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# Nitrogen increased aboveground biomass of *Leymus chinensis* grown in semi-arid grasslands of inner Mongolia, China

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**Abstract**
There is controversy over whether the addition of nitrogen (N) is the key to the rapid growth of Chinese rye grass *Leymus chinensis* (Trin.) Tzvel. in natural, semiarid grasslands. We investigated yearly impact of various N additions (0, 91, 183, and 274 kg N $ha^{-1}$) on the relationships between nutrient traits (plant carbon [C], N, and phosphorous [P]), morphological traits (plant height, leaf number, leaf length, leaf width, stem length and stem diameter) and aboveground biomass in *L. chinensis*. Results showed that most of the growth characteristics of *L. chinensis* increased with N rate except for leaf number and stem length. Nitrogen addition increased aboveground biomass, plant height, leaf length and stem length of *L. chinensis*, which was related to high precipitation during the critical period for the growth of *L. chinensis*. Nitrogen addition increased the N concentration and N to P ratio of *L. chinensis* tissue, but decreased the C to N ratio in the leaf and stem of *L. chinensis*. Compared to the control, N addition increased C and N concentrations and the N to P ratio, but decreased P concentration and the C to N ratio.

## 1 | INTRODUCTION

The meadow steppe areas in northeast Asia are the largest remaining grasslands in the world (Kawamura et al., 2005). In the past 20 yr, extensive hay harvesting in the Mongolian steppe has resulted in export of nutrients, affecting nutrient availability by decreasing soil nutrient content, and leading to significant changes in both aboveground and belowground components of the ecosystem (Bardgett, Wardle, & Yeates, 1998; Kuzyakov, Kretzschmar, & Stahr, 1999; Olde Venterink, Kardel, Kotowski, Peeters, & Wassen, 2009). Nitrogen (N) is an important nutrient in terrestrial ecosystems that affects the uptake efficiency of phosphorus (P) by plant and therefore, N fertilization is needed for low-N ecosystems (Galloway et al., 2004; Lü & Han, 2010; Steffens, Kölbl, Totsche, & Kögel-Knabner, 2008; Yahdjian, Gherardi, & Sala, 2011). However, N application may decrease soil pH and the subsequent soil acidification has negative effects on plant communities (Basso et al., 2016; Liu et al., 2017). Therefore, determining appropriate rate of N addition is essential for sustainable management of grassland ecosystems.

Plant functional traits represent ecological strategies and determine how plants respond to environmental factors, affect other trophic levels and influence ecosystem properties (Liu et al., 2012). Changes in plant functional traits, can effectively signify shifts in ecosystem functions and processes, and can be more sensitive to disturbance from N addition (Gu, Liu, Guo, & Zhang, 2008; Huang et al., 2015). Plasticity in plant nutrient traits, including the content, uptake, stoichiometry, and allocation of nutrients, is predicted to play a key role in regulating different traits, indirectly affecting ecosystem stability (Lü, Freschet, Flynn, & Han, 2012a). In semiarid grasslands, studies have found that dominant grass species displayed decreasing nutrient uptake with the duration of N addition (Liu et al., 2011; Lü et al., 2013). These results showed that carbon (C), N, P and their stoichiometry in plant tissues are generally associated with plant growth strategies, which were strongly influenced by external environmental disturbances (He et al., 2006; Liu et al., 2018). Studies on Chinese terrestrial plants have found that leaf P is considerably lower than the global average (excluding Chinese species), resulting in a markedly higher N to P ratios than the global average, and previous studies observed correlation between C to N and climate (He et al., 2006; He et al., 2008). These ratios could also be changed in response to global change factors such as atmospheric N deposition, and may exert strong controls on community composition and ecosystem functions in arid and semiarid areas. Plant stoichiometric characteristics play a significant role in driving several fundamental ecosystem processes, so better understanding of plant stoichiometric responses to N addition is critical for predicting related positive and negative feedback in ecosystem processes.

The Chinese rye grass (*Leymus chinensis*) is a native perennial rhizomatous grass, and has been considered one of the most promising grass species for typical steppe rehabilitation and restoration in semiarid regions of northern China (Huang et al., 2015; Liu & Han, 2008). This dominant grass species in the Mongolian steppe is also very palatable for grazing animals and has high forage value and drought tolerance (Bai, Han, Wu, Chen, & Li, 2004; Huang et al., 2015). Therefore, *L. chinensis* is recognized as an economically and ecologically important fodder crop. In our study, *L. chinensis* was chosen as a model plant for exploring ecological processes under N addition. We examined the effects of N addition on morphological plasticity, nutrient concentration, allocation, and ecological stoichiometry of *L. chinensis* within a meadow steppe in Hulunbuir, Inner Mongolia, Northern China. In frigid semiarid grasslands similar to Hulunbeier our findings should be applicable. This study was designed to address the following three questions: (i) How does short-term N addition affect the morphological plasticity and biomass allocation change of *L. chinensis*? (ii) How does N addition influence concentrations of C, N, and P in leaves and stems of *L. chinensis*? (iii) How does N addition affect the C to N to P stoichiometry and the relationship with morphological plasticity and plant nutrients?

**Core Ideas**

- Higher nitrogen (N) addition increased aboveground biomass of *Leymus chinensis*.
- High precipitation at the critical growth period enhances the increased (biomass, height, leaf/stem length) caused by N fertilizer.
- Compared to the control, N addition increased N concentration and N to P ratio of the whole plant.
- Compared to the control, N addition decreased the C to N ratio in leaves and stems of *L. chinensis*.

## 2 | MATERIALS AND METHODS

### 2.1 | Study sites and experimental design

This experiment was conducted in semiarid grassland of Inner Mongolia, Northern China (49°23′ N, 120°02′ E). The steppe has a semiarid continental climate characterized by mean annual precipitation (1985–2017) of 355 mm, with approximately 70% falling during the growing season from April to August. The soil type is Kastanozem (FAO classification). The plant community is dominated by *Leymus chinensis* (Trin.) Tzvel. and *Stipa baicalensis* (Roshev.), which together account for 60% of total aboveground biomass, accompanied by *Vicia amoena* (Fisch. ex DC.), *Thalictrum squarrosum* (Steph.), *Pulsatilla turczaninovii* (Kryl. et Serg.), *Cleistogenes squarrosa* (Trin.) Keng, *Carex duriuscula* (C. A. Mey.), *Allium bidentatum* (Allium L.), and *Artemisia tanacetifolia* (Linn.). Mowing for forage is a traditional grassland use strategy in this area.

The site was selected in 2013 at a number of permanent plots within an enclosure to prevent grazing. The experiment was performed as a completely randomized block with four treatments and three replicates. The local soil nutrient survey results in 2013 (total N content in 0 to ∼30 cm soil was 2.86 g $kg^{-1}$, total P was 0.49 g $kg^{-1}$, total K was 22.96 g $kg^{-1}$, total C was 35 g $kg^{-1}$, pH was 6.98, and bulk density was 1.11 g $cm^{-3}$) were used along with consideration of local traditional fertilization practices to select four amount of N fertilizer: CK (0 kg N $ha^{-1}$), N1 (91 kg N $ha^{-1}$), N2 (183 kg N $ha^{-1}$) and N3 (274 kg N $ha^{-1}$). Twelve 6-m by 10-m plots were randomly placed on site with an average separation distance of approximately 2 m in 2014. Nitrogen fertilizer was urea ($CON_2H_4$, total nitrogen content ≥46.4%)

and fertilizer was broadcast on the surface by hand once a year, beginning in May 2014.

### 2.2 | Biomass sampling

Field sampling was conducted from August 1st to 3rd, 2015 to 2017, which corresponds with annual peak standing biomass. Three 0.25-$m^2$ quadrats were selected randomly in each plot and 20 *L. chinensis* plants were randomly selected for testing phenotypic functional traits. Phenotypic functional traits (plant height, leaf number, leaf length, leaf width, stem length, stem diameter, and whole-plant biomass) of each *L. chinensis* plant were measured in the laboratory. Leaf width and stem diameter were measured using vernier calipers (precision = 0.01 mm). Stem, leaf, and whole plant biomass of *L. chinensis* were measured after being oven-dried at 65°C for at least 48 h. Leaves, stems, and whole plants of *L. chinensis* in each quadrat were separated and pulverized using a mechanical mill (Retsch MM 400, Retsch GmbH & Co KG, Haan, Germany). Total C was determined using the $H_2SO_4$–$K_2Cr_2O_7$ oxidation method (Bennett, Judd, & Adams, 2003). Total N was measured with the Kjeldahl method using a Kjeltec System 1026 distilling unit (Tecator AB, Höganäs, Sweden) (AOCS, 1989), and total P was measured colorimetrically at 880 nm after reaction with molybdenum blue. All stoichiometric ratios of C to N to P are reported as mass ratios (Lü, Kong, Pan, Simmons, & Han, 2012b).

### 2.3 | Statistical analyses

Statistical analyses were conducted on the average functional traits of the 20 plants for each quadrat in the field experiment. Significant differences in plant traits between the four nitrogen fertilizer treatments were evaluated by two-way analysis of variance (ANOVA) procedures. The plasticity index for the different plant traits of *L. chinensis* was calculated as follows:

$$\text{Plasticity index} = (\text{maximum value} - \text{minimum value}) / \text{maximum value}.$$

Values of the four treatments (CK, N1, N2, and N3) were used for each indicator to calculate the plasticity index. The plasticity index is always between 0 and 1. A mean plant traits plasticity index was calculated, as the mean of all the plant traits plasticity indexes (Valladares, Sanchez-Gomez, & Zavala, 2006).

To summarize the effect of nitrogen fertilizer on plant traits we calculated the fertilization effect as follows:

$$\text{Fertilization effect (FE)} = \text{mean plant traits } i \text{ under fertilization/mean root trait } i \text{ under control treatment.}$$

A value of the fertilizer effect >1 means that fertilizer increased the value of this trait, while a value <1 means that fertilizer decreased the value of this trait. The fertilizer effect and plasticity indexes reflect different aspects. Plasticity index values close to zero indicate that the plant traits show little plasticity in response to changes in environmental conditions, regardless of the direction of change. The fertilizer effect indicates the effect of fertilizer (increase or decrease), and its magnitude, on the plant traits.

A principal component analysis (PCA) was performed to determine relationships among plant traits and the effect of nitrogen fertilizer on these (Fort et al., 2015). Before analysis, we centered and normalized all variables with their standard deviations, and the importance of a trait in a given component is indicated by its relative loading on a component.

Two-way analysis of variance (ANOVA) was completed using SPSS 20.0 software (SPSS statistical package, Chicago, IL). An $\alpha$ of 0.05 was used for statistical significance. Statistical graphs were prepared using Excel 2010. The relationships between all the plant traits under nitrogen fertilizer treatments were analyzed by principal component analysis (PCA) using R package 'vegan'.

## 3 | RESULTS

### 3.1 | Environmental conditions during the fertilization experiment

During fertilization, the average growing-season monthly temperature from April to August was similar from 2015 to 2017, but there were large differences in precipitation (Figure 1). Precipitation during the most critical period for grass growth (April to June) was 27, 43, and 28 mm, for 2015, 2016, and 2017, respectively.

### 3.2 | Morphological plasticity response to N addition

Short-term effects on the plant traits of *L. chinensis* had a significant interaction for nitrogen treatments and years. The majority of traits were significantly increased by N fertilizer addition except for leaf number and stem length (Table 1, $P < .001$; Figure 2, $P < .05$). The sorting of morphological traits indicated that the morphological traits of *L. chinensis* could be distinguished into sensitive traits (leaf mass, stem mass, and stem diameter) and insensitive traits (leaf width, plant height, leaf length, stem length, and leaf number) in response to different N rate (Figure 3). Aboveground biomass of *L. chinensis* tended to increase with the increase of N rate especially in 2016 that N3 was significantly higher than other treatments ($P < .05$) (Figure 4). This was associated with the

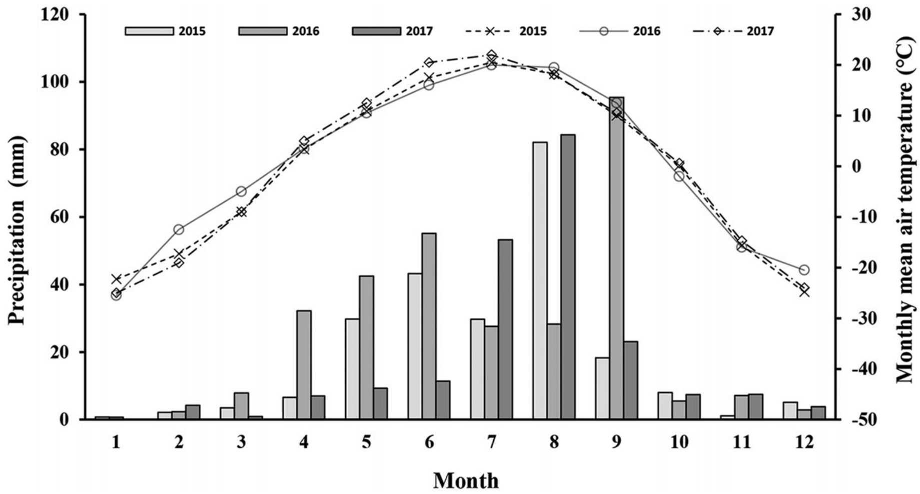


**FIGURE 1** Monthly (numbers 1 to 12 represent January to December) mean air temperature (lines) and rainfall (bars) received during the study period in 2015 to 2017 at the experiment site near Hulunbuir Grassland Ecosystem National Field Observation Station

**TABLE 1** Results (*P*-values) of ANOVA for the effects of fertilizer on *Leymus chinensis* plant traits: plant height (PH), leaf number (LN), leaf length (LL), leaf width (LW), stem length (SL), stem diameter (SD), stem mass (SM), leaf mass (LM), whole-plant aboveground biomass (AG), and steam to leaf ratio (SLR)

| Source of variance | df | PH | LN | LL | LW | SL | SD | SM | LM | AG | SLR |
|---|---|---|---|---|---|---|---|---|---|---|---|
| N-rate (N)[a] | 3 | <.001 | .976 | <.001 | <.001 | .320 | <.001 | <.001 | <.001 | <.001 | <.001 |
| Year (Y)[b] | 2 | <.001 | <.001 | <.001 | .198 | <.001 | .543 | <.001 | .476 | <.001 | <.001 |
| Block (B) | 2 | .177 | .364 | .382 | .406 | .263 | .575 | <.05 | .679 | .141 | <.05 |
| N × B | 6 | .884 | <.05 | .440 | <.05 | .310 | .513 | .065 | .387 | .992 | .256 |
| Y × B | 4 | .688 | .726 | .655 | .993 | .487 | .956 | .197 | .774 | .987 | .154 |
| N × Y | 6 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | .053 |
| N × Y × B | 12 | .833 | <.05 | .823 | .316 | .173 | .145 | <.05 | .168 | 1.000 | .078 |

[a]Nitrogen fertilizer treatments: CK (0 kg N ha$^{-1}$), N1 (91 kg N ha$^{-1}$), N2 (183 kg N ha$^{-1}$), and N3 (274 kg N ha$^{-1}$).
[b]Year: 2015, 2016, and 2017.

high precipitation from April to June compared to the other 2 yr (Figure 1), indicating that the effect of N fertilizer rate on biomass production can be more realized if the moisture condition is good. Although treatment differences in leaf mass and stem mass were not consistent over the 3 yr (Figure 4), more N input (N3 and N2) tended to result in more leaf mass and stem mass than less N input (N1 and CK). There was no N by year interaction for stem to leaf ratio and N1 was significantly lower than other three N treatments ($P < .05$). For plant height, the highest N treatment (N3) was the highest compared to other treatments in 2016, but no significant treatment difference was observed in the other 2 yr. Similar results were found for leaf length and stem length indicating that the impact of moisture condition on the effectiveness of N input on plant height, leaf length and stem length. Less N input (CK and N1) tended to increase the leaf number in 2017 compared to more N input treatments of N2 and N3, but no difference was found for 2015 or 2016 between N treatments. No significant treatment difference was found in leaf width in all 3 yr except that N2 was higher than other treatments in 2016. No obvious effects of N input on stem diameter were observed in all years except that N2 had the highest stem diameter than other treatments in 2015. Overall, N inputs tended to increase biomass, plant height, leaf length and stem length especially under the good moisture condition (2016), but the effect of N fertilizer rate on other traits were not significant or consistent.

In all plant traits and biomass, only three sets of indicators had significant correlation, stem mass and stem length ($r = 0.97$, $P < .05$), leaf number and leaf width ($r = 0.95$, $P < .05$), plant height and stem length ($r = 0.96$, $P < .05$), respectively. Additionally, the first two axes of PCA could explain 56.8% of the total variances observed in plant traits of *L. chinensis*.

### 3.3 | Nutrient allocation responses to N addition

The interaction of nitrogen treatments and years were significant on C, N and P rates except for C on leaf and P on

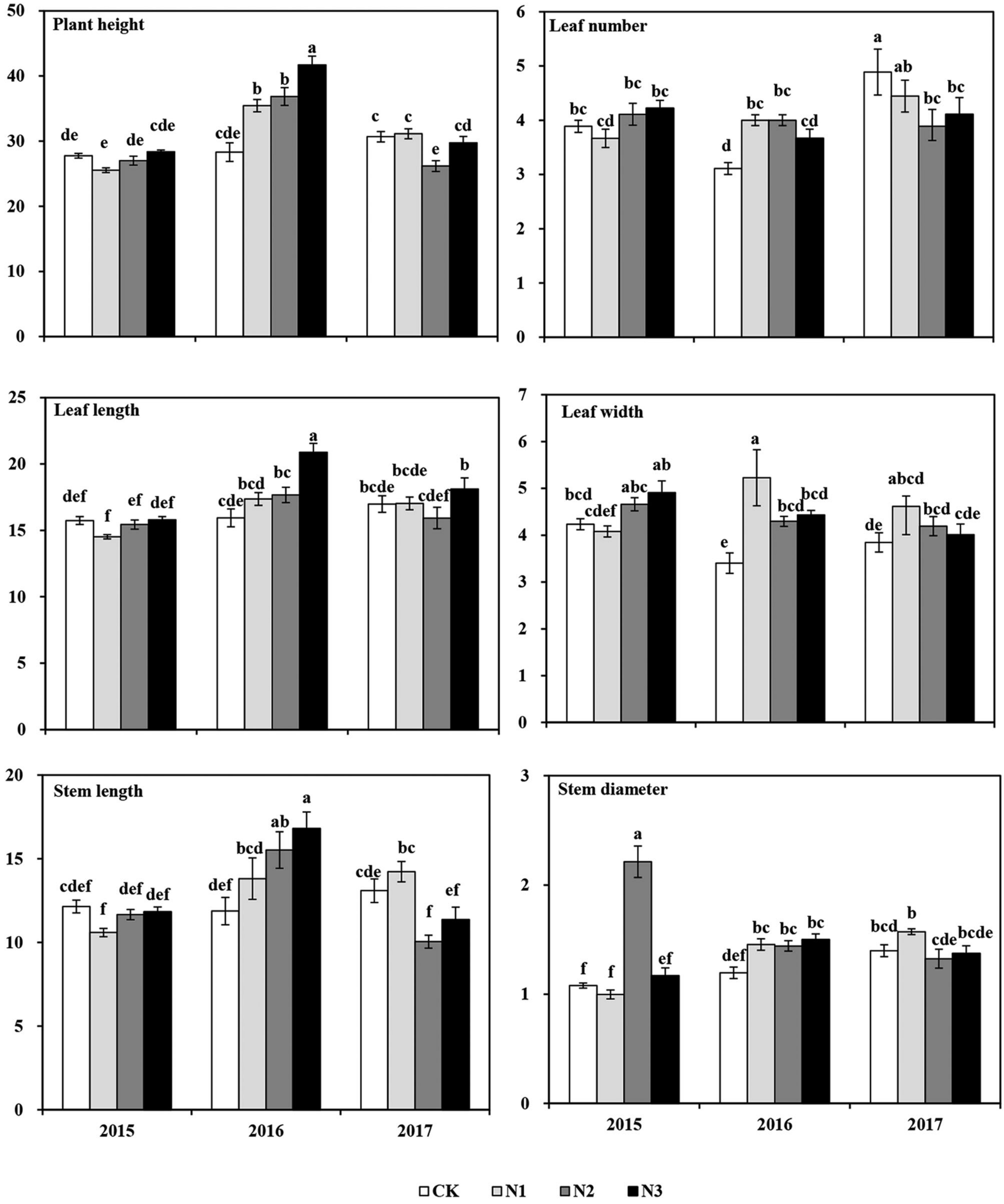


**FIGURE 2** Effects of N fertilizer addition on plant traits of *Leymus chinensis* from 2015 to 2017; treatments were: CK, (0 kg N ha$^{-1}$), N1 (91 kg N ha$^{-1}$), N2 (183 kg N ha$^{-1}$) and N3 (274 kg N ha$^{-1}$). The vertical bars represent standard errors. Values not sharing a common lowercase letter were significantly different according to the LSD test at $P < .05$

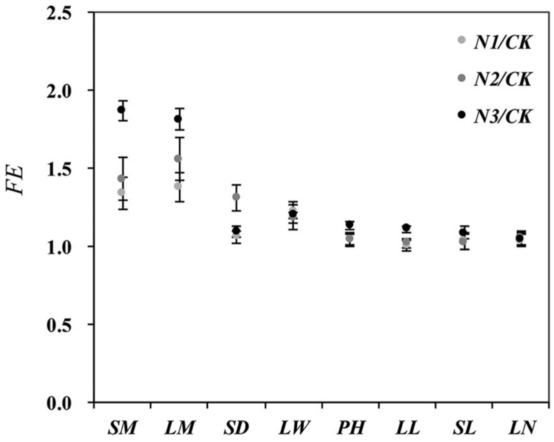


**FIGURE 3** Fertilization effect (FE) of *Leymus chinensis* in response to N fertilizer addition with standard error bars. Nitrogen fertilizer treatments were: CK, (0 kg N ha$^{-1}$), N1 (91 kg N ha$^{-1}$), N2 (183 kg N ha$^{-1}$) and N3 (274 kg N ha$^{-1}$). SM, stem mass; LM, leaf mass; SD, stem diameter; LW, leaf width; PH, plant height; LL, leaf length; SL, stem length; LN, leaf number

aboveground biomass of *L. chinensis* (Table 2; $P < .05$). For C concentration, the high values of aboveground biomass and Stem were apparent in N3, while significant higher on C of leaf in 2015 was found across different fertilizer years (Figure 5a, b, c). We found that there was a steady increase of N in aboveground biomass and leaf until N3 in 2017 reached the peak value, while N3 of stem in 2016 was higher (Figure 5d, e, f; $P < .05$). However, the P of aboveground biomass, leaf and stem were found to be significantly higher in CK across different N rate, but appeared in different years (Figure 5g, h, i; $P < .05$).

## 3.4 | Stoichiometric responses

The interaction of nitrogen treatments and years was significant on stoichiometric except for C to P on aboveground biomass of *L. chinensis* (Table 3; $P < .05$). Nitrogen fertilizer slowly decreased the C to N in leaf, stem, and aboveground biomass of *L. chinensis* except for aboveground biomass in 2015 (Figure 6a, b, c; $P < .05$). In addition, the higher values of C to P ratio on leaf and stem were found on low N rate fertilizer in 2017, while medium N rate performed better on aboveground biomass (Figure 6d, e, f; $P < .05$). In contrast,

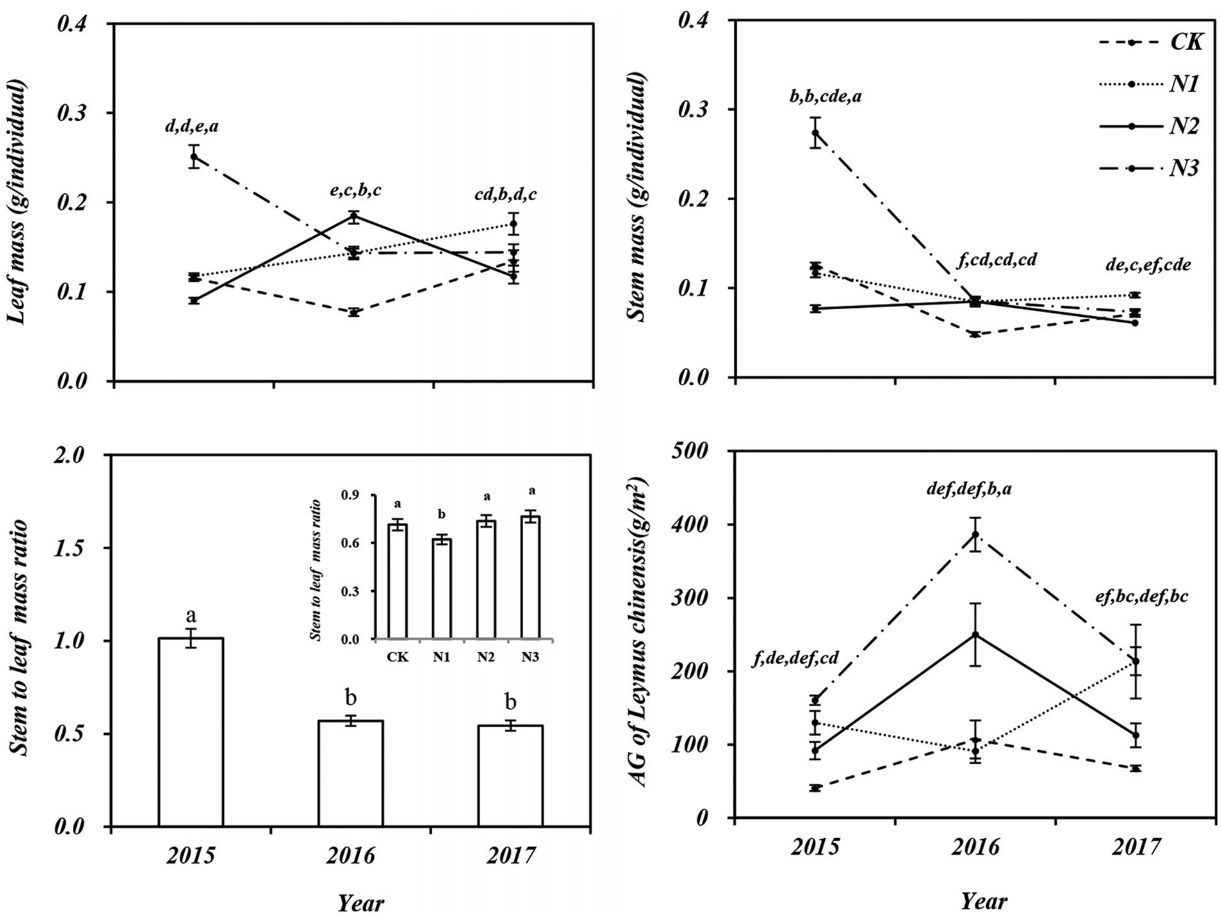


**FIGURE 4** Effects of N fertilizer addition on the aboveground biomass (AG) of *Leymus chinensis*, and in leaf mass, and stem mass, and stem to leaf mass ratio. Nitrogen fertilizer treatments were: CK, (0 kg N ha$^{-1}$), N1 (91 kg N ha$^{-1}$), N2 (183 kg N ha$^{-1}$) and N3 (274 kg N ha$^{-1}$). Values not sharing a common lowercase letter were significantly different according to the LSD test at $P < .05$

**TABLE 2** Results (*P*-values) of ANOVA for the effects of fertilizer on nutrient concentrations of *Leymus chinensis*: whole-plant aboveground biomass (AG), leaf mass (LM), and stem mass (SM)

| | | C concentration | | | N concentration | | | P concentration | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Source of variance** | **df** | **AG** | **LM** | **SM** | **AG** | **LM** | **SM** | **AG** | **LM** | **SM** |
| N-rate (N)[a] | 3 | .553 | .480 | <.05 | <.001 | <.001 | <.001 | <.05 | <.001 | <.001 |
| Year (Y)[b] | 2 | <.01 | <.01 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| Block (B) | 2 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.01 | <.001 | <.001 |
| N × B | 6 | .895 | .639 | .880 | .840 | .491 | .726 | .821 | .141 | .092 |
| Y × B | 4 | <.05 | .066 | <.05 | .066 | .222 | .268 | .593 | <.001 | .093 |
| N × Y | 6 | <.001 | .672 | <.05 | <.001 | <.001 | <.001 | .225 | <.001 | <.001 |
| N × Y × B | 12 | .966 | .876 | .946 | .935 | .336 | .832 | .560 | .726 | .441 |

[a]Nitrogen fertilizer treatments: CK (0 kg N $ha^{-1}$), N1 (91 kg N $ha^{-1}$), N2 (183 kg N $ha^{-1}$), and N3 (274 kg N $ha^{-1}$).
[b]Year: 2015, 2016, and 2017.

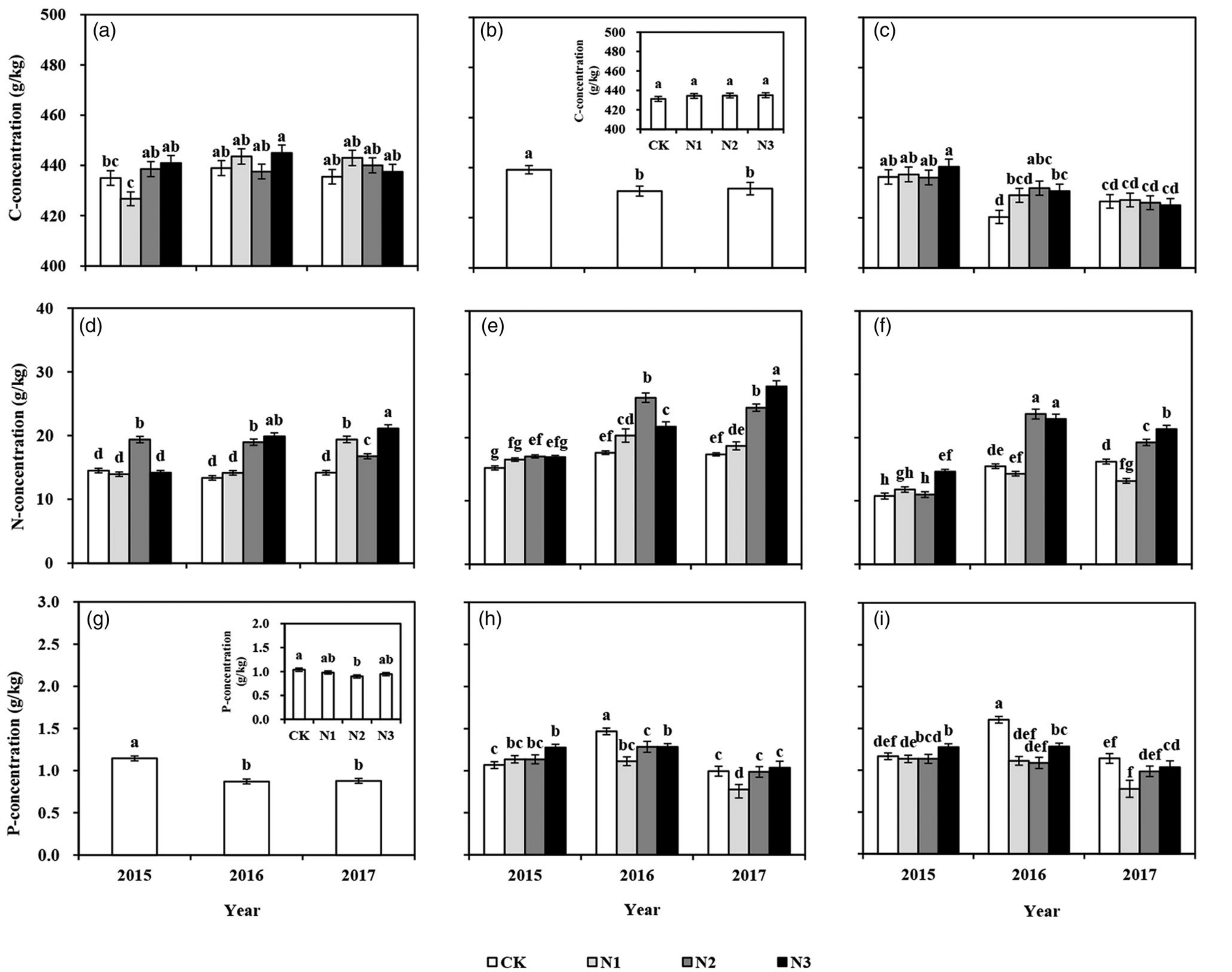


**FIGURE 5** Effects of N fertilizer addition on C, N, and P concentrations in the aboveground portions of *Leymus chinensis* (a, d, g, respectively), and in leaves (b, e, h, respectively), and stems (c, f, i, respectively). Nitrogen fertilizer treatments were: CK, (0 kg N $ha^{-1}$), N1 (91 kg N $ha^{-1}$), N2 (183 kg N $ha^{-1}$) and N3 (274 kg N $ha^{-1}$). Values not sharing a common lowercase letter were significantly different according to the LSD test at $P < .05$

**TABLE 3** Results (*P*-values) of ANOVA for the effects of fertilizer on nutrient stoichiometry of *Leymus chinensis*: whole-plant aboveground biomass (AG), leaf mass (LM), and stem mass (SM)

| | | C to N ratio | | | C to P ratio | | | N to P ratio | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Source of variance** | **df** | **AG** | **LM** | **SM** | **AG** | **LM** | **SM** | **AG** | **LM** | **SM** |
| N-rate (N)[a] | 3 | <.001 | <.001 | <.001 | <.05 | <.05 | <.001 | <.001 | <.001 | <.001 |
| Year (Y)[b] | 2 | <.001 | <.001 | <.001 | <.001 | <.001 | <.05 | <.001 | <.001 | <.001 |
| Block (B) | 2 | <.05 | <.001 | <.001 | .120 | <.05 | <.001 | .088 | <.05 | .694 |
| N × B | 6 | .432 | .213 | .827 | .081 | .665 | .431 | .644 | .621 | .600 |
| Y × B | 4 | .376 | .773 | <.05 | .334 | .074 | .225 | .313 | <.05 | .981 |
| N × Y | 6 | <.001 | <.001 | <.001 | <.001 | <.05 | <.001 | <.001 | <.05 | <.001 |
| N × Y × B | 12 | .252 | .869 | .866 | <.05 | .853 | .688 | .012 | .686 | <.05 |

[a]Nitrogen fertilizer treatments: CK (0 kg N ha$^{-1}$), N1 (91 kg N ha$^{-1}$), N2 (183 kg N ha$^{-1}$), and N3 (274 kg N ha$^{-1}$).
[b]Year: 2015, 2016, and 2017.

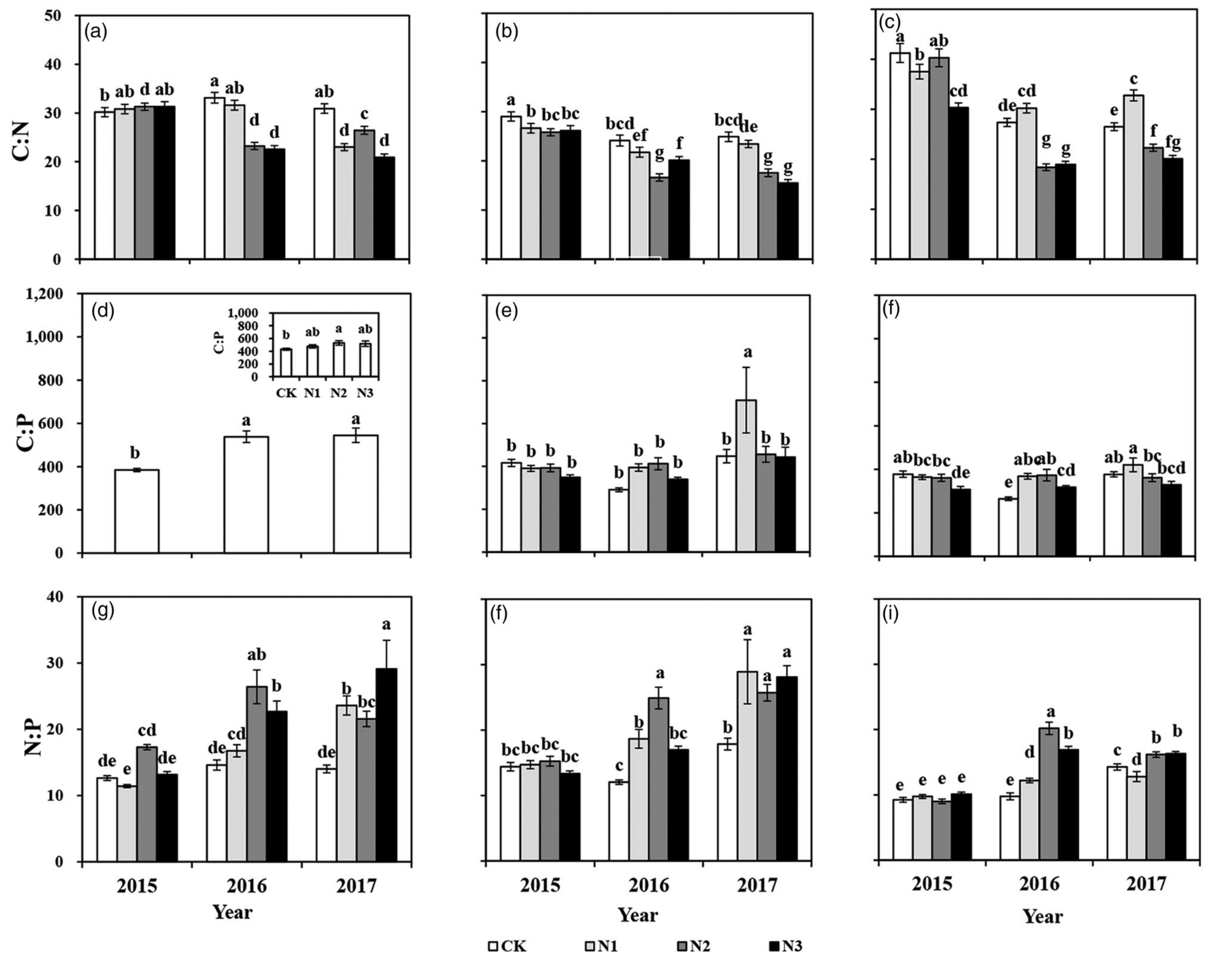


**FIGURE 6** Effects of N fertilizer addition on C to N, C to P, and N to P ratios in the aboveground portions of *Leymus chinensis* plants (a, d, g, respectively), and in leaves (b, e, h, respectively), and stems (c, f, i, respectively). Nitrogen fertilizer treatments were: CK, (0 kg N ha$^{-1}$), N1 (91 kg N ha$^{-1}$), N2 (183 kg N ha$^{-1}$) and N3 (274 kg N ha$^{-1}$). Values not sharing a common lowercase letter were significantly different according to the LSD test at $P < .05$

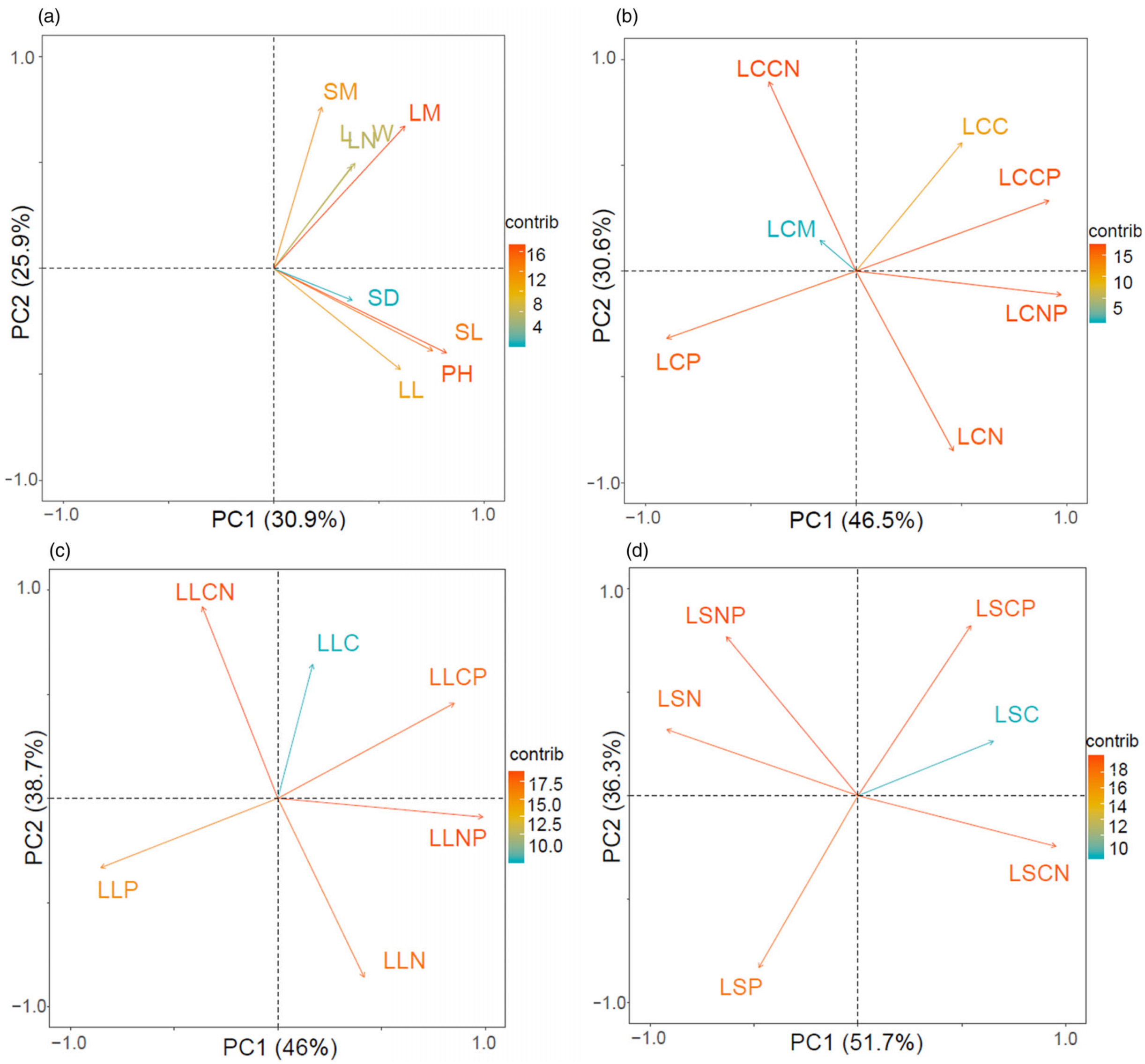


**FIGURE 7** Principal component analysis showing (a) the relationships between plant traits, (b) *Leymus chinensis* whole-plant nutrient concentration and stoichiometry, (c) leaf nutrient concentration and stoichiometry, and (d) stem nutrient concentration and stoichiometry. (a) PH, plant height; LN, leaf number; LL, leaf length; LW, leaf width; SL, stem length; SD, stem diameter; SM, stem mass; LM, leaf mass. (b) LCC/LCN/LCP, *Leymus chinensis* whole-plant carbon, nitrogen, and phosphorus; LCCN/LCNP/LCCP, *Leymus chinensis* whole-plant C to N, N to P, and C to P. (c) LLC/LLN/LLP, leaf carbon, nitrogen, and phosphorus; LLCN/LLNP/LLCP, leaf C to N, N to P, and C to P. (d) LSC/LSN/LSP, stem carbon, nitrogen, and phosphorus; LSCN/LSNP/LSCP, stem C to N, N to P, and C to P

the high value of N to P on aboveground biomass was found on high N rate in 2017 (Figure 6g; $P < .05$). Leaf N to P has similar result, but fertilized treatments performed better than CK in 2017 (Figure 6h; $P < .05$). Interestingly, the higher peak value of stem N to P was found on N2 in 2016 (Figure 6i; $P < .05$). There were significantly negative correlation between leaf C to P and leaf P ($r = -0.95$, $P < .05$), stem C to N and stem N ($r = -0.99$, $P < .05$), *L. chinensis* C to P and *L. chinensis* P ($r = -0.97$, $P < .05$), while *L. chinensis* C to N and *L. chinensis* P ($r = 0.95$, $P < .05$), *L. chinensis* C to P and *L. chinensis* N ($r = 0.99$, $P < .05$) had significantly positive correlations. The first two axes of PCA could explain 77.1, 84.7, and 88% of the total variances observed in whole plants, leaves, and stems of *L. chinensis* respectively (Figure 7).

## 4 | DISCUSSION

### 4.1 | Morphological plasticity of *L. chinensis*

The plant functional traits, which exist in various terrestrial plant species, can effectively signify shifts in ecosystem functions and processes (De Vries, Brown, & Stevens, 2016). In semiarid regions, N addition affects native grassland in the macro-scale performance of ecosystems and the plant function at the micro-scale (Sanjuán et al., 2018). Our results demonstrated that the aboveground biomass of *L. chinensis* tended to be larger under N addition compared with CK. This is consistent with the findings described in several previous studies (He et al., 2016; Shen et al., 2018). We found a dramatic change in the plasticity of different functional traits with the increase of nitrogen rate—the higher sensitivity of stems than leaves—indicated that *L. chinensis* can exhibit rapid pant growth by promoting stem elongation. In our results, N addition increased plant height, leaf length, stem length, especially N3 in 2016. Primary production was co-limited by available water and N in semiarid ecosystems, as well as plant functional traits are affected (Bai et al., 2008; He et al., 2016; Hooper & Johnson, 1999). Hence, water availability might mediate the response of productivity and plant functional traits to N addition. Although these three fertilization years were dry years, aboveground biomass of *L. chinensis* and some plants traits performed better in 2016 because of much more precipitation in May and June and fertilizer accumulation.

### 4.2 | Effects of N addition on nutrient allocation

We initially hypothesized that plant biomass, N and P are in response to soil nutrient addition, and this was supported by our results that nutrient addition significantly increased total plant C concentration and N concentration of *L. chinensis* compared to CK (Aerts & Chapin, 1999; Hessen, Elser, Sterner, & Urabe, 2013; Lü, Lü, Zhou, Han, & Han, 2012c). Notably, N addition significantly decreased P concentration in leaf, stem and whole plants regardless of water available in the soil, not in line with other studies (Güsewell, 2004; Lü et al., 2012b; Shen et al., 2018). Theoretically, this may be explained by the effects of N inputs on P uptake are modulated by environment factors (Lü & Han, 2010). Nitrogen addition is predicted to enhance plant P concentration due to its positive impacts on soil P availability by stimulating the activities of soil P-mineralizing enzymes (Lü, Reed, Yu, & Han, 2016; Marklein & Houlton, 2012). Water stress can also result in plant down regulating nutrient uptake, and increasing N can increase water use which reduces soil water and slows P uptake. However, N addition will drive P limitation if there is no additional external P input in the long-term (Vitousek, Porder, Houlton, & Chadwick, 2010; Wang et al., 2018). The negative impacts of N addition on P concentration in *L. chinensis* would result from the growth dilution effects, because N addition substantially increased growth of *L. chinensis* (Van Heerwaarden, Toet, & Aerts, 2003).

### 4.3 | Effects of N addition on stoichiometry

Nitrogen addition often results in higher availability of soil inorganic N, thereby increased N rates and decreased C to N ratios in green plant tissue (AbdElgawad et al., 2014; Xia & Wan, 2008). Consistent with previous research, N addition significantly decreased C to N ratio in our study, and there were significant negative correlation between C to N ratio and N concentration in green plant tissue (Henry, Cleland, Field, & Vitousek, 2005; Lü et al., 2012c). Previous studies suggest that the increased concentration of nutrients in plant tissues is mediated by positive fertilizer effects on soil N cycling, which leads to greater plant N uptake (Hofer, Suter, Buchmann, & Lüscher, 2017; Zhou, Bowker, Tao, Wu, & Zhang, 2018). Other studies showed that N was released and then taken up by the plant, resulting in more N remaining in senesced leaves and thus lower C to N ratio (Lü & Han, 2010; Lü et al., 2012b). The result in our study was in line with our expectations, increased N to P ratios with increased N, especially in leaves and *L. chinensis* plants. We attribute the increased N to P ratio to N addition. Nitrogen addition would lead to higher N uptake and lower P uptake in the same community. In contrast, in a study conducted in the similar area, it was found that N to P ratios of *L. chinensis* remained constant after N addition. These inconsistencies indicated a need to conduct an analysis of other environment factors to assess to underlying mechanisms accounting for the site differences (Lü & Han, 2010). Another important result in this study was that N addition led to a lower P in whole plants and consequently a higher C to P ratio (Billings et al., 2003). Additionally, we found significant positive correlation between *L. chinensis* N and *L. chinensis* C to P ratio (AbdElgawad et al., 2014). In previous studies, higher N availability with water added treatments resulted in a lower C to P ratio (Lü & Han, 2010). We suggest the difference in precipitation might be a reason of decreased C to P ratio in our study.

## 5 | CONCLUSION

We conclude that an increased N rate could have a significantly positive effect with most plant morphological traits, and nutrient concentrations and stoichiometry were also strongly impacted. As hypothesized, we showed that the response of leaf mass and stem mass uptake to N addition were extremely sensitive. The patterns of biomass allocation of *L. chinensis* were significantly correlated with increased N rate. However, the increase in N to P ratio and decreased C to N ratio would attribute the change of stoichiometry to N addition; this led to higher N and C uptake, and lower P uptake of *L. chinensis*. Our results suggest that sustainable development of *L. chinensis* temperate grasslands requires not only control of N addition, but also a knowledge of the processes and mechanisms of vegetation dynamics in response to variations in the environment.

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## REFERENCES

AbdElgawad, H., Peshev, D., Zinta, G., Van den Ende, W., Janssens, I. A., & Asard, H. (2014). Climate extreme effects on the chemical composition of temperate grassland species under ambient and elevated CO2: A comparison of fructan and non-fructan accumulators. *PLoS One*, *9*, E92044. https://doi.org/10.1371/journal.pone.0092044

Aerts, R., & Chapin, F. S. (1999). The mineral nutrition of wild plants revisited: A re-evaluation of processes and patterns. In A. H. Fitter & D. G. Raffaelli (Eds.), *Advances in ecological research* (Vol. 30, pp. 1–67). London: Academic Press.https://doi.org/10.1016/S0065-2504(08)60016-1

AOCS. 1989. Official methods and recommended practices of the American Oil Chemists' Society (4th ed.). Champaign, IL: AOCS.

Bai, Y., Han, X., Wu, J., Chen, Z., & Li, L. (2004). Ecosystem stability and compensatory effects in the Inner Mongolia grassland. *Nature*, *431*, 181–184. https://doi.org/10.1038/nature02850

Bai, Y., Wu, J., Xing, Q., Pan, Q., Huang, J., Yang, D., & Han, X. (2008). Primary production and rain use efficiency across a precipitation gradient on the Mongolia plateau. *Ecology*, *89*, 2140–2153. https://doi.org/10.1890/07-0992.1

Bardgett, R. D., Wardle, D. A., & Yeates, G. W. (1998). Linking aboveground and below-ground interactions: How plant responses to foliar herbivory influence soil organisms. *Soil Biology and Biochemistry*, *30*, 1867–1878. https://doi.org/10.1016/S0038-0717(98)00069-8

Basso, B., Dumont, B., Cammarano, D., Pezzuolo, A., Marinello, F., & Sartori, L. (2016). Environmental and economic benefits of variable rate nitrogen fertilization in a nitrate vulnerable zone. *Science of The Total Environment*, *545–546*, 227–235. https://doi.org/10.1016/j.scitotenv.2015.12.104

Bennett, L. T., Judd, T. S., & Adams, M. A. (2003). Growth and nutrient content of perennial grasslands following burning in semi-arid, sub-tropical Australia. *Plant Ecology*, *164*, 185–199. https://doi.org/10.1023/A:1021253600712

Billings, S. A., Zitzer, S. F., Weatherly, H., Schaeffer, S. M., Charlet, T., Arnone, J. A., III, & Evans, R. D. (2003). Effects of elevated carbon dioxide on green leaf tissue and leaf litter quality in an intact Mojave Desert ecosystem. *Global Change Biology*, *9*, 729–735. https://doi.org/10.1046/j.1365-2486.2003.00608.x

De Vries, F. T., Brown, C., & Stevens, C. J. (2016). Grassland species root response to drought: Consequences for soil carbon and nitrogen availability. *Plant Soil*, *409*, 297–312. https://doi.org/10.1007/s11104-016-2964-4

Fort, F., Cruz, P., Catrice, O., Delbrut, A., Luzarreta, M., Stroia, C., & Jouany, C. (2015). Root functional trait syndromes and plasticity drive the ability of grassland Fabaceae to tolerate water and phosphorus shortage. *Environmental and Experimental Botany*, *110*, 62–72. https://doi.org/10.1016/j.envexpbot.2014.09.007

Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W., Seitzinger, S. P., … Vosmarty, C. J. (2004). Nitrogen cycles: Past, present, and future. *Biogeochemistry*, *70*, 153–226. https://doi.org/10.1007/s10533-004-0370-0

Gu, J., Liu, G.-S., Guo, J., & Zhang, J. (2008). Effects of vitamin E on the activities of protective enzymes and membrane lipid peroxidation in Leymus chinensis under drought stress. *Chemical Research in Chinese Universities*, *24*, 80–83. https://doi.org/10.1016/S1005-9040(08)60017-3

Güsewell, S. (2004). N to P ratios in terrestrial plants: Variation and functional significance. *New Phytologist*, *164*, 243–266. https://doi.org/10.1111/j.1469-8137.2004.01192.x

He, J.-S., Fang, J., Wang, Z., Guo, D., Flynn, D. F. B., & Geng, Z. (2006). Stoichiometry and large-scale patterns of leaf carbon and nitrogen in the grassland biomes of China. *Oecologia*, *149*, 115–122. https://doi.org/10.1007/s00442-006-0425-0

He, J.-S., Wang, L., Flynn, D. F. B., Wang, X., Ma, W., & Fang, J. (2008). Leaf nitrogen: Phosphorus stoichiometry across Chinese grassland biomes. *Oecologia*, *155*, 301–310. https://doi.org/10.1007/s00442-007-0912-y

He, K., Qi, Y., Huang, Y., Chen, H., Sheng, Z., Xu, X., & Duan, L. (2016). Response of aboveground biomass and diversity to nitrogen addition–A five-year experiment in semi-arid grassland of Inner Mongolia, China. *Scientific Reports*, *6*, 31919. https://doi.org/10.1038/srep31919

Henry, H. A. L., Cleland, E. E., Field, C. B., & Vitousek, P. M. (2005). Interactive effects of elevated CO2, N deposition and climate change on plant litter quality in a California annual grassland. *Oecologia*, *142*, 465–473. https://doi.org/10.1007/s00442-004-1713-1

Hessen, D. O., Elser, J. J., Sterner, R. W., & Urabe, J. (2013). Ecological stoichiometry: An elementary approach using basic principles. Limnol. *Oceanogr*, *58*, 2219–2236. https://doi.org/10.4319/lo.2013.58.6.2219

Hofer, D., Suter, M., Buchmann, N., & Lüscher, A. (2017). Nitrogen status of functionally different forage species explains resistance to severe drought and post-drought overcompensation. *Agriculture, Ecosystems & Environment*, *236*, 312–322. https://doi.org/10.1016/j.agee.2016.11.022

Hooper, D. U., & Johnson, L. (1999). Nitrogen limitation in dryland ecosystems: Responses to geographical and temporal variation in precipitation. *Biogeochemistry*, *46*, 247–293. https://doi.org/10.1007/BF01007582

Huang, L., Liang, Z., Suarez, D. L., Wang, Z., Ma, H., Wang, M., … Liu, M. (2015). Continuous nitrogen application differentially affects growth, yield, and nitrogen use efficiency of Leymus chinensis in two saline–sodic soils of Northeastern China. *Agronomy Journal*, *107*, 314–322. https://doi.org/10.2134/agronj14.0250

Kawamura, K., Akiyama, T., Yokota, H.-O., Tsutsumi, M., Yasuda, T., Watanabe, O., & Wang, S. (2005). Quantifying grazing intensities using geographic information systems and satellite remote sensing in the Xilingol steppe region, Inner

Mongolia, China. *Agriculture, Ecosystems & Environment*, *107*, 83–93. https://doi.org/10.1016/j.agee.2004.09.008

Kuzyakov, Y., Kretzschmar, A., & Stahr, K. (1999). Contribution of Lolium perenne rhizodeposition to carbon turnover of pasture soil. *Plant Soil*, *213*, 127–136. https://doi.org/10.1023/A:1004566027237

Liu, G. X., & Han, J. G. (2008). Seedling establishment of wild and cultivated Leymus chinensis (Trin.) Tzvel. under different seeding depths. *Journal of Arid Environments*, *72*, 279–284. https://doi.org/10.1016/j.jaridenv.2007.06.008

Liu, J., Wang, L., Wang, D., Bonser, S. P., Sun, F., Zhou, Y., … Teng, X. (2012). Plants can benefit from herbivory: Stimulatory effects of sheep saliva on growth of Leymus chinensis. *PLoS One*, *7*, E29259. https://doi.org/10.1371/journal.pone.0029259

Liu, X., Yang, Y., Li, W., Li, C., Duan, D., & Tadano, T. (2011). Interactive effects of sodium chloride and nitrogen on growth and ion accumulation of a halophyte. *Communications in Soil Science and Plant Analysis*, *35*, 2111–2123. https://doi.org/10.1081/LCSS-200028936

Liu, Y., Taxipulati, T., Gong, Y., Sui, X., Wang, X., Parent, S.-É., … Guan, K. (2017). N-P fertilization inhibits growth of root hemiparasite Pedicularis kansuensis in natural grassland. *Frontiers in Plant Science*, *8*. https://doi.org/10.3389/fpls.2017.02088

Liu, Z., Baoyin, T., Duan, J., Yang, G., Sun, J., & Li, X. (2018). Nutrient Characteristics in Relation to Plant Size of a Perennial Grass Under Grazing Exclusion in Degraded Grassland. *Frontiers in Plant Science 9*. https://doi.org/10.3389/fpls.2018.00295

Lü, X.-T., Freschet, G. T., Flynn, D. F. B., & Han, X.-G. (2012a). Plasticity in leaf and stem nutrient resorption proficiency potentially reinforces plant-soil feedbacks and microscale heterogeneity in a semi-arid grassland. *Journal Ecology*, *100*, 144–150. https://doi.org/10.1111/j.1365-2745.2011.01881.x

Lü, X.-T., & Han, X.-G. (2010). Nutrient resorption responses to water and nitrogen amendment in semi-arid grassland of Inner Mongolia, *China. Plant Soil*, *327*, 481–491. https://doi.org/10.1007/s11104-009-0078-y

Lü, X.-T., Kong, D.-L., Pan, Q.-M., Simmons, M. E., & Han, X.-G. (2012b). Nitrogen and water availability interact to affect leaf stoichiometry in a semi-arid grassland. *Oecologia*, *168*, 301–310. https://doi.org/10.1007/s00442-011-2097-7

Lü, X.-T., Lü, F. M., Zhou, L. S., Han, X., & Han, X.-G. (2012c). Stoichiometric response of dominant grasses to fire and mowing in a semi-arid grassland. *Journal of Arid Environments*, *78*, 154–160. https://doi.org/10.1016/j.jaridenv.2011.11.008

Lü, X.-T., Reed, S., Yu, Q., He, N.-P., Wang, Z.-W., & Han, X.-G. (2013). Convergent responses of nitrogen and phosphorus resorption to nitrogen inputs in a semiarid grassland. *Global Change Biology*, *19*, 2775–2784. https://doi.org/10.1111/gcb.12235

Lü, X.-T., Reed, S. C., Yu, Q., & Han, X.-G. (2016). Nutrient resorption helps drive intra-specific coupling of foliar nitrogen and phosphorus under nutrient-enriched conditions. *Plant Soil*, *398*, 111–120. https://doi.org/10.1007/s11104-015-2642-y

Marklein, A. R., & Houlton, B. Z. (2012). Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytologist*, *193*, 696–704. https://doi.org/10.1111/j.1469-8137.2011.03967.x

Olde Venterink, H., Kardel, I., Kotowski, W., Peeters, W., & Wassen, M. J. (2009). Long-term effects of drainage and hay-removal on nutrient dynamics and limitation in the Biebrza mires, *Poland. Biogeochemistry*, *93*, 235–252. https://doi.org/10.1007/s10533-009-9300-5

Sanjuán, Y., Arnáez, J., Beguería, S., Lana-Renault, N., Lasanta, T., Gómez-Villar, A., … García-Ruiz, J. M. (2018). Woody plant encroachment following grazing abandonment in the subalpine belt: A case study in northern Spain. *Regional Environmental Change*, *18*, 1103–1115. https://doi.org/10.1007/s10113-017-1245-y

Shen, Y., Yang, X., Sun, X., Chen, W., Yang, G., Liu, N., … Zhang, Y. (2018). Increased precipitation modulates the influence of nitrogen and litter inputs on the nutrient resorption proficiency rather than efficiency of Leymus chinensis. *Plant Ecology*, *219*, 217–230. https://doi.org/10.1007/s11258-017-0790-2

Steffens, M., Kölbl, A., Totsche, K. U., & Kögel-Knabner, I. (2008). Grazing effects on soil chemical and physical properties in a semiarid steppe of Inner Mongolia (P.R. China). *Geoderma*, *143*, 63–72. https://doi.org/10.1016/j.geoderma.2007.09.004

Valladares, F., Sanchez-Gomez, D., & Zavala, M. A. (2006). Quantitative estimation of phenotypic plasticity: Bridging the gap between the evolutionary concept and its ecological applications. *Journal of Ecology*, *94*, 1103–1116. https://doi.org/10.1111/j.1365-2745.2006.01176.x

Van Heerwaarden, L. M., Toet, S., & Aerts, R. (2003). Nitrogen and phosphorus resorption efficiency and proficiency in six sub-arctic bog species after four years of nitrogen fertilization. *Journal of Ecology*, *91*, 1060–1070. https://doi.org/10.1046/j.1365-2745.2003.00828.x

Vitousek, P. M., Porder, S., Houlton, B. Z., & Chadwick, O. A. (2010). Terrestrial phosphorus limitation: Mechanisms, implications, and nitrogen–phosphorus interactions. *Ecological Applications*, *20*, 5–15. https://doi.org/10.1890/08-0127.1

Wang, H.-Y., Wang, Z.-W., Ding, R., Hou, S.-L., Yang, G.-J., Lü, X.-T., & Han, X.-G. (2018). The impacts of nitrogen deposition on community N to P stoichiometry do not depend on phosphorus availability in a temperate meadow steppe. *Environmental Pollution*, *242*, 82–89. https://doi.org/10.1016/j.envpol.2018.06.088

Xia, J., & Wan, S. (2008). Global response patterns of terrestrial plant species to nitrogen addition. *New Phytologist*, *179*, 428–439. https://doi.org/10.1111/j.1469-8137.2008.02488.x

Yahdjian, L., Gherardi, L., & Sala, O. E. (2011). Nitrogen limitation in arid-subhumid ecosystems: A meta-analysis of fertilization studies. *Journal of Arid Environments*, *75*, 675–680. https://doi.org/10.1016/j.jaridenv.2011.03.003

Zhou, X., Bowker, M. A., Tao, Y., Wu, L., & Zhang, Y. (2018). Chronic nitrogen addition induces a cascade of plant community responses with both seasonal and progressive dynamics. *Science of The Total Environment*, *626*, 99–108. https://doi.org/10.1016/j.scitotenv.2018.01.025

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