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Precipitation pattern alters the effects of nitrogen deposition on the growth of alien species *Robinia pseudoacacia*

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A R T I C L E I N F O

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A B S T R A C T

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 access how the growth of *Robinia pseudoacacia*, a widely distributed and cultivated N_2-fixing alien species, is affected by both the pattern of precipitation and N supplies.
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, despite receiving the same amount of precipitation. Higher N supply reduced biomass allocation to nodules. Under low precipitation level, nodule growth and N_2 fixation of *R. pseudoacacia* was more inhibited by high N deposition compared with plants under higher precipitation level. Even slightly N deposition under higher precipitation inhibited N_2 fixation but it was insufficient to meet the N needs of the plants.
Conclusions: Even at low levels, N deposition might inhibit N_2 fixation of plants but low N in soil cannot meet the N requirements of plants, and caused N_2 fixation limitation in plants during seedling stage. There was likely a transition from N_2 fixation to acquisition of N from soil directly with root when N supply was increased.

1. Introduction

Anthropogenic nitrogen (N), which largely comes from industrialized production of fertilizer, has doubled the input of N into the global N cycle [1]. Due to human activities, N deposition has been increasing in the past century [2]; which including. ammonium (NH_4^+), mostly derived from agriculture, and N oxides (NO_x), mostly released during fossil fuel combustion [3,4]. Nitrogen deposition

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is predicted to reach a level as high as 200 Tg year^{-1} by 2050 globally [2], however N deposition is observed to be more complicated in China, which peaked in 2011 and then went down [5]. A lack of N limits plant productivity in many ecosystems with young soils [6,7], and has affected the net primary productivity globally [8]. However, increased N deposition would not only decrease biodiversity in many ecosystems [9], inhibit N_2 fixation in many legume species [10]; but also promote invasive plant growth more than native plant [11–13]. Invasive plants increase N pool in soil, consequently accelerating N cycle of ecosystem; while removing N from soil would lower the risk of invasion [14–16]. Thus, it is very important to study how complicated N deposition scenario is affecting invasive plant growth, especially in China.

The effect of N on plants is related to precipitation in many ways [3,17]. First, wet deposition of N, as a major deposition pathway [18,19], happens with precipitation. Second, water availability in soil directly affects ion mobility and availability [20,21] and decreased soil moisture could lead to N deficiency [22]. Water is also significantly related to N mineralization by influencing the activity of microorganisms in soil [17]. In some dry places, water and N co-limit plant growth, but appropriate levels of N can enhance a plant's water use efficiency [23]. On the other hand, N fertilization or deposition affect the sensitivity of stromata to water potential, and plant water use efficiency [24,25].

Precipitation has been changing over the past decades due to climatic change [26,27]. Extreme precipitation events and extreme droughts have happened more frequently in recent years [28,29]. Increased precipitation amount and frequency increases seed germination [30,31], plant growth [32,33] and yield [34], and it is also worth noting that the number of heavy precipitation events increased in many regions [35], and some plants are sensitive to these large rainfall events [36]. Moreover, the same amount of annual mean precipitation may lead to different performance of plants under different precipitation pattern (frequencies) [37].

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 been naturalized on other continents [38], and has been growing in wide range of areas including China [39–41]; and in China, the area of *R. pseudoacacia* exceeded 70,000 ha just in Loess Plateau [42]. The species is drought resistant and possesses N_2-fixing nodules [43]. Nitrogen fixation makes it more competitive in many natural ecosystems, especially in arid habitats [44]. So, it remains an open question how *R. pseudoacacia* would perform both under elevated N supply and altered precipitation regimes [38,43,45]. 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 and frequency would promote the growth of *R. pseudoacacia* under both precipitation frequencies.
(2) Due to the less availability of N under water stress, N supply promotes the growth of *R. pseudoacacia* and reduce nodule biomass especially under low precipitation level.

2. Materials and methods

2.1. 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 brown loam (the parent material is granite wash) 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.

2.2. 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, 2.5 (current N deposition level), 10 and 20 g m^{-2} N, that is 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, current N deposition, high N deposition and very high N deposition or N contamination [46]. NH_4NO_3 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 [47]. 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.

2.3. 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 [48].

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 [49] and a colorimetric analysis for leaf P concentration [50].

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 [51], while plant-available P was measured colorimetrically after extraction by sodium bicarbonate [52].

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:

$$\text{Total biomass} = \text{root biomass} + \text{stem biomass} + \text{leafbiomass Root biomass}$$
$$= \text{main root biomass} + \text{lateral root biomass} + \text{nodule biomassRoot to shoot ratio} = \text{root biomass}/(\text{stem biomass} + \text{leaf biomass})$$

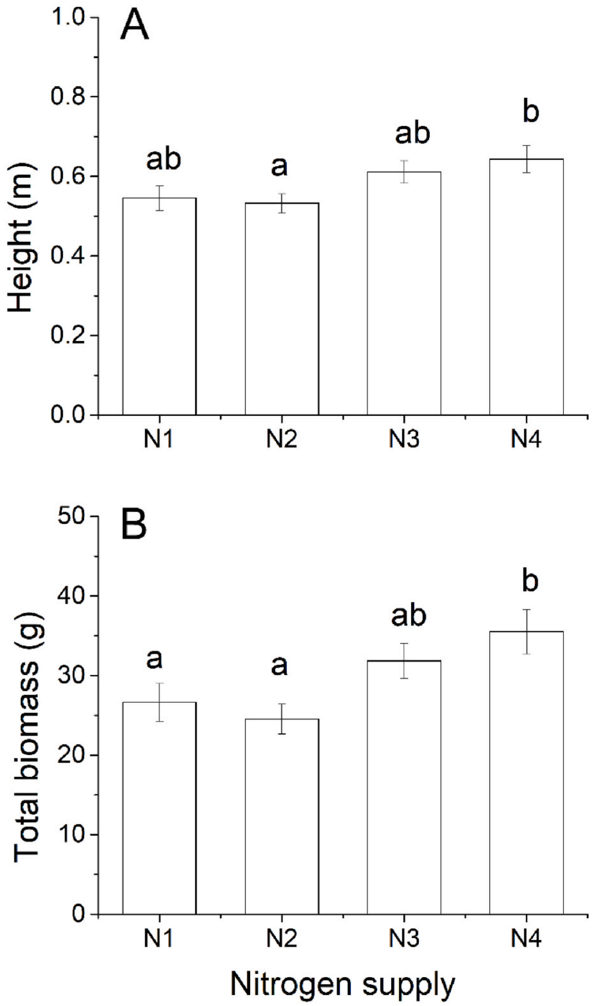

Fig. 1. Effects of different levels of nitrogen (N) supply (N1: 0 mg per pot, N2: 22.5 mg per pot, N3: 89 mg per pot and N4: 179 mg per pot for each treatment) under averaged precipitation regimes on plant growth of *Robinia pseudoacacia*. A: Height, B: Total biomass, mean ± SE, n = 48. Different lower-case letters for each bar denote significant differences of different N level under certain precipitation regime ($p < 0.05$).

Nodule mass fraction = nodules biomass/total biomass

2.4. 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).

3. Results

3.1. 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).

3.2. 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 W1, while no significant effect was observed under W2 and W3 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 under W1, while no significant difference was observed between different precipitation amount under certain N treatment (Fig. 2B). Nodule mass fraction of *R. pseudoacacia* decreased as more N deposition added, especially with W1 or W2 (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 W1, plant-available soil N increased when more N deposition added, but no significant effect of N deposition was observed under W2 and W3 (Fig. 2D).

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

Robinia pseudoacacia under W2 and W3 had significantly larger height and crown area than that under W1, while high precipitation frequency (F1) only increased plant height and crown area under high precipitation amount (Fig. 3A and B). Plants under W2 and W3 had significantly greater total biomass than that under W1, while high precipitation frequency increased plant total biomass under W2 and W3(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 under W2 and W3 had no significant difference with that under W1 (Fig. 3E). Nodule mass fraction of *R. pseudoacacia* significantly increased as more precipitation was applied, especially under F2, while nodule mass fraction of plants that received medium and high precipitation amount had no significant difference under high precipitation frequency (Fig. 3F).

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 pseodoacacia*.

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 * $P < 0.05$.

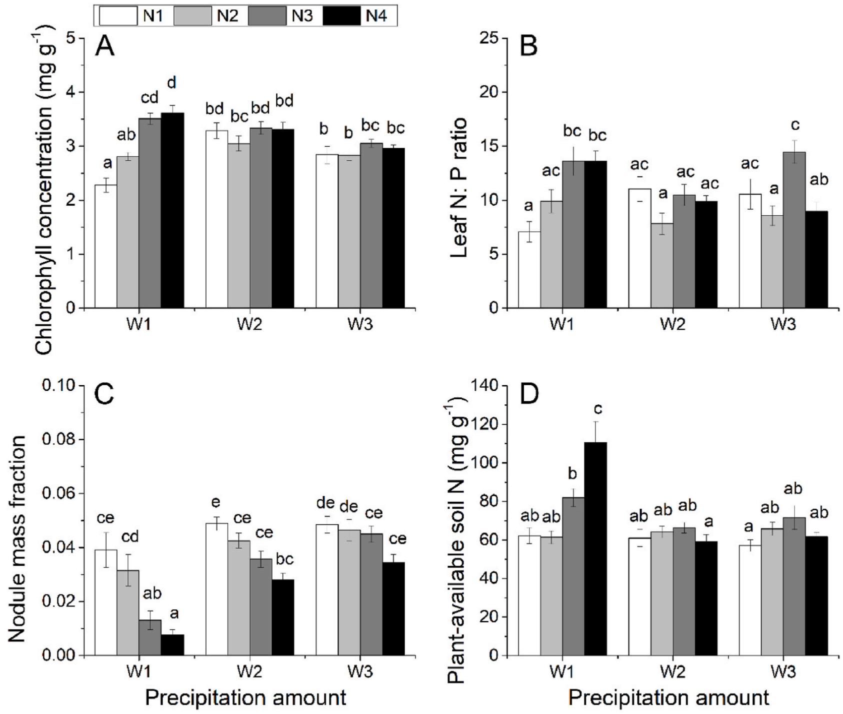

Fig. 2. Effects of different levels of nitrogen (N) supply (N1: 0 mg per pot, N2: 22.5 mg per pot, N3: 89 mg per pot and N4: 179 mg per pot for each treatment) under various precipitation amounts (W1: 90 ml per pot, W2: 270 ml per pot and W3: 450 ml per pot average to a day) under averaged precipitation 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 ± 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 ($p < 0.05$).

3.4. 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 and C).

4. Discussion

This 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.

4.1. 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 might be due to the water stress, first, inhibited leaf expansion [53], and then photosynthesis [54]. 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 [55]. 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 [56], 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 [37]. 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

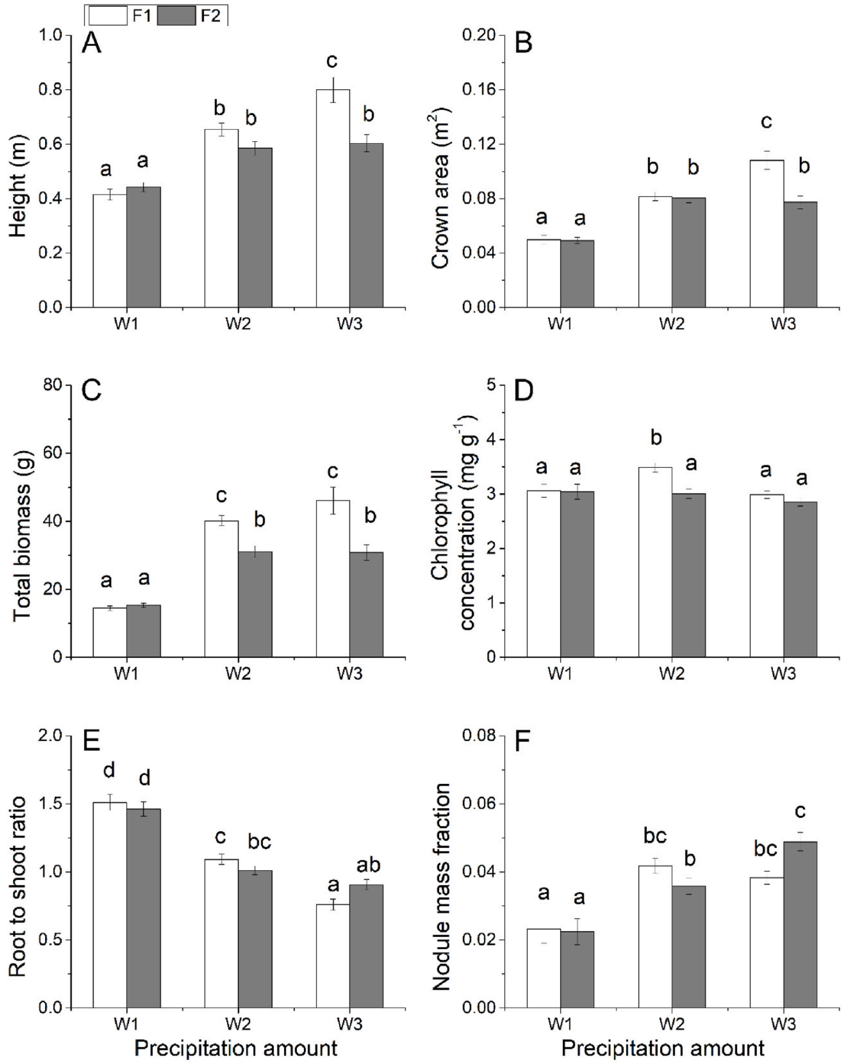

Fig. 3. Effects of different precipitation amounts (W1: 90 ml per pot, W2: 270 ml per pot and W3: 450 ml per pot average to a day) and different precipitation frequencies (F1: watered once a day and F2: watered once every five days) under averaged nitrogen (N) supply on plant growth and plant traits of *Robinia pseudoacacia*. A: Height, B: Crown area, C: Total biomass, D: Chlorophyll concentration, E: Root to shoot ratio, F: Nodule mass fraction; mean ± SE, n = 32. Different lower-case letters for each bar denote significant differences of different precipitation regimes under averaged N supply ($p < 0.05$).

precipitation level. So, it is suggested 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.

4.2. 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. pseudoacacia* was not significantly affected by N fertiliser [57,58]. 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* [58,59], the growth of *R. pseduoacacia* 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. pseduoacacia* also lowered the soil N during growth [60]. Though *R. pseduoacacia* is an N_2-fixing species, its N_2-fixation still cannot compensate the N loss in soil and absorbing N from soil

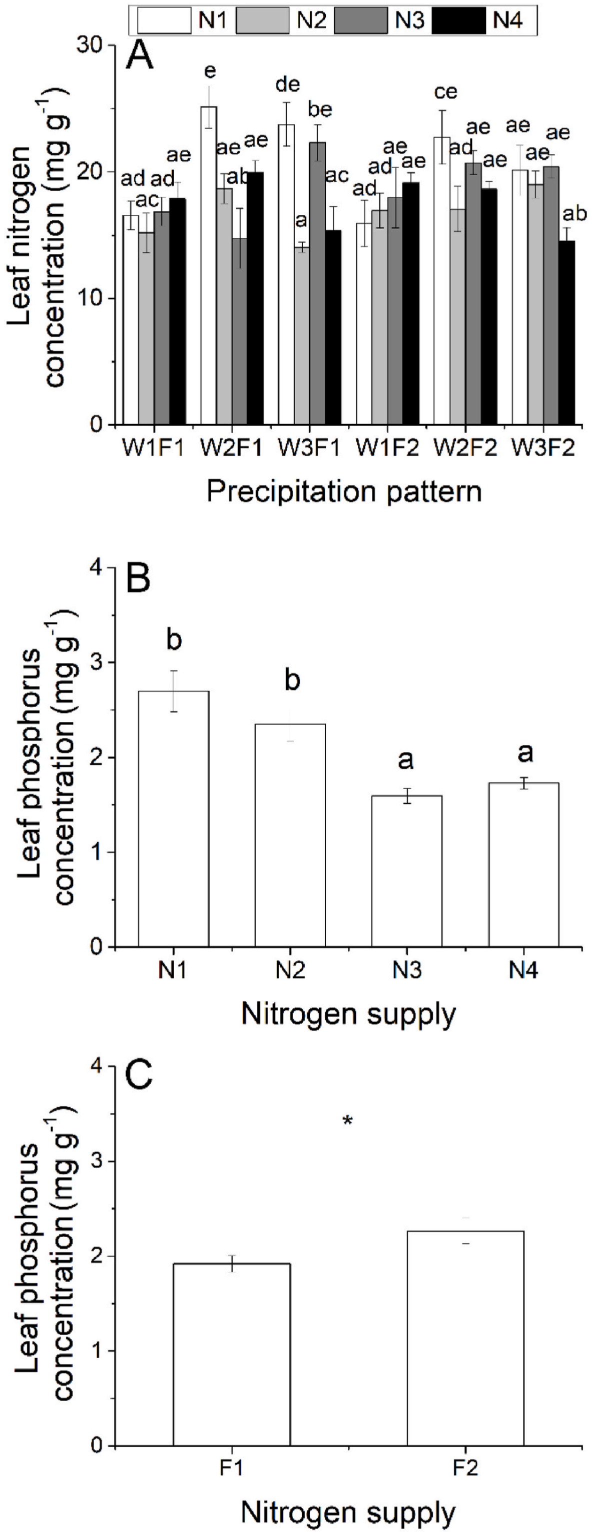

Fig. 4. Effects of different levels of nitrogen (N) supply (N1: 0 mg per pot, N2: 22.5 mg per pot, N3: 89 mg per pot and N4: 179 mg per pot for each treatment), various precipitation amounts (W1: 90 ml per pot, W2: 270 ml per pot and W3: 450 ml per pot average to a day) and different precipitation frequencies (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 ($p < 0.05$).

is still needed, which may in turn lower the risk of other invasions due to a lower soil N [61].

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 [62]. 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 [20,21]. So, plants under low precipitation amount were more sensitive to N deposition.

Nodule mass fraction of *R. pseudoacacia* significantly decreased with more N deposition under all precipitation amounts. Nitrogen fixation, which costs more energy compared with absorbing N from soil [63], is only favourable when absorbing N from soil is becoming more expensive for satisfying the plant N needs [64]; nitrate in soil can also inhibit nodulation and N_2 fixation [65,66]. 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 [67]. 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. pseudoacacia* was significantly higher at a high precipitation amount (Fig. 3F). This might be because increased precipitation amount increased plant relative growth rate [53,54] 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.

4.3. 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 [68], 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). As the plant P-utilisation efficiency is the inverse ratio of P concentration [68], 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 N_2 fixation because nodules are P sinks [69,70] and N_2-fixing processes are P consuming [71]. A lack of P in legumes would lead to cease of N_2 fixation [69,72]. 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 N_2-fixing species on the relationship between the P-utilisation efficiency and water-utilisation efficiency.

4.4. 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 N_2 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 N_2-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 N_2-fixation is more energy costing, and is less likely to be undertaken when more N is in soil [63]. This result gave us a new insight of the effects of low N deposition in environment, which may not only decrease plant N_2-fixation rate, but also did not provide enough N for plants. Slightly N deposition might inhibit N_2 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 N2 which is similar to the N deposition level in China right now (with N around 2 g m^{-2}), *R. pseudoacacia* receiving medium or high precipitation amount might not be facilitated, while very high N deposition would increase soil N pool.

However, plants under low precipitation levels performed very differently from plants received higher precipitation level. 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 current N deposition level, which might indicate a different N utilisation strategy compared with plants under medium and high precipitation level.

5. 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 N_2 fixation by the current nitrogen deposition level (with yearly N around 2 g m^{-2}) led to lower leaf N concentration of *R. pseudoacacia* which may cause a further inhibition of growth, especially under sufficient water treatments. Alteration between N_2 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 increase the growth of *R. pseudoacacia*.

6. 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*.

Data availability statement

The related data is not available now, and would be available on request.

CRediT authorship contribution statement

Xiao Wang: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Xiao Guo:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Wenli Ding:** Data curation, Formal analysis, Methodology, Software, Writing – review & editing. **Ning Du:** Conceptualization, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Weihua Guo:** Conceptualization, Funding acquisition, Methodology, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing. **Jiayin Pang:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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