Ecological stoichiometry and biomass response of Agropyron michnoi Roshev. under simulated N deposition in a sandy grassland, China (Postprint)

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Abstract

Sandy grassland in northern China is a fragile ecosystem with poor soil fertility. Exploring how plant species regulate growth and nutrient absorption under the background of nitrogen (N) deposition is crucial for the management of the sandy grassland ecosystem. We carried out a field experiment with six N levels in the Hulunbuir Sandy Land of China from 2014 to 2016 and explored the Agropyron michnoi Roshev. responses of both aboveground and belowground biomasses and carbon (C), N and phosphorus (P) concentrations in the plant tissues and soil. With increasing N addition, both aboveground and belowground biomasses and C, N and P concentrations in the plant tissues increased and exhibited a single-peak curve. C:N and C:P ratios of the plant tissues first decreased but then increased, while the trend for N:P ratio was opposite. The peak values of aboveground biomass, belowground biomass and C concentration in the plant tissues occurred at the level of 20 g N/(m2?a), while those of N and P concentrations in the plant tissues occurred at the level of 15 g N/(m2?a). The maximum growth percentages of aboveground and belowground biomasses were 324.2% and 75.9%, respectively, and the root to shoot ratio (RSR) decreased with the addition of N. N and P concentrations in the plant tissues were ranked in the order of leaves>roots>stems, while C concentration was ranked as roots>leaves>stems. The increase in N concentration in the plant tissues was the largest (from 34% to 162%), followed by the increase in P (from 10% to 33%) and C (from 8% to 24%) concentrations. The aboveground biomass was positively and linearly correlated with leaf C, N and P, and soil C and N concentrations, while the belowground biomass was positively and linearly correlated with leaf N and soil C concentrations. These results showed that the accumulation of N and P in the leaves caused the increase in the aboveground biomass, while the accumulation of leaf N resulted in the increase in the belowground biomass. N deposition can alter the allocation of C, N and P stoichiometry in the plant tissues and has a high potential for increasing plant biomass, which is conducive to the restoration of sandy grassland.

Full Text

Abstract

Sandy grasslands in northern China represent fragile ecosystems with poor soil fertility. Understanding how plant species regulate growth and nutrient absorption under nitrogen (N) deposition is crucial for managing these ecosystems. We conducted a field experiment with six N addition levels in the Hulunbuir Sandy Land of China from 2014 to 2016, examining aboveground and belowground biomass responses and carbon (C), N, and phosphorus (P) concentrations in both plant tissues and soil. With increasing N addition, aboveground and belowground biomasses and C, N, and P concentrations in plant tissues all increased following a single-peak curve. C:N and C:P ratios of plant tissues initially decreased then increased, while the N:P ratio showed the opposite trend. Peak values for aboveground biomass, belowground biomass, and C concentration in plant tissues occurred at 20 g N/($m^2 \cdot a$), whereas N and P concentration peaks occurred at 15 g N/($m^2 \cdot a$). Maximum growth increases were 324.2% for aboveground biomass and 75.9% for belowground biomass, while the root-to-shoot ratio (RSR) decreased with N addition. N and P concentrations in plant tissues ranked as leaves > roots > stems, while C concentration ranked as roots > leaves > stems. The increase in N concentration in plant tissues was the largest (34% to 162%), followed by P (10% to 33%) and C (8% to 24%). Aboveground biomass showed positive linear correlations with leaf C, N, and P concentrations and soil C and N concentrations, while belowground biomass was positively linearly correlated with leaf N and soil C concentrations. These results demonstrate that accumulation of N and P in leaves drove aboveground biomass increase, while leaf N accumulation drove belowground biomass increase. N deposition can alter C, N, and P stoichiometric allocation in plant tissues and has high potential to increase plant biomass, which is conducive to sandy grassland restoration.

Keywords: biomass; nitrogen deposition; plant tissue; C, N and P stoichiometry; sandy grassland

1 Introduction

Since the Industrial Revolution, fossil fuel combustion and agricultural fertilizer use have caused dramatic increases in atmospheric nitrogen (N) deposition, reaching two to seven times preindustrial levels in some developed countries (Galloway et al., 2004). In China, the N deposition increase rate has reached 0.041 g/($m^2 \cdot a$) over the last 30 years (Liu et al., 2013), making it the region with the highest N deposition rate worldwide. Increased global atmospheric N deposition directly impacts regional N cycling, human and ecosystem health, greenhouse gas balance, and biodiversity (Clark and Tilman, 2008).

Previous studies show that in N-deficient ecosystems, increasing N inputs can promote plant growth and increase ecosystem primary productivity, but long-term N inputs may eventually reduce plant growth (Magill et al., 2000). In N-saturated ecosystems, excessive N inputs can inhibit plant growth and cause ecosystem degradation (Song et al., 2017). In sandy grassland ecosystems characterized by low soil N availability and critical N deposition loads (Duan et al., 2002; Yahdjian et al., 2011), N deposition serves as the main N input pathway and plays a positive role in significantly increasing plant productivity, thereby facilitating vegetation restoration (Fenn et al., 2003; Huang et al., 2018).

Many studies demonstrate that under low-N conditions, plants increase allocation of photosynthetic products to belowground parts (Grechi et al., 2007). With exogenous N addition, aboveground biomass in grassland ecosystems increases significantly (Lee et al., 2010), while belowground biomass decreases (Bai et al., 2001). Nevertheless, biomass of plants adapted to specific environments can increase substantially, making them dominant community species (Bai et al., 2010).

Carbon (C), N, and phosphorus (P) are structural and major nutrient elements that maintain biogeochemical cycles and ensure nutrient cycling and energy flow within ecosystems (Elser et al., 2000, 2010). Many studies have investigated N and P levels in plant leaves, using the N:P ratio to assess nutrient limitations for individual species and entire ecosystems (Güsewell, 2004; Zhu et al., 2016). According to dynamic balance theory, organisms can control many characteristics, including nutrient balance and pH stability, maintaining their internal environment within relatively narrow ranges despite external changes (Zhang et al., 2003). However, higher plants continually alter C, N, and P concentrations and their relative ratios (N:P, C:N, and C:P) in response to biotic and abiotic factors such as soil nutrients, functional groups, growth rates, and biomass accumulation (Elser et al., 2010; Wu et al., 2012). Soil solution N:P ratio, as a key environmental factor, influences both plant biomass and leaf N:P ratio (Güsewell, 2004; Wu et al., 2012). Previous studies have focused on nutrient dynamics and relationships between soil and plant leaves (Lee et al., 2010). However, how plant tissues regulate C, N, and P concentrations and ratios in response to N deposition, and their relationships with biomass in sandy grasslands, remain unclear.

Grasslands are important terrestrial ecosystems, accounting for approximately one-third of total ecosystem productivity (Hoekstra et al., 2005) and providing crucial ecological services (Wrage et al., 2011). However, long-term imbalanced human utilization has caused large-scale degradation and desertification of grassland ecosystems. Currently, desertified grasslands in China account for 16.7% of the total land area, severely threatening ecological security in northern China. *Agropyron michnoi* Roshev. is a typical rhizomatous grass species highly adaptable to sandy grasslands. In recent years, *A. michnoi* has been used for establishing windbreaks and sand fixation in China. In this study, we conducted a three-year field experiment with *A. michnoi* under N addition, analyzing plant biomass and C, N, and P stoichiometry in plant tissues and soil. Our objectives were to determine: (1) how plant tissues and soil stoichiometry respond to N addition, (2) how aboveground and belowground plant biomasses vary in response to N addition, and (3) how plant biomass and tissue stoichiometry are related.

2.1 Study Area

The sandy grassland research station (2001-2018) is located in the Hulunbuir Sandy Land in northern China (49.05°-49.25°N, 118.20°-118.40°E; 560-670 m a.s.l.). This area has a typical continental climate, with mean annual precipitation of 250-300 mm, over 70% occurring from June to September. Mean annual evaporation is 4-6 times precipitation. Annual sunshine duration is approximately 3150 h, and annual mean temperature is -1°C. The major soil type is sandy soil. Fixed sandy grassland, consisting of soil covered by sand, has relatively low species richness and vegetation cover. Species composition is dominated by drought-tolerant plants: *A. michnoi*, *Leymus chinensis*, *Carex duriuscula*, and *Melissitus ruthenius*. *Thymus vulgaris* constitutes perennial species, while *A. japonica*, *Setaria viridis*, *Salsola foliosa*, *Agriophyllum squarrosum*, and *Agriophyllum arenarium* are annual species. Vegetation coverage is less than 40%, with *A. michnoi* as the single dominant species. No differences exist in topography, soil type, or spatial heterogeneity across the study area.

2.2 Experimental Design

The field was divided into three blocks separated by 2-m buffer zones. Each block contained 12 plots (6 m × 6 m) separated by 1-m buffer zones for different treatments. N addition experiments ran from 2014 to 2016 using NH_4NO_3 fertilizer (46% pure N). Six N levels were applied: 0 (control, CK), 5, 10, 15, 20, and 25 g $N/(m^2 \cdot a)$, referenced from historical N deposition data in northern China (Ti and Yan, 2010; Zhu et al., 2016). These are hereafter designated N0, N5, N10, N15, N20, and N25 treatments. N fertilizer was applied four times during the first 10 days of May, June, July, and August, with one-quarter of the annual amount each time. NH_4NO_3 solutions were sprayed onto plants from foliage to soil surface. The water amount applied to CK treatment plants (3 $L/(plot \cdot month)$, equal to 0.08 mm/month) matched the solution amount in other treatments. This value is much less than average precipitation (40 mm in May, up to 110 mm in July). Pests and weeds were controlled manually.

2.3 Field Sampling

In late August 2016, plants were randomly selected from each treatment using a sampling frame (50 cm × 50 cm × 30 cm). Plants were harvested to determine aboveground and belowground biomasses. Soil was randomly sampled with an auger at 0-10 cm depth. Soil samples were mixed in triplicate for each treatment for composition determination. Additionally, thirty plants with fully connected aboveground and belowground organs were randomly selected from each treatment, stored in insulated cans, and immediately transported to the laboratory for further analysis.

2.4 Methods of Measurement

Belowground organs (rhizomes and fibrous roots) were rinsed with distilled water, and aboveground organs (green leaves and stems) were oven-dried at 75°C for 48 h and 65°C for 48 h, respectively, to measure organ biomasses. The root-to-shoot ratio (RSR) formula is as follows:

Total C, N, and P concentrations were analyzed after samples were finely ground in a Wiley mill and passed through a 40-mesh sieve. Total C concentration was measured using the K_2MnO_4 volumetric method, and total N concentration via the Kjeldahl method using an Alpkem autoanalyzer (Kjektec System 2300 Distilling Unit, Sweden). Total P concentration was determined colorimetrically at 700 nm after reaction with a molybdenum-antimony solution. After air-drying, soil samples were separated into three groups to measure total C, N, and P concentrations.

2.5 Statistical Analysis

Statistical analysis was performed using SPSS version 23.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA was used to test N addition effects on biomass, RSR, and C:N:P stoichiometry of plants and soil. Linear regression analysis determined relationships between plant C:N:P stoichiometry and biomass, soil C:N:P stoichiometry and biomass, and soil and leaf C:N:P stoichiometry. Data are presented as mean ± standard error (SE; $n = 3$).

3.1 Changes in Plant Biomass and RSR

With increasing N addition, both aboveground and belowground biomasses of *A. michnoi* significantly increased following a single-peak curve, with peak values under N20 treatment [Figure 1: see original paper]. RSR tended to decrease under low N addition (N0 to N15) but remained stable under high N addition (N15 to N25) [Figure 1: see original paper]. The highest increases in aboveground and belowground biomasses were 324.2% and 75.9%, respectively, under N20 treatment compared with N0. RSR of *A. michnoi* varied from 2.6 to 8.2 across N treatments.

3.2 Changes in C, N, and P Concentrations and C:N:P Ratios of Plant

C, N, and P concentrations in roots, stems, and leaves of *A. michnoi* significantly increased with N addition [Figure 2: see original paper]. Highest values occurred under N20 for C concentration and under N15 for N and P concentrations. The increase in N concentration was largest (34% to 162%), followed by P (10% to 33%) and C (8% to 24%). Under all treatments, N and P concentrations in plant tissues ranked as leaves > roots > stems, while C concentration ranked as roots > leaves > stems. With increasing N addition, C:N ratios in roots, stems, and leaves and C:P ratios in stems and leaves initially decreased then increased, while C:P ratio in roots increased under high N treatments. N:P ratios in roots, stems, and leaves initially increased then decreased [Figure 2: see original paper].

3.3 Changes in C, N, and P Concentrations and C:N:P Ratios of Soil

Soil total C concentration exhibited an increasing trend with N addition [Figure 3: see original paper]. Soil total N and P concentrations significantly increased then decreased following a single-peak curve, with highest values under N15 treatment [Figure 3: see original paper]. Soil C:N ratios under all N addition treatments were significantly lower than under N0, with few differences among N addition treatments [Figure 3: see original paper]. Soil C:P and N:P ratios initially increased then decreased [Figure 3: see original paper].

3.4 Relationships Between Plant Biomass and C:N:P Ratios of Plant

Relationships between plant biomass and C:N:P ratios are shown in [Figure 4: see original paper]. Positive linear relationships were found between aboveground biomass and C concentrations in leaves, stems, and roots, as well as N and P concentrations in leaves. Strong positive relationships occurred between aboveground biomass and C concentrations in leaves and roots. In contrast, negative relationships were detected between aboveground biomass and C:N and C:P ratios in leaves. However, positive linear relationship was found only between belowground biomass and leaf N concentration.

3.5 Relationships Between Plant Biomass and C:N:P Ratios of Soil

Relationships between plant biomass and soil C:N:P ratios are shown in [Figure 5: see original paper]. Positive linear relationships were detected between both aboveground and belowground biomasses of *A. michnoi* and soil C and N concentrations. In contrast, negative linear relationships were found between aboveground biomass and soil C:N ratio and between belowground biomass and soil C:N ratio.

3.6 Relationships Among C:N:P Ratios of Plant and Soil

Relationships among plant and soil C:N:P ratios are shown in [Figure 6: see original paper]. A strong positive linear relationship was detected between leaf C:N ratio and soil C:N ratio, and a positive linear relationship was found between leaf N:P ratio and root N:P ratio.

4.1 Changes in Plant Biomass in Response to N Addition

N and water are two important factors limiting grassland ecosystem productivity. In this ecosystem, N has different effects on plant photosynthesis and growth, leading to varied productivity responses. In dry environments, nutrient migration and transmission are limited. However, increased precipitation can relieve soil nutrient limitations, improving nutrient availability, promoting plant nutrient absorption and use, and ultimately increasing ecosystem productivity (Li et al., 2013). Therefore, N and water interactions tend to be synergistic (Niu et al., 2009).

In this study, with increasing N addition (maximum 20 g $N/(m^2 \cdot a)$), aboveground and belowground biomasses tended to increase, but plant biomass decreased under high N levels (25 g $N/(m^2 \cdot a)$) [Figure 1: see original paper]. This indicates that appropriate N addition benefited *A. michnoi* biomass accumulation, but excessive N addition had no effect. These results align with other grassland N addition studies. N can improve plant growth within a certain range, but when N addition exceeds the minimum N saturation for regional plants, they become less sensitive to N deposition (Hedwall et al., 2013), and P limitation in grassland increases, reducing plant biomass (Menge and Field, 2007).

Studies show that plants can increase allocation of photosynthetic products to belowground organs to obtain N under low N conditions (Grechi et al., 2007). Moreover, with increasing exogenous N addition, aboveground biomass and productivity of grassland communities can significantly increase, reducing RSR (Lee et al., 2010). In this study, belowground biomass always exceeded aboveground biomass under N addition, while RSR decreased with increasing N addition. However, when N addition exceeded 15 g $N/(m^2 \cdot a)$, RSR tended to stabilize [Figure 1: see original paper]. This may occur because *A. michnoi* clones increase allocation to belowground components to facilitate resistance against harsh environmental conditions and expand populations via vegetative propagation, thus maintaining this species' dominant position in the community (Jin et al., 2012).

4.2 Linkage Between Plant Biomass and C:N:P Ratios of Plant

C, N, and P concentrations in plant leaves were reported as 436.0, 26.8, and 1.8 g/kg, respectively, in Inner Mongolia, China (He et al., 2006). These values exceed the 413.0, 17.6, and 1.47 g/kg, respectively, observed in our study under no N addition [Figure 2: see original paper]. This occurs because plants growing in low-fertility soils have relatively low nutrient concentrations, while plant nutrient concentrations increase with soil nutrient concentrations (Xu et al., 2016). Analysis of plant stoichiometric characteristics in response to N addition in a *Stipa baicalensis* grassland in Inner Mongolia revealed that leaf C and N concentrations in Gramineae plants increased with N addition while leaf P concentration decreased (Liu et al., 2018). Similar results were obtained in a *Schima superba* study, where increased N availability from N deposition was associated with increased N concentration and decreased P concentration (Zhang et al., 2018). In our study, C, N, and P concentrations in roots, stems, and leaves of *A. michnoi* significantly increased under different N treatments [Figure 2: see original paper]. These increases may have occurred because N addition increased available soil N concentration during the growing season, enhancing N absorption by plant roots (Liu et al., 2018). Moreover, increased N uptake results in increased uptake of other nutrients such as P, which may become limiting over time (Lu et al., 2018). However, leaf P concentration increased, possibly because: (1) plants increased expression of genes encoding acid phosphatase and other genes expressed outside epidermal cells to adapt to low P stress, and increased acid phosphatase activity benefited organic P decomposition in soil (Liu et al., 2004); (2) differences in grassland types and species; and (3) P inputs may have increased with increasing belowground root biomass and plant litter (Zhou et al., 2015).

In this study, aboveground biomass was positively related to C concentrations in leaves, stems, and roots, as well as N and P concentrations in leaves [Figure 4: see original paper]. Negative relationships were detected between aboveground biomass and leaf C:N and C:P ratios [Figure 4: see original paper]. However, positive relationships were found only between belowground biomass and leaf N concentration [Figure 4: see original paper]. These results indicate that aboveground biomass increases in response to N addition depended on increases in both leaf N and P concentrations, while belowground biomass increases depended only on leaf N concentration.

According to the plant growth rate hypothesis, relative growth rate and C:N ratio are negatively correlated when N is limiting and positively correlated when P is limiting. When N or P is no longer limiting, plants absorb these elements in excess and store them, reducing use efficiency (Ågren, 2004). Previous studies suggest that differences in nutrient storage and metabolic function make plant stems and roots less sensitive to environmental changes than leaves, and N addition has little effect on C:N, C:P, and N:P ratios of stems and roots. However, we detected significant changes in C, N, and P concentrations and C:N, C:P, and N:P ratios not only in leaves but also in roots and stems of *A. michnoi*. We also found that C, N, and P concentrations in plant tissues increased following a single-peak curve with increasing N addition [Figure 2: see original paper], while C:N and C:P ratios initially decreased then increased, and N:P ratio showed the opposite trend [Figure 2: see original paper]. Furthermore, aboveground biomass was negatively correlated with C:N and C:P ratios [Figure 4: see original paper]. These results indicate that *A. michnoi* adapts to environmental changes by coordinating C, N, and P concentrations and ratios in roots, stems, and leaves to achieve high potential biomass in response to N deposition, which is conducive to sandy grassland restoration.

Leaf N:P ratio typically indicates N or P limitation. Plant growth is limited by N, P, or both when the ratio is <21, >23, or 21–23, respectively. With increasing N deposition, N limitation in degraded grasslands is alleviated and leaf N:P ratio increases. However, long-term or excessive N deposition can cause N saturation, resulting in imbalanced N:P ratios and P limitation for plant growth (Wardle et al., 2013). Critical N:P ratio indices were proposed through N addition experiments with *Leymus chinensis* and *Carex korshinskyi* in a steppe ecosystem in Inner Mongolia (Zhang et al., 2004). Our findings showed that *A. michnoi* growth was N-limited under low N addition (N0 and N5), P-limited under moderate N addition (N10 and N15), and limited by both N and P under high N addition (N20 and N25). This occurs because sandy grassland soils are poor in N and P, especially N. N tended toward saturation with increasing N addition, and plant growth became P-limited. Additionally, plant biomass increased with N addition, increasing plant P requirements and resulting in both N and P limitations. We also found that the strong positive response of leaf N:P ratio to N addition likely caused the greater root N:P ratio [Figure 6: see original paper]. These results indicate that *A. michnoi* can regulate N:P ratios in roots and leaves, as described in previous studies (Huang et al., 2018).

4.3 Linkage Between Plant Biomass and C:N:P Ratios of Soil

Soil nutrient distribution is associated with plant characteristics such as nutrient stoichiometry, aboveground and belowground biomass partitioning, biomass cycling rate, and rooting depth (Jobbágy and Jackson, 2001), particularly soil C and N storage (Li et al., 2013). Analysis of 0–10 cm soil layer C:N:P ratios showed that surface soil C:N and N:P ratios could adequately indicate soil N supply status (Tian et al., 2010). Desert steppe ecosystems have relatively low soil N:P ratios (Delgado-Baquerizo et al., 2013), and soil N:P ratio can increase with long-term, large-scale N deposition, gradually shifting the relationship between soil N and P (Vitousek et al., 2010). Soil C:N and C:P ratios importantly affect plant growth (Wang and Yu, 2008; Yu et al., 2014). Our results showed that soil total C, total N, and N:P ratio increased with N addition, while soil C:N and C:P ratios decreased [Figure 3: see original paper]. Moreover, positive relationships were detected between aboveground and belowground biomasses and soil N and C concentrations, respectively, while negative relationships were found between aboveground and belowground biomasses and soil C:N ratio [Figure 5: see original paper]. These results indicate that N deposition alleviated plant N limitation, promoted plant growth and biomass accumulation, and increased soil total C concentration. Therefore, N deposition can effectively increase soil C and N storage and promote sandy grassland restoration.

5 Conclusions

Sandy grasslands of northern China are fragile ecosystems with poor soil fertility. Understanding how plants regulate growth and nutrient absorption under N deposition is crucial for sandy grassland ecosystem management. We conducted an N addition field experiment with *A. michnoi* in the Hulunbuir Sandy Land of China. With increasing N addition, aboveground and belowground biomasses and C, N, and P concentrations in plant tissues increased following a single-peak curve. Aboveground biomass accumulated faster than belowground biomass, N accumulated faster than P in plant tissues, and C accumulated relatively slowly. Aboveground biomass was positively and linearly correlated with leaf C, N, and P concentrations and soil C and N concentrations, while belowground biomass was positively and linearly correlated with root N and soil C concentrations. These results show that accumulations of N and P in leaves played a major role in increasing aboveground biomass, while root N accumulation played a major role in increasing belowground biomass. N deposition can alter C, N, and P stoichiometric allocation in plant tissues and has high potential to increase plant biomass, which is conducive to sandy grassland restoration. However, this three-year field study requires comprehensive confirmation in other sandy grasslands where vertical soil nutrient distribution is dominated by plant cycling.

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Note: Figure translations are in progress. See original paper for figures.

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