Growth Responses of Agropyron Mongolicum Keng to Nitrogen Addition Is Linked to Root Morphological Traits and Nitrogen-Use Efficiency.

Aiyun XU (✉ xaynxu@163.com)

Ningxia University

Xing Wang

Ningxia University

Xiaojia Wang

Ningxia University

Dongmei Xu

Ningxia University

Bing Cao

Ningxia University

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Abstract

Background and aims

Nitrogen (N) is the primary limiting factors for plant growth and development, and increasingly N deposition alters plant composition, consequently affecting ecosystem function have been widely acknowledged. However, the effects of N fertilization on native species in desert grassland ecosystem and underlying mechanisms of these effects are still poorly understood. This study was conducted to examine the growth response of *Agropyron mongolicum* keng to N addition and potential mechanisms underlying this effect.

Methods

A.mongolicum Keng was subjected to five N addition levels (0, 0.8, 1.6, 2.4, and 4.0g N m⁻² yr⁻¹) for six months under greenhouse conditions. A combination of linear and structural equation modelling was used to examine growth response of *A.mongolicum* Keng to N addition and test whether its response related to root morphological traits and N-use efficiency.

Results

Growth responses of *A. mongolicum* Keng to increasing N addition appeared a unimodal-shaped with a N saturation threshold at 3.2g N m⁻¹ yr⁻¹. Its response closely related to the root surface area, volume, length, and forks number, N uptake and utilization efficiency. Besides, N-induced changes in soil available nutrient have an indirect impact on biomass of *A. mongolicum* Keng via regulation of root morphological traits and N-use efficiency.

Conclusions

These findings highlight the sensibility of *A. mongolicum* Keng to N addition and the importance of root morphological traits and N-use efficiency in affecting biomass. Therefore, these can provide important insights into potential changes of native species survival and development in nutrient-limited desert grassland caused by N deposition.

Introduction

Nitrogen(N) is one of essential and limiting factor to growth and development of terrestrial plants (Elser et al. 2007; Luo et al. 2019; Rubio-Asensio and Bloom 2017), and in no ecosystem is nitrogen more sensitive than in desert ecosystems (Bai et al. 2020). Desert steppe ecosystems are highly vulnerable to global climate changes because species are surviving at their upper biological limits (Bai et al. 2020; Tielborger et al. 2014). A large of fossil-fuel combustion and fertilizer applications have been and are continuing to exacerbate global and regional atmospheric nitrogen deposition rate (Galloway et al. 2008), and the increased N inputs will likely alter soil nutrient availability and subsequently affecting plant community composition and ecosystem functions (Galloway et al. 2008; Maestre et al. 2016; Zhao et al. 2019).

Native species are the main components of species composition and the basis of communities stability, its changes have been used to provide specific signals from environmental change and make sensitive environmental monitors (Philippi et al. 1998). Thus, understanding the sensitivity of native species to nitrogen addition (e.g. N critical loads or saturation thresholds) is key to predicting ecological impacts of climate change in desert ecosystems.

N saturation threshold, which have been widely considered with signals of ecosystem responses to N enrichment, is defined as the point when ammonium and nitrate availability exceed plant and microbial nitrogen demand (Aber et al. 1989; Bowman et al. 2006; Zong et al. 2016). Indicators of N saturation have been documented by soil-based measures, including changes in N mineralization, nitrification, and NO_3^- -N leaching rates (Aber et al. 1989; Bowman et al. 2006; Zong et al. 2016) or changes in sensitive biota, such as plant productivity (e.g. biomass and cover) and community composition. Previous studies showed that plant biomass responses to multi-level N additions generally is not linear response but may occur linearly response as thresholds (i.e., saturation) of N inputs are reached and exceeded (Bai et al. 2010; Bowman et al. 2012; Yahdjian et al. 2011; Yang et al. 2011b; Zhu et al. 2016). Once reaching N saturation threshold, plants cease to take up N entering the system from deposition, leaching of nutrient base cations, increases in soluble aluminum, and soil acidification can occur, eventually leading to decreases in plant productivity and continued losses in diversity (Bai et al. 2010; Bowman et al. 2012; Tian et al. 2016). However, most of these studies have focused on the responses of plant community-level to N addition, although evidence also indicates that the nonlinear responses in individual biomass to N enrichment (Bowman et al. 2012; Gong et al. 2010), the underlying mechanisms of these responses are still far from being fully understood.

The nonlinear responses of plant species/functional group to N addition has been broadly reported from global scale studies (Yuan and Chen 2015), regional surveys (Bai et al. 2010; Yang et al. 2011b), meta-analyses (Deng et al. 2017; Li et al. 2016; Peng et al. 2020; Tian et al. 2016) and manipulative experiments (Luo et al. 2019; Wang et al. 2018). These responses are associated with the effects of N addition on soil nutrients availability: N addition could enhance the soil net N mineralization and produce more available N; Simultaneously, additional N inputs may increase phosphatase activity and increase soil phosphorus (P) availability (Marklein and Houlton 2012), thereby promoting plant growth. However, the rise of soil acidification with high levels of N addition could result in the N-induced increase of P availability is insufficient to balance the plant increasing P demand (Deng et al. 2017; Gong et al. 2010; Li et al. 2016), thus further affecting plant nutrient uptake and inhibiting plant growth. In addition, plant responses to N addition may also be related to N-use strategy (Harpole et al. 2016), because different intrinsic N utilization strategies can enable plant to access sufficient N quantities or to more effectively use their N uptake to adapt variable nutrient conditions (Harpole et al. 2016; Xu et al. 2012). For instance, the root is the main organ of plant N uptake, therefore, its morphological characteristics, such as root length and surface area, determine N utilizing zones and capacity of plants to acquire N (Jiang et al. 2017). Plants can uptake inorganic (NH_4^+ and NO_3^-) N forms from the soil by roots (Xu et al. 2012). After uptake, a small proportion of the absorbed NO_3^- is assimilated locally into the roots, and the larger part is

transported to the shoots (Luo et al. 2019). Plant N metabolism enzymes play central roles during the process (Iqbal et al. 2020b). For example, with the help of nitrate reductase (NR) in cytosolic and nitrite reductase (NiR) in chloroplastic, cytosolic NO_3^- is reduced to NO_2^- , which can be further converted into NH_4^+ in plastids, glutamine synthetase (GS) and glutamate synthase (GOGAT) use NH_4^+ to synthesize glutamine (Gln) and glutamate (Glu) (He et al. 2013) in plastids (Luo et al. 2015). In the mitochondria, NH_4^+ can also be converted into Glu with the help of glutamate dehydrogenase (GDH) (Iqbal et al. 2020b). Gln and Glu can be further converted into an amino acid or other organic N molecules, thereby providing precursors for the biosynthesis of N-containing compounds in plants (Luo et al. 2019; Luo et al. 2015). Likewise, these N-containing compounds (amino acids, proteins, etc.) are also used to detect plant responses to the multi-level N additions (Iqbal et al. 2020b; Quan et al. 2017). Plant regulates their ability of N uptake, assimilation and metabolism depending to the internal N demands and external N availability, as which together determining plant's N-use efficiency (NUE) (Iqbal et al. 2020b; Xu et al. 2012) and subsequently affect plant responses to various N addition concentrations via direct and indirect pathways. NUE is a complex trait with two main components of N-uptake efficiency (NUpE) and N-utilization efficiency (NUE) (Iqbal et al. 2020b). However, few studies that explicitly examines the roles of morphophysiological, biochemical traits and NUE in mediating plant responses to various N addition have been reported, particularly in desert ecosystems (Mamashita et al. 2015; Yang et al. 2011a; Yuan et al. 2020). Hence, the responses of desert plant to N addition are still required to investigate. In addition, elucidating the relative importance of each direct and indirect effects pathway in causing the response of native plant to increasing N enrichment may help to clarify and explain the mechanisms involved in changes to the plant community.

The desert steppe in Ningxia, located in the central part of southern rim area of the Mu Us Desert of northern China, is the transitional zone between typical temperate grassland and desert (Gao et al. 2014). Structure and function in plant communities of desert steppe are vulnerable to climate changes and anthropogenic disturbance due to a harsh environment with an arid climate and barren soils (Gao et al. 2014; Ye et al. 2020). *A. mongolicum* Keng, also known as Mongolian wheatgrass, is an important poaceae forage in desert steppe (Li et al. 2019; Zhao et al. 2018). *A. mongolicum* Keng, as a typical xeric plant and grows well on sand dunes, gravel slopes and sandy soil, has strong tolerance to wind, sand, drought and cold stress (Che and Li 2006). It is often used for restoring deteriorated rangeland and for ecological construction in arid and semi-arid regions (Zhao et al. 2018). Moreover, as a perennial forage, *A. mongolicum* Keng regrows in early springs, making it palatable for grazing with good forage quality and high yield (Ma et al. 2018; Zhao et al. 2018). However, this region is currently experiencing significant N deposition, which is projected to increase in the coming decades (Bai et al. 2020; Liu et al. 2013), it remains unclear how *A. mongolicum* Keng responses to N enrichment and the underlying mechanisms. Here, we tested the following hypotheses: (H₁) *A. mongolicum* Keng has a N saturation threshold with increasing N addition rates; (H₂) N addition would alter soil N availability, and then directly or indirectly influences the biomass of *A. mongolicum* Keng; (H₃) the growth response of the *A. mongolicum* Keng to N addition was regulated by root morphological traits and N-use efficiency.

Materials And Methods

Experimental conditions and treatments

The experiment was conducted from 02 July 2020 to 8 January 2021 in a climate-controlled greenhouse at the Agricultural Research Center of Ningxia University (38°30'11"N, 106°8'25"E), Yinchuan, Ningxia Province, China. The relative humidity in the glasshouse was about 60%, and day/night temperature was 28/15±2°C. The average light intensity ranged from 22.6 to 184.2 mmol m⁻²s⁻¹, and the photoperiod was set at 16 hours (natural light was supplemented with high-pressure sodium lamps on cloudy days, and early in the mornings and late in the evenings).

The seeds of native *A. mongolicum* keng were collected, originating from desert steppe within Sidunzi Village (37°20'30"N, 107°15'38"E) in Yanchi County in Ningxia, China, a location at which these species are commonly found. Ten healthy seeds of *A. mongolicum* were surface sterilized in 5% NaClO₃ for 10 min, rinsed three times in distilled water and then sown in plastic pots (25 cm top diameter×27 cm height) filled with 6 kg of sandy soil collected from the top 30 cm of soil from vacant land on desert steppe, where was similar to where collected seeds of *A. mongolicum* keng. The organic matter content of the untreated soil was 2.01 g kg⁻¹, the pH was 9.07, and the alkali-hydrolyzable nitrogen, available phosphorus and available potassium concentrations were 8.26 mg kg⁻¹, 8.04 mg kg⁻¹ and 119.2 mg kg⁻¹, respectively.

The experiment comprised five N addition treatments (N0, ambient; N0.8, ambient + 0.8 g N m⁻¹ yr⁻¹; N1.6, ambient + 1.6 g N m⁻¹ yr⁻¹; N2.4, ambient + 2.4 g N m⁻¹ yr⁻¹; and N4, ambient + 4 g N m⁻¹ yr⁻¹) and six replicate pots. The low level of N addition at the N1.2 treatment (1.2 g N m⁻¹ yr⁻¹, data not shown) was nearly equivalent to the background deposition rate (1.2 g N m⁻¹ yr⁻¹) in the study area (Bai et al. 2020). N additions were applied through Urea [CO(NH₂)₂] at twice equal applications, which dissolved in 300 ml of tap water to add at the time of seeding and at the time of tillering on, respectively. The same amount of tap water was added to the N control pots during each fertilization event. All plants were irrigated every 2 days with plain tap water (300–500 mL per pot) and maintained soil water content at 70–80% of field capacity during the experiment. All pots were randomly arranged in the greenhouse. All the plants were harvested for further analysis on January 8th, (the five weeks after the last N application).

plant and soil sampling

Whole tillers of individuals from the different treatments (six replicated pots) were dug up and separated into roots and shoots (leaves and stem). The roots were washed using deionized water, and the water on the surface of the roots was wiped with lab tissue paper. Then, all the harvest samples were divided into two subsamples. One subsample was packed into tinfoil and immediately immersed in liquid N₂ for transportation to the laboratory, and stored at -80°C for further physiological analysis. The other subsample was taken to the laboratory and stored at 4 °C for morphological properties analysis.

For each treatment, four of the six replicated pots were randomly selected for soil analysis after the plants harvested ($n = 4$). Soil samples were sieved through a 2-mm mesh to remove roots and placed in individual plastic bags and then immediately stored in a portable refrigerator to take the laboratory. To determine the response of soil N availability, including $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$, to N addition, 30 g of each soil composite sample was packed into a small plastic bag, and then immediately stored at 4 °C in the laboratory by using standard methods. The rest of each soil composite sample was air-dried for analyzing soil available phosphorus (AP).

Measurement of NUE traits and soil available nutrients

Dried samples of root and shoot biomass in each treatment were milled in a ball-mixer mill (MM200, Retsch, Haan, Germany) and then kept in a 1.5-ml vial at 60 °C to avoid water sorption. The samples 2.5 mg of pulverized material was placed in a tin capsule and then analyzed for total N concentration using elemental analyzer (Flash EA1112, Thermo Scientific, West Palm Beach, USA). The total N accumulation was obtained as the product of total N concentrations and plant total dry weight (Iqbal et al. 2020b). NUE was measured as total plant dry weight divided by N concentrations and NUpE was determined as total N accumulation divided by root dry weight, as described by Iqbal et al. (2020b).

Soil available N, a subsample of 10 g fresh soil was extracted with 50 ml of 2 M KCl solution, and then $\text{NH}_4^+\text{-N}$ was measured by using indophenol blue spectrophotometric method (UV-1601, Shimadzu Inc.), while $\text{NO}_3^-\text{-N}$ was determined using UV spectrophotometry (UV-1601, Shimadzu Inc.) at 220 and 275 nm. AP was extracted with 50 ml of 0.5 M NaHCO_3 solution at pH 8.5 from 5 g airdried soil subsample and measured colorimetrically with the molybdate-ascorbic acid method (Yuan et al. 2019).

Measurements of biomass and root morphological traits

After the plants were harvested, five plants per treatment from six replicated pots were randomly selected and separated into roots and shoots (leaves and stem), and then the root parts were carefully washed with tap water. The parts of each plant were packed in separate envelopes and put in the oven at 105 °C for 15min followed by 80 °C for 48 h. The root and shoot dry weight of each plant was measured with the help of an electronic balance. Total plant dry weight of each plant was equal to root dry weight plus shoot dry weight. Root morphological parameters of 4 plants from different replicated pots in each treatment were measured and analyzed by a scanner (Epson Perfection V700; Seiko Epson Corp., Suwa, Japan) and the WinRHIZO root image analysis system (Regent Instrument Inc., Quebec, QC, Canada), respectively, as described by Iqbal et al. (2020b).

Determination of activities of N metabolic enzymes

NR activity (NR: EC 1.7.1.3) was measured according to the method of Silveira et al. (2001), with some modification (Iqbal et al. 2020b). GS activity (EC 6.3.1.2), GOGAT (EC 1.4.7.1) as well as GDH activity (EC 1.4.1.2) were determined as described by Iqbal et al. (2020b).

Determination of total soluble protein, free amino acid, starch and total soluble sugar content

Total soluble protein content (SPC) in roots and shoots was measured according to the supposed method by (Marion et al. 1976) using Coomassie Brilliant Blue (G-250) as a dye and albumin as a standard (Balasubramanian and Sadasivam 1987). For the determination of total free amino acid content (FAAC), the ninhydrin method was used as reported previously (Yokoyama et al. 2003) with some modifications (Iqbal et al. 2020b). The total amounts of soluble sugar content (SSC) and starch content (SC) in tissues were determined using the anthrone method as described previously (He et al. 2013).

Statistical analyses

Data were checked for normality and homogeneity of the residuals using Levene's Test prior to analyses. Log transformation was applied when data did not meet the criteria for normality and homogeneity. One-way analysis of variance (ANOVA) and Tukey's post hoc test was performed to examine the effects of N addition on root and shoot biomass, soil available nutrient properties, root morphological traits, NUE and the potential activity of enzymes and N-containing compounds related to NUE. A polynomial dose response curve was estimated the response function for the *A. mongolicum* Keng to the N deposition rate, with the apex of fitting curve providing the N saturation threshold for *A. mongolicum* Keng (Bowman et al. 2012; Zong et al. 2016). We also used simple linear regression to test the relationships of biomass and root morphological traits and NUE across all N addition treatments. Finally, a structural equation modelling (SEM) was performed to identify how N-induced changes in soil available nutrients regulated *A. mongolicum* Keng to uptake and utilize N, and subsequently affected plant biomass. Before performing SEM, we first reduced the number of variables for soil available nutrient properties, root morphological traits and NUE relevant properties using principal component analyses (PCAs) (Yuan et al. 2020; Zhang et al. 2019). The first principal component (PC1) was used in the subsequent SEM analysis to represent soil available nutrient (PC1 explained 79.19% of the variance; Fig.S1), root morphological characteristic (PC1 explained 75.48% of the variance; Fig.S2), N metabolism enzymatic activity (PC1 explained 86.09% of the variance; Fig.S3), N assimilation product (PC1 and PC2 explained 75.33% of the variance) and N-use efficiency (PC1 explained 73.42% of the variance; Fig.S5). The rest of the variables were transformed into a score for final SEM analysis (Yuan et al. 2020). Goodness of fit for SEM were evaluated by χ^2 tests, Goodness of Fit Index (GFI), Normal Fit Index (NFI) and the Root Square Mean Error of Approximation (RMSEA). We improved the goodness of fit for SEM by removing pathways between observed variables based on Modification Indices (Yuan et al. 2020; Zhang et al. 2019).

All the data were analyzed using SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). Figures were drawn using Origin 16.0 software (Origin Lab Co., Northampton, MA, USA). SEM analysis was performed in Amos 21.0 (Amos Development, Spring House, Pennsylvania, USA).

Results

Effects of N addition on *A. mongolicum* Keng biomass

Shoot biomass of *A. mongolicum* Keng were significantly increased with the increasing N addition. Compared to the control group (N0), shoot biomass was significantly improved by 100.51% under N1.6 and 219.76% under N2.4, respectively, with the highest value at N2.4 treatment ($P < 0.001$; Fig. 1a). Although no significant effects of N addition on the root biomass of *A. mongolicum* Keng were observed, tending to increase from N0 to N2.4 but decrease from N2.4 to N4.0 ($P = 0.058$; Fig. 1a). Meanwhile, a quadratic equation can also be used to describe the relationship ($R^2 = 0.657$) between the total biomass and the N addition gradients exhibited a unimodal response with the increasing N addition gradient, and the N saturation threshold was around $3.2\text{g N m}^{-1}\text{ yr}^{-1}$ (Fig. 1b).

N addition effects on soil available nutrient properties and root morphological traits

We found that there were significant effects of N addition on soil NO_3^- -N concentration ($P = 0.000$; Table 1) and available phosphorus concentration (AP) ($P = 0.000$; Table 1), respectively. Both of them significantly increased with increasing N addition from N0 to N4 and showed linear response to N addition. While soil NH_4^+ -N concentration showed negative response to N addition, the difference is not significant ($P = 0.14$; Table 1). Simultaneously, the root morphological traits response to N addition was observed (Table 1 and Fig. 2). Root surface area (RSA) and volume (RV) showed extremely significant responses to N addition (both $P = 0.000$; Table 1), total root length (RL) and root forks number (RF) showed significant response to N addition (both $P = 0.002$; Table 1), as well as all of them tended to increase with increasing N addition rates from N0 to N2.4 but decreased above N2.4, with the higher value at N2.4 treatment (Table 1). However, N addition did not affect root diameter (RD) ($P = 0.23$; Table 1). In addition, linear regressions showed that root morphological traits were significantly and positively related to biomass of *A. mongolicum* Keng (all $P < 0.01$; Fig. 2).

Responses of activities of N metabolism enzymes and assimilation products in shoot and root to N addition

The activities of N metabolism enzymes in shoot and root were altered by increasing N addition (all $P < 0.001$; Fig. 3). N addition significantly improved the NR, NiR, GOGAT, GS and GDH activities in both the shoot and root (Fig. 3a, b, c, d and e). The N2.4 treatment significantly increased all the N metabolism enzymes activity compared with the N0 treatment, while there was not significantly changed when N addition treatment were more than N2.4 (Fig. 3).

For N assimilation products, N addition had significantly effect on both shoot ($F = 5.847$, $P = 0.005$; Fig. 4a) and root ($F = 3.224$, $P = 0.043$; Fig. 4a) of SPC, with its highest value (14.82 ± 0.81 ; 2.78 ± 0.13) at N2.4 treatment, respectively. FAAC in shoot and root also had similar response to increasing N addition, up to its highest value also at N2.4 level ($F = 4.269$, $P = 0.017$; $F = 6.258$, $P = 0.004$; Fig. 4b). In addition, across all N addition treatments, Non-structural carbohydrates in shoot and root, including SSC and SC, were significantly affected by N addition. Relative to N0 treatment, both SSC ($F = 4.269$, $P = 0.017$; $F = 6.258$, $P = 0.004$; Fig. 4c) and SC ($F = 4.269$, $P = 0.017$; $F = 6.258$, $P = 0.004$; Fig. 4d) were significantly increased when N addition treatment were lower than N2.4 but then not changed significantly (Fig. 3c, d).

Responses of N-use efficiency in *A. mongolicum* Keng to N addition

N-use efficiency, like N uptake efficiency (NUpE) and N utilization efficiency (NUE), was significantly affected by N addition. NUpE ($F = 16.597$, $P = 0.000$; Fig. 5a) and NUE ($F = 15.068$, $P = 0.000$; Fig. 5a) significantly increased with increasing N addition rates from N0 to N2.4 but significantly decreased more than N2.4. We also estimated the relationships between biomass *A. mongolicum* Keng and NUE, and found biomass was increased linearly by NUpE and NUE, respectively (Fig. 5b).

Direct and indirect effects soil available nutrient and N-use efficiency on biomass

Our structural equation models fitted the data well, indicating that N-induced changes in soil available nutrient other than direct affect, but have an indirect impact on biomass of *A. mongolicum* Keng via regulation of root morphological traits and N-use efficiency ($\chi^2 = 2.292$, $P = 0.994$, GFI=0.969, RMSEA < 0.001; Fig. 6). This model showed that N addition directly explained 80.5% of variance in soil available nutrient (Fig. 6). In accordance with the results of ANOVA (Table 1), N addition had positive effects on soil NO_3^- -N and AP concentration (All $P < 0.05$; Table 1, Fig. 5). It also showed that pathways of soil available nutrient, root morphological characteristic, N metabolism enzymatic activity, N assimilation products and N-use efficiency together explained 71.7% of the total variance in biomass (Fig. 6). All of them had a strong and direct effect on biomass (Fig. 6). Additionally, soil available nutrient contributed weights of up to 16.2% and 84.4% to biomass via influencing the root morphological characteristic and N-use efficiency, respectively (Fig. 6), indicating that soil available nutrient also had an indirect positive effect on biomass. However, N metabolism enzymatic activity displayed a significantly negative effect on biomass, but this effect became strong positive via the pathway of N-use efficiency (Fig. 6).

Discussion

4.1 Nonlinear responses of *A. mongolicum* Keng to N addition

Many studies have confirmed that N addition has a positive effect on plant growth, but this effect will decrease and even diminish when the N inputs surpass the N saturation threshold (Tian et al. 2016; Zong et al. 2016). In this study, N addition promoted the root and shoot biomass of *A. mongolicum* Keng at low levels of N addition but decreased at high levels of N addition (Fig.1a), and total biomass exhibited a nonlinear response to the increasing N addition gradient, with a threshold at $3.2 \text{ g N m}^{-2} \text{ yr}^{-1}$ (Fig. 1b), this result was in agreement with this study's initial hypothesis and consistent with previous studies. For instance, a study based on meta-analysis found nonlinearity in plant biomass to continuous N enrichment, with a N saturation threshold of $5\text{--}6 \text{ g N m}^{-2} \text{ yr}^{-1}$ (Tian et al. 2016). Similarly, Bai et al. (2010) carried out a long-term N enrichment experiment in temperate steppe and found where N saturation level was more than $10.5 \text{ g N m}^{-2} \text{ yr}^{-1}$. However, this biomass changes was generally accompanied by species richness and diversity loss (Harpole et al. 2016). Song et al. (2011) also conducted a field experiment in temperate steppe with six N rates, he found that grasses species richness showed a weak change across N treatments, but species richness of forbs declined more strongly. Furthermore, our results also showed

that shoot biomass revealed more significant changes than root after N fertilization, indicating that shoot was more sensitive to N addition. As documented previously, most terrestrial ecosystems have received an accumulation of 2–5 g N m⁻² ambient N deposition (Tian et al. 2016), which suggests that plant species in global terrestrial ecosystems likely to have the risk of N saturation and loss in the face of potential future climatic and environmental changes. Although the response of whole community cannot be evaluated by individual species in this study, our result that this N saturation threshold (3.2g N m⁻¹ yr⁻¹) in *A. mongolicum* Keng may provide specific signals for restoring and managing the biodiversity and ecosystem functioning of desert steppe ecosystem in the future.

Analysis of mechanisms underlying for biomass nonlinear responses to N addition

The soil on the desert grassland ecosystem in Yanchi are poor and N-limited (Gao et al. 2014), and external N inputs can adjust to this situation. Results from our study showed that N addition enhanced soil available nutrient (Table 1). Soil NO₃⁻-N and AP showed a significant, linear increase with N addition (Fig.6) and no an appearance of threshold, consistent with that of a previous study (Bowman et al. 2006) which showed that plant responses rather than soil responses may provide more sensitive signal of the influences of atmosphere N deposition. Simultaneously, results from SEM analyses showed that soil available nutrient generated a significant positive effect on biomass of *A. mongolicum* Keng ($P < 0.001$; Fig.6), indicating that response of *A. mongolicum* Keng to N addition may be partly and directly explained by soil available nutrient. Additionally, in our experiment, N:P ratio of soil available nutrient had a positive relationship with dry biomass of *A. mongolicum* Keng (Fig.S6a), implying that nonlinear responses of *A. mongolicum* Keng to N addition affected by changes in other resource availability. Since the rise of soil acidification with high levels of N addition could result in the N-induced increase of P availability is insufficient to balance the plant increasing P demand (Deng et al. 2017; Gong et al. 2010; Li et al. 2016). In our study, we also found that soil pH values were significantly decreased with N addition (Fig.S6b), suggesting that the response of *A. mongolicum* Keng to N addition may be associated with soil pH (Yuan et al. 2020). For nonlinear responses to N addition, the evidence are also usually observed in soil microbial biomass, microbial reallocation of C and some other soil biogeochemistry processes (Zhang et al. 2019; Zhou et al. 2012), therefore, additional targets should be investigated to provide robust evidence.

Roots play a crucial role in response to diverse nutrient fluctuations and can signal the plant to alter their morphology traits to adapt environmental changes (Iqbal et al. 2020b; Luo et al. 2019). A remarkable level of variation in root morphology observed in this study (Table.1; Fig.2) was consistent with the widely demonstrated changes in root morphological traits with N enrichment occurring in other plant species (De Pessemer et al. 2013; Jiang et al. 2017; Luo et al. 2019). Similarly, root morphological traits can influence plant dry matter accumulation because it can directly increase plant nutrient absorption and indirectly affect N-use utilization efficiency through altering root length, root surface area and so on (Dupas and Monteiro 2018; Jiang et al. 2017; Yang et al. 2011a). Consistent with the above expectation, our results showed that the response of *A. mongolicum* Keng was partly induced by root morphological characteristics changes following N addition (Fig.6), which was confirmed by our linear regressions between biomass and root morphological traits (Fig. 2a, b, c, d). This means, like other plants, that *A.*

mongolicum Keng supplied with external N will tend to have a root system that is larger and more efficient in absorbing nutrients and subsequently promote its growth. The root growth may become limited by other resources (e.i., carbohydrates) and finally ceases when the nutrients in the soil exceed an optimal level (Dupas and Monteiro 2018; Xu et al. 2012). In addition, growth adaptation to N fluctuations is paralleled by the adjustment of N metabolism (Luo et al. 2019). Therefore, determination only of root morphological characteristics does not contribute much information for assessment of plant response to N addition.

Given that plant growth was tightly linked NUE by biochemistry process, N-induced changes in NUE can be regarded as potential mechanisms interpreting the responses of plants to N addition. The NO_3^- absorbed by the root system is converted to ammonium by NR and NiR, and then is assimilated to Gln and Glu via the activities of GS and GOGAT. Alterations in N metabolism-related enzyme activities under different N levels cause the discrepant absorption and formation of N-containing compounds (Wang et al. 2020), and finally affect NUE (Iqbal et al. 2020a). In the present study, the increase of NR, NiR, GS, GOGAT and GDH at low N addition rates and decrease at high N addition rates were observed in the roots and shoots of *A. mongolicum* Keng (Fig.3), similar response to increasing N addition had also been found in the N-containing compounds (Fig.4), indicating that response of *A. mongolicum* Keng to N addition may be related to changes in N metabolism and assimilation. This is further supported by both of them negative effect on biomass and this effect became somewhat positive via the pathway of NUE (Fig. 6). Furthermore, we also found that N addition altered NUE. Specifically, N addition caused a significant decrease in NU_PE and NU_tE when N addition rates are more than N_{2.4} treatment (Fig.5a), and this phenomenon has been widely considered as one of the most important mechanisms underlying the effects of N deposition on plant productivity (Dupas and Monteiro 2018; Iqbal et al. 2020b; Yang et al. 2011a). In support of this view, results from the SEM analyses showed that N addition generated a significant indirect effect on biomass via the pathway of N-use efficiency ($P < 0.01$; Fig.6). Meanwhile, linear regressions also demonstrated there are well-correlated relationships between biomass and NUE (Fig.5b). Therefore, the lower NUE as evidenced by declines of N metabolism and assimilation at high N addition rates may explain the nonlinear response of *A. mongolicum* Keng as observed in the current study. In addition, physiological and morphological adaptation to environmental conditions also have been driven by changes in gene regulation (Iqbal et al. 2020a; Luo et al. 2015), hence, genome research is needed to test this response of *A. mongolicum* Keng to N addition in the next research.

Conclusion

Our results indicated that the growth responses of *A. mongolicum* Keng to increasing N addition rates appeared a unimodal-shaped response with a N saturation threshold at $3.2\text{g N m}^{-1} \text{yr}^{-1}$. Besides, N-induced changes in soil available nutrient other than direct affect, have an indirect impaction on biomass of *A. mongolicum* Keng via regulation of root morphological traits and N-use efficiency. All these can provide important insights into potential changes of native species survival and development in a nutrient-limited desert steppe ecosystem caused by atmospheric N deposition.

Declarations

Author's contribution

A. Y., B. C., and D.M. designed the experiment. A.Y conducted the experiment and X.W and X.J. assisted the field measurements. A.Y. analyzed the data and wrote the manuscript, and B.C revised the manuscript. All authors provided comments on the manuscript and the revisions and approved the final version.

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References

1. Aber, John, D., Nadelhoffer, Knute, J., Steudler, Paul, Melillo, Bioscience JJ (1989) Nitrogen Saturation in Northern Forest Ecosystems:hypotheses revisited. BioScience 11: 921-934. doi: 10.2307/1313296.
2. Bai Y, She W, Zhang Y, Qiao Y, Fu J, Qin S (2020) N enrichment, increased precipitation, and the effect of shrubs collectively shape the plant community in a desert ecosystem in northern China. Sci Total Environ 716: 135379. doi: 10.1016/j.scitotenv.2019.135379.
3. Bai Y, Wu J, Clark CM, Naeem S, Pan Q, Huang J, Zhang L, Han X (2010) Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: evidence from inner Mongolia Grasslands. Global Change Biology 16: 358-372. doi: 10.1111/j.1365-2486.2009.01950.x.
4. Balasubramanian T, Sadasivam SJPFfHN (1987) Changes in starch, oil, protein and amino acids in developing seeds of okra (*Abelmoschus esculentus* L. Moench). Plant Foods for Human Nutrition 37: 41-46. doi: 10.1007/BF01092299.
5. Bowman WD, Gartner JR, Holland K, Wiedermann MJEAAPotESoA (2006) Nitrogen Critical Loads For Alpine Vegetation And Terrestrial Ecosystem Response: Are We There Yet? Ecological Applications 16: 1183-1193. doi: 10.1890/1051-0761(2006)016[1183:NCLFAV]2.0.CO;2.
6. Bowman WD, Murgel J, Blett T, Porter E (2012) Nitrogen critical loads for alpine vegetation and soils in Rocky Mountain National Park. J Environ Manage 103: 165-171. doi: 10.1016/j.jenvman.2012.03.002.
7. Che YH, Li LH (2006) Genetic diversity of prolamines in Agropyron mongolicum Keng indigenous to northern China. Genetic Resources and Crop Evolution 54: 1145-1151. doi: 10.1007/s10722-006-9006-7.
8. De Pessemier J, Chardon F, Juraniec M, Delaplace P, Hermans C (2013) Natural variation of the root morphological response to nitrate supply in Arabidopsis thaliana. Mech Dev 130: 45-53. doi: 10.1016/j.mod.2012.05.010.

9. Deng Q, Hui D, Dennis S, Reddy KC (2017) Responses of terrestrial ecosystem phosphorus cycling to nitrogen addition: A meta-analysis. *Global Ecology and Biogeography* 26: 713-728. doi: 10.1111/geb.12576.
10. Dupas E, Monteiro FA (2018) Nitrogen and potassium, but not boron, change the morphology, production and nutrient concentration of Tanzania guineagrass roots. *Journal of Plant Nutrition* 41: 2222-2231. doi: 10.1080/01904167.2018.1500588.
11. Elser JJ, Bracken ME, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol Lett* 10: 1135-1142. doi: 10.1111/j.1461-0248.2007.01113.x.
12. Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320: 889-892. doi: 10.1126/science.1136674.
13. Gao G-L, Ding G-D, Zhao Y-Y, Wu B, Zhang Y-Q, Qin S-G, Bao Y-F, Yu M-H, Liu Y-D (2014) Fractal approach to estimating changes in soil properties following the establishment of *Caragana korshinskii* shelterbelts in Ningxia, NW China. *Ecological Indicators* 43: 236-243. doi: 10.1016/j.ecolind.2014.03.001.
14. Gong XY, Chen Q, Dittert K, Taube F, Lin S (2010) Nitrogen, phosphorus and potassium nutritional status of semiarid steppe grassland in Inner Mongolia. *Plant and Soil* 340: 265-278. doi: 10.1007/s11104-010-0577-x.
15. Harpole WS, Sullivan LL, Lind EM, Firn J, Adler PB, Borer ET, Chase J, Fay PA, Hautier Y, Hillebrand H, MacDougall AS, Seabloom EW, Williams R, Bakker JD, Cadotte MW, Chaneton EJ, Chu C, Cleland EE, D'Antonio C, Davies KF, Gruner DS, Hagenah N, Kirkman K, Knops JMH, La Pierre KJ, McCulley RL, Moore JL, Morgan JW, Prober SM, Risch AC, Schuetz M, Stevens CJ, Wragg PD (2016) Addition of multiple limiting resources reduces grassland diversity. *Nature* 537: 93-96. doi: 10.1038/nature19324.
16. He J, Ma C, Ma Y, Li H, Kang J, Liu T, Polle A, Peng C, Luo ZB (2013) Cadmium tolerance in six poplar species. *Environ Sci Pollut Res Int* 20: 163-174. doi: 10.1007/s11356-012-1008-8.
17. Iqbal A, Dong Q, Wang X, Gui H, Zhang H, Zhang X, Song M (2020a) Variations in Nitrogen Metabolism are Closely Linked with Nitrogen Uptake and Utilization Efficiency in Cotton Genotypes under Various Nitrogen Supplies. *Plants (Basel)* 9. doi: 10.3390/plants9020250
18. Iqbal A, Qiang D, Zhun W, Xiangru W, Huiping G, Hengheng Z, Nianchang P, Xiling Z, Meizhen S (2020b) Growth and nitrogen metabolism are associated with nitrogen-use efficiency in cotton genotypes. *Plant Physiol Biochem* 149: 61-74. doi: 10.1016/j.plaphy.2020.02.002.
19. Jiang S, Sun J, Tian Z, Hu H, Michel EJS, Gao J, Jiang D, Cao W, Dai T (2017) Root extension and nitrate transporter up-regulation induced by nitrogen deficiency improves nitrogen status and plant

- growth at the seedling stage of winter wheat (*Triticum aestivum* L.). *Environmental and Experimental Botany* 141: 28-40. doi: 10.1016/j.envexpbot.2017.06.006.
20. Li G, Zhao P, Shao W, Jin C, Song L, Chen Y (2019) Effect of enclosure on reproductive allocation of wheatgrass *Agropyron mongolicum* populations in desert steppes. *Ecol Evol* 9: 14023-14030. doi: 10.1002/ece3.5839.
  21. Li Y, Niu S, Yu G (2016) Aggravated phosphorus limitation on biomass production under increasing nitrogen loading: a meta-analysis. *Glob Chang Biol* 22: 934-943. doi: 10.1111/gcb.13125.
  22. Liu X, Zhang Y, Han W, Tang A, Shen J, Cui Z, Vitousek P, Erisman JW, Goulding K, Christie P, Fangmeier A, Zhang F (2013) Enhanced nitrogen deposition over China. *Nature* 494: 459-462. doi: 10.1038/nature11917.
  23. Luo J, Zhou J-J, Masclaux-Daubresse C, Wang N, Wang H, Zheng B (2019) Morphological and physiological responses to contrasting nitrogen regimes in *Populus cathayana* is linked to resources allocation and carbon/nitrogen partition. *Environmental and Experimental Botany* 162: 247-255. doi: 10.1016/j.envexpbot.2019.03.003.
  24. Luo J, Zhou J, Li H, Shi W, Polle A, Lu M, Sun X, Luo ZB (2015) Global poplar root and leaf transcriptomes reveal links between growth and stress responses under nitrogen starvation and excess. *Tree Physiol* 35: 1283-1302. doi: 10.1093/treephys/tpv091.
  25. Ma Y, Yu X, Yu Z, Sun F, Li X, Li X (2018) RNA-Seq of *Agropyron mongolicum* Keng in response to drought stress. *Grassland Science* 64: 3-15. doi: 10.1111/grs.12176.
  26. Maestre FT, Eldridge DJ, Soliveres S, Kefi S, Delgado-Baquerizo M, Bowker MA, Garcia-Palacios P, Gaitan J, Gallardo A, Lazaro R, Berdugo M (2016) Structure and functioning of dryland ecosystems in a changing world. *Annu Rev Ecol Evol Syst* 47: 215-237. doi: 10.1146/annurev-ecolsys-121415-032311.
  27. Mamashita T, Larocque GR, DesRochers A, Beaulieu J, Thomas BR, Mosseler A, Major J, Sidders D (2015) Short-term growth and morphological responses to nitrogen availability and plant density in hybrid poplars and willows. *Biomass and Bioenergy* 81: 88-97. doi: 10.1016/j.biombioe.2015.06.003.
  28. Marion, M., *Biochemistry BJA* (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. 72: 248-254.
  29. Marklein AR, Houlton BZ (2012) Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytol* 193: 696-704. doi: 10.1111/j.1469-8137.2011.03967.x.
  30. Peng Y, Chen HYH, Yang Y, Stevens C (2020) Global pattern and drivers of nitrogen saturation threshold of grassland productivity. *Functional Ecology* 34: 1979-1990. doi: 10.1111/1365-2435.13622.
  31. Philippi TE, Dixon PM, Taylor BEJEA (1998) Detecting Trends in Species Composition. *Ecological Applications* 8: 300–308. doi: 10.2307/2641069.
  32. Quan X, Zeng J, Han Z, Zhang G (2017) Ionomeric and physiological responses to low nitrogen stress in Tibetan wild and cultivated barley. *Plant Physiol Biochem* 111: 257-265. doi: 10.1016/j.plaphy.2016.12.008.

33. Rubio-Asensio JS, Bloom AJ (2017) Inorganic nitrogen form: a major player in wheat and Arabidopsis responses to elevated CO₂. *J Exp Bot* 68: 2611-2625. doi: 10.1093/jxb/erw465.
34. Silveira J, Matos J, Ce Catto VM, Viegas RA, Oliveira JJE, Botany E (2001) Nitrate reductase activity, distribution, and response to nitrate in two contrasting Phaseolus species inoculated with Rhizobium spp. *Environ Exp Bot* 46: 37-46. doi: 10.1016/S0098-8472(01)00082-X.
35. Song L, Bao X, Liu X, Zhang Y, Christie P, Fangmeier A, Zhang F (2011) Nitrogen enrichment enhances the dominance of grasses over forbs in a temperate steppe ecosystem. *Biogeosciences* 8: 2341-2350. doi: 10.5194/bg-8-2341-2011.
36. Tian D, Wang H, Sun J, Niu S (2016) Global evidence on nitrogen saturation of terrestrial ecosystem net primary productivity. *Environmental Research Letters* 11. doi: 10.1088/1748-9326/11/2/024012.
37. Tielborger K, Bilton MC, Metz J, Kigel J, Holzapfel C, Lebrija-Trejos E, Konsens I, Parag HA, Sternberg M (2014) Middle-Eastern plant communities tolerate 9 years of drought in a multi-site climate manipulation experiment. *Nat Commun* 5: 5102. doi: 10.1038/ncomms6102.
38. Wang Q, Liu C-h, Huang D, Dong Q-l, Li P-m, van Nocker S, Ma F-w (2020) Physiological evaluation of nitrogen use efficiency of different apple cultivars under various nitrogen and water supply conditions. *Journal of Integrative Agriculture* 19: 709-720. doi: 10.1016/s2095-3119(19)62848-0.
39. Wang X, Guo X, Yu Y, Cui H, Wang R, Guo W (2018) Increased nitrogen supply promoted the growth of non-N-fixing woody legume species but not the growth of N-fixing Robinia pseudoacacia. *Sci Rep* 8: 17896. doi: 10.1038/s41598-018-35972-6.
40. Xu G, Fan X, Miller AJ (2012) Plant nitrogen assimilation and use efficiency. *Annu Rev Plant Biol* 63: 153-182. doi: 10.1146/annurev-arplant-042811-105532.
41. Yahdjian L, Gherardi L, Sala OE (2011) Nitrogen limitation in arid-subhumid ecosystems: A meta-analysis of fertilization studies. *Journal of Arid Environments* 75: 675-680. doi: 10.1016/j.jaridenv.2011.03.003.
42. Yang H, Li Y, Wu M, Zhang ZHE, Li L, Wan S (2011a) Plant community responses to nitrogen addition and increased precipitation: the importance of water availability and species traits. *Global Change Biology* 17: 2936-2944. doi: 10.1111/j.1365-2486.2011.02423.x.
43. Yang H, Wu M, Liu W, Zhang ZHE, Zhang N, Wan S (2011b) Community structure and composition in response to climate change in a temperate steppe. *Global Change Biology* 17: 452-465. doi: 10.1111/j.1365-2486.2010.02253.x.
44. Ye R, Liu G, Chang H, Shan Y, Mu L, Wen C, Te R, Wu N, Shi L, Liu Y, Wang H, Yun X, Liu G, Li F (2020) Response of plant traits of Stipa breviflora to grazing intensity and fluctuation in annual precipitation in a desert steppe, northern China. *Global Ecology and Conservation* 24. doi: 10.1016/j.gecco.2020.e01237.
45. Yokoyama S, Hiramatsu JIJJoB, Bioengineering (2003) A modified ninhydrin reagent using ascorbic acid instead of potassium cyanide. *Journal of bioscience and bioengineering* 95: 204-205. doi: 10.1016/S1389-1723(03)80131-7.

46. Yuan X, Niu D, Gherardi LA, Liu Y, Wang Y, Elser JJ, Fu H (2019) Linkages of stoichiometric imbalances to soil microbial respiration with increasing nitrogen addition: Evidence from a long-term grassland experiment. *Soil Biology and Biochemistry* 138: 107580. doi: 10.1016/j.soilbio.2019.107580.
47. Yuan X, Niu D, Weber-Grullon L, Fu H (2020) Nitrogen deposition enhances plant-microbe interactions in a semiarid grassland: The role of soil physicochemical properties. *Geoderma* 373: 114446. doi: 10.1016/j.geoderma.2020.114446.
48. Yuan ZY, Chen HYH (2015) Decoupling of nitrogen and phosphorus in terrestrial plants associated with global changes. *Nature Climate Change* 5: 465-469. doi: 10.1038/nclimate2549.
49. Zhang J, Ai Z, Liang C, Wang G, Liu G, Xue S (2019) How microbes cope with short-term N addition in a *Pinus tabulaeformis* forest-ecological stoichiometry. *Geoderma* 337: 630-640. doi: 10.1016/j.geoderma.2018.10.017.
50. Zhao Y, Gao C, Shi F, Yun L, Jia Y, Wen J (2018) Transcriptomic and proteomic analyses of drought responsive genes and proteins in *Agropyron mongolicum* Keng. *Current Plant Biology* 14: 19-29. doi: 10.1016/j.cpb.2018.09.005.
51. Zhao Y, Yang B, Li M, Xiao R, Rao K, Wang J, Zhang T, Guo J (2019) Community composition, structure and productivity in response to nitrogen and phosphorus additions in a temperate meadow. *Sci Total Environ* 654: 863-871. doi: 10.1016/j.scitotenv.2018.11.155.
52. Zhou X, Zhang Y, Downing A (2012) Non-linear response of microbial activity across a gradient of nitrogen addition to a soil from the Gurbantunggut Desert, northwestern China. *Soil Biology and Biochemistry* 47: 67-77. doi: 10.1016/j.soilbio.2011.05.012.
53. Zhu K, Chiariello NR, Tobeck T, Fukami T, Field CB (2016) Nonlinear, interacting responses to climate limit grassland production under global change. *Proc Natl Acad Sci U S A* 113: 10589-10594. doi: 10.1073/pnas.1606734113.
54. Zong N, Shi P, Song M, Zhang X, Jiang J, Chai X (2016) Nitrogen Critical Loads for an Alpine Meadow Ecosystem on the Tibetan Plateau. *Environ Manage* 57: 531-542. doi: 10.1007/s00267-015-0626-6.

Tables

Table 1 Results of one-way ANOVAS testing the effects of N addition on soil available nutrient properties and root morphological traits of *A. mongolicum* Keng.

Parameters	N addition gradients (g N m ⁻¹ yr ⁻¹)					P-value
	N0.0	N0.8	N1.6	N2.4	N4.0	
NO ₃ ⁻ -N (mg kg ⁻¹)	1.57±0.01e	4.26±0.02d	6.04±0.04c	8.08±0.05b	9.12±0.09a ***	
NH ₄ ⁺ -N (mg kg ⁻¹)	2.52±0.22a	2.25±0.28a	2.1±0.42a	1.38±0.30a	1.55±0.10a ns	
Available phosphorus (mg kg ⁻¹)	2.56±0.09c	2.66±0.07bc	2.79±0.13bc	2.99±0.11b	3.39±0.08a ***	
Total root length (cm)	2.93±0.06b	2.98±0.03b	3.10±0.07ab	3.28±0.06a	3.04±0.04b **	
Root surface area (cm ²)	1.95±0.04b	2.08±0.04b	2.11±0.05b	2.34±0.04a	2.07±0.03b ***	
Root volume (cm ³)	0.75±0.02c	1.22±0.11b	1.08±0.09bc	2.04±0.12a	1.04±0.11bc ***	
Root diameter (mm)	0.34±0.02a	0.35±0.01a	0.37±0.02a	0.39±0.01a	0.38±0.02a ns	
Root forks number	3.27±0.06c	3.36±0.08bc	3.55±0.06ab	3.69±0.06a	3.45±0.06abc **	

Significant differences ($P < 0.05$) among N addition treatments ($n = 4$) are indicated with lowercase letters. Data express as mean $\pm$ SE ($n = 4$). P-values are expressed as: *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ns: not significant.

Figures

Figure 1

Biomass of *A. mongolicum* Keng in response to N addition treatments. Means $\pm$ SE (n=5). Different letters indicate significant difference at $P < 0.05$. The arrow indicates the N saturation threshold for biomass of *A. mongolicum* Keng.

Figure 2

Root phenotype of *A. mongolicum* Keng under various N addition gradients (left) and the relationship between these root morphological parameters and total biomass (a-d).

Figure 3

N metabolism enzymatic activities in shoot and root of *A. mongolicum* Keng in response to N addition treatments. Different letters above bars indicate significant differences among the N addition treatments ($P < 0.05$). The values are presented as mean $\pm$ SE ($n = 4$).

Figure 4

N assimilation products in shoot and root of *A. mongolicum* Keng in response to N addition treatments. Different letters above bars indicate significant differences among the N addition treatments ($P < 0.05$). The values are presented as mean $\pm$ SE ($n = 4$).

Figure 5

N-use efficiency of *A. mongolicum* Keng in response to N addition treatments. Different letters above bars indicate significant differences among the N addition treatments ($P < 0.05$). The values are presented as mean $\pm$ SE ($n = 4$).

Figure 6

Structural equation model of the effects of N addition on biomass of *A. mongolicum* Keng. The model showing the direct effect of N addition on soil available nutrient and indirect effects on the biomass via the pathways of the soil available nutrient, root morphological traits and N-use efficiency. Green solid arrows and red solid arrows indicate positive and negative effects, respectively. The standardized path coefficients are adjacent to the arrows and indicate the size of this effect. The symbols “↑” and “↓” denote a significant increase and decrease ($p < 0.05$), respectively, in response to N addition. The letters “S” and “R” indicate the response of shoot and root to N addition, respectively. The numbers included in variables represent the slope of the linear model of each index with increasing N addition rates as a continuous predictor. The Percentages beside the response variables refer to the proportion of variance explained by the model (R^2). *, ** and *** indicate statistically significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

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