N addition alters non-structural carbohydrates and C:N:P stoichiometry in different organs of *Reaumuria soongorica* seedlings in Gobi Desert of the Northwest, China

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Abstract

Reaumuria soongorica is an important biological barrier for ecological protection in Gobi desert of the Northwest, China, where soil nitrogen availability is low. N deposition increasing significantly in Gobi desert, so that the response of *R. soongorica* to N enrichment may become a problem. However, little is known the effects of N addition on biomass, non-structural carbohydrates (NSC), and carbon:nitrogen:phosphorus (C:N:P) stoichiometry of *R. soongorica* in this region. Here, we examined changes in biomass, NSC and C:N:P ratios of different organs for one year growth of *R. soongorica* at four N treatments which is 0 (N_0), 4.6 (N_1), 9.2(N_2), and 13.8 (N_3) $\text{g m}^{-2} \text{ year}^{-1}$. The N addition (reach to $9.2 \text{ g m}^{-2} \text{ year}^{-1}$) significantly enhanced the biomass of different organs, meanwhile the belowground:aboveground ratio was also significantly increased. The NSC concentration of root significantly enhanced under N addition treatments, but the stem and leaf NSC concentration was only increased significantly at N_1 and N_2 addition. N addition only enhanced the soluble sugar concentration of leaf and root, and reduced starch concentration of different organs. The N concentration of stem and root were significantly enhanced at N_2 and N_3 addition and the leaf N concentration was only increased in N_3 addition, but N addition had no significant effect on C and P concentrations. The stem and leaf C:N ratio were reduced significantly in N_2 and N_3 treatments, but the root C:N ratio was decreased significantly in N addition. The N_3 addition significantly increased N:P ratio of different organs. Our results suggested N addition changed the biomass, NSC, N concentration, C:N and N:P ratio of different organs, meanwhile the root responded more strongly than stem or leaf, this is beneficial for absorbing more water in arid soil in this region, then to ensure the survival of *R. soongorica* seedlings.

Introduction

Since the industrial revolution, atmospheric nitrogen (N) deposition has increased rapidly due to the widespread use of N-containing synthetic fertilizers and increased fossil fuel combustion, and the influence of N deposition on land ecosystems has become one of the research hotspots^{1,2}. The study showed that N deposition affected plant photosynthesis and nutrient transport³, but as the main product of photosynthesis, the existence form and distribution of carbohydrates in plant organs will be affected by N deposition⁴. Meanwhile N deposition has also been shown to affect the stoichiometry(C, N, P) of plant in terrestrial ecosystems and thus alter the physiological activity and growth rates⁵.

The non-structural carbohydrate(NSC) include soluble sugars and starch, it is the main energy supply substrate for plant growth, development and reproduction, and it plays the function of metabolism, transporting water and assimilation products, osmotic regulation and reservoir pool^{6,7}. So understanding the NSC concentration of plants and its changes in various organs is the ideal entry point to explore the response and adaptation of plant growth and physiological ecological process. Facing environmental stress, plants will change C allocation among different organs. For example, drought either significantly increased or maintained the total NSC concentration in the above-ground organs, but reduced the total NSC concentration in the sapling roots⁸. Compared to without any fertilization, the NSC in leaf, stem, and

root of *Moringa oleifera* seedlings were greatly reduced by N and P fertilization which could be explained by the dilution effects of increased biomass following fertilization⁹. It is seen that NSC and its components (soluble sugar and starch) showed different responses to different environmental factors among different organs^{10,11}. So understanding the change pattern of NSC under different environmental stress, has become an important means to explore the plant adaptation strategy under different environmental stress.

Carbon (C), nitrogen (N) and phosphorus (P) are structural elements and the major nutrient elements that play an important role in maintaining the biogeochemical cycle and ensuring both nutrient cycle and energy flow within ecosystem¹². Many researches found N addition significantly increased the C, N and P contents of plant organs^{13,14,15}. Plant C:N and C:P ratios usually increased¹⁶ or decreased¹⁷ under N addition, but plant N:P ratio maybe increased¹⁵, decreased¹⁸ or maybe not affected¹⁷ depending on species¹⁸ and soil nutrient conditions¹⁹. However, most studies have focused on aboveground stoichiometries rather than the level of whole plants^{20,21}, the responses of plant different organs to N addition remain largely unexplored in Gobi desert region.

Under natural conditions, *R. soongorica* can reproduced by seeds, The success of its natural renewal mainly depends on the seed germination and seedling survival conditions. For Gobi desert regions, nitrogen was a key factor which restricted vegetation growth, distribution and the restoration of damaged ecosystems, so the germination of *R. soongorica* seeds and the survival of seedlings are bound to be affected by nitrogen. N deposition maybe is beneficial for the *R. soongorica* growth, but the researches about *R. soongorica* had focused on growth^{22,23}, photosynthetic physiological properties^{24,25} and genes²⁶ in recent years, however little is known about the biomass, C:N:P stoichiometry and NSC distribution in *R. soongorica* seedlings under N addition in Gobi desert region of the northwest China, especially the differences among different organs. So in order to evaluate the distribution of C:N:P stoichiometry and NSC content for different organs and how nitrogen changes may alter these distribution for *R. soongorica*, we hypothesized that (1) N addition would significantly enhance the different organs biomass, but the aboveground (stem and leaf) biomass would respond more strongly than belowground (root) which result in lower belowground:aboveground ratio; (2) N addition would significant enhanced N concentration of plant organs, and have stronger effect on N concentration and C:N ratio of root than shoot (3) N addition significantly enhanced the NSC content of different organs. The results are important to reveal the desert plants response and adaptation mechanisms under global climate change.

Results

Biomass in different organs

With increasing N addition, the total, stem and root biomass was increased significantly and reached the maximum value at N₂ addition treatment ($P<0.05$). Compared with N₀ treatment, the total, stem, leaf and

root biomass increased by 60.49%, 48.86%, 42.85% and 97.5% in N₂ addition treatment, respectively. The ratio of belowground to aboveground biomass was also significantly increased in N₁ and N₂ treatment because the increase of root biomass was more rapid than that of stem and leaf biomass ($P<0.05$), but then reduced in N₃ addition treatment (**Table1**).

NSC concentration in different organs

The soluble sugar concentration, starch concentration and NSC concentration for different organs was different with increasing N addition. With increasing N addition, the soluble sugar concentration for stem, leaf and root were all increased significantly at the N₁ and N₂ addition treatments, but then it decreased significantly at the highest N₃ treatment except of the stem (Fig. 1a) ($P<0.05$). The starch concentration for stem, leaf and root were reduced significantly in N₂ and N₃ addition treatments (Fig. 1b) ($P<0.05$). Compared with N₀, the N addition (N₁-N₃) significantly enhanced the NSC concentration of root and reached the maximum value at N₂ addition treatment, but the stem and leaf NSC concentration was only increased significantly at N₁ and N₂ addition (Fig. 1c) ($P<0.05$).

Nutrient concentrations and stoichiometry in different organs

The N addition had no significant effect on C concentration and P concentration of different organs (Fig. 2a, 2c). Compared with N₀, The N concentration of stem and root significantly increased in N₂ and N₃ addition treatments; the leaf N concentration significantly enhanced in N₃ addition treatment ($P<0.05$). The N concentration of different organs had no significant difference between N₀ and N₁ addition treatment (Fig. 2b).

The stem and leaf C:N ratio was all reduced significantly in N₂ and N₃ addition treatments, but the root C:N ratio was decreased significantly with increasing N addition ($P<0.05$) (Fig. 2d). Compared with N₀, the C:P ratio of different organs all had some decreasing, but it had no significant difference among N addition treatments ($P>0.05$) (Fig. 2e). The higher N addition (N₂-N₃) significantly enhanced the stem N:P ratio, but the leaf and root N:P ratio was only enhanced in N₃ addition treatment (Fig. 2f) ($P<0.05$).

Discussion

Effect of N addition on plant biomass. Plant growth is closely related to nutrients, especially for nutrient-deficient soil. Soil nitrogen content is lower and it becomes one of the main limiting factors for plant productivity in arid and semi-arid region of northwest China²⁷. Contrary to our first hypothesis, we found N addition (reach to $9.2\text{ g m}^{-2}\text{ year}^{-1}$) had significantly enhanced the biomass of different organs, but it was reduced significantly at higher level ($13.8\text{ g m}^{-2}\text{ year}^{-1}$), this is consistent with the results of Jin et al.¹³ this finding indicates that appropriate N addition is beneficial to the growth of *R. soongorica* in Gobi desert region, but if the N addition exceeded the minimum N demanded of plant growth, the plants can be less sensitive to N addition²⁸.

The previous study showed that plants can increase their allocation of photosynthetic products to belowground organs under lower 0.15mM N condition²⁹, but with the N addition, the biomass allocation are species-specific^{4,30}. We found the response of roots biomass to N addition(reach to 9.2g m⁻² year⁻¹) was significantly higher than the shoot biomass, thus result in higher belowground:aboveground ratio, this was inconsistent with the previous study⁴ and our hypothesis. The reason maybe is in order to absorbed more water from the soil in Gobi desert region, the root biomass of *R. soongorica* was increased more than the stem and leaf under N addition condition.

Effect of N addition on NSC. NSC is the product of photosynthesis and provide energy for plant growth and metabolism, meanwhile it play an important role in the response of plants to environmental changes³¹. Some studies reported that N addition increased the NSC accumulation³⁰, but other studies found N addition had no effect on or even decreased NSC concentration^{4,9}. In this study, we found a significantly increase in NSC concentration of different organs of *R. soongorica* within a certain range of N addition (0 -9.2 g N m⁻² year⁻¹), but it had some decreasing in highest N addition (13.8g N m⁻² year⁻¹), this mainly due to significant increasing in soluble sugar concentration and reducing in starch concentration, but this finding not supported our third hypothesis and was consistent with the results of other studies^{4,32}. Meanwhile some research reported the accumulation of higher NSC concentrations can balance the osmotic pressure of cells³³ and be used to cope with environmental stress, thus the highest NSC concentration in different organs under middle N addition (9.2g m⁻² year⁻¹) in our study indicated adding proper amount of N will benefit for the NSC accumulation and improve the resistance of *R. soongorica* to water stress in Gobi desert region, and then it is beneficial for the successful settlement of *R. soongorica* in this region .

In this study, significant NSC increasing was observed in roots under N addition condition than stem and leaf NSC, this is consistent with other research^{4,30}, but the difference is the root NSC was higher than stem and leaf NSC in our study, Ai et al⁴ found the aboveground NSC was higher than belowground NSC, this difference may be related to the characteristics of carbon allocation and transport³⁴. In order to facilitate water absorption and survive in Gobi desert region, the more carbon of *R. soongorica* allocated to the root than stem and leaf³⁵.

Effect of N addition on plant stoichiometry. Stoichiometry reflected the utilization ability of C, N and P for plant, which are susceptible to environmental changes³⁶. Many studies have concluded that N addition will resulted in higher soil N availability, and thus enhanced N concentrations and declined C:N ratios in many species^{37,38}, some researches also found N addition significantly increased the C and N contents of plants and no significantly effect on C:N ratio^{14,39}. In this study, we found that N addition increased the N concentration and reduced C:N ratio of *R. soongorica*, but no significantly effect on N and P concentrations and C:P ratio, this is consistent with our second hypothesis and many previous studies^{17,40}. N:P ratio has been interpreted as an indicator of N and/or P limitation^{41,42}. It is widely accepted that N:P ratios < 10 indicate N limitation. Therefore, *R. soongorica* shows very low N:P ratios due

to relatively high P concentrations in higher N addition treatment, it is indicated this plant would still be N limited despite the massive N doses that were applied. this is consistent with the result of Wang et al.⁴³ who found the N:P ratio of *R. soongorica* was lower in the arid desert region, but it is inconsistent with the result of Niu et al.⁴⁴ who found the N:P ratios of six shrubs(including *R. soongorica*) in the desert of the Alxa Plateau all were higher. This inconsistent results maybe related with ecosystem types and environmental factors⁴⁵.

The responses of stoichiometry of different organs was different when environmental changes⁴⁶. For example, some studies showed that leaf N and P concentration and N:P were higher than those of the other organs under N addition¹⁵. Xiao et al.³⁰ reported that N addition increased N concentration in roots than shoots, but decreased P concentration and increased N:P in all organs. In this study, we found N addition significantly increased N concentrations and decreased C:N the in roots than aboveground organs, this supporting our second hypothesis, the reason maybe is that N addition enhanced soil N availability, but the absorbed N by roots can not be transferred to the aboveground organs because of lower soil water in Gobi desert region.

Conclusions

The total and other organs biomass significantly enhanced with a range of N addition($0-9.2\text{ g m}^{-2}\text{ year}^{-1}$), and the increasing of root biomass more rapidly than the stem and leaf biomass which resulted in higher belowground:aboveground ratio. N addition enhanced the soluble sugar concentration, but reduced the starch concentration of all organs, and the NSC concentration of root significantly enhanced at all N addition, but the stem and leaf NSC concentration was only increased significantly at N_1 and N_2 addition. The N concentration was increased at higher N addition($9.2-13.8\text{ g m}^{-2}$), but it had no significant effect on C and P concentrations, thus resulted in the C:N ratio reducing significantly. This indicated N addition changes the biomass, NSC, N concentration, C:N and N:P ratio of different organs, meanwhile the root responded more strongly than stem or leaf.

Materials And Methods

Plant materials and growth conditions. Seeds of *R. soongorica* were harvested in October 2017 from the Gobi experiment fields at the Linze Inland River Basin Research Station ($39^{\circ}21'$ N, $100^{\circ}02'$ E, 1400 m asl), Chinese Academy of Sciences, the seeds were saved in the sealed plastic bags at a laboratory.

The experiment was also conducted at the Linze Inland River Basin Research Station. The average annual precipitation of 117 mm and average temperature is 7.6°C . The brown desert soil was used in our experiment which was collected from the upper 20 cm layer of Gobi experiment field in October 2017, the organic matter concentration of soil is 3.9 g kg^{-1} and the PH is 9.0, the available N and P is 0.19 and 0.21 g kg^{-1} , respectively. The soil was sieved through a 5 mm mesh before the experiment.

Experimental design. In the March 2018, the soil was added to pots with an diameter of 34 cm and a height of 40 cm, then the seeds of *R. soongorica* were sown into the pots, the soil water content was maintained at the above 80% of field capacity during the seedling establishing. After emergence, two seedlings of *R. soongorica* were retained in each pot for one year growth. The experiment was started in May 2019 and the excess plant was removed to maintain one seedling in each pot.

The nitrogen level was designed according to the nitrogen deposition level in the desert region⁴⁷, four treatments received additional N in the form of urea at the rates of 0 (N_0), 4.6 (N_1), 9.2 (N_2), and 13.8 (N_3) g N m⁻² year⁻¹. Each treatment had six replicates. The urea was selected as N source and the N content is 46.7% , so the amount of urea was 0.8865, 1.7730, 2.6595g for N_1 , N_2 , N_3 treatments. The N was applied in the beginning of May, June, July, August, September and October in 2019, as a solution of urea in the same volume deionised water, and the N_0 received the same volume of water in the same time.

Sampling and chemical analysis. The six seedlings of *R. soongorica* for each treatment were sampled in October. The whole seedlings of *R. soongorica* were taken out from the pots when sampling, then the roots were wrapped with plastic wrap to prevent water loss, and all samples were taken back to the laboratory. The seedlings were divided into 3 parts: leaves, stems and roots, then every part was washed by distilled water and excess water in the surface was removed by blotting paper. All samples were weighed, then they were placed in labeled envelopes at 120°C for 30 min in an oven and oven-dried at 80°C for 48 h to assess the dry mass. At last, the dried samples ground through a 40-mesh sieve and stored for chemical analysis.

The NSC concentration was calculated as the soluble sugar concentrations plus starch concentrations in this study⁴ and the concentrations of the soluble sugars and starch were determined using an anthrone method⁴⁸. Total N concentrations were determined using the Kjeldahl method. Total P concentrations were determined using the molybdenum blue colorimetric method. Total C concentrations were determined using the dichromate oxidation method⁴⁹.

Data analysis. A one-way analysis of variance (ANOVA) to test the effects nitrogen on biomass, the concentrations and stoichiometries of NSC, C, N and P of different organs. Duncan's multiple range tests was used for multiple comparisons among each treatment. Data analyses were performed using SPSS Statistics procedure (version 13.0) and figures were drawn by Origin 8.

Research involving plants. Experimental research and field studies on *Reaumuria soongorica* plants were complied with relevant institutional guidelines.

Declarations

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Author contributions

TTX analyzed the data and wrote the manuscript. WTZ conducted the experiment and performed the measurements. SLS participated in the planning of the study, data analysis, and writing of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Tables

Table 1 Effects of N addition on biomass of *R. soongarica*. The data was mean $\pm$ SE. Different lowercase letters in each column indicate significant differences among N addition at P=0.05.

N treatments	Total biomass(g)	Aboveground		Belowground	Belowground to Aboveground ratio
		Stem biomass(g)	Leaf biomass(g)	Root biomass(g)	
N ₀	2.86±0.10d	0.88 ±0.05c	1.19 ±0.01c	0.8 ±0.06d	0.39 ±0.02c
N ₁	3.53±0.08c	1.18 ±0.03b	1.30±0.09bc	1.08 ±0.06c	0.44 ±0.02bc
N ₂	4.59±0.04a	1.31±0.04a	1.70 ±0.08a	1.58 ±0.05a	0.53±0.03a
N ₃	4.05±0.11b	1.26 ±0.05ab	1.42 ±0.13b	1.36 ±0.07b	0.51 ±0.03ab

Figures

Figure 1

Effects of N addition on soluble sugar, starch, and NSC concentrations of *R. soongorica*. Error bars are SE (n = 6). Different lowercase letters above bars indicate significant difference at P = 0.05.

Figure 2

Effects of N addition on plant biomass C, N, and P concentrations and their stoichiometric ratios of C:N, C:P, and N:P of *R. soongorica*. Error bars are SE (n = 6). Different lowercase letters above bars indicate significant difference at P = 0.05

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [DATA.xls](#)