved the highest P_{Nmax} and PNUE under N50 combined with P addition, while the highest values of *S. bungeana* occurred at N100 (Table 1, Fig. 4). The photosynthetic capacity and N-use efficiency of *L. davurica* appeared to decrease under N100 addition because of light competition (Ma *et al.* 2017, Chen *et al.* 2020). This is a partial consequence of relatively more N allocation in chlorophyll to trap more light and maintain electron transport capacity (Evans and Poorter 2001, Hikosaka 2004). Considering photosynthetic capacity and nutrient-use efficiency, N50P20 was the optimal combination for the community to improve photosynthetic capacity and nutrient-use efficiency. Our results implied that the physiological response of the three different functional species exhibited obvious asynchrony and complementary interaction, which is important for community stability and diversity (Xu *et al.* 2015, 2016).

Conclusion: Contrasting photosynthetic N- and P-use strategies were employed by the three different functional species in response to N and P addition. Among the three species, *B. ischaemum* had low N requirements and maintained relatively stable leaf N and P content with the high N- and P-use efficiency. *B. ischaemum* achieved higher photosynthetic capacity through the improvement of nutrient-use efficiency rather than leaf nutrient content, while the increased P_{Nmax} in *S. bungeana* might be due to the improved stomatal conductance and increased leaf N and nutrient-use efficiency. *L. davurica* tended to retain the greater photosynthetic capacity and nutrient-use efficiency at high leaf P than at high leaf N content. N50P20 was the optimal combination for the three species to maintain high photosynthetic capacity and nutrient-use efficiency. These results indicated that *S. bungeana* is more sensitive to further N deposition than the other two species in terms of leaf N content and photosynthetic capacity. In the long term, *B. ischaemum* and *L. davurica* may not be able to take the advantage of elevated N availability, resulting in replacement by *S. bungeana*. Anthropogenic P addition can greatly improve photosynthetic capacity and N-use efficiency in *B. ischaemum* and *L. davurica* under further N deposition. These contrasting interspecific differences in response to N and P addition reflect the compensatory adaptation of different functional species to environmental changes, and benefit grassland community stability and diversity in semiarid region.

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