Precipitation Pattern Alters the Effects of Nitrogen Deposition on the Growth of Alien Species *Robinia Pseudoacacia*

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Abstract

Aims

Nitrogen (N) supply and precipitation pattern (amount and frequency) both affect plant growth. However, N deposition is increasing and precipitation regimes are changing in the context of global change. An experiment was conducted to determine the effect of precipitation pattern and N supplies on the growth of a widely distributed and planted N₂-fixing alien species *Robinia pseudoacacia*.

Methods

Seedlings were grown in a glasshouse at four different N levels combined with different precipitation regimes, including three precipitation amounts, and two precipitation frequencies. After treatment for 75 days, plant height, biomass allocation, leaf and soil nutrient concentrations were measured.

Results

Plants under high precipitation frequency had greater biomass compared with plants lower precipitation frequency with the same amount of precipitation. Higher N supply decreased biomass allocation to nodules. Nodule growth and N₂ fixation of *R. pseudoacacia* with low precipitation amount was more inhibited by high N deposition compared with plants received higher precipitation amount. Slightly N deposition under higher precipitation inhibited N₂ fixation but did not fulfil the N need in plants.

Conclusions

Nitrogen deposition might inhibit N₂ fixation of plants even in low level but low nitrogen in soil cannot fulfil the N need of plants, and caused N₂ fixation limitation in plants during seedling stage. There was likely a shift from acquiring N mainly from N₂ fixation to acquisition of N directly with root when N supply was increased. High N deposition level, and increased precipitation frequency might increase the invasion risk of *R. pseudoacacia*.

Introduction

Anthropogenic nitrogen (N), which largely comes from industrialized production of fertilizer, has doubled the input of N into the global N cycle (Schlesinger 2009). Due to human activities, N deposition have been increasing in the past century (Galloway et al. 2008); which including. ammonium (NH₄⁺), mostly derived from agriculture, and N oxides (NO_x), mostly released during fossil fuel combustion (Fowler et al. 2013; Liu et al. 2016). Nitrogen deposition is predicted to reach a level as high as 200 Tg year⁻¹ by 2050 globally (Galloway et al. 2008), however N deposition is observed to be more complicated in China, which

peaked in 2011 and then went down (Wen et al. 2020). A lack of N limits plant productivity in many ecosystems with young soils (Crawford 1995; Laliberté et al. 2012), and has affected the net primary productivity globally (Lebauer and Treseder 2008). However, increased N deposition would not only decrease biodiversity in many ecosystems (Bai et al. 2010), inhibit N₂ fixation in many legume species (Leidi and Rodriguez-Navarro 2000); but also promote invasive plant growth more than native plant (Dukes et al. 2011; Eskelinen and Harrison 2014; Liu et al. 2017). Alien plants, especially invasive plants, can not only increase N cycle but also increase N pool in soil; while removing N from soil would lower the risk of invasion (Mack and D'Antonio 2003; Rippel et al. 2020). Thus, it is very important to study how complicated N deposition scenario is affecting alien plant growth, especially in China.

The effect of N on plants is related to precipitation in many ways (Liu et al. 2016; Manzoni et al. 2012). First, wet deposition of N, as a major deposition pathway (Sheng et al. 2013; Zhan et al. 2015), happens with precipitation. Second, water availability in soil directly affects ion mobility and availability (Chapin 1991; Kreuzwieser and Gessler 2010) and decreased soil moisture could lead to N deficiency (Cramer et al. 2010). Water is also significantly related to N mineralization by influencing the activity of microorganisms in soil (Manzoni et al. 2012). In some dry places, water and N co-limit plant growth, but appropriate levels of N can enhance a plant's water use efficiency (Wu et al. 2008). For invasive plant species, water and nutrient-rich soils also promote invasions (Eskelinen and Harrison 2014).

Precipitation has been changing over the past decades due to climatic change (Ge et al. 2016; Konapala et al. 2020). Extreme precipitation events and extreme droughts have happened more frequently in recent years (Ault 2020; Trenberth 2011). Increased precipitation amount and frequency increases seed germination (Carón et al. 2015; O'Brien et al. 2013), plant growth (Gao et al. 2015; Huang and Zhang 2016) and yield (Schneider et al. 2014), and it is also worth noting that the number of heavy precipitation events increased in many regions (IPCC 2014), and some plants are sensitive to these large rainfall events (Zhang et al. 2012). Moreover, the same amount of annual mean precipitation may lead to different performance of plants under different precipitation pattern (frequencies) (Coe and Sparks 2014).

This study analysed the effects of N deposition and changed precipitation amount and frequency on the growth of *Robinia pseudoacacia*. *Robinia pseudoacacia*, which is native to the Appalachian uplands in North America, has naturalized on other continents (Cierjacks et al. 2013), and has invaded into a wide range of areas including China (Boring and Swank 1984; Grünewald et al. 2009; Vítková et al. 2015); and in China, the area of *R. pseudoacacia* exceeded 70000 ha just in Loess Plateau (Wang et al. 2012). The species is drought resistant and possesses N₂-fixing nodules (Wurzburger and Miniat 2014). Nitrogen fixation makes it more competitive in many natural ecosystems, especially in arid habitats (Rice et al. 2004). In previous studies, global changes including increased N deposition and increased precipitation have been reported to cause more positive effects on invasive species than native species (Dukes et al. 2011; Eskelinen and Harrison 2014; Liu et al. 2017), but other studies reported that climate change is not invariably the driver of invasive species' success (González-Muñoz et al. 2014). So, it remains an open question how *R. pseudoacacia* would perform both under elevated N supply and altered precipitation regimes (Cierjacks et al. 2013; Johnsen and Bongarten 1992; Wurzburger and Miniat 2014). Therefore, in

a greenhouse experiment, we grew seedlings of *R. pseudoacacia* under four levels of N supply, three levels of precipitation amount and two levels of precipitation frequency. We addressed the following hypotheses:

1. Increased precipitation amount would promote the growth of *R. pseudoacacia* under both precipitation frequencies.
2. With the same precipitation amount, *R. pseudoacacia* under high precipitation frequency would have faster growth than that under low precipitation frequency.
3. Nitrogen supply promotes the growth of *R. pseudoacacia* under all precipitation regimes.

Materials And Methods

Plant materials

On May 20th 2014, seeds of *R. pseudoacacia* were germinated in a growth chamber after washing and soaking in water for 24 h. One week later, 300 seedlings of similar size were transplanted into the plastic pots. Each pot (25 cm in height and 24 cm in diameter) was filled with a mixture of 6 kg loam and 2 kg sand. Loam and sand were carefully sifted and fully mixed before filling pots. The substrate's chemical properties were: 87.7 mg kg⁻¹ plant-available soil N and 25.2 mg kg⁻¹ plant-available soil phosphorus (P) (n = 4). After one and a half months of cultivation, 200 seedlings in similar size were selected to conduct the experiments and 8 of 200 seedlings were used to measure the initial biomass (initial biomass was 1.51 ± 0.16 g). Each pot was planted with one seedling.

Experimental design

Our experiment was carried out in a greenhouse at Fanggan Research Station of Shandong University (36°26' N, 117°27' E), China. The top and four sides of the greenhouse were covered with plastic film and the film around can be rolled up to keep ventilation. We applied four N levels in the experiment, i.e. 0, 22.5, 90 and 180 mg N per pot every 15 days as N1, N2, N3 and N4, respectively which represented no N deposition, low N deposition, high N deposition and very high N deposition or N contamination (Liu et al. 2013). NH₄NO₃ solution was added as the source of N. Water was given at three levels, on average 90, 270 and 450 mL per pot each day (W1, W2 and W3), which represents the precipitation of drought (200 mm precipitation annually), semi-moist (600 mm precipitation annually) and moist (1000 mm precipitation annually) conditions in eastern China (Wang et al. 2006). Water was also supplied with two frequencies, once a day (F1 with 90, 270 and 450 ml per pot each time) or once every five days (F2, with 450, 1350 and 2250 ml per pot each time). For all treatments, there were eight replicates and all pots were randomly arranged and pots were also randomly re-arranged fortnightly. There were 192 pots (8 replicates × 4 N treatment × 3 precipitation amount levels × 2 precipitation frequencies) in total for the experiment. Treatments lasted for 75 days, from 15/07/2014–28/09/2014. Pests and weeds were removed regularly by hand.

Measurements and calculations

Plant height and crown area were measured at the beginning and the end of the experiment. On 25th Sept 2014, the fourth fully-expanded mature leaves from the top were collected for the measurement of leaf chlorophyll concentration. Leaf chlorophyll was extracted using 95% (v/v) ethanol and measured spectrophotometrically (Lichtenthaler and Wellburn 1983).

During the harvest, all plants were carefully washed and divided into main root, lateral roots, root nodules, stems, petioles and leaf blades. Each part was oven-dried for 48 h under 80°C. Biomass of different parts was weighed after drying. We used the Kjeldahl method to measure leaf N (Lang 1958) and a colorimetric analysis for leaf P concentration (King 1932).

At the end of the experiment, we also collected soil samples from every pot for N and P analyses. Soil chemical analyses were done at Beijing Academy of Agriculture and Forestry Science. Plant-available soil N was analysed with the alkaline hydrolysis diffusion method (Zhang et al. 2013), while plant-available P was measured colorimetrically after extraction by sodium bicarbonate (Olsen et al. 1954).

Crown area was calculated as:

$$0.5a * b$$

Where a and b were the length and width of diagonal of crown

Biomass production and allocation parameters were calculated as:

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Biomass production and allocation parameters were calculated as:

$$\text{Total biomass} = \text{main root biomass} + \text{lateral root biomass} + \text{nodule biomass} + \text{stem biomass} + \text{leaf blade biomass} + \text{petiole biomass}$$

$$\text{Root to shoot ratio} = (\text{main root biomass} + \text{lateral root biomass} + \text{nodule biomass}) / (\text{stem biomass} + \text{lamina biomass} + \text{petiole biomass})$$

$$\text{Nodule mass fraction} = \text{root nodules biomass} / \text{total biomass}$$

Data analysis

We first tested the homogeneity of variance, and then checked the normality of data. Data that did not meet normality were log transformed to improve normality and homogeneity. We used three-way ANOVA, T test and Tukey's test to analyse our data. We conducted the analyses in IBM SPSS Statistics 19 (SPSS

Inc., Chicago, IL, USA) and drew figures using Original 8.0 software (Originlab Co., Northampton, MA, USA).

Results

Effect of N deposition on plant growth

Robinia pseudoacacia had the least height and total biomass under N2 and largest under N4 (Fig. 1). Nitrogen did not significantly affect root to shoot ratio of *R. pseudoacacia* (Table 1). No two-way interactions between N level × precipitation amount or N level × precipitation frequency was found for height, total biomass, crown area and root to shoot ratio (Table 1).

Table 1

Three-way ANOVA of the effects of nitrogen (N) supply, precipitation amount (W) and precipitation frequency (F) on plant growth, biomass allocation and soil condition of *Robinia pseudoacacia*.

Measurements	F and significance						
	N	W	F	N·W	N·F	W·F	N·W·F
Height	5.409**	50.573***	12.099**	1.785	1.385	8.117***	0.865
Crown area	2.794*	59.914***	10.583**	1.584	0.580	9.010***	0.980
Total biomass	9.895***	88.135***	24.436***	2.048	1.223	8.764***	1.343
Root to shoot ratio	2.433	118.234***	0.028	1.795	1.041	4.069*	1.005
Nodule mass fraction	22.474***	37.049***	0.371	2.174*	1.545	5.567**	1.300
Chlorophyll concentration	16.126***	8.346***	10.381**	8.471***	0.770	4.761*	1.063
Leaf N concentration	7.657***	6.377**	0.139	5.877***	2.361	0.332	2.174*
Leaf P concentration	12.612***	2.016	5.532*	0.894	2.051	0.157	0.249
Leaf N:P ratio	8.863***	1.521	2.267	4.539***	0.939	0.746	0.909
Plant-available soil N concentration	7.977***	13.760***	0.679	8.167***	2.325	0.020	0.189
Plant-available soil P concentration	0.375	2.868	1.450	1.363	0.479	0.592	1.562

P refers to phosphorus in the table. Numbers in the table represent F values; asterisks indicate significant effects: *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$

Effect of N deposition × precipitation amount on leaf traits, biomass allocation and soil properties

Chlorophyll concentration of *R. pseudoacacia* was the lowest under N1 and highest under N4 with low precipitation amount, while no significant effect was observed under medium and high precipitation amount with various N deposition, which value was in between N1W1 and N4W1 (Fig. 2A). Leaf N:P ratio of *R. pseudoacacia* was the lowest at N1W1, N2W2 and N2W3, and highest at N3W3 (Fig. 2B). Leaf N:P ratio increased with increased N level in plants with low precipitation amount (Fig. 2B). Nodule mass fraction of *R. pseudoacacia* decreased as more N deposition added, especially with low or medium precipitation amount (Fig. 2C). Plant received highest N deposition and low precipitation amount had the least nodule mass fraction. Moreover, increased precipitation amount decreased the effect of N deposition on nodule mass fraction (Fig. 2C). Plant-available soil N reached the highest under N4W1 (Fig. 2D). Under low precipitation amount, plant-available soil N increased when more N deposition added, but no significant effect of N deposition was observed in medium or high precipitation amount (Fig. 2D).

Effect of precipitation amount × precipitation frequency on plant growth, biomass allocation and leaf traits

Robinia pseudoacacia received medium and high precipitation amount had significantly larger height and crown area than that received low precipitation amount, while high precipitation frequency only increased plant height and crown area under high precipitation amount (Fig. 3A, B). Plants received medium and high precipitation amount had significantly greater total biomass than that received low precipitation amount, while high precipitation frequency increased plant total biomass under medium and high precipitation amount (Fig. 3C). Plants under W2F1 had higher chlorophyll concentration than other groups (Fig. 3D). Root to shoot ratio of *R. pseudoacacia* significantly decreased as more precipitation was applied, especially under high precipitation frequency, while root to shoot ratio of plants that received medium and high precipitation amount had no significant difference under low precipitation frequency (Fig. 3E). Nodule mass fraction of *R. pseudoacacia* significantly increased as more precipitation was applied, especially under low precipitation frequency, while nodule mass fraction of plants that received medium and high precipitation amount had no significant difference under high precipitation frequency (Fig. 3F).

Responses of leaf N and P to different treatments

Leaf N concentration of *R. pseudoacacia* was higher under N1W2F1, N1W3F1 and N1W2F2; and the lowest under N3W2F1, N2W3F1 and N4W3F2 (Fig. 4A). Leaf P concentration of *R. pseudoacacia* significantly decreased when more N added and it was higher under low precipitation frequency than under high precipitation frequency (Fig. 4B, C).

Discussion

Our study demonstrated the complex interactions among N deposition, precipitation amount and precipitation frequency in affecting the growth of *R. pseudoacacia*. Growth of *R. pseudoacacia* was observed to be promoted by increased precipitation amount, increased N deposition, as well as high precipitation frequency under medium or high precipitation level. Water stress made *R. pseudoacacia* more sensitive to be promoted by N deposition.

Increased precipitation amount promoted growth of *R. pseudoacacia*, so does high precipitation frequency except for under low precipitation amount

Higher precipitation amounts significantly promoted the growth of *R. pseudoacacia* under both watering frequencies (Fig. 3). This was due to the water stress, first, inhibited leaf expansion (Hsiao 1973), and then photosynthesis (Tezara et al. 1999). With a higher precipitation amount, plants had a significantly lower root to shoot ratio under both F1 and F2, as have demonstrated in optimal partitioning theory, that plants allocate more resource to organ that acquires the most limiting resource (Ledo et al. 2018).

High precipitation frequency also significantly promoted plant total biomass and other plant growth traits with a relatively high precipitation amount (W2 and W3 in Fig. 3). So, as plants tend to increase their net photosynthesis rate after each precipitation (Yang et al. 2014), we suspect that *R. pseudoacacia* may have accumulated more biomass when plants received more times of precipitation. The above may also explain why plants had more growth under high precipitation frequency compared with low precipitation frequency with the same precipitation amount. Thus for *R. pseudoacacia*, high precipitation frequency promoted plant growth more effectively than a large precipitation event followed by a few dry days, with the same or even lower precipitation amount. With the same precipitation amount, plants received higher precipitation frequency would have better growth (Coe and Sparks 2014). On the other hand, plants responded similarly under both precipitation frequencies with low precipitation amount, as plants may keep high water-use efficiency even after precipitation due to the low precipitation level. So, we suggest that global climate change, especially extremely low precipitation frequency, regardless of the amount, would lead to a lower productivity of *R. pseudoacacia* forests, and how the extreme precipitation would affect the invasion of *R. pseudoacacia* still need further study. Meanwhile, precipitation frequency change didn't affect plant growth under drought conditions thus won't affect the invasion of *R. pseudoacacia* especially during the seedling stage.

High N deposition promoted the growth of *R. pseudoacacia*, and water stressed plants were more sensitive to N deposition

Robinia psueduacacia had significantly higher total biomass under N4 (33% and 45% higher respectively, Fig. 1B) than under N1 and N2 after combining different precipitation regimes. This may be different from previous studies on the effect of N fertiliser on *R. psuedoacacia*, in which biomass of *R. psuedoacacia* was not significantly affected by N fertiliser (Wang et al. 2021a; Wang et al. 2018b). However, unlike previous studies that are watered in accordance with plants requirement, our plants were supplied with various precipitation regimes, and high precipitation may have magnified the effect of N to some extent. In the present experiment, like many non-fixing species such as *Calluna vulgaris* and *Senna surattensis* (Carroll et al. 1999; Wang et al. 2018b), the growth of *R. psuedoacacia* was significantly promoted by N deposition.

Compared with the soil before starting experiment (87.7 mg kg^{-1}), soil after plant growth still contained about 60 - 70 mg kg^{-1} plant-available N on average (Fig. 2D). Like invasive species *Bromus tectorum*, *R. psuedoacacia* also lowered the soil N during growth (Evans et al. 2001). Though *R. psuedoacacia* is an N_2 -fixing species, its N_2 -fixation still cannot compensate the N loss in soil and absorbing N from soil is still needed, which may in turn lower the risk of other invasions due to a lower soil N (Perry et al. 2010).

Under low precipitation amount, plants received higher N deposition had significantly higher chlorophyll concentration and higher leaf N:P ratio than received lower N deposition, and a balanced N:P ratio around 15 (Koerselman and Meuleman 1996) under high N deposition. This stronger response of leaf traits under low precipitation amount may be explained by the fact that water availability affect ion mobility in soil, thus plants growing in dry places are more likely to be nutrient limited than in wet places, and nutrient supply under dry area are more effectively promoting plant growth (Chapin 1991; Kreuzwieser and Gessler 2010). So, plants under low precipitation amount were more sensitive to N deposition.

Nodule mass fraction of *R. psuedoacacia* significantly decreased with more N deposition under all precipitation amounts. Nitrogen fixation, which costs more energy compared with absorbing N from soil (Lambers et al. 2002), is only favourable when absorbing N from soil is becoming more expensive for satisfying the plant N needs (Rastetter et al. 2001); nitrate in soil can also inhibit nodulation and N_2 fixation (Cowie et al. 1990; Hansen et al. 1992). In condition of facultative N_2 fixation, plant allocation to bacterial symbionts is controlled by the cost of fixation compared with directly absorbing N with root (Menge et al. 2009). So, when more N was added in our experiment, plants allocated less biomass to nodule thus decreased N_2 -fixation rate. As to the effects of precipitation amount on N_2 fixation, nodule mass fraction of *R. psuedoacacia* was significantly higher at a high precipitation amount (Fig. 3F). This might be because increased precipitation amount increased plant relative growth rate (Hsiao 1973; Tezara et al. 1999) thus increased the demand of N and the investment of nodule biomass. Plants under low precipitation amount had more dramatic decrease in nodule ratio as N deposition increased compared with under medium and high precipitation amount, so high N deposition may cause a stronger inhibition of nodule growth and N_2 fixation under dry condition than plants under well-watered condition.

Phosphorus-utilisation efficiency was affected by both N deposition and precipitation frequency

Leaf P concentration in our experiment significantly increased when plants received higher precipitation frequency (Fig. 4C). Leaf P concentration equals to the reciprocal of P-utilisation efficiency, so in our experiment (Wang et al. 2021b), plant P-utilisation efficiency increased when precipitation frequency decreased. Interestingly, precipitation amount did not significantly affect P-utilisation efficiency of *R. pseudoacacia*. It is also worth noting that leaf P concentration in our experiment significantly decreased when more N was added to plants (Fig. 4B). So, in our experiment, plant P-utilisation efficiency increased when N deposition increased. With higher precipitation, P would be a limitation for *R. psuedoacacia*, rather than water and N. Moreover, many previous studies have shown that P is very important in N₂ fixation because nodules are P sinks (Schulze et al. 2006; Wang et al. 2018a) and N₂-fixing processes are P consuming (Raven 2013). A lack of P in legumes would lead to cease of N₂ fixation (Sa and Israel 1991; Schulze et al. 2006). Only N limitation in soil would promote N-fixation thus won't lead to N limitation in legumes, but both N and P limitation in soil would lead to N limitation. So, further studies should still be conducted in N₂-fixing species on the relationship between the P-utilisation efficiency and water-utilisation efficiency.

Altered N absorbing mechanism of *R. pseudoacacia* under various N and water status

Leaf N concentration of *R. pseudoacacia* under N1W2F1, N1W3F1 and N1W2F2 was significantly higher than that under N3W2F1, N2W3F1 and N2W2F2 (Fig. 4A). The total biomass of plants under both N1 and N2 group was very similar (Fig. 1B), so *R. pseudoacacia* with no N deposition (N1) absorbed more N than that with low N deposition (N2). Leaf N:P ratio of *R. pseudoacacia* under N2W2 and N2W3 was also slightly lower than that in other groups (Fig. 2B). However, as plant-available soil N was very similar under N1 and N2 with medium and high precipitation amount, the more N that *R. pseudoacacia* absorbed under N1 was mainly from N₂ fixation. This meant that extra N added to plants in N2 not only had no significant promotion effect on its growth, but also decreased its N₂-fixation rate and N concentration in leaves. However, as more N added in group N3 and N4, growth of *R. pseudoacacia* significantly increased and leaf N concentration was similar to or higher than that in N2 especially under medium and high precipitation amount. This was due to the fact that N₂-fixation is more energy costing, and is less likely to be undertaken when more N is in soil (Lambers et al. 2002). This result gave us a new insight of the effects of low N deposition in environment, which may not only decrease plant N₂-fixation rate, but also did not provide enough N for plants. Slightly N deposition might inhibit N₂ fixation rate, but haven't reached the threshold of promoting plant growth under medium or high precipitation amount in our experiment. Plants under this condition might have lower leaf N concentration and leaf N:P ratio, compared with plants received no or high N supply, thus may led to a limited growth due to N limitation at least in the early growing stage. Due to this strategy, under the level of low N deposition (N2), which is similar to the N deposition level in China right now, *R. pseudoacacia* as an alien species receiving

medium or high precipitation amount might not be facilitated, and its invasion won't be promoted until N deposition reached certain level; while very high N deposition would not only increase soil N pool but also increase the risk of *R. pseudoacacia* invasion.

However, plants under low precipitation levels had another story. Nodule biomass was more significantly decreased when receiving more N deposition, and leaf N concentration and leaf N:P ratio was not significantly affected by N deposition. No significant low leaf N were observed under slightly N supply, which might indicate a different N utilisation strategy compared with plants under medium and high precipitation level.

Conclusions

Larger precipitation amount, higher precipitation frequency and higher N deposition all promoted plant growth in present study. With a high precipitation amount (1000 mm precipitation annually), plant growth under high precipitation frequency was greater than under low precipitation frequency. More significant response of *R. pseudoacacia* leaf traits and nodule traits to N under low precipitation was observed compared with plants with medium and high precipitation amount. The inhibition of N₂ fixation by slightly N supply led to lower leaf N concentration of *R. pseudoacacia* which may cause a further inhibition of growth, especially under sufficient water treatments. Alteration between N₂ fixation and N absorption from soil may exist in *R. pseudoacacia* depending on the amount of N supply. Plant growth of *R. pseudoacacia* in dry places would more likely to be promoted by N deposition, and extreme precipitation events (larger precipitation amount with low precipitation frequency) would lower the growth of plants. High N deposition level, and increased precipitation frequency would higher the risk of *R. pseudoacacia* invasion.

Declarations

Author contributions

XW, ND and WG conceived and designed the experiments. XW performed the experiments. XW, XG, JP and WD analysed the data. XW, XG, WD, ND, WG, JP wrote the manuscript.

Conflict of interest

The authors declare that they have no financial or non-financial conflict of interest. And this article does not contain any studies with human participants or animals performed by any of the authors.

Contribution to the field statement

Robinia pseudoacacia is one of the most widely spread planted tree species in the world. It is very important that how climatic change would affect this species especially in a non-native ecosystem. We grew *R. pseudoacacia* seedlings at four different N levels combined with different precipitation regimes,

including three precipitation amounts, and two precipitation frequencies. The results showed that growth of *R. pseudoacacia* was promoted by increased precipitation amount, increased N deposition, as well as high precipitation frequency under medium or high precipitation level. Water stress made *R. pseudoacacia* more likely to be promoted by N deposition. High N deposition level, and increased precipitation frequency may increase the invasion risk of *R. pseudoacacia*.

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Figures

Figure 1

Effects of different levels of nitrogen (N) supply (N1: 0 mg per pot, N2: 22.5 mg per pot, N3: 89 mg per pot and N4: 179 mg per pot for each treatment) under averaged precipitation regimes on plant growth of *Robinia pseudoacacia*. A: Height, B: Total biomass, mean $\pm$ SE, n = 48. Different lower-case letters for each bar denote significant differences of different N level under certain precipitation regime (p < 0.05)

Figure 2

Effects of different levels of nitrogen (N) supply (N1: 0 mg per pot, N2: 22.5 mg per pot, N3: 89 mg per pot and N4: 179 mg per pot for each treatment) under various precipitation amounts (W1: 90 ml per pot, W2: 270 ml per pot and W3: 450 ml per pot average to a day) under averaged precipitation frequency on plant and soil traits of *Robinia pseudoacacia*. A: Chlorophyll concentration, B: Leaf N:P ratio, C: Nodule mass fraction, D: Plant-available soil N; mean $\pm$ SE, $n = 16$. Different lower-case letters for each bar denote significant differences of different N levels and different precipitation amount under averaged precipitation frequency ($p < 0.05$)

Figure 3

Effects of different precipitation amounts (W1: 90 ml per pot, W2: 270 ml per pot and W3: 450 ml per pot average to a day) and different precipitation frequencies (F1: watered once a day and F2: watered once every five days) under averaged nitrogen (N) supply on plant growth and plant traits of *Robinia pseudoacacia*. A: Height, B: Crown area, C: Total biomass, D: Chlorophyll concentration, E: Root to shoot

ratio, F: Nodule mass fraction; mean $\pm$ SE, n = 32. Different lower-case letters for each bar denote significant differences of different precipitation regimes under averaged N supply ($p < 0.05$)

Figure 4

Effects of different levels of nitrogen (N) supply (N1: 0 mg per pot, N2: 22.5 mg per pot, N3: 89 mg per pot and N4: 179 mg per pot for each treatment) , various precipitation amounts (W1: 90 ml per pot , W2: 270 ml per pot and W3: 450 ml per pot average to a day) and different precipitation frequencies (F1: watered

once a day and F2: watered once every five days) on plant leaf N concentration (A) of Robinia pseudoacacia and effect of different precipitation amount (B) and precipitation frequency (C) on plant leaf phosphorus (P) concentration of Robinia pseudoacacia. Different lower-case letters for each bar denote significant differences of different treatments ($p < 0.05$)