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Effects of long-term nitrogen addition on the $\delta^{15}N$ and $\delta^{13}C$ of *Larix gmelinii* and soil in a boreal forest

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Abstract

Background: Natural abundance of carbon (C) and nitrogen (N) stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) has been used to indicate the state and cycle of ecosystem C and N. However, it is still unclear how C and N cycle of boreal forests respond to the N deposition.

Results: We conducted an 8-year continuous N addition field experiment in a *Larix gmelinii* forest in Greater Khingan Mountains, Northeast China. Four N treatments (0, 25, 50, 75 kg N ha^{-1} year^{-1}) were built. The effects of N addition on the $\delta^{13}C$ and $\delta^{15}N$ of needle, branch, bark, and fine root of *Larix gmelinii* and soil were studied. The result of the balance between the N input and output flux showed that N addition significantly increased the $\delta^{15}N$ in each organ of *Larix gmelinii*, but did not change the $\delta^{15}N$ of soil. We also found that the N absorption by needles of *Larix gmelinii* could increase the needle photosynthesis rate and $\delta^{13}C$ by increasing carboxylation, but N addition had no significant effect on the $\delta^{13}C$ of soil and other organs. In addition, both the soil $\delta^{15}N$ and $\delta^{13}C$ increased with the soil depth.

Conclusions: Long-term N addition may lead to more open C and N cycles and further affect plant nutrient acquisition strategies in boreal forest ecosystems.

Keywords: Nitrogen deposition, Stable isotope technology, Natural abundance of carbon, Natural abundance of nitrogen, Boreal forest

Background

Due to fossil fuel combustion and agricultural emissions, anthropogenic nitrogen (N) deposition has been increased dramatically in the past century and is expected to intensify further in the coming decades (Galloway et al. 2008; Liu et al. 2013). Excessive N input can lead to many negative effects, including increased N leaching to water, soil acidification, increased N gas losses from soil and changes in biodiversity (Gao et al. 2014; Chen et al. 2016; Lucander et al. 2021). Therefore, it is necessary to understand the impact direction, intensity and future trend of N deposition on ecosystem C and N cycle, and formulate effective C and N management measures under enhanced global N deposition.

The natural abundance of stable C and N isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) is an effective method to explore the C and N dynamics of different ecosystems (Liu et al. 2017; Rao et al. 2017). ^{15}N natural abundance ($\delta^{15}N$) is the comprehensive result of the dynamic process of N cycle (Högberg 1997; Zhang et al. 2018). However, the fractionation of N isotopes in the process of N transformation, absorption, assimilation, distribution, and loss, leads to plants $\delta^{15}N$ being different from the $\delta^{15}N$ of N source (Bai et al. 2012). Therefore, $\delta^{15}N$ of plants can reflect the $\delta^{15}N$ of source N and the isotopic fractionation during N cycling

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processes (Robinson 2001; Kahmen et al. 2008). For example, during nitrification, when the chemical bond of the substrate breaks, the heavier ^{15}N will be transformed into the product more slowly than the lighter ^{14}N, so that the product will have a lower ^{15}N/^{14}N ratio than that of the remaining residual nitrate (Niu et al. 2016; Reis et al. 2016). Therefore, when N saturation causes increased nitrification, soil and plant will become more and more enriched in ^{15}N (Wang et al. 2014). For ecosystem N pool, the N transformation rate is determined by the input and output rates (Högberg et al. 2014). If the increased N input leads to increased N transformation, stronger fractionation and loss of ^{15}N-depleted N, it would affect enrichment of soil and subsequently plant tissue. However, if both the rates of N input and output changes with the increase of N deposition, the responses of plant and soil δ^{15}N values would be complex. Results showed that N additions increased N losses, and the change in plant δ^{15}N after N addition might be related to the balance between higher soil δ^{15}N and ^{15}N depleted N from atmospheric deposition (Kriszan et al. 2009; Gurmesa et al. 2017; Hobbie et al. 2017). Therefore, evaluating the changes in ecosystem δ^{15}N under N addition treatments can provide valuable information on how N flux, assimilation and distribution respond to the increase in N input in forest ecosystems.

Atmospheric air represents a constant C pool with relatively stable isotopic composition and concentration (Galewsky et al. 2016). Therefore, the change in ^{13}C reflects plant physiology: the balance between CO_2 supply (conduction) and CO_2 demand (assimilation) (Ehleringer 1993). For example, the decrease in stomatal conductance and the restriction of photosynthetic enzyme activity will eventually lead to an increase in plant δ^{13}C value (Jia et al. 2016; Lavergne et al. 2020). Several studies have suggested that foliar uptake of atmospheric reactive N could enhance the rates of photosynthesis and altered foliar δ^{13}C through increased carboxylation (Siegwolf et al. 2001; Guerrieri et al. 2011), although some studies reported no changes in plant δ^{13}C as a result of N enrichment (Wang et al. 2021). Carbon isotope exchange reaction enriches ^{13}C in inorganic salts (such as carbonate), while the dynamic fractionation effect of photosynthesis in the organic cycle of C leads to the ^{13}C-depletion in biogenic C (organic matter), leading to a change in C isotope composition during the exchange of CO_2 between organisms and terrestrial ecosystems (Cao et al. 2012; Busch et al. 2020). In the process of microbial decomposition and mineralization of soil organic carbon (SOC) into CO_2, the isotope effect is small (only 1–3‰), while strong fraction (10–70‰) occurs during methane production from SOC under anaerobic condition (Conrad et al. 2010). Compared with the fractionation of CO_2 fixation during plant photosynthesis, the fractionation during SOC decomposition is almost negligible (Crow et al. 2006). Therefore, by measuring the change in C isotope ratio in each C pool, we can quantify the source, distribution and turnover of C in the "atmosphere–plant–soil" continuum.

Boreal forests account for about 15% of the earth's total land area and 30% of the forest area, making them the second largest forest biota on land (Vitousek et al. 2002). The boreal forest plays an important role in regulating the global C and N balance and slowing down the rise of greenhouse gas concentration in the atmosphere, and it is also considered to be one of the ecosystems seriously limiting N (Randerson et al. 2006; Houghton 2007). The Greater Khingan Mountains is the only boreal forest zone of China. The widely distributed *Larix gmelinii* forest is one of the most sensitive vegetation types to global climate change. However, the impacts of N enrichment on the C and N cycle in this ecosystem need to be evaluated. Therefore, we carried out N addition field experiment in *Larix gmelinii* forest in Greater Khingan Mountains. In 2011, According to the current N deposition rate in northern China (25 kg N ha^{-1} $year^{-1}$) (Liu et al. 2013), we established four N addition treatments (0, 25, 50, 75 kg N ha^{-1} $year^{-1}$) to simulate the increase of atmospheric N deposition by 0, 1, 2, 3 times in the future. In this experiment, we measured the total C (TC), total N (TN), δ^{13}C and δ^{15}N of needle, branch, bark, and fine root of *Larix gmelinii* and soil under different N addition treatments. We also measured the photosynthetic rate and photosynthetic enzyme activity. The purpose of this study is to answer the following questions: (1) Whether N addition will change the composition of δ^{13}C and δ^{15}N in plants and soil? (2) What are the effects of different N addition levels on the changes of δ^{13}C and δ^{15}N composition in plants and soil? We hypothesized that (1) N addition will increase the loss of N through gaseous pathway and increase the soil δ^{15}N value. (2) N addition will increase the absorption of N by *Larix gmelinii* and increase δ^{15}N value in each organ. (3) N addition can increase the δ^{13}C value of *Larix gmelinii* needles by increasing related processes of photosynthesis.

Methods

This study was conducted in a natural larch forest of Nanwenghe National Nature Reserve in the Greater Khingan Mountains, Northeast China (51° 05′–51° 39′ N, 125° 07′–125° 50′ E) (Fig. 1). The region has a typical temperate continental climate, with an average temperature of − 2.7 ℃, an annual precipitation of about 500 mm, a plant growth period of about 110 days and an annual sunshine time of 2500 h. The soil is dark brown sandy gravel soil with an average depth of 20 cm. The dominant tree

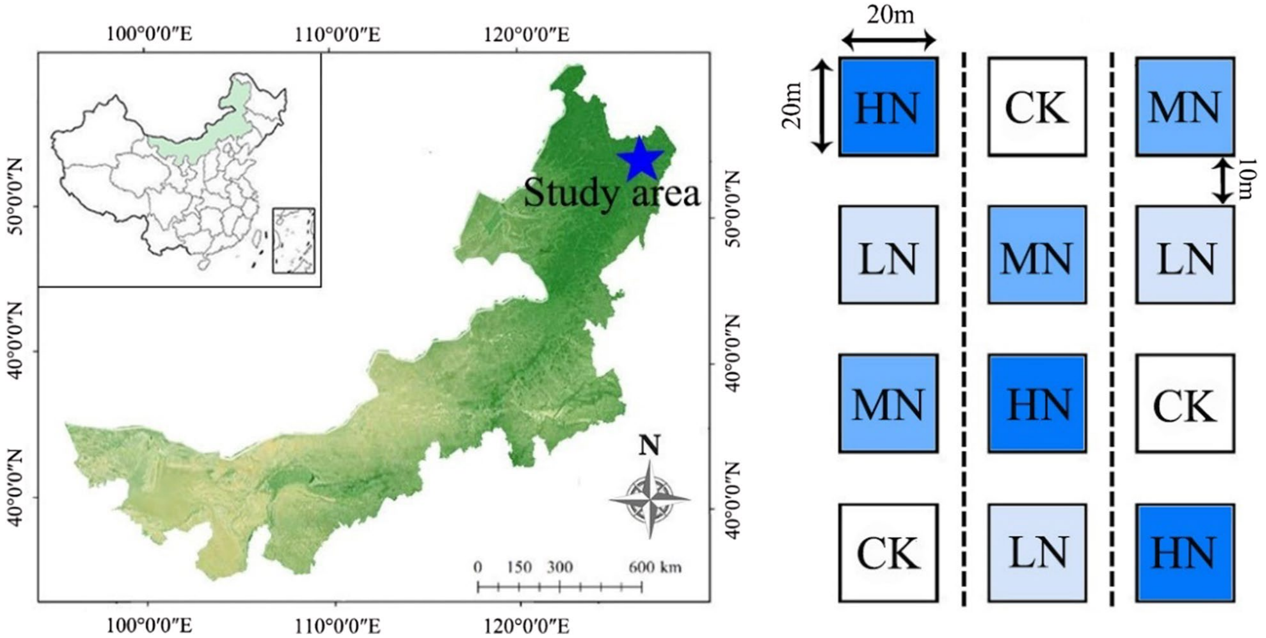


Fig. 1 Diagram of the experimental design in the Greater Khingan Mountains northeastern China. CK, LN, MN, HN represent the control, low N treatment, medium N treatment, and high N treatment, respectively

species is *Larix gmelinii*, whose growth density is 2852 (±99) trees/ha, and the average diameter at breast height (DBH) was 8.98 (±0.32) cm, a stand age of 40 years and a canopy density of 0.76±0.30. The vegetation under the forest includes *Rhododendron dauricum* and *Rosa davurica*.

In 2011, we randomly set up three sample blocks (Fig. 1), and each sample block includes four 20 m × 20 m plots. The buffer between any two plots is greater than 10 m to avoid interference with nearby plots. We randomly set up four levels of N addition treatment, control (CK, 0 kg N ha^{-1} $year^{-1}$), low N (LN, 25 kg N ha^{-1} $year^{-1}$), medium N (MN, 50 kg N ha^{-1} $year^{-1}$), and high N (HN, 75 kg N ha^{-1} $year^{-1}$). The N deposition levels were chosen to simulate a future N deposition of 1–3 folds of the current N deposition level in the study region (25 kg N ha^{-1} $year^{-1}$) (Liu et al. 2013). For each N addition plot, NH_4NO_3 was dissolved in 32 L water in the growing season (from May to September, a total of 5 times each year), and sprayed evenly on the forest floor of each plot using a prayer. To compensate water supply, the control plot was sprayed with the same amount of pure water. The natural abundance of the N fertilizer (NH_4NO_3) in this study was 0.21%±0.43‰.

Sampling method

The sample collection was carried out in mid July 2019. Three trees with similar height and DBH (diameter at breast height) in each plot were randomly selected. Current-year needle, branch, and bark of *Larix gmelinii* from the top most were cut with a pole pruner. The sampled tree species were marked with numbers. Under the marked trees in each plot, we randomly drilled three soil cores with a soil auger (5 cm in diameter and 20 cm in length), then picked out the root. To obtain soil samples, we randomly selected three points under each labeled tree species, and used a soil auger to get the soil (divided into O_{a+e} layer and mineral layer, in which the "O" layer is the surface layer of the soil, composed of forest litter, the "a" layer represents the humus layer, and the "e" layer represents the leaching layer). The samples of the same soil layer from each plot were evenly combined to obtain a representative soil sample in each plot. All samples were dried to constant weight at 65 °C and then ground into a fine powder using a Tecator sample mill (Subang, Shanghai, China) for analysis.

Photosynthetic parameter

Three *Larix gmelinii* trees with similar DBH and height were randomly selected in each plot, with a total of 36 trees (trees for sampling). The healthy needles were selected at different positions (from upper, middle, and lower canopy layers) of the tree crown, and the photosynthetic rate was measured in situ by a hand-held photosynthesis measurement system (CI-340, CID, USA). For each tree, the photosynthesis measurement was done three times in each layer to produce average photosynthetic rate of the whole plant. The photosynthetic rate of all leaves was measured from 9:00 to 11:00 every morning. Because the needles of *Larix gmelinii* are irregular, it is difficult to accurately determine its leaf area. It is inappropriate to use the photosynthesis per unit area of leaves to express the photosynthetic rate. Therefore, after each measurement of photosynthesis, the measured needles were removed with scissors and weighed to calculate the photosynthetic rate per unit mass. The following equation was used (Qiu 2011):

$$P_n(\mathrm{FW})\left(\upmu\mathrm{mol\ CO_2 kg^{-1} FW\ s^{-1}}\right) = P_n \times S/\mathrm{FW}$$

where P_n (FW) is the photosynthetic rate per unit fresh weight; P_n is the measured value displayed by photosynthetic instrument; S is the leaf chamber area; FW is the fresh weight of leaves in leaf chamber.

Chemical analysis

The C, ^{13}C, N, and ^{15}N contents of all samples were measured on an isotope ratio mass spectrometer (Isoprime 100, Isoprime Ltd.) coupled to an automatic, online elemental analyzer (Vario ISOTOPE cube). The reproducibility of the isotope determination was analyzed in multiple runs at several laboratories using an internal standard soil and plant sample from boreal forests. The accuracy of element content and isotope test is 0.01‰, and the repeated test error ($\pm 2\sigma$) is $<0.02\%$ and <0.05‰, respectively. Natural abundance δ^{15}N and δ^{13}C in samples were reported in the conventional delta (δ) notation, with units of per mil (‰). When measuring the ^{13}C abundance of the soil, we acidified the soil with 1 N HCl at room temperature for 24 h to remove any inorganic C. The stable isotope ratio (δ^{15}N and δ^{13}C) is expressed as parts per thousand differences from a standard: $\delta^{15}\mathrm{N}/\delta^{13}\mathrm{C} = (R_{\mathrm{sample}}/\mathrm{R}_{\mathrm{standard}} - 1) \times 1000$ (‰). R_{sample} represents the isotope ratio of ^{15}N/^{14}N or ^{13}C/^{12}C in the plant or soil sample, and R_{standard} represents the standard ratio of ^{15}N/^{14}N or ^{13}C/^{12}C, usually expressed as ^{15}N/^{14}N of N_2 in the atmosphere, $R_{\mathrm{standard}} = 0.011237$ is the ^{13}C/^{12}C ratio of international standard (PeeDee Belemnite—PDB).

To determine the content of NH_4^+–N and NO_3^-–N in the soil, 5 g fresh soil samples were extracted with 100 mL 0.2 M KCl, oscillated 60 min in the oscillator, and then collected the supernatant. All analyses were performed on a Smartchem instrument 200 (Westco Scientific Instruments, Inc., Italy). Stomatal conductance was measured by hand-held photosynthesis measurement system (CI-340, CID, USA), chlorophyll content and Rubisco activity were measured by ultraviolet–visible spectrophotometer (SHIMADZU UV-1780, Japan).

Data analysis

The Kolmogorov–Smirnov test was used to detect whether the data conformed to the normal distribution, and data homogeneity was detected with Levene's test. One-way analysis of variance (ANOVA) was used to analyze the effects of N addition on different components C %, δ^{13}C, N %, and δ^{15}N of the soil and *Larix gmelinii*, and the effects of N addition on needle photosynthesis related parameters. Pearson correlation analysis was used to analyze the correlation between different components C %, δ^{13}C, N %, and δ^{15}N of the soil and *Larix gmelinii*, and the correlation between photosynthetic related parameters and needle δ^{13}C. All analyses were performed with SPSS 22.0 software package (SPSS, Inc., Chicago, Illinois, USA). Diagrams were drawn using Sigmaplot 13.0 software (Systat Software Inc., Chicago, IL, USA). Tukey's post hoc test was used to test differences between treatments. Statistically significant differences were set at $p<0.05$.

Results

Effects of N addition on the N content and δ^{15}N of plant and soil

Compared with the control, the δ^{15}N and N content of O_{a+e} layer showed a significant increase under MH and HN treatments ($p<0.05$, Table 1). There was no significant difference in the δ^{15}N between 0–10 cm and 10–20 cm soil, but MN and HN treatments significantly increased TN content in 0–10 cm soil ($p<0.05$). HN treatment significantly increased NH_4^+–N and NO_3^-–N in 0–10 cm soil, while all the three levels of N addition had no significant effect on TN, NH_4^+–N, and NO_3^-–N in 10–20 cm soil (Table 1). Under different N addition treatments, the concentration of NH_4^+–N in O_{a+e} layer showed significant differences, and the concentration of NH_4^+–N under HN treatment was significantly higher than that of the control ($p<0.05$, Table 1). Besides, MN treatment significantly increased NO_3^-–N in O_{a+e} layer ($p<0.05$, Table 1).

The δ^{15}N of each organ of *Larix gmelinii* increased with N addition. MN and HN treatments significantly increased δ^{15}N of needle, bark, and fine root ($p<0.05$), while HN treatment significantly increased the δ^{15}N of branch and litter ($p<0.05$, Fig. 2A). HN treatment significantly increased TN of needle, bark, and litter ($p<0.05$), MN and HN treatments significantly increased TN of fine root ($p<0.05$), while N addition had no significant effect on TN of branch (Fig. 2B). The soil δ^{15}N and litter δ^{15}N were significantly negatively correlated with the needle δ^{15}N, while the needle δ^{15}N was significantly positively correlated with the δ^{15}N of O_{a+e}, bark, branch, litter, and fine root. There was a significant positive correlation

Table 1 Soil N concentrations, δ^{15}N values, C concentrations, δ^{13}C in the O_{a+e} horizon and mineral horizon (0–20 cm) in four treatments

Soil horizons	**Soil properties**	**N addition treatments**			
		CK	**LN**	**MN**	**HN**
O_{a+e} horizons	Total N (g kg^{-1})	1.65 ± 0.12b	1.77 ± 0.07b	1.85 ± 0.32a	1.96 ± 0.10a
	δ^{15}N (‰)	−1.47 ± 0.52b	−0.89 ± 0.19ab	−0.25 ± 0.58a	−0.48 ± 0.10a
	Total C (g kg^{-1})	39.92 ± 4.21a	42.64 ± 1.90a	39.12 ± 6.21a	42.60 ± 1.12a
	δ^{13}C (‰)	−29.13 ± 0.11a	−28.96 ± 0.28a	−29.16 ± 0.28a	−29.15 ± 0.20a
	NH_4^+–N (mg kg^{-1})	45.54 ± 12.39b	85.12 ± 15.99ab	186.28 ± 85.75a	166.32 ± 85.43a
	NO_3^-–N (mg kg^{-1})	9.88 ± 0.90b	9.66 ± 0.54b	17.52 ± 3.21a	14.11 ± 3.46ab
Mineral horizons	Total N (g kg^{-1})	0.19 ± 0.03b	0.21 ± 0.03b	0.29 ± 0.04a	0.26 ± 0.04ab
0–10 cm	δ^{15}N (‰)	5.54 ± 0.93a	4.77 ± 0.69a	5.03 ± 0.70a	4.47 ± 0.55a
	Total C (g kg^{-1})	5.15 ± 1.29a	4.07 ± 1.61a	4.02 ± 1.13a	5.13 ± 2.67a
	δ^{13}C (‰)	−26.54 ± 0.30a	−26.75 ± 0.25a	−26.28 ± 0.19a	−26.70 ± 0.17a
	NH_4^+–N (mg kg^{-1})	6.14 ± 0.72b	8.01 ± 3.01b	13.47 ± 3.47a	12.64 ± 2.19a
	NO_3^-–N (mg kg^{-1})	1.70 ± 0.21c	2.74 ± 0.16bc	3.14 ± 0.42b	5.02 ± 2.06a
10–20 cm	Total N (g kg^{-1})	0.08 ± 0.01a	0.08 ± 0.03a	0.08 ± 0.02a	0.09 ± 0.04a
	δ^{15}N (‰)	5.93 ± 0.36a	5.90 ± 0.87a	5.87 ± 0.72a	5.13 ± 0.52a
	Total C (g kg^{-1})	1.41 ± 0.71a	1.26 ± 0.07a	1.18 ± 0.48a	1.18 ± 0.44a
	δ^{13}C (‰)	−26.00 ± 0.44a	−25.98 ± 0.03a	−26.11 ± 0.38a	−26.12 ± 0.25a
	NH_4^+–N (mg kg^{-1})	3.52 ± 1.59a	4.05 ± 1.46a	4.78 ± 0.73a	4.54 ± 1.52a
	NO_3^-–N (mg kg^{-1})	2.23 ± 0.75a	2.47 ± 0.41a	2.78 ± 0.52a	2.37 ± 0.45a

Alphabet indicates the significant difference among different N addition treatments. Tukey's HSD test ($p < 0.05$)

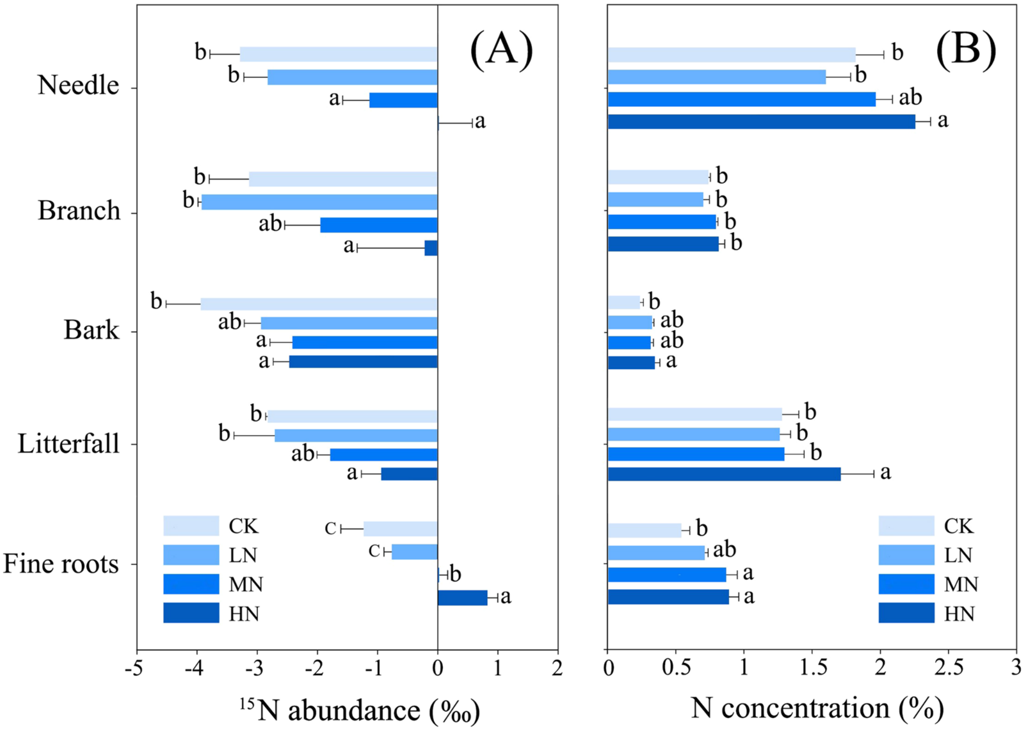


Fig. 2 Effects of nitrogen addition on δ^{15}N value and N concentration of different components of *Larix gmelinii*. **A** The δ^{15}N value of different components of *Larix gmelinii*. **B** The N concentration of different components of *Larix gmelinii*. Error bars represent 1 SE of the mean. CK, LN, MN, HN represent the control, low N treatment, medium N treatment, and high N treatment, respectively

between the $\delta^{15}N$ of fine root and litter, branch, and bark (Fig. 5).

Effects of N addition on the C content and $\delta^{13}C$ of plant and soil

Nitrogen addition did not significantly change TC and $\delta^{13}C$ in O_{a+e} and 0–20 cm soil layers (Table 1). For *Larix gmelinii*, N addition significantly increased the $\delta^{13}C$ of the needle ($p < 0.05$). The $\delta^{13}C$ of branch and bark decreased with N addition, while the $\delta^{13}C$ of litter and fine root increased with N addition (Fig. 3A). The N addition had no significant effect on the TC of organs of *Larix gmelinii* (Fig. 3B). The TC and TN of O_{a+e} were significantly positively correlated, and the TC of litter was significantly positively correlated with the $\delta^{13}C$ of litter (Fig. 5).

Effects of N addition on the photosynthetic rate of *Larix gmelinii*

MN treatment significantly increased the photosynthetic rate of *Larix gmelinii* (Fig. 4A). HN treatment significantly increased the chlorophyll content (Fig. 4C). LN and MN treatments significantly increased the activity of Rubisco enzyme (Fig. 4D). Nitrogen addition had no significant effect on the stomatal conductance of the needle (Fig. 4B). The photosynthetic rate was significantly positively correlated with Rubisco enzyme, chlorophyll, and needle $\delta^{13}C$ ($p < 0.05$), and chlorophyll and stomatal conductance were significantly positively correlated with the needle $\delta^{13}C$ ($p < 0.05$, Table 2).

Discussion

Effects of N addition on the N content and $\delta^{15}N$ of plant and soil

The input process (e.g., N deposition and biological N fixation) and output process (e.g., denitrification, leaching and plant absorption) of N in soil determine the size of N pool (Dawson et al. 2002; Fernandez-Alonso et al. 2018; Rivero-Villar et al. 2021). In this study, addition of N with $\delta^{15}N$ close to that of atmospheric N_2 (≈ 0), significantly increased the $\delta^{15}N$ of *Larix gmelinii* (Fig. 2). Our results showed that the N addition significantly increased the soil TN (Table 1), but had no significant effect on the $\delta^{15}N$ of soil mineral horizon, which is inconsistent with previous studies (Gurmesa et al. 2017; Wang et al. 2021) and our first hypothesis. The $\delta^{15}N$ of soil is regulated by N input and N output fluxes (Liu et al. 2017). Based on the mass balance model of Brenner et al. (2001), under the condition of constant N output flux, N addition reduces the $\delta^{15}N$

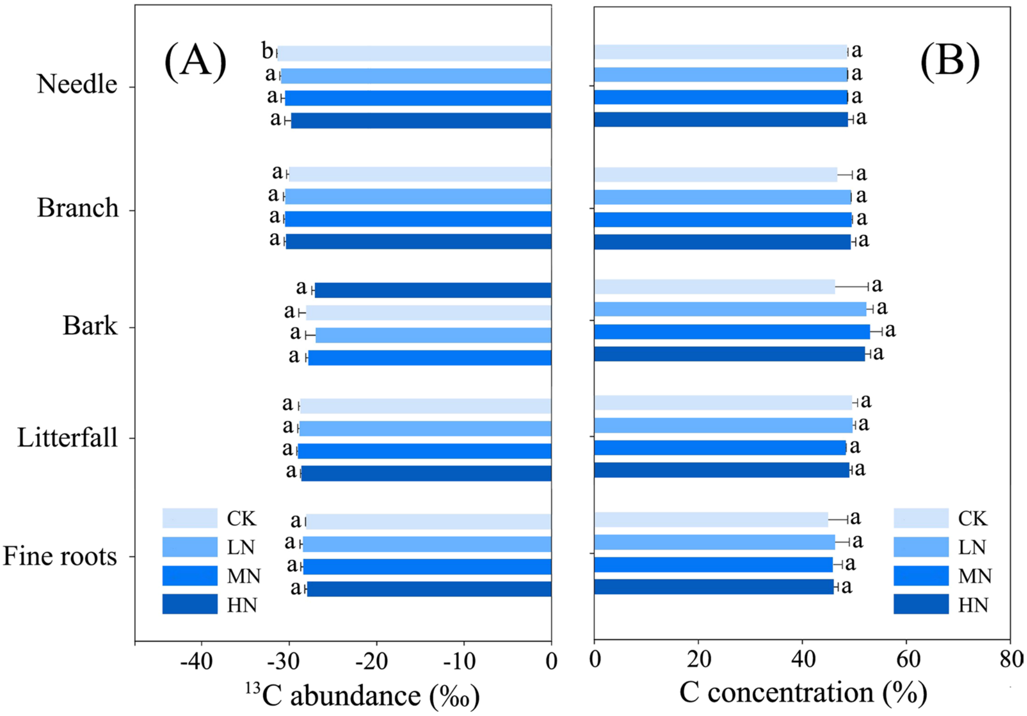


Fig. 3 Effects of nitrogen addition on $\delta^{13}C$ value and C concentration of different components of *Larix gmelinii*. **A** The $\delta^{13}C$ value of different components of *Larix gmelinii*. **B** The C concentration of different components of *Larix gmelinii*. Error bars represent 1 SE of the mean. CK, LN, MN, HN represent the control, low N treatment, medium N treatment, and high N treatment, respectively

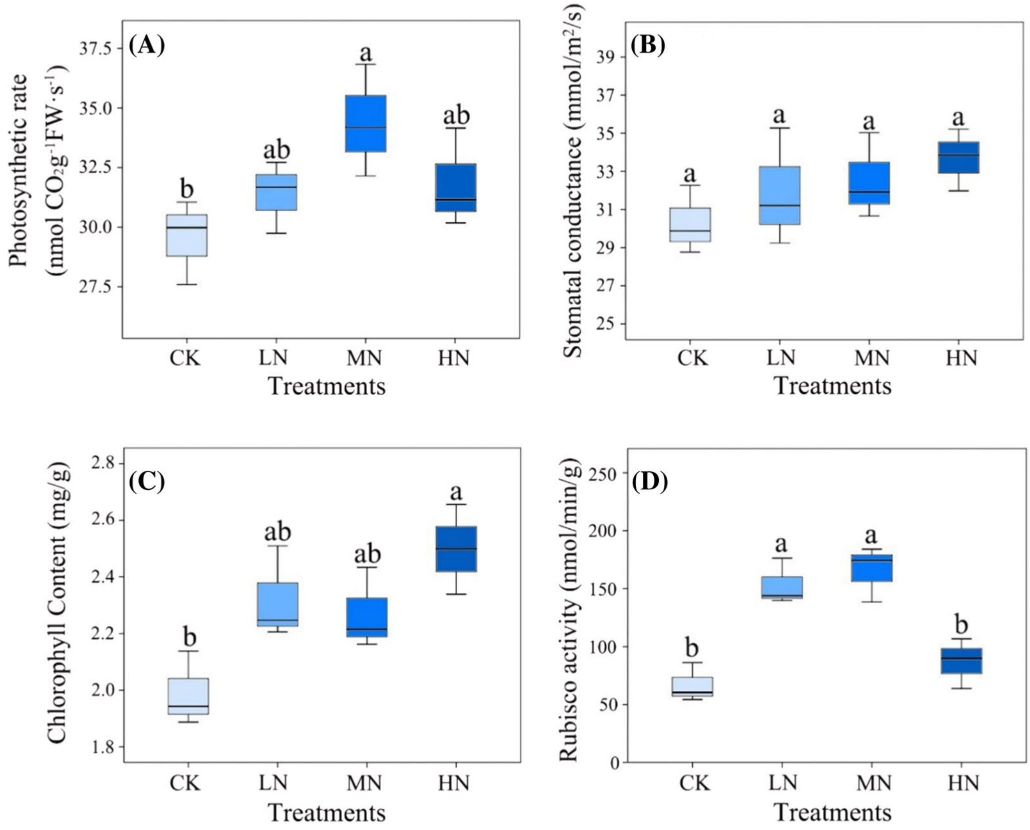


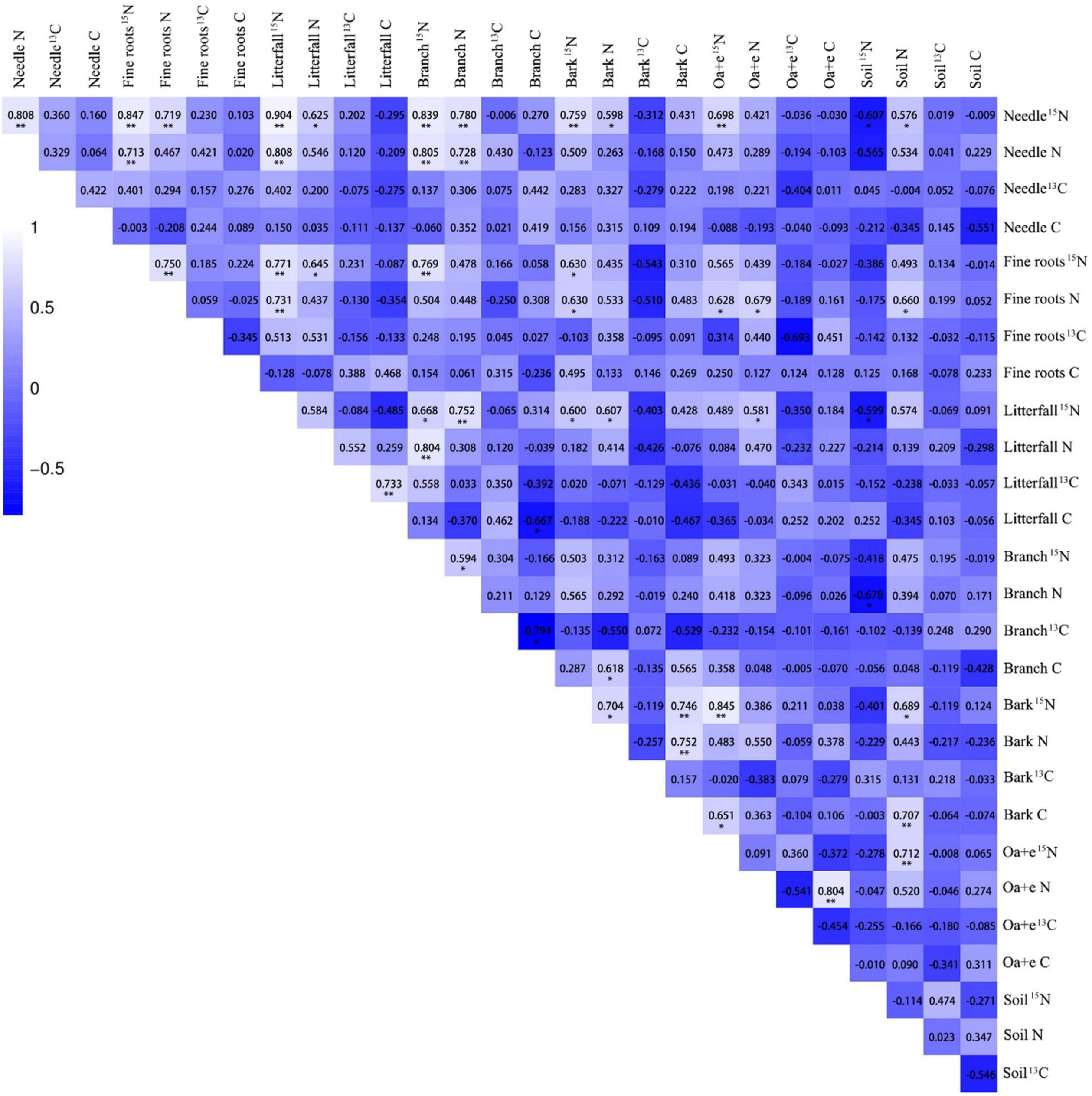
Fig. 4 Effects of nitrogen addition on photosynthetic related indexes of *Larix gmelinii*. Error bars represent 1 SE of the mean. **A** The photosynthetic rate of *Larix gmelinii*. **B** The stomatal conductance of *Larix gmelinii*. **C** The chlorophyll content of *Larix gmelinii*. **D** The Rubisco activity of *Larix gmelinii*. CK, LN, MN, HN represent the control, low N treatment, medium N treatment, and high N treatment, respectively

Table 2 Pearson correlation coefficients between the photosynthesis related parameters and TC, $\delta^{13}C$ of *Larix gmelinii*

	P_n	Rubisco	chlorophyll	Stomatal conductance	Needle $\delta^{13}C$
Rubisco	0.603*				
Chlorophyll	0.637*	0.304			
Stomatal conductance	0.526	0.23	0.714**		
Needle $\delta^{13}C$	0.624*	− 0.06	0.818**	0.039	
Needle TC	− 0.222	0.268	− 0.228	− 0.191	0.422

* represents $p < 0.05$, ** represents $p < 0.01$

of soil. Therefore, the lack of significant changes in soil $\delta^{15}N$ after long-term N addition is likely to be the result of the balance between input and output flux. This is probably due to the loss of ^{15}N-depleted N through denitrification and leaching. Under such condition, the residual soil (and plant) would be ^{15}N-enriched. MN and HN treatments significantly increased NH_4^+–N and NO_3^-–N, and increased soil mineralization rate (Table 1), which might lead to increased denitrification and leaching. Gaseous losses of ^{15}N-depleted N by denitrification would enrich ^{15}N in soil N (Houlton et al. 2006). It has been proved that NH_3 volatilization process will cause 40–60‰ isotope fractionation effect (Robinson 2001; Liu et al. 2017). MN and HN treatments significantly increased the $\delta^{15}N$ of O_{a+e}, which might be because we added a relatively ^{15} N-enriched N to the O_{a+e} that was relatively ^{15}N-depleted. In addition, soil organic matter rich in ^{14}N was decomposed during soil microbial decomposition, resulting in soil ^{15}N enrichment (Bostrm et al. 2007). ^{15}N enriched microorganisms and microbial residues are produced in the process of microbial assimilation for organic



Needle N	Needle ^{13}C	Needle C	Fine roots ^{15}N	Fine roots N	Fine roots ^{13}C	Fine roots C	Litterfall ^{15}N	Litterfall N	Litterfall ^{13}C	Litterfall C	Branch ^{15}N	Branch N	Branch ^{13}C	Branch C	Bark ^{15}N	Bark N	Bark ^{13}C	Bark C	Oa+e ^{15}N	Oa+e N	Oa+e ^{13}C	Oa+e C	Soil ^{15}N	Soil N	Soil ^{13}C	Soil C	
0.808**	0.360	0.160	0.847**	0.719**	0.230	0.103	0.904**	0.625*	0.202	-0.295	0.839**	0.780**	-0.006	0.270	0.759**	0.598*	-0.312	0.431	0.698**	0.421	-0.036	-0.030	-0.607*	0.576*	0.019	-0.009	Needle ^{15}N
	0.329	0.064	0.713**	0.467	0.421	0.020	0.808**	0.546	0.120	-0.209	0.805**	0.728**	0.430	-0.123	0.509	0.263	-0.168	0.150	0.473	0.289	-0.194	-0.103	-0.565	0.534	0.041	0.229	Needle N
		0.422	0.401	0.294	0.157	0.276	0.402	0.200	-0.075	-0.275	0.137	0.306	0.075	0.442	0.283	0.327	-0.279	0.222	0.198	0.221	-0.404	0.011	0.045	-0.004	0.052	-0.076	Needle ^{13}C
			-0.003	-0.208	0.244	0.089	0.150	0.035	-0.111	-0.137	-0.060	0.352	0.021	0.419	0.156	0.315	0.109	0.194	-0.088	-0.193	-0.040	-0.093	-0.212	-0.345	0.145	-0.551	Needle C
				0.750**	0.185	0.224	0.771**	0.645*	0.231	-0.087	0.769**	0.478	0.166	0.058	0.630*	0.435	-0.543	0.310	0.565	0.439	-0.184	-0.027	-0.386	0.493	0.134	-0.014	Fine roots ^{15}N
					0.059	-0.025	0.731**	0.437	-0.130	-0.354	0.504	0.448	-0.250	0.308	0.630*	0.533	-0.510	0.483	0.628*	0.679*	-0.189	0.161	-0.175	0.660*	0.199	0.052	Fine roots N
						-0.345	0.513	0.531	-0.156	-0.133	0.248	0.195	0.045	0.027	-0.103	0.358	-0.095	0.091	0.314	0.440	-0.693	0.451	-0.142	0.132	-0.032	-0.115	Fine roots ^{13}C
							-0.128	-0.078	0.388	0.468	0.154	0.061	0.315	-0.236	0.495	0.133	0.146	0.269	0.250	0.127	0.124	0.128	0.125	0.168	-0.078	0.233	Fine roots C
								0.584	-0.084	-0.485	0.668*	0.752**	-0.065	0.314	0.600*	0.607*	-0.403	0.428	0.489	0.581*	-0.350	0.184	-0.599*	0.574	-0.069	0.091	Litterfall ^{15}N
									0.552	0.259	0.804**	0.308	0.120	-0.039	0.182	0.414	-0.426	-0.076	0.084	0.470	-0.232	0.227	-0.214	0.139	0.209	-0.298	Litterfall N
										0.733**	0.558	0.033	0.350	-0.392	0.020	-0.071	-0.129	-0.436	-0.031	-0.040	0.343	0.015	-0.152	-0.238	-0.033	-0.057	Litterfall ^{13}C
											0.134	-0.370	0.462	-0.667*	-0.188	-0.222	-0.010	-0.467	-0.365	-0.034	0.252	0.202	0.252	-0.345	0.103	-0.056	Litterfall C
												0.594*	0.304	-0.166	0.503	0.312	-0.163	0.089	0.493	0.323	-0.004	-0.075	-0.418	0.475	0.195	-0.019	Branch ^{15}N
													0.211	0.129	0.565	0.292	-0.019	0.240	0.418	0.323	-0.096	0.026	-0.678*	0.394	0.070	0.171	Branch N
														-0.794*	-0.135	-0.550	0.072	-0.529	-0.232	-0.154	-0.101	-0.161	-0.102	-0.139	0.248	0.290	Branch ^{13}C
															0.287	0.618*	-0.135	0.565	0.358	0.048	-0.005	-0.070	-0.056	0.048	-0.119	-0.428	Branch C
																0.704*	-0.119	0.746**	0.845**	0.386	0.211	0.038	-0.401	0.689*	-0.119	0.124	Bark ^{15}N
																	-0.257	0.752**	0.483	0.550	-0.059	0.378	-0.229	0.443	-0.217	-0.236	Bark N
																		0.157	-0.020	-0.383	0.079	-0.279	0.315	0.131	0.218	-0.033	Bark ^{13}C
																			0.651*	0.363	-0.104	0.106	-0.003	0.707**	-0.064	-0.074	Bark C
																				0.091	0.360	-0.372	-0.278	0.712**	-0.008	0.065	Oa+e ^{15}N
																					-0.541	0.804**	-0.047	0.520	-0.046	0.274	Oa+e N
																						-0.454	-0.255	-0.166	-0.180	-0.085	Oa+e ^{13}C
																							-0.010	0.090	-0.341	0.311	Oa+e C
																								-0.114	0.474	-0.271	Soil ^{15}N
																									0.023	0.347	Soil N
																										-0.546	Soil ^{13}C

Fig. 5 Pearson correlation coefficients among the C, N, δ^{13}C, and δ^{15}N of different compartments of *Larix gmelinii* and soil. * represents $p < 0.05$, ** represents $p < 0.01$

matter, which will also lead to the gradual enrichment of ^{15}N in soil organic matter over time (Dijkstra et al. 2010).

Nitrogen addition significantly increased the δ^{15}N in plant compartments (Fig. 2), and the δ^{15}N in needles was positively correlated with branch, bark, fine root, litter, and humus (Fig. 5), which was consistent with our second hypothesis. The increased plant δ^{15}N as a result of N addition could be the imprint of the added fertilizer N which was ^{15}N-enriched compared to the plant tissues (Gurmesa et al. 2017). Our results also showed that the increase of δ^{15}N of *Larix gmelinii* was directly related to the soil δ^{15}N (Fig. 5). In the process of N cycle, N addition leads to an increase in plant N utilization rate, and then increases the δ^{15}N of each organ of *Larix gmelinii* due to isotope fractionation (Sheng et al. 2019). In addition, when the soil N availability is low, plants may be more dependent on mycorrhizal fungi (Vallano and Sparks

2013). Mycorrhizal fungi usually transfer ^{15}N depleted N to plants, resulting in high enrichment of ^{15}N in mycorrhizal mycelium, resulting in ^{15}N depletion in plant tissues (Schweiger, 2016). The $\delta^{15}N$ of fine roots of *Larix gmelinii* is significantly lower than that of needle, branch, and bark (Fig. 2), which may be caused by the fractionation effect of mycorrhizal. The increase of $\delta^{15}N$ in needles can also be used to indicate the increase of N cycle rate. Previous studies have also shown that there is a strong correlation between the enrichment level of ^{15}N in leaves and the increase in N cycle rate (Garten 1993; Emmett et al. 1998). The greater the difference in $\delta^{15}N$ between the shoot and the root, the greater the proportion of inorganic N absorbed by the leaves (Kalcsits et al. 2014).

We also found that the soil $\delta^{15}N$ increased with an increase in soil depth, which is consistent with a large number of studies (Kramer et al. 2017; Högberg et al. 2019; Potapov et al. 2019). This may be because ^{15}N depleted litter and plant residues enter the surface soil, and the accumulation of ^{15}N enriched mycorrhizal fungal residues in the deep soil can lead to a large enrichment of ^{15}N in the deep soil relative to the surface litter.

Effects of N addition on the C content and $\delta^{13}C$ of plant and soil and photosynthetic rate

Our results showed that N addition significantly increased the $\delta^{13}C$ of needle, but had no significant effect on the $\delta^{13}C$ of other plant organs (Fig. 3). The C isotope fractionation caused by photosynthesis mainly takes place in the following two steps: first, there is a certain resistance when $^{13}CO_2$ enters mesophyll cells. Due to the influence of kinetic fractionation effect, $^{12}CO_2$ diffuses faster and is easily absorbed by plants; Second, under the action of carboxyl polypeptidase, $^{12}CO_2$ dissolved in cells and Rubisco carboxylase are preferentially combined into phosphoglyceric acid, while the remaining CO_2 is enriched in ^{13}C (Park and Epstein 1960; Flexas et al. 2006; Ulli et al. 2008). Previous studies have shown that the decrease in plant stomatal conductance leads to a decrease of CO_2 concentration in plant leaves and the increase of $\delta^{13}C$ in leaves (Morecroft and Woodward 1990; Rao et al. 2017). In our study, N addition had no significant effect on stomatal conductance (Fig. 4B). Therefore, we speculate that the increase in needle $\delta^{13}C$ may be caused by the assimilation of $^{12}CO_2$. The increase of ^{13}C in needles could be explained by the addition of N increased Rubisco activity (Fig. 4D) and chlorophyll (Fig. 4C), increased photosynthetic rate (Fig. 4A), and gradually increased the consumption of $^{12}CO_2$, which is consistent with our third hypothesis. Tomaszewski and Sieving (2007) also showed that N addition increased photosynthetic rate, which could enhance CO_2 assimilation but also reduce the thermally dissipated light.

Our previous results found that N addition significantly increased CH_4 and CO_2 emissions from the *Larix gmelinii* forest (Yan et al. 2018), and the increase of C output flux usually led to the enrichment of soil ^{13}C. The lack of significant change in soil ^{13}C in response to N addition (Fig. 3A) could be a trade-off between increased levels of plant residue (more ^{13}C depleted than soil C) inputs and ^{13}C enrichment from enhanced soil C decomposition. Litter, fine root, and root exudates are the main sources of C input to soil (Waldrop et al. 2004; Cusack et al. 2010). The decrease of litter $\delta^{13}C$ (Fig. 3A) may be related to the fractionation effect of C release during litter decomposition. Easily decomposable organic compounds (such as sugar, starch and cellulose) in litter usually show high $\delta^{13}C$ value. In the process of microbial decomposition, this leads to the loss of ^{12}C faster than ^{13}C, resulting in the enrichment of ^{13}C in litter (Cotrufo et al. 2005; Bowling et al. 2008). The retention efficiency of root C in soil is much higher than that of litter C (Clemmensen et al. 2013). Therefore, the change in equilibrium $\delta^{13}C$ output may mainly come from the input of roots and root exudates. Gougherty et al. (2018) found that the greater allocation of relatively ^{13}C depleted (i.e., lower $\delta^{13}C$ values) non-structure carbohydrate (NSC) from leaves to roots when the photosynthesis was high. Our previous studies also showed that N addition increased the biomass of underground roots of *Larix gmelinii* and strengthened the transport function of fine roots to overcome the P limitation (Liu et al. 2021). A meta-analysis by Finzi et al. (2015) showed that the contribution of root C input to the C–N mineralization process of forest soil was as high as 33%. The study by Bais et al. (2006) also found that up to 40% of photosynthetic fixed C was secreted into the rhizosphere. And high nutrient availability can promote the release of fine root exudates (Neumann and Romheld 2000). Although we have not measured root exudates, the fractionation effect of root exudates on soil C input cannot be ignored.

Gessler et al. (2012) showed that the difference of $\delta^{13}C$ in leaves and $\delta^{13}C$ in phloem of branch reached 30%, due to the fractionation effect of photosynthesis. In our results, although there is no significant change in $\delta^{13}C$ of branch and bark, it shows a decreasing trend with the N addition, and the $\delta^{13}C$ of branch and bark is also 8.4–13% lower than that of needle (Fig. 3A), which may be due to the increased transport of organic matter rich in ^{12}C to the aboveground.

Conclusions

Our study shows that long-term N addition changes the C and N cycle of boreal forest ecosystem. The N addition increased the $\delta^{15}N$ value and TN of each organ of *Larix gmelinii* and also increased the N mineralization of soil, but had no significant effect on the soil δ^{15}N. Compared with N, the C concentration and ^{13}C of *Larix gmelinii* were less affected. The N addition only increased the δ^{13}C of needle, which was the result of the increase of photosynthetic efficiency of needle. By exploring the stable C and N isotopic signatures in plant–soil compartments in boreal forest ecosystems, this study confirms that the C and N state and cycle of ecosystem may be fluctuated under the projected N enrichment.

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Author contributions
QW and YX designed the study, were awarded funding, supervised data collection and contributed to and edited manuscripts. QW, GL, YX, ZY, GY, SL and XW contributed the whole manuscript preparation and design and wrote the main manuscript text. QW, GL, YX, ZY, GY and SL prepared all figures, GL, YX, ZY, GY, SL, XW and QW prepared field experiments, tables and collected literatures. All authors read and approved the final manuscript.

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Availability of data and materials
Data are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflict of interests.

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