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Root responses to nitrogen pulse frequency under different nitrogen amounts

Qing-Ye Yuan [a], Pu Wang [a], Lu Liu [a], Bi-Cheng Dong [a, *], Fei-Hai Yu [a, b]

[a] School of Nature Conservation, Beijing Forestry University, Beijing 100083, China
[b] Zhejiang Provincial Key Laboratory of Plant Evolutionary Ecology and Conservation, Taizhou University, Taizhou 318000, China

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ABSTRACT

Responses of morphology and biomass allocation of roots to frequency of nitrogen (N) pulse potentially influence the fitness of plants, but such responses may be determined by root size. We grew 12 plant species of three functional groups (grasses, forbs, and legumes) under two N pulse frequencies (high *vs.* low supply frequency) and two N amounts (high *vs.* low supply amount). Compared to low-amount N supply, high-amount N supply stimulated biomass accumulation and root growth by either increasing the thickness and length of roots or decreasing the root mass fraction. Compared to low-frequency N supply, high-frequency N supply improved biomass accumulation and root growth in forbs or grasses, but not in legumes. Furthermore, the magnitude of the response to N frequency was significantly negatively correlated with root size at the species scale, but this was only true when the N amount was high. We conclude that root responses to N frequency are related to plant functional types, and non-legume species is more sensitive to N frequency than legume species. Our results also suggest that root size is a determinant of root responses to N frequency when N supply amount is high.

1. Introduction

Nitrogen (N) is a key nutrient element for plant growth (Elser et al., 2007; Vitousek and Howarth, 1991). As the root is commonly the most important organ by which plants acquire N from soils (Hodge, 2009; Hodge et al., 2009; Johnson and Biondini, 2001), its morphological plasticity and biomass allocation are important for plants to adapt to changing soil N concentration. When soil N is limited, plants may maximize N uptake efficiency by increasing the investment in root mass and/or modifying root morphology by, for example, enhancing lateral root length and root surface area (Johnson and Biondini, 2001; Mou et al., 2013; Robinson and Rorison, 1988). When soil N is abundant, plants may optimize N utilization efficiency by increasing leaf production or nitrogen concentration per leaf area (Bloom et al., 1985; Thornley, 1972).

There is increasing evidence that the response of roots to soil N is constrained by the inherent root structure and function of different species (Campbell et al., 1991; Cantarel et al., 2015; Fransen et al., 1998; Johnson and Biondini, 2001; Song et al., 2015; Wijesinghe et al., 2001; Yu et al., 2015). Species with large root systems have been reported to be more sensitive to temporal or spatial variation in N supply and possibly adjust root morphology and allocation more rapidly than do species with smaller root systems (Fransen et al., 1998; Johnson and Biondini, 2001). For instance, species with large root size (e.g., *Anthoxanthum odoratum*, *Holcus lanatus*, *Lolium perenne*) increased root mass in the high than in the low nutrient patches in a heterogeneous soil environment, but species with a smaller root size (e.g., *Festuca rubra*) did not (Fransen et al., 1998). Furthermore, morphological plasticity related to N availability is not only species-specific, but also varies among plant functional groups. For instance, plant species and functional groups often diverge in their N chemical form preference in response to fertilization (e.g., legumes sometimes show an apparent preference of nitrate over ammonium in an alpine meadow system; Song et al., 2015), which may influence the uptake efficiency of resources among functional groups, and also their morphological plasticity in response to changing in N availability. Besides, legumes often have the weaker growth vigor and morphological plasticity in response to N availability than grasses or forbs, especially when they fail to establish a plant-rhizobia mutuality during the seedling development period (Craine et al., 2003; Hodge et al., 2009). Therefore, comparing

* Corresponding author.
E-mail address: bcdong@bjfu.edu.cn (B.-C. Dong).

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morphological plasticity between species and functional groups is necessary for a better understanding of the adaptive strategies of plants in response to changing N availability.

N loading is often considered as a discrete event in natural habitats, especially following unpredictable disturbance such as fire, rainfall and fertilization (Esque et al., 2010; Ivans et al., 2003). N loading of different frequencies increases the challenge for plants to acquire resources, but to some extent also provides the opportunity for plants to acclimatize to resource fluctuation (Bilbrough and Caldwell, 1997; Osone et al., 2014). One plausible prediction is that if high-frequency N supply may continually stabilize N availability in soils compared to low-frequency N supply, high-frequency N supply will improve plant performance. Meanwhile, plants with larger root systems may also exhibit a better adaptation to high-frequency N supply, because they are often larger in whole body size, are supported by a larger amount of photosynthates and N, and require more flexible root morphology in response to pulsing N supply (e.g., James and Richards, 2006). Another prediction is that the effect of N pulse frequency on plant performance may be modified by the amount of N supply. For instance, *Alternanthera sessilis* tended to increase leaf production and biomass accumulation under high-frequency than under low-frequency N supply only when N amount is lower, but the response of *Alternanthera philoxeroides* to N frequency was not related to N amount (Wang et al., 2015). To our knowledge, the effect of N pulse has rarely been investigated at the functional group scale (James and Richards, 2005; Jankju-Borzelabad and Griffiths, 2006; Osone et al., 2014).

We selected 12 common grassland species of three functional groups (i.e., grasses, forbs and legumes) to investigate effects of N frequency and amount on biomass production and root performance at both species and functional group scales. Additionally, we examined the relationship between root responses to N frequency and root size and between biomass production and root morphology. We proposed the following specific hypotheses: (1) different amounts of N supply trigger contrasting root morphology and biomass allocation, and high-amount N supply enhances plant performance; (2) high-frequency N supply is more beneficial for plant performance than low-frequency supply, and such an effect of N frequency depends on N amount; (3) the effects of N amount and N frequency vary at both species and functional group scales; (4) the magnitude of root responses to N frequency depends upon the root system size in the term of final root mass.

2. Materials and methods

2.1. Experimental design

The 12 species (4 legumes, 4 grasses and 4 forbs) are common grassland species native to and also widespread in China. They produce roots over a wide range of size. The seeds of these species were all provided by Golden Earth Agricultural Technology Research Institute, Beijing (website: http://www.jtdseed.com). More detailed descriptions of each species are shown in Table 1, and available at http://www.floraofchina.org.

The experiment took a factorial design, with two levels of N amount (low amount *vs*. high amount) crossed with two levels of N pulse frequency (low-frequency *vs*. high-frequency), resulting in a total of four treatments. There were five replicates for each treatment. Low-amount N supply was set to 2.59 g m^{-2} yr^{-1}, presenting the average level of current N deposition in the eastern China, and high-amount N supply was 10.36 g m^{-2} yr^{-1}, reflecting a possible level of future N deposition (Lu and Tian, 2007).

On June 1, 2014, 250 seeds of each species were sown in 50-cell trays filled with a 1:1 (v:v) mixture of sand and peat (Pindstrup Seedling; Pindstrup Mosebrug A/S, Pindstrup, Denmark). The peat was specially designed for cultivation of seedlings, containing both macronutrients (4.341 mg N L^{-1}, 2.138 mg P L^{-1}, 7.128 mg K L^{-1} and 0.518 Mg mg L^{-1}) and micronutrients (data from the instruction of “Pindstrup Seedling” peat). Total N in the sand-peat mixture was 5.74 ± 1.56 mg N g^{-1} (mean ± SE, $N = 4$). After one month of growth, 20 similar-sized seedlings (approx. 15 cm tall) of each species were randomly selected, and there were 240 plants in total for the 12 species. These plants were transplanted into 240 pots (14 cm in diameter × 12 cm in height) filled with the sand-peat mixture, with one plant per pot. N was supplied in the form of the NH_4NO_3 solution. In the low-amount treatment each pot was supplied with a total of 12 mg N during the experiment, and in the high-amount treatment each pot was supplied with a total of 48 mg N. In the low-frequency treatment, each pot was supplied with N (0.75 g for the low-amount treatment and 3 g for the high-amount treatment) for 16 times (once every three days) during the 48-day experiment, and in the high-frequency treatment it was supplied with N (6 g for the low-amount treatment and 24 g for the high-amount treatment) for only twice (once every 24 days).

The experiment was conducted over a period of 48 days in a greenhouse at Forest Science Company of Beijing Forestry University in Beijing, China. During the experiment, daily mean temperature was 29.44 °C (SE: 0.20 °C) and mean humidity was 64.23% (SE: 0.63%), as measured by iButtons (model DS1923; Maxim Integrated Products, Sunnyvale, CA, USA). During the experiment, the day length in Beijing was about 14 h per day (http://www.wunderground.com).

2.2. Measurements and data analyses

At the end of the experiment, all plants were harvested and the whole root length of each plant was measured using the WinRHIZO Pro 2004a software (Regent Instruments, Inc., Québec, Canada). The plants were then divided into leaves, stems and roots, oven-dried at 70 °C for 48 h, and weighed. Before analysis, we calculated total mass (i.e., the sum of leaves, stems and roots), root mass, root length, root mass fraction (i.e., root mass/total mass; RMF), and specific root length (i.e., root length/root mass; SRL). Total mass and root mass were natural log-transformed to meet the assumption of homogeneity of variance. Root biomass was also used as a measure of the size of the root system (Campbell et al., 1991; Wijesinghe et al., 2001).

We used three-way nested ANOVAs to test the effects of functional group (fixed factor), N amount (fixed factor), N frequency (fixed factor), and species identity (nested within the functional group factor) on total mass, root mass, root length, RMF, and SRL. Linear contrasts were conducted to determine the differences between treatments.

Regressions were employed to examine the relationships between biomass and root related measures within each of the four N treatments. The log response ratio of root mass was calculated to measure the relative response strength of each species to the N frequency treatment within each N amount treatment, i.e., LnRR = Ln (value in the low-frequency treatment/value in the high-frequency treatment). The distribution of LnRR is symmetrical around zero, with negative values indicating that the plant benefits more from the high-frequency treatment and positive values indicating the plant benefits more from the low-frequency treatment. One-sample *t*-tests were used to examine whether LnRR differed significantly from zero. All analyses were conducted using SPSS 22.0 (SPSS, Inc., Chicago, IL, USA).