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 (LNCₐ and LPCₐ), photosynthetic capacity (Pₘₐₓ), 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 LNCₐ and LPCₐ/LPCₐ ratio of Stipa bungeana (C₄ grass) only, while Pₘₐₓ increased significantly in all three species. Under N addition, P addition caused significantly lower LNCₐ, but higher PNUE and Pₘₐₓ in Bothriochloa ischaemum (C₄ grass) and Stipa bungeana. The LNCₐ, Pₘₐₓ, and PNUE of Lespedeza davurica (C₃ legume) increased significantly after P addition regardless of N application. The LNCₐ/LPCₐ for optimum PNUE changed with species. The Pₘₐₓ and PNUE of Bothriochloa ischaemum and Stipa bungeana had a peak when LNCₐ/LPCₐ attained ~ 11 and ~ 20. Lespedeza davurica tended to retain greater Pₘₐₓ and PNUE at lower LNCₐ/LPCₐ. Our results indicated that the C₄ grass was more sensitive to N and P addition than the C₃ grass and the C₃ legume in terms of leaf N and P contents and Pₘₐₓ. 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) CO₂ 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 et al. 2018).
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, Katoh et al. 2009, Mao et al. 2018). Firstly, photosynthetic pathway affects PNUE and PPUE. For example, C₄ plants perform better than C₃ plants under nutrient-limited environment due to its superior PPUE. For example, C₄ plants perform better than C₃ plants (Hikosaka 2004, Ghamnoum et al. 2005). This is because Rubisco of C₄ plants has carboxylation rates up to double of those of C₃ plants (Ghamnoum et al. 2005, Ma et al. 2017). In addition, Rubisco operates at or close to substrate-saturated enzyme activity (V_{max}) in C₄ plants due to CO₂ concentrating mechanism, while Rubisco in C₃ plants only operates at about 25% of V_{max} (Sage and Pearcy 1987, Ghamnoum et al. 2005). C₄ plants need relatively less N allocation to photosynthetic enzymes to achieve high photosynthetic capacity (Ghamnoum 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 N₂-fixture. P addition would improve their PNUE and photosynthetic capacity by stimulating nodulation and nitrogenase activity (Naecm 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 (C₄ grass), Stipa bungeana (C₃ grass), and Lespedeza davurica (C₃ 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_{nax}, 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_{nax} 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 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 (H₂O) 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

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**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(NO₃)₂·NH₄NO₃·10 H₂O]. The main plots within each block were separated by a 2.0-m buffer strip. Each main plot was divided into four 1.0-m buffer strip. Each main plot was divided into four subplots randomly as triple superphosphate [Ca(H₂PO₄)₂·H₂O]. 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.

**P₆-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(phonon) m⁻² s⁻¹ with the leaf chamber at Cᵢ of 360–370 (365 ± 3.1) μmol(CO₂) 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 P₆-PAR response curves measurement on the same leaves. Net photosynthetic rate (P₆ [μmol(CO₂) m⁻² s⁻¹]), transpiration rate (E [mmol(H₂O) m⁻² s⁻¹]), stomatal conductance (gs [mmol(H₂O) m⁻² s⁻¹]), and intercellular CO₂ concentration (Ci [μmol(CO₂) mol⁻¹]) of the three species were measured at a saturating irradiance [1,600 μmol(phonon) m⁻² s⁻¹]. Instantaneous water-use efficiency (WUE [μmol(CO₂) mmol(H₂O)⁻¹]) was calculated by determining P₆/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 [m² 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 (Nₘₑₜ) was determined using the auto-Kjeldahl method (Kjektec System 2300 Distilling Unit, Foss, Sweden). Mass-based leaf P concentration (Pₘₑₜ) was analyzed using a molybdenum–antimony colorimetric method after H₂SO₄-H₂O₂ digestion quantified by reference to a national standard material with a known P concentration (UV-2600 spectrophotometer, Japan). Area-based leaf N concentration (LNCₑₜ [g(N) m⁻²]) and leaf P concentration (LPCₑₜ [g(P) m⁻²]) were calculated as Nₘₑₜ and Pₘₑₜ divided by SLA, respectively. The LNCₑₜ/LPCₑₜ
ratio was calculated as the LNCa divided by the LPCa. PNUE and PPUE [μmol(CO₂) (nutrient)^1 s^-1] were calculated as maximum photosynthetic rate (P_{\text{max}}) divided by LNC and LPC, 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_{\text{max}} 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,) and above PAR_{\text{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:

\[ P_n = \frac{1 - \beta \text{PAR}}{1 + \gamma \text{PAR}}(\text{PAR} - \text{LCP}) \]  

where PAR is irradiance, LCP is light-compensation point, \( \alpha \) is the initial slope of \( P_n \)-PAR curves when irradiance approaches zero, \( \beta \) and \( \gamma \) 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 \( P_{\text{max}} \) were given by following formulae:

\[ \text{LSP} = \frac{(\beta + \gamma)(1 + \gamma \text{LCP})}{\gamma} - 1 \]  

\[ P_{\text{max}} = \frac{1 - \beta \text{LSP}}{1 + \gamma \text{LSP}}(\text{LSP} - \text{LCP}) \]  

The main and interaction effects of N and P addition on \( P_{\text{max}}, \) AQE, LSP, LPC, LNC, LPC, 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≤0.05, p≤0.01, and p≤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≤0.05). Statistical analyses were performed with GenStat 18th edition (VSN International Ltd., Rothamsted, UK). Relationships between \( P_{\text{max}} \) and LNC, LPC 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 \( P_{\text{max}}, \) LNC, LPC, 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, LPC, LNC/LPC, and \( g_\text{c} \) significantly affected the \( P_{\text{max}}, \) 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 \( P_{\text{max}}, \) PNUE, and PPUE explained by LNC, LPC, LNC/LPC, and \( g_\text{c} \) 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, \( B. \text{ischaemum} \) had the highest \( P_{\text{max}} \) [14.4 μmol(CO₂) m⁻² s⁻¹] and \( S. \text{bungeana} \) had the lowest \( P_{\text{max}} \) (9.1 μmol m⁻² s⁻¹), while \( L. \text{davurica} \) possessed intermediate \( P_{\text{max}} \) [13.5 μmol(CO₂) m² s⁻¹]. \( P_{\text{max}} \) and AQE of \( B. \text{ischaemum} \) and \( S. \text{bungeana} \) showed an increasing trend as N addition increased regardless of P addition, while those of \( L. \text{davurica} \) showed significant decreases under N100 compared to those under N25 and N50 addition. \( P_{\text{max}} \) and AQE increased significantly only in \( L. \text{davurica} \) after P addition alone. Under combined N and P addition, \( P_{\text{max}} \) and AQE of all three species significantly increased. The highest \( P_{\text{max}} \) value of \( B. \text{ischaemum} \) [32.7 μmol(CO₂) m⁻² s⁻¹], \( S. \text{bungeana} \) [26.6 μmol(CO₂) m² s⁻¹], and \( L. \text{davurica} \) [29.7 μmol(CO₂) m² s⁻¹] appeared at N50P80, N100P80, and N25P80 treatments, respectively. N and P significantly affected the \( P_{\text{max}} \) of all three species, while N × P interactions significantly affected \( P_{\text{max}} \) of \( B. \text{ischaemum} \) and \( S. \text{bungeana} \) only (Table 1). The \( g_\text{c} \) of \( S. \text{bungeana} \) and \( L. \text{davurica} \) improved significantly after P addition with or without N addition, while P addition had no effects on that of \( B. \text{ischaemum} \). The \( g_\text{c} \) of \( S. \text{bungeana} \) and \( L. \text{davurica} \) increased significantly under all N addition treatments, whereas those of \( B. \text{ischaemum} \) exhibited significant increases only under N50 and N100 (Table 2).

The LSP of \( B. \text{ischaemum} \) showed a significant increase only under N50 and N100, while those of \( S. \text{bungeana} \) and \( L. \text{davurica} \) increased significantly under all N addition treatments. LSP increased significantly only in \( L. \text{davurica} \) after P addition regardless of N addition (Fig. 2; Table 1S, supplement). The LSP of \( B. \text{ischaemum} \) and \( L. \text{davurica} \) increased to ~1,750 μmol(photon) m⁻² s⁻¹ under N100 combined with P addition, while that of \( S. \text{bungeana} \) was only ~1,650 μmol(photon) m⁻² s⁻¹. The LCP of \( S. \text{bungeana} \) decreased significantly after N addition with or without P addition, while the LCP of \( L. \text{davurica} \) 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 \( S. \text{bungeana} \) and \( L. \text{davurica} \) (Table 1S).
Leaf N, and P concentration (LNCa and LPCa): In N0P0 treatments, B. ischaemum (0.64 and 0.062 g m⁻²) had lower LNCa and LPCa 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 LNCa of S. bungeana showed an increasing trend as N addition increased. LNCa increased significantly under P addition alone only in L. davurica. Under combined N and P addition, LNCa of B. ischaemum and S. bungeana decreased significantly, while it increased significantly in L. davurica (Fig. 3). N addition alone had no effects on LPCa of all three species. Under P addition, LPCa 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). LNCa/LPCa ratio of B. ischaemum and S. bungeana increased significantly with N addition level, while N addition had no effect on those of L. davurica. LNCa/LPCa 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 LPCa and LNCa/LPCa, and only P significantly affected LNCa, N, P, and their interaction had significant effects on LNCa, LPCa, and their ratio of S. bungeana. In L. davurica, only P significantly affected LNCa, LPCa, and their ratio (Fig. 3).

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

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
INTERSPECIFIC DIFFERENCES IN RESPONSE TO N AND P ADDITION

Table 1. Leaf light-saturated net photosynthetic rate ($P_{\text{Nmax}}$) 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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>$P_{\text{Nmax}}$ $\mu$mol(CO$_2$) m$^{-2}$ s$^{-1}$</th>
<th>AQE $\mu$mol(CO$_2$) μmol(photon)$^{-1}$</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>P0</td>
<td>P20</td>
</tr>
<tr>
<td>B. ischaemum</td>
<td>N0</td>
<td>14.41 ± 0.44</td>
<td>14.21 ± 0.24</td>
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<tr>
<td></td>
<td>N25</td>
<td>16.42 ± 0.41</td>
<td>20.70 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>N50</td>
<td>21.81 ± 0.77</td>
<td>25.22 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>N100</td>
<td>24.51 ± 0.52</td>
<td>29.63 ± 0.19</td>
</tr>
<tr>
<td>S. bungeana</td>
<td>N0</td>
<td>9.10 ± 0.64</td>
<td>8.61 ± 0.34</td>
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<tr>
<td></td>
<td>N25</td>
<td>10.40 ± 0.59</td>
<td>13.32 ± 0.67</td>
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<tr>
<td></td>
<td>N50</td>
<td>15.30 ± 0.29</td>
<td>21.34 ± 0.51</td>
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<tr>
<td></td>
<td>N100</td>
<td>17.55 ± 0.25</td>
<td>26.56 ± 0.67</td>
</tr>
<tr>
<td>L. davurica</td>
<td>N0</td>
<td>13.48 ± 0.27</td>
<td>21.30 ± 1.10</td>
</tr>
<tr>
<td></td>
<td>N25</td>
<td>21.03 ± 0.27</td>
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<td></td>
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<td>21.20 ± 0.52</td>
<td>29.52 ± 1.21</td>
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<tr>
<td></td>
<td>N100</td>
<td>20.27 ± 0.36</td>
<td>25.83 ± 0.34</td>
</tr>
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B. ischaemum
N" (0.52) P" (0.85) N × P" (1.52)
S. bungeana
N" (0.77) P" (0.82) N × P" (1.55)
L. davurica
N" (1.53) P" (1.01) N × P"
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≤0.05). *, **, and *** indicate statistically significant difference at p≤0.05, p≤0.01, and p≤0.001, respectively. *ns* means no significant difference.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>g_s [mmol(H2O) m⁻² s⁻¹]</th>
<th>P0</th>
<th>P20</th>
<th>P40</th>
<th>P80</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. ischaemum</em></td>
<td>N0</td>
<td>205.24 ± 2.78</td>
<td>200.46 ± 9.19</td>
<td>192.94 ± 17.42</td>
<td>196.43 ± 10.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N25</td>
<td>196.64 ± 8.30</td>
<td>195.94 ± 9.62</td>
<td>193.97 ± 4.87</td>
<td>217.97 ± 5.77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N50</td>
<td>234.20 ± 12.72</td>
<td>237.09 ± 30.32</td>
<td>248.67 ± 13.29</td>
<td>233.88 ± 14.74</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N100</td>
<td>267.42 ± 25.69</td>
<td>236.86 ± 20.79</td>
<td>281.77 ± 7.26</td>
<td>287.01 ± 18.29</td>
<td></td>
</tr>
<tr>
<td><em>S. bungeana</em></td>
<td>N0</td>
<td>279.73 ± 4.31</td>
<td>374.36 ± 16.19</td>
<td>383.45 ± 26.12</td>
<td>358.85 ± 18.37</td>
<td></td>
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<tr>
<td></td>
<td>N25</td>
<td>364.90 ± 27.43</td>
<td>425.89 ± 23.29</td>
<td>464.71 ± 18.71</td>
<td>426.45 ± 21.01</td>
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<tr>
<td></td>
<td>N50</td>
<td>418.54 ± 16.62</td>
<td>434.23 ± 40.96</td>
<td>463.16 ± 19.53</td>
<td>498.47 ± 16.13</td>
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<tr>
<td></td>
<td>N100</td>
<td>401.57 ± 7.26</td>
<td>465.18 ± 8.28</td>
<td>437.58 ± 13.03</td>
<td>470.56 ± 20.05</td>
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<tr>
<td><em>L. davurica</em></td>
<td>N0</td>
<td>280.93 ± 11.67</td>
<td>291.48 ± 4.22</td>
<td>290.95 ± 9.53</td>
<td>353.43 ± 15.85</td>
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<tr>
<td></td>
<td>N25</td>
<td>387.58 ± 17.41</td>
<td>466.03 ± 25.23</td>
<td>464.75 ± 9.97</td>
<td>462.47 ± 10.48</td>
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<tr>
<td></td>
<td>N50</td>
<td>351.44 ± 32.99</td>
<td>481.32 ± 19.12</td>
<td>487.09 ± 8.16</td>
<td>459.19 ± 23.26</td>
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<tr>
<td></td>
<td>N100</td>
<td>343.18 ± 30.34</td>
<td>462.19 ± 22.36</td>
<td>446.87 ± 11.55</td>
<td>471.44 ± 12.20</td>
<td></td>
</tr>
</tbody>
</table>

*B. ischaemum* N*** (20.40) Pns N × Pns
*S. bungeana* N*** (31.47) P*** (30.34) N × P**
*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≤0.05). *, **, and *** indicate statistically significant difference at p≤0.05, p≤0.01, and p≤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 P addition significantly increased the photosynthetic nitrogen-use efficiency (PNUE) and photosynthetic phosphorus-use efficiency (PPUE) of the three species (*B. ischaemum*, *S. bungeana*, and *L. davurica*).
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*\(_{\text{max}}\), PNUE, and PPUE upon LNC\(_a\), LPC\(_a\), and \(g_s\): The RDA analysis illustrates the divergent responses of *P*\(_{\text{max}}\), PNUE, and PPUE of the three species to N and P addition in relation to changes in LNC\(_a\), LPC\(_a\), 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*\(_{\text{max}}\), PNUE, and PPUE of *B. ischaemum* was explained by LPC\(_a\) (48.2%), followed by \(g_s\) and LNC\(_a\)/LPC\(_a\), explained 30.6, 30.3, and 14.1% of the variability in *P*\(_{\text{max}}\), PNUE, and PPUE of *S. bungeana*, respectively. \(g_s\) and LPC\(_a\) explained 46.5 and 26.5% of the variability in *P*\(_{\text{max}}\), PNUE, and PPUE of *L. davurica*, respectively, while 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 (Naeeem 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, Ghannoun 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, Naeeem 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 stimulating effects of P addition through benefiting symbiotic N\(_2\)-fixation (Chaudhary et al. 2008, Naeeem 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*\(_{\text{max}}\)–N relationship and increase PNUE by influencing Rubisco activity and N partition (Hikosaka et al. 2004, Reich et al. 2009). The *P*\(_{\text{max}}\)–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 ($P_{\text{Nmax}}$), 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 (LNCa), and leaf P concentration per unit area (LPCa). ▲ 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 $P_{\text{max}}$ increased with the increase of leaf P content (Reich et al. 2009). Second, N and P combined addition further improved $g_\text{c}$ in *S. bungeana* and *L. davurica* with better photosynthetic capacity (dos Santos et al. 2004, Naem 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 (Shanggguan 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 $g_\text{c}$, leaf nutrient, and nutrient-use efficiency. In *L. davurica*, the relationship of $P_{\text{max}}$--P was stronger than that of $P_{\text{max}}$--N, and $P_{\text{max}}$ exhibited negative relationships with LNC--LPC, indicating that *L. davurica* tended to achieve higher PNUE and $P_{\text{max}}$ at higher LPC, than at higher LNC. 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 $P_{\text{max}}$ 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 $P_{\text{max}}$ 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.

**References**


INTERSPECIFIC DIFFERENCES IN RESPONSE TO N AND P ADDITION


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