Intervention of Nitrogen Evolution on Species Diversity of Broad-leaved Forest Plants in Xianwen Mountain

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Abstract. Nitrogen evolution is one of the important factors driving biodiversity change. It is generally believed that the species diversity structure will be changed, and under the conditions of exogenous nitrogen addition, grasses and deciduous shrubs have a competitive advantage over hybrid grasses and evergreen shrubs. For the competition between plants in the same life type, the interaction between different life-type plants is not involved. Therefore, from the changes in forest ecosystem structure and nitrogen elements, the response of different life-type plants to nitrogen evolution can be further explored. The lower plant species of the deciduous broad-leaved forest-Mongolian carp are studied, and different gradients of nitrogen (0, 40, 80, 120 kg N·ha⁻¹·yr⁻¹) are added to the four gradients of extraneous nitrogen effects of nitrogen evolution on forest biodiversity in the north temperate zone. The results of five-year continuous nitrogen addition experiments showed that nitrogen addition significantly reduced the species richness and diversity of understory plants, and changed the species composition of the community. Nitrogen addition increased the species richness of shrub plants and diversity, reduced the richness of herbaceous plants, and reduced the important value of grasses and increased the important value of hybrid grasses. The study showed that long-term nitrogen addition significantly changed the species composition of understory plants, and the response of different life-type plants to nitrogen addition was also different.

1. Introduction
Biodiversity is one of the important indicators for measuring the structure and function of ecosystems, and it is also the basis for the survival and development of human society [1]. Increased rates of nitrogen evolution are important drivers of changes in biodiversity, long-term low levels of nitrogen evolution (eg: 10kg N·ha⁻¹·yr⁻¹) or intermediate levels of nitrogen evolution (eg: 25kg N·ha⁻¹·yr⁻¹) affects species diversity [2]. At present, due to environmental pollution and an increased range of agricultural activities, the average rate of nitrogen evolution is 0.42-24.2 kg N·ha⁻¹·yr⁻¹, and in some areas even more than 60 kg N·ha⁻¹·yr⁻¹[3]. Therefore, it is of great significance to study the effects of nitrogen evolution on species diversity in China, and to clarify the changes in regional ecosystem structure and function under the continuous increase of atmospheric nitrogen evolution [4]. Due to the differences in the characteristics of the species and the ability to compete with resources, different species respond differently to the evolution of nitrogen, which in turn leads to changes in biodiversity. Under the condition of exogenous exogenous nitrogen addition, grasses and deciduous shrubs have
more competitive advantages than hybrid grasses and evergreen shrubs\[5\]. Therefore, it is necessary to carry out relevant experiments in forest ecosystems to further understand the response mechanism of biodiversity to nitrogen evolution. As the main body of terrestrial ecosystems, forest ecosystems play an extremely important role in the global carbon cycle. Forests have a higher rate of nitrogen evolution, and nitrogen evolution has a greater impact on forest ecosystems than other terrestrial ecosystems\[6\]. The structure and function of forest ecosystems are generally complex, and their diversity changes are more related to changes in plant diversity under the forest\[7\]. At present, the average nitrogen evolution level of temperate forests in the world is 5.5-15 kg N·ha\(^{-1}\)·yr\(^{-1}\), and it is increasing year by year. Since the demand for nitrogen in organisms is often greater than the mineralization rate of soil organic nitrogen in temperate forests, the addition of exogenous nitrogen affects the supply and demand of nitrogen to organisms, making the species diversity of temperate forests more sensitive to exogenous nitrogen addition. Therefore, it is of great significance to discuss the impact of exogenous nitrogen evolution on temperate forest biodiversity \[8\]. *Quercus mongolica* is one of the common deciduous broad-leaved tree species in the northern temperate zone of China, and is widely distributed in the northeast and Inner Mongolia. The forest community with *Quercus mongolica* as the main dominant species or the grouped species is a relatively stable vegetation type in the northern warm temperate zone, which plays an important role in soil and water conservation and maintaining the stability of the community structure. In this study, the *Quercus mongolica* forest was used as the research object, and CK (0 kg N·ha\(^{-1}\)·yr\(^{-1}\)), N40 (50 kg N·ha\(^{-1}\)·yr\(^{-1}\)) and N80 were set according to the nitrogen concentration gradient of the existing fertilization platforms at home and abroad. (100 kg N·ha\(^{-1}\)·yr\(^{-1}\)) 3 gradients, through 5 years of field control of exogenous nitrogen addition experiments, to explore the effects of exogenous nitrogen addition on plant species diversity of *Quercus mongolica* forests and different ecological types of plants Response to exogenous nitrogen addition.

2. Materials and methods

2.1 Experimental plot

The Liangshui National Nature Reserve is located in the centre of the belting area of Yichun City, Heilongjiang Province. It is on the east slope of the Daling Mountains in the south of Xiaoxing’anling. It covers an area of 1.2×10\(^5\) hm\(^2\). The whole territory is mountainous, with an average elevation of 400 mm and a slope of 10-15°. Temperate continental climate, average annual precipitation of 676 mm, annual average evaporation of 805 mm; annual average temperature of -0.3°C, an annual average minimum temperature of -6.5°C, annual average temperature of 7.5°C; the soil is mainly dark brown soil. The forest cover rate is 98%. The main dominant species of the arbor layer in the study area is *Quercus mongolica*. It belongs to the deciduous tree and *Quercus* species. It is one of the main species of coniferous and broad-leaved mixed forest in temperate regions of China. It belongs to the most distributed deciduous forest in the north, and it supports water conservation and soil and water conservation in China. And fire and wind have played a significant role. Among the flora of Liangshui Nature Reserve, there are 86 genera of Salix, such as *Calix*, *Saussurea*, *Potuteilla*, *Vicia*, and *Viola*, which are a dominant genus. There are 280 species of plants, accounting for 63.64% of the total number of plants in cold water vascular plants, and 15 species of genus. The order from large to small was: *Falipeudula*, *Circaea*, *Pseudostellaria*, and genus *Cocopus*, *Equisetum diestrum*, *Patricia*, *Cimicifum R-a*, *Larch*, etc.

2.2 Experimental setup

The establishment of the experimental plot began in the spring of 2015, and in the same year began the experiment of exogenous nitrogen addition gradient. Three nitrogen addition levels were set up in the experiment: CK (0 kg N·ha\(^{-1}\)·yr\(^{-1}\)), N40 (50 kg N·ha\(^{-1}\)·yr\(^{-1}\)), and N80 (50 kg N·ha\(^{-1}\)·yr\(^{-1}\)). According to the similar site conditions, the experimental plots were randomly divided into 9 sample plots according to the specifications of 3 treatments × 3 replicates, each plot was 20m×20m, and a transition zone of 10m was set between the sample plots. The nitrogen fertilizer used in the experiment was urea (CO(NH\(_2\))\(_2\)).
Since 2015, it has been fertilized 6 times a year, and it is carried out at the beginning of each month from May to October. The amount of urea used in N40 treatment was 725 g/time, and the amount of urea treated by N80 was 1450 g/time. Before fertilizing, according to the concentration requirements, the corresponding quality of urea is dissolved in 30L of water, completely dissolved, and evenly sprayed on the experimental sample with a sprayer, and the same amount of water is sprayed only by the control (CK) sample. Before the start of the experiment in 2015 and at the beginning of May 2019 (6 months from the last fertilization time), randomly take 5 points on each sample to collect 0-20 cm surface soil with a 5 cm inner diameter soil drill. Bring back to the laboratory with 50mL KCl (1mol/L) solution, and then use a pH meter to determine the pH; soil total nitrogen (TN) and total carbon (TC) content were determined using an elemental analyzer (Elementar Vario EL III, Hanau, Germany), and soil total phosphorus (TP) content was determined by molybdenum antimony colorimetric method. At the beginning of August 2018 (30 days from the last nitrogen addition). Twenty healthy leaves were randomly collected from each plant and brought back to the laboratory for drying to a constant weight in a 65℃ oven.

2.3 Data Processing
The larger the Gini coefficient, the more obvious the difference in the competitiveness of light between functional groups; species richness, coverage, abundance, altitude, relative importance, and Gini coefficient are calculated as follows:

\[ S = \frac{\sum_{i=1}^{n} P_i}{S} \quad (2) \quad H = -\sum P_i \ln P_i \quad (3) \quad Gini = \frac{\sum_{i=1}^{n} \sum_{j=1}^{n} |X_i - X_j|}{2n^2X} \]

2.4 Data statistics means
Using Microsoft Excel to organize the data, one-way ANOVA was used to compare the species richness, height, and coverage of different fertilization treatments. The variance was first tested by Levene. If the data met the test requirements, the least significant difference was used. The Least significant difference (LSD) was used to compare the physical and chemical properties, species richness, height, and coverage of the sample soil (P<0.05), otherwise it was analyzed by Tamhane’s T2.

3. Results and analysis
3.1 Investigation of species in the experimental plot
The results showed that there were 43 species of undergrowth plants in the Quercus mongolica plot, including 32 species of herbaceous plants, mainly Gramineae, Cyperaceae, Compositae, and Ranunculaceae, 11 species of shrub plants, mostly deciduous shrubs, mainly. It belongs to the family of Leguminosae, Rosaceae, Rhododendron and Saxifragaceae. The species composition of each treated plant community was compared. The addition of nitrogen changed the species composition of the plants under the Quercus mongolica forest. The specific performance is as follows: there are 28 kinds of herbaceous plants and 8 species of shrub plants in the control sample (CK) without added nitrogen, and the number of species is greater than the fertilization treatment. N40 treated 23 herbaceous plants and 9 shrub plants, N80 treated 20 herbaceous plants and 10 shrub plants. The number of species in the sample decreases with the increase of plant nitrogen concentration, especially for non-grass plants. Nitrogen addition significantly altered the dominant or dominant species of understory plants. There was no significant difference in the important values of the main species of herbaceous and shrub plants in 5 years (in Table 1).
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Table 1. Conditions of understory plants during the experiment in 2018

| Species              | Average Height | Average Cover | CK  | N40 | N80 | Species              | Average Height | Average Cover | CK  | N40 | N80 |
|----------------------|----------------|---------------|-----|-----|-----|----------------------|----------------|---------------|-----|-----|-----|----------------------|----------------|---------------|-----|-----|-----|
| Herbs                |                |               |     |     |     |                      |                |               |     |     |     |                      |                |               |     |     |     |
| Carex rigescens      | 15.56          | 14.91         | +  | +  | +  | Rabdosia amethystoides | 68.27          | 0.20          | +  | +  | +  | Sedum aizoon          | 28.12          | 0.10          | +  | +  | +  |
| D.arundinacea        | 30.32          | 33.21         | +  | +  | +  | Adenophora divaricata  | 43.55          | 0.06          | +  | +  | +  | Artemisia tanacetifolia | 58.28          | 0.15          | +  | +  | +  |
| Saussurea nivea       | 15.47          | 19.68         | +  | +  | +  | Viola variegata        | 18.34          | 0.32          | –  | +  | +  | Patrinia scabiosaefolia | 30.78          | 0.28          | +  | +  | +  |
| T. petaloideum       | 23.25          | 9.43          | +  | +  | +  |                      |                |               |     |     |     |                      |                |               |     |     |     |
| Phlomis ambozeroa    | 45.27          | 1.96          | +  | +  | –  |                      |                |               |     |     |     |                      |                |               |     |     |     |
| Dioscorea nipponica  | 23.43          | 2.28          | +  | +  | +  |                      |                |               |     |     |     | Viola dissecta        | 15.38          | 0.58          | –  | +  | +  |
| D.chamnii            | 13.79          | 2.71          | +  | +  | +  |                      |                |               |     |     |     | Paraxieris denticulata | 18.68          | 0.30          | –  | +  | +  |
| Bletilla strataia    | 26.67          | 1.43          | +  | +  | –  | Shrubs                |                |               |     |     |     |                      |                |               |     |     |     |
| Aster ageratoides    | 33.36          | 1.65          | +  | +  | +  |                      |                |               |     |     |     | Spirea pubescens      | 143.78         | 10.96         | +  | +  | +  |
| Clematis hexapetala  | 29.38          | 1.25          | +  | +  | +  |                      |                |               |     |     |     | Deutzia Grandiflora   | 108.33         | 13.68         | +  | +  | +  |
| Veratum nigrum       | 31.63          | 1.67          | +  | –  | –  |                      |                |               |     |     |     | Lespedeza bicolor     | 98.82          | 17.45         | +  | +  | +  |
| Atractylodes lancea  | 34.02          | 1.18          | +  | +  | +  |                      |                |               |     |     |     | R.micranthum          | 102.96         | 22.78         | +  | +  | +  |
| S. officinals        | 27.58          | 1.37          | +  | +  | –  |                      |                |               |     |     |     | Aebilia biflora       | 272.94         | 15.33         | +  | +  | +  |
| A. schoberoides      | 35.55          | 0.52          | +  | +  | +  |                      |                |               |     |     |     | Spirea trifolata      | 77.39          | 5.61          | +  | +  | +  |
| A.tetraphylla        | 44.08          | 0.35          | +  | –  | –  |                      |                |               |     |     |     | Deutzia parviflora    | 129.48         | 5.29          | +  | +  | +  |
| Saussurea monolica    | 36.25          | 1.24          | +  | +  | +  |                      |                |               |     |     |     | R. macronulatum       | 124.76         | 3.24          | +  | +  | +  |
| Rubia cordifolia     | 23.14          | 0.62          | +  | +  | +  |                      |                |               |     |     |     | Lonicera maackii      | 176.50         | 4.36          | –  | +  | +  |
| Galium bungei        | 18.85          | 0.33          | +  | +  | +  |                      |                |               |     |     |     | Rhamnus globosa       | 85.43          | 0.50          | –  | +  | +  |

Note: ‘+’ means plots have this species, ‘–’ means plots do not have this species. CK, N40, N80 denote nitrogen addition 0, 50, 100 kg N·ha⁻¹·yr⁻¹, respectively.

3.2 Effects of exogenous nitrogen addition on species diversity of understory plants

The long-term exogenous nitrogen addition significantly reduced the species richness of understory plant species, and the experimental treatment was significantly different from the control treatment (P<0.05). The species richness of CK, N40 and N80 treatments were 6.49, 5.21 and 4.72, respectively; the Shannon-Wiener index was significantly different between exogenous nitrogen addition treatment and control treatment, and the indices of CK, N40, and N80 treatment were 1.25, 1.04 and 0.98, respectively. As the concentration of exogenous nitrogen increased, the biodiversity of understory plants decreased (in Fig.1-A). The results showed that the addition of exogenous nitrogen had an inhibitory effect on the species richness of herbaceous plants, and N40, N80, and CK were significantly different (P<0.05). The species richness of the three treatments were 5.70, 4.67 and 4.41, respectively (Fig.1-A). The Shannon-Wiener index decreased with increasing levels of exogenous nitrogen, but the statistical difference was not significant (in Fig.1-B). There was no significant difference in plant height between treatments (in Fig.1-C). The coverage increased with increasing concentration, and the difference was significant (P<0.05). The addition of exogenous nitrogen for 8 years significantly increased the species richness and diversity of shrub plants. The species richness of the three treatments was 6.00, 6.58 and 6.92, respectively, and the Shannon-Wiener index was 1.03, 1.20, and 1.39 (in Fig.1-B). As the concentration of exogenous nitrogen increased, the height and coverage of shrub plants increased, and the experimental treatment was significantly different from the control treatment (P<0.05) (in Fig.1-C, D).
3.3 Comparison of nitrogen content in leaves and soils of dominant species

Long-term exogenous nitrogen addiction treatment did not significantly change the element content in the leaves of dominant species under the forest, except for the total carbon content of the leaves of *Spiraea Chinensis* and *P. Sinensis* in the N80 treatment and the CK treatment. There were no significant differences in leaf element content (P>0.05). The experiment starts before 2015. The contents of total carbon, total nitrogen and total phosphorus in the soil were 20.79, 1.79, 0.38 mg/g, and the soil pH was 7.07. After 5 years of exogenous nitrogen addition treatment, the effects of total carbon and total nitrogen addition in each treated soil on the changes of important values of different dominant species in 5 years. The contents of nitrogen and total phosphorus were significantly different (P<0.05), and the nitrogen addition significantly reduced the pH value of soil. The pH values of soil treated with CK, N40, and N80 were 6.83, 6.50 and 6.18, respectively (In Table 2).

Table 2. Influence of nitrogen addition on dominant species importance value of different treatments in 2015 and 2019

| Species               | 2015       | 2019       |
|-----------------------|------------|------------|
|                       | CK         | N40        | N80        | CK         | N40        | N80        |
| Herbaceous            |            |            |            |            |            |            |
| Carex rigescens       | 15.53±3.93a| 14.25±1.95a| 20.53±1.11a| 10.16±0.98a| 8.29±0.36a | 4.17±0.21b |
| Deyeuxia arundinacea  | 40.82±5.15a| 36.64±4.11a| 32.14±3.66a| 39.9±7.41a | 35.94±3.91a| 34.24±4.66a|
| Saussurea Nivea       | 8.26±3.99a | 9.73±1.28a | 8.49±2.29a | 9.77±2.45a | 12.49±4.78a| 8.78±1.75a |
| Thalictrum petioleum  | 3.73±1.31a | 4.17±2.45a | 7.18±1.74a | 4.73±1.53a | 9.84±4.12a | 8.97±3.93 |
| Spiraec pubescens     | 22.32±3.53a| 18.17±5.41a| 18.74±6.22a| 21.19±3.03a| 20.81±2.48a| 9.30±3.63b |
| Deutzia grandiflora   | 12.57±2.57a| 11.64±2.39a| 12.53±0.26a| 7.00±2.12a | 15.80±4.64b| 26.12±2.52b|
| Lespedez abicolor     | 15.18±2.10a| 13.09±3.72a| 16.08±4.64a| 15.15±6.16a| 20.10±1.91a| 22.33±6.26a|
| Rhododendron micranthum| 11.56±5.66a| 13.99±2.81a| 16.50±1.78a| 15.83±4.44a| 22.50±3.73a| 21.59±2.82a|
| Abelia biflora       | 9.31±1.54a | 9.93±5.22a | 6.84±1.06a | 8.80±5.55a | 13.50±4.8a  | 12.50±3.73a|

Note: mean ± SE, n=3. Different lowercase letters indicate significant difference among treatments (P < 0.05).

4. Conclusion

The addition of exogenous nitrogen significantly increased the abundance of shrub plants (mainly deciduous shrubs) and reduced the abundance of herbaceous plants. The herbaceous plants were divided into grasses and weeds. It was found that the addition of exogenous nitrogen reduced the
important value of grasses and increased the important value of weeds. Causes of this phenomenon, in addition to the effects of soil environmental changes mentioned above light may also be an important factor affecting the growth of different life-type plants. Long-term exogenous nitrogen addition experiments reduced the species diversity of understory plants, and the responses of different life forms to exogenous nitrogen were different. The effects of exogenous nitrogen addition on plants may be related to the change of soil environment and the ability of plants to obtain the light. In the future, when discussing the effects of nitrogen evolution on understory biodiversity, it may be necessary to distinguish between different ecosystems and different life forms. Due to the small number of understory plants in this experimental plot, other life-type plants such as green shrubs and moss, the interaction between species may not be sufficient, and the experiment did not analyze the species changes of understory plants year by year. Therefore, the mechanism of the effect of the nitrogen evolution rate on understory plants still needs further study.

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