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 (N₀), 4.6 (N₁), 9.2(N₂), and 13.8 (N₃) g m⁻² year⁻¹. The N addition (reach to 9.2 g m⁻² 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 N₁ and N₂ 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 N₂ and N₃ addition and the leaf N concentration was only increased in N₃ addition, but N addition had no significant effect on C and P concentrations. The stem and leaf C:N ratio were reduced significantly in N₂ and N₃ treatments, but the root C:N ratio was decreased significantly in N addition. The N₃ 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. The study showed that N deposition affected plant photosynthesis and nutrient transport, but as the main product of photosynthesis, the existence form and distribution of carbohydrates in plant organs will been affected by N deposition. 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 rates.

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. 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 roots. 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 fertilization\(^9\). 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 ecosystem\(^12\). 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 increased\(^16\) or decreased\(^17\) under N addition, but plant N:P ratio maybe increased\(^15\), decreased\(^18\) or maybe not affected\(^17\) depending on species\(^18\) and soil nutrient conditions\(^19\). 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 genes\(^26\) 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 \(N_2\) addition treatment \((P<0.05)\). Compared with \(N_0\) treatment, the total, stem, leaf and
root biomass increased by 60.49%, 48.86%, 42.85% and 97.5% in N sub 2 addition treatment, respectively. The ratio of belowground to aboveground biomass was also significantly increased in N sub 1 and N sub 2 treatment because the increase of root biomass was more rapid than that of stem and leaf biomass (P<0.05), but then reduced in N sub 3 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 N sub 1 and N sub 2 addition treatments, but then it decreased significantly at the highest N sub 3 treatment except of the stem (Fig. 1a) (P<0.05). The starch concentration for stem, leaf and root were reduced significantly in N sub 2 and N sub 3 addition treatments(Fig. 1b)(P<0.05). Compared with N sub 0, the N addition(N sub 1-N sub 3) significantly enhanced the NSC concentration of root and reached the maximum value at N sub 2 addition treatment, but the stem and leaf NSC concentration was only increased significantly at N sub 1 and N sub 2 addition(Fig. 1c) (P<0.05).

**Nutrient concentrations and stoichiometry in different organs**

The N addition had no significant effect on concentration and P concentration of different organs (Fig. 2a, 2c). Compared with N sub 0, The N concentration of stem and root significantly increased in N sub 2 and N sub 3 addition treatments; the leaf N concentration significantly enhanced in N sub 3 addition treatment (P<0.05). The N concentration of different organs had no significant difference between N sub 0 and N sub 1 addition treatment (Fig. 2b).

The stem and leaf C:N ratio was all reduced significantly in N sub 2 and N sub 3 addition treatments, but the root C:N ratio was decreased significantly with increasing N addition(P<0.05) (Fig. 2d). Compared with N sub 0, 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 (N sub 2-N sub 3) significantly enhanced the stem N:P ratio, but the leaf and root N:P ratio was only enhanced in N sub 3 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 become one of the main limiting factors for plant productivity in arid and semi-arid region of northwest China. Contrary to our first hypothesized, we found N addition( reach to 9.2g m^{-2} year^{-1}) had significantly enhanced the biomass of different organs, but it was reduced significantly at higher level(13.8 g m^{-2} year^{-1}), this is consistent with the results of Jin et al. 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 addition.
The previous study showed that plants can increase their allocation of photosynthetic products to belowground organs under lower 0.15mM N condition\textsuperscript{29}, but with the N addition, the biomass allocation are species-specific\textsuperscript{4,30}. We found the response of roots biomass to N addition (reach to 9.2g m\textsuperscript{−2} year\textsuperscript{−1}) was significantly higher than the shoot biomass, thus result in higher belowground:aboveground ratio, this was inconsistent with the previous study\textsuperscript{4} and our hypothesis. The reason maybe is in order to absorbed more water from the soil in Gobi desert region, the root biomass of \textit{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 changes\textsuperscript{31}. Some studies reported that N addition increased the NSC accumulation\textsuperscript{30}, but other studies found N addition had no effect on or even decreased NSC concentration\textsuperscript{4,9}. In this study, we found a significantly increase in NSC concentration of different organs of \textit{R. soongorica} within a certain range of N addition (0 -9.2 g N m\textsuperscript{−2} year\textsuperscript{−1}), but it had some decreasing in highest N addition (13.8g N m\textsuperscript{−2} year\textsuperscript{−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\textsuperscript{4,32}. Meanwhile some research reported the accumulation of higher NSC concentrations can balance the osmotic pressure of cells\textsuperscript{33} and be used to cope with environmental stress, thus the highest NSC concentration in different organs under middle N addition (9.2g m\textsuperscript{−2} year\textsuperscript{−1}) in our study indicated adding proper amount of N will benefit for the NSC accumulation and improve the resistance of \textit{R. soongorica} to water stress in Gobi desert region, and then it is beneficial for the successful settlement of \textit{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\textsuperscript{4,30}, but the difference is the root NSC was higher than stem and leaf NSC in our study, Ai et al\textsuperscript{4} found the aboveground NSC was higher than belowground NSC, this difference may be related to the characteristics of carbon allocation and transport\textsuperscript{34}. In order to facilitate water absorption and survive in Gobi desert region, the more carbon of \textit{R. soongorica} allocated to the root than stem and leaf\textsuperscript{35}.

**Effect of N addition on plant stoichiometry.** Stoichiometry reflected the utilization ability of C, N and P for plant, which are susceptible to environmental changes\textsuperscript{36}. 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\textsuperscript{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\textsuperscript{14,39}. In this study, we found that N addition increased the N concentration and reduced C:N ratio of \textit{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\textsuperscript{17,40}. N:P ratio has been interpreted as an indicator of N and/or P limitation\textsuperscript{41,42}. It is widely accepted that N:P ratios < 10 indicate N limitation. Therefore, \textit{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. 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. 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 factors.

The responses of stoichiometry of different organs was different when environmental changes. For example, some studies showed that leaf N and P concentration and N:P were higher than those of the other organs under N addition. Xiao et al. 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 g m⁻² 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 N₁ and N₂ addition. The N concentration was increased at higher N addition (9.2-13.8 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°21′ N, 100°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 region, four treatments received additional N in the form of urea at the rates of 0 (N₀), 4.6 (N₁), 9.2(N₂), and 13.8 (N₃) 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 N₁, N₂, N₃ 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 N₀ 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 study and the concentrations of the soluble sugars and starch were determined using an anthrone method. 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 method.

**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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References

1. Galloway, J. N., Townsend, W. H., Erisman, J. W., Bekunda, M. & Cai, Z. Transformation of the nitrogen cycle: recent trends, questions and potential solutions. Science 320, 889–892 (2008).
2. Phoenix, G. K. et al. Impacts of atmospheric nitrogen deposition: responses of multiple plant and soil parameters across contrasting ecosystems in long-term field experiments. Glob. Chang. Biol. 18, 1197–1215 (2012).
3. Pons, T. L., van der Werf, A., Lambers, H. Photosynthetic nitrogen use efficiency of inherently slow- and fast-growing species: Possible explanations for observed differences//Roy J, Garnier E, eds. A Whole Plant Perspective on Carbon-Nitrogen Interactions. The Hague: SPB Academic Publishing: 61–77 (1994).

4. Ai, Z. M., Xue, S., Wang, G.L. & Liu, G. B. Responses of Non-structural carbohydrates and C:N:P stoichiometry of Bothriochloa ischaemum to nitrogen addition on the Loess Plateau, China. J. Plant Growth Regul. 36, 714–722 (2017).

5. Marklein, A. R. & Houlton, B. Z. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. New Phytol. 193, 696–704 (2012).

6. Dietze, M. C. et al. Nonstructural carbon in woody plants. Annu. Rev. Plant Biol. 65, 667–687 (2014).

7. Hartmann, H. & Trumbore, S. Understanding the roles of nonstructural carbohydrates in forest trees - from what we can measure to what we want to know. New Phytol. 211, 386–403 (2016).

8. Yang, Q. P. et al. Different responses of non-structural carbohydrates in above-ground tissues/organisms and root to extreme drought and re-watering in Chinese fir (Cunninghamia lanceolata) saplings. Trees 30, 1863–1871 (2016).

9. Peng, Z. T. et al. Non-Structural Carbohydrates Regulated by Nitrogen and Phosphorus Fertilization Varied with Organs and Fertilizer Levels in Moringa oleifera Seedlings. J. Plant Growth Regul. 40, 1777–1786 (2021).

10. Nardini, A. et al. Rooting depth, water relations and non-structural carbohydrate dynamics in three woody angiosperms deferentially affected by an extreme summer drought. Plant Cell Environ. 39, 618–627 (2016).

11. Wang, F. C. et al. Effects of experimental nitrogen addition on nutrients and nonstructural carbohydrates of dominant understory plants in a Chinese Fir plantation. Forests 10, 155 (2019).

12. Elser J. J. et al. Growth rate-stoichiometry couplings in diverse biota. Ecol. Lett. 6, 936–943 (2003).

13. Jinm X. M. et al. Ecological stoichiometry and biomass response of Agropyron michnoi under simulated N deposition in a sandy grassland, China. J. Arid Land 12, 741–751 (2020).

14. Jing, H. et al. Nitrogen Addition Changes the Stoichiometry and Growth Rate of Different Organs in Pinus tabuliformis Seedlings. Front. Plant Sci. 8, 1922 (2017).

15. Zhan, S. X., Wang, Y., Zhu, Z. C., Li, W. H. & Bai, Y. F. Nitrogen enrichment alters plant N: P stoichiometry and intensifies phosphorus limitation in a steppe ecosystem. Environ. Exp. Bot. 134, 21–32 (2017).

16. Stiling, P. & Cornelissen, T. How does elevated carbon dioxide (CO₂) affect plant–herbivore interactions? A field experiment and meta-analysis of CO₂-mediated changes on plant chemistry and herbivore performance. Glob. Change Biol. 13, 1823–1842 (2007).

17. Wang, X. G. et al. Responses of C:N:P stoichiometry of plants from a Hulunbuir grassland to salt stress, drought and nitrogen addition. Phyton-Int. J. Exp. Bot. 87, 123–132 (2018).

18. Liu, X. J. et al. Enhanced nitrogen deposition over China. Nature 494, 459–462 (2013).
19. Huang, W. J., Houlton, B. Z., Marklein, A. R., Liu, J. X. & Zhou, G. Y. Plant stoichiometric responses to elevated CO2 vary with nitrogen and phosphorus inputs: evidence from a global-scale meta-analysis. Sci. Rep. 5, 18225 (2015).

20. Wang, X. et al. Effects of nutrient addition on nitrogen, phosphorus and non-structural carbohydrates concentrations in leaves of dominant plant species in a semiarid steppe. Chin. J. Ecol. 33, 1795–1802 (2014).

21. Yang, D. X., Song, L. & Jin, G. Z. The soil C:N:P stoichiometry is more sensitive than the leaf C:N:P stoichiometry to nitrogen addition: a four-year nitrogen addition experiment in a Pinus koraiensis plantation. Plant Soil 442, 183–198 (2019).

22. Chong, P. F., Zhan, J., Li, Y. & Jia, X. Y. Carbon dioxide and precipitation alter Reaumuria soongorica root morphology by regulating the levels of soluble sugars and phytohormones. Acta Physiol. Plant 41, 184 (2019).

23. Ma, X. Z. & Wang, X. P. Biomass partitioning and allometric relations of the Reaumuria soongorica shrub in Alxa steppe desert in NW China. Forest Ecol, Manag. 468, 118–178 (2020).

24. He, F. L., Bao, A. K., Wang, S. M. & Jin, H. X. NaCl stimulates growth and alleviates drought stress in the salt-secreting xerophyte Reaumuria soongorica. Environ Exp. Bot. 162, 433–443 (2019).

25. Xu, D. H. et al. Photosynthetic parameters and carbon reserves of a resurrection plant Reaumuria soongorica during dehydration and rehydration. Plant Growth Regul. 60, 183–190 (2010).

26. Zhang, H. et al. miRNA–mRNA integrated analysis reveals roles for miRNAs in a typical halophyte, Reaumuria soongorica, during seed germination under salt stress. Plants 9, 351 (2020).

27. Bai, Y. M., Li, Y., Shan, L. S., Su, M. & Zhang, W. T. Effects of precipitation change and nitrogen addition on root morphological characteristics of Reaumuria soongorica. Arid Zone Res. 37, 1284–1292 (2020).

28. Hedwall, P.O., Nordin, A., Strengbom, J., Brunet, J., & Olsson, B. Does background nitrogen deposition affect the response of boreal vegetation to fertilization? Oecologia 173, 615–624 (2013).

29. Grechi, I. et al. Effect of light and nitrogen supply on internal C:N balance and control of root-to-shoot biomass allocation in grapevine. Environ. Exp. Bot. 59, 139–149 (2007).

30. Xiao, L., Liu, G., Li, P. & Xue, S. Nitrogen addition has a stronger effect on stoichiometries of non-structural carbohydrates, nitrogen and phosphorus in Bothriochloa ischaemum than elevated CO2. Plant Growth Regul. 83, 325–334 (2017).

31. Quentin, A. G. et al. Non-structural carbohydrates in woody plants compared among laboratories. Tree Physiol. 35, 1146–1165 (2015).

32. White, L. M. Carbohydrate reserves of grasses: a review. J. Range Manag. 26, 13–18 (1973).

33. Millard, P., Sommerkorn, M. & Grelet, G. A. Environmental change and carbon limitation in trees: a biochemical, ecophysiological and ecosystem appraisal. New Phytol. 175, 11–28 (2007).

34. Zhang, T., Cao, Y., Chen, Y. M. & Liu, G. B. Non-structural carbohydrate dynamics in Robinia pseudoacacia saplings under three levels of continuous drought stress. Trees 29, 1837–1849 (2015).
35. Chapin, F. S., Schulze, E. D., Mooney, H. A. The ecology and economics of storage in plants. Annu. Rev. Ecol. Syst. 21, 423–447 (1990).

36. Sardans, J., Rivas-Ubach, A. & Peñuelas, J. The C:N:P stoichiometry of organisms and ecosystems in a changing world: a review and perspectives. Perspect Plant Ecol. Evolut. Syst. 14, 33–47 (2012).

37. Xia, J. Y. & Wan, S. Q. Global response patterns of terrestrial plant species to nitrogen addition. New Phytol. 179, 428–439 (2008).

38. Yang, Y. H., Luo, Y. Q., Lu, M., Schädel, C. & Han, W. X. Terrestrial C: N stoichiometry in response to elevated CO\textsubscript{2} and N addition: a synthesis of two meta-analyses. Plant Soil 343, 393–400 (2011).

39. Liu, H. M. et al. Effects of nitrogen addition on the stoichiometric characteristics of plants and soil in the Stipa baicalensis grassland of Inner Mongolia, China. Acta Prataculturae Sin. 27, 25–35 (2018).

40. Cui, Q., Lü, X. T., Wang, Q. B. & Han, X. G. Nitrogen fertilization and fire act independently on foliar stoichiometry in a temperate steppe. Plant Soil 334, 209–219 (2010).

41. Koerselman, A., Meuleman, A. F. The vegetation ratio: A new tool to detect the nature of nutrient limitation. J. Applied Ecol. 33, 1441–1450 (1996).

42. Gusewell, S. N:P ratios in terrestrial plants: variation and functional significance. New Phytol. 164, 243–266 (2004).

43. Wang, S., Shan, L. S., Li, Y., Zhang, Z. Z. & Ma, J. Effect of Precipitation on the Stoichiometric Characteristics of Carbon, Nitrogen and Phosphorus of Reaumuria soongarica and Salsola passerina. Acta Bot. Boreal.-Occident Sin. 40, 0335–0344 (2020).

44. Niu, D. C., Li, Q., Jiang, S. G., Chang, P. J. & Fu, H. Seasonal variations of leaf C:N:P stoichiometry of six shrubs in desert of China’s Alxa Plateau. Chin. J. Plant Ecol. 37, 317–325 (2013).

45. Mayor, J. R., Wright, S. J. & Turner, B. L. Species-specific responses of foliar nutrients to long-term nitrogen and phosphorus additions in a lowland tropical forest. J Ecol. 102, 36–44 (2014).

46. Kleyer, M. & Minden, V. Why functional ecology should consider all plant organs: an allocation-based perspective. Basic Appl. Ecol. 16, 1–9 (2015).

47. Liu, J. X. et al. Nitrogen to phosphorus ratios of tree species in response to elevated carbon dioxide and nitrogen addition in subtropical forests. Global Change Biol. 19, 208–216 (2013).

48. Yemm, E. & Willis, A. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57, 508–514 (1954).

49. Bao, S. D. Soil and Agricultural Chemistry Analysis. 3 rd edn. Beijing, China Agriculture Press. (2000).

**Tables**

**Table 1** Effects of N addition on biomass of *R. soongorica*. The data was mean ± 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<sub>0</sub> | 2.86±0.10d | 0.88 ±0.05c | 1.19 ±0.01c | 0.8 ±0.06d | 0.39 ±0.02c |
| N<sub>1</sub> | 3.53±0.08c | 1.18 ±0.03b | 1.30±0.09bc | 1.08 ±0.06c | 0.44 ±0.02bc |
| N<sub>2</sub> | 4.59±0.04a | 1.31±0.04a | 1.70±0.08a | 1.58 ±0.05a | 0.53±0.03a |
| N<sub>3</sub> | 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