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Effect of nitrogen addition on carbon and nitrogen stable isotopes in temperate forest litter and soil

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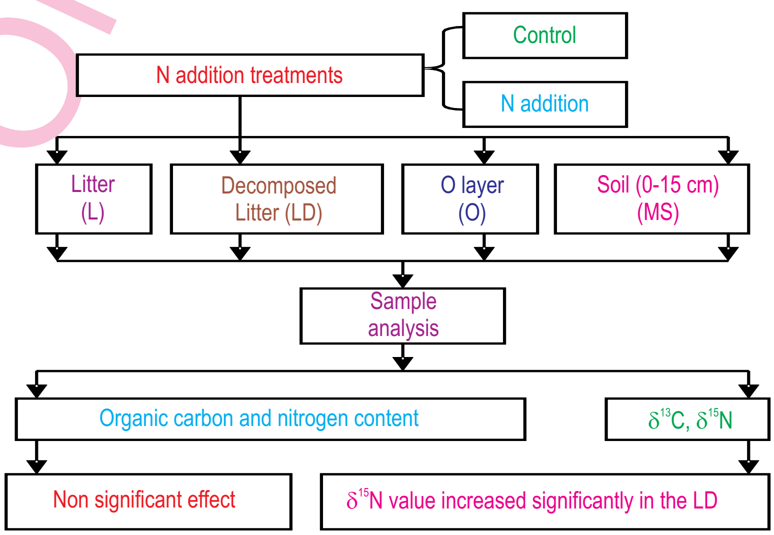
Abstract

Aim : The aim of this study was to investigate the changes of litter and soil carbon and nitrogen stable isotopes in temperate forest ecosystem under nitrogen addition.

Methodology : In a mixed broad-leaf Korean pine forest in Changbai Mountain, China after 6 years of nitrogen addition (50 $kg \cdot ha^{-2} \cdot yr^{-1}$) treatment, the content of organic carbon and nitrogen, stable isotopes of carbon and nitrogen in litter and soil layer were determined by elemental analyzer and isotope ratio mass spectrometer.

Results : The results showed that the carbon concentration decreased with soil depth in the control and treatment samples. The nitrogen concentration had a tendency to increase in the decomposed litter layer. Nitrogen addition did not show significant effect on carbon and nitrogen concentration. The carbon stable isotope ($\delta^{13}C$) was about -27‰ in forest cover layer and organic matter layer, while it was as high as -25.3‰ in mineral soil layer. Nitrogen addition had no significant effect on the value of $\delta^{13}C$. The range of nitrogen stable isotope ($\delta^{15}N$) was -1.6 ~ 5.5‰, which increased with the depth of soil. $\delta^{15}N$ value increased significantly ($P = 0.039$) in the decomposed litter layer under nitrogen addition.

Interpretation : $\delta^{15}N$ enrichment implied that more ^{15}N was remaining under nitrogen addition. This indicated that nitrogen deposition enhanced the decomposition of organic matter implied by $\delta^{15}N$ enrichment in the decomposed litter layer, although carbon content did not show significant change. This study provides a data support for further studies on the effects of nitrogen deposition on forest ecosystems.



Introduction

Nitrogen (N) is an essential component of the organism (Högberg, 2007; Houlton *et al.,* 2015). When the amount of inorganic nitrogen deposition is within a certain range, it is generally believed that most of the nitrogen is retained in the ecosystem (Luo *et al.,* 2015). 20 kg N ha^{-1} yr^{-1} is usually considered as a critical point of nitrogen saturation (McNulty *et al.,* 2017) that will affect the biological diversity of the forest ecosystem. In nitrogen-deficient ecosystem, the nitrogen input through atmospheric deposition could increase the primary productivity (Wu *et al.,* 2015), biomass of the ecosystem and the accumulation of soil organic matter (Whittinghill *et al.,* 2012; Dai *et al.,* 2013). In the nitrogen-saturated ecosystem, the external input of organic nitrogen can not play the role of nutrient. It will aggravate the nitrogen loss of terrestrial ecosystems, leading to water eutrophication (Girolamo *et al.,* 2017). ^{14}N and ^{15}N are two stable isotopes of nitrogen. The relative abundance of ^{14}N is 99.633% in the air, while the relative abundance of ^{15}N is only 0.366%. Abundance ratio of $^{15}N/^{14}N$ is constant that is 1/272 even at different elevations (Phillips *et al.,* 2002; Kramer *et al.,* 2017). Therefore, atmosphere can be used as a reference standard while detecting the nitrogen isotopic composition ($\delta^{15}N$) of different substances. Isotope distribution varies with ecosystem process due to isotope fractionation. The $\delta^{15}N$ values of different substances are different.

Soil carbon (C) storage accounts for 73%, up to 1500 Pg C (Post *et al.,* 1982) in terrestrial ecosystems, twice the atmospheric carbon storage capacity and three times the terrestrial vegetation carbon pool. Thus, the soil carbon pool is considered to be the largest carbon stock of the terrestrial ecosystem (Siegenthaler and Sarmiento, 1993). The CO_2 released from soil respiration is ten times higher than CO_2 concentration in atmosphere (Raich and Potter, 1995). Therefore, small changes in soil carbon pool will cause significant fluctuations in the concentration of CO_2 in the atmosphere (Schlesinger and Andrews, 2000). Forest ecosystem accounts for 30% of the land area and the soil carbon pool is 45% of total carbon stocks (Dixon and Turner, 1991). Soil carbon mainly contains labile components (such as proteins, carbohydrates) and refractory components (such as lignin, waxes). The ages of labile and refractory components are several days and hundreds years, respectively. Therefore, one of the carbon sequestration mechanisms is the biochemical protection of refractory components in soil. The study of dynamic changes in soil carbon stock is significant to the global carbon cycle.

Carbon exists in the form of three isotopes of ^{12}C, ^{13}C and ^{14}C in nature, among which the first two are carbon stable isotopes ($\delta^{13}C$), the relative abundances are 98.89% and 1.11%, respectively. ^{14}C is a radioactive isotope with a half-life of 5730 yr, but its content is minimal (Wotherspoon *et al.,* 2017). The $\delta^{13}C$ value of defined standard material is 0‰, which is the United States South Carolina Cretaceous Pee Dee group of rocket fossils (PDB). Thus, the carbon isotopic composition of CO_2 is -7.4‰ in the atmosphere (Keeling *et al.,* 1979). Atmospheric CO_2 is the carbon sources of plant photosynthesis process. In the process of material transformation, there are obvious isotopic fractionation, resulting in different carbon stocks with their typical carbon stable isotope composition. Carbon isotopes can then be used to research carbon cycle process in the ecosystem. Elevated nitrogen deposition may increase litter and soil carbon storage. Minor changes of soil C storage may significantly affect the atmospheric CO_2 concentration, and thereby global warming. However, previous reports of the response of soil carbon and nitrogen stable isotope have demonstrated complicated and seemingly contradictory results. In order to investigate the process of carbon and nitrogen cycles in forest ecosystem, a broad-leaf Korean pine forest in Changbai Mountain in North Eastern China was selected as study area, belonging to temperate forest ecosystem. This is to explore the changes of litter and soil carbon and nitrogen stable isotope in temperate forest ecosystem under nitrogen addition.

Materials and Methods

Site description : The research site was based on the broad-leaf Korean pine forest established by Changbai Mountain Forest Ecosystem Research Station of Chinese Academy of Sciences (42°24'N, 128°5'E). It is the representative top-level vegetation of temperate zone. The broad-leaf Korean pine forest in Changbai Mountain is distributed in the range of 500 ~ 1100 m elevation (average elevation of 738 m). The annual average temperature and rainfall are 3.8°C and 700 mm, respectively. The soil type is dark brown soil with volcanic ash as parent material. Broad-leaf species are more numerous than conifers in this area.The main tree species include *Pinus koraiensis, Tilia amurensis, Quercus mongolica, Fraxinus mandshurica, Acer mono* and *Acer barbinerve.* The average age is 200 yrs. The average canopy height and the DBH are 15 m and 34.2 cm, respectively (Cai *et al.,* 2017). The main shrub species include *Philadelphus schrenkii, Euonymus alatus, Lonicera japonica, Corylus mandshurica* and *Deutzia scabra.* The main herbaceous species include *Anemone raddeana, Cyperus microiria, Funaria officinalis, Adonis vernalis, Brachybotrys paridiformis, Anemone cathayensis* and *Filipendula palmata*.

Sample collection : The experiment of nitrogen addition was established in Changbai Mountain in 2006, consisting of four control and four N addition plots, 25 m × 25 m. In N addition plots, ammonium nitrate (NH_4NO_3) 50 kg N ha^{-1} yr^{-1} was used to fertilize during the growing season (from May to October) with a sprayer. The rate of atmospheric N deposition was 23 kg N ha^{-1} yr^{-1} in this area. The fertilization rate was two times the current N inputs of atmosphere. In control plots, the same rate of deionized water was applied during the same periods. In order to avoid N leaching and exchange between N addition and control plots, iron plates were inserted in the soil at the depth of 1.5 m between plots.

In control and nitrogen treatment plots, samples were collected separately in litter (L), decomposed litter (LD), O horizon

(O) and mineral soil (MS) in June 2012. To create a composite sample, four samples were randomly collected and mixed together in each plot. The mineral soil was sampled at 0-15 cm using a 5 cm diameter impact corer. All samples were brought to the laboratory as soon as possible. The L and LD samples were dried and then ground into powder using an automated grinder. To remove roots, rocks and other coarse debris, 2 mm sieve was used to process O horizon and MS samples. The samples were then dried in a convection oven at 50°C and ground into powder for further chemical analysis.

Elemental and stable isotope composition analyses : Total C and N contents, carbon and nitrogen stable isotope composition of L, LD, O and MS samples were determined in duplicate, using a Sercon GSL (Crewe, UK) elemental analyzer combined with isotope ratio mass spectrometer. In this experiment, peach leaves (PLs) were used as standard substance and the external standard method was used to determine the value of the parameters. The values of the standard substances were: $\delta^{15}N$ = 1.5‰, $\delta^{13}C$ = -26.1‰, %C = 46.8 and %N = 2.84. The accuracy of this method for $\delta^{13}C$ and $\delta^{15}N$ was 0.2‰ and 0.35‰, respectively. The pH value of this study area decreased to 5.47. The reason may be the increase of soil ammonium nitrogen concentration (10.40 mg N kg^{-1} soil) and nitrate nitrogen concentration (22.56 mg N kg^{-1} soil), which could enhance soil acidification under nitrogen addition (Bai *et al.,* 2014). So the measured total carbon content was equivalent to organic carbon (OC) content.

Statistical analyses : The null hypothesis is that N addition had no effect on the concentration of C and N, stable isotope composition of C and N. One way ANOVA was performed to assess the effect of N addition in SPSS (v 16.0). When the P-value was ≤0.050, differences were considered significant. When P-value was between 0.050 and 0.100, it was called trends. It indicated no difference when a P-value > 0.100.

Results and Discussion

The C and N contents were used for calculating compounds percentage in previous study (Wu *et al.,* 2015). The concentration of C (%) decreased with the increase of soil depth: L (46.6%) > LD (42.8%) > O (18.3%) > MS (2.0%) in control samples and L (45.9%) > LD (43.7%) > O (20.2%) > MS (2.4%) in the treatment samples (Table 1). The concentration of N (%) increased from L (1.03% in control and 1.24% in treatment, respectively) to LD layer (1.70% in control and 1.67% in treatment, respectively). And then N content decreased in O layer (1.31% in control and 1.44% in treatment, respectively) and MS (0.18% in control and 0.22% in treatment, respectively). There was no significant difference in the effects of nitrogen addition on C concentration in the four layers, L (P = 0.157), LD (P = 0.212), O (P = 0.170) and MS (P = 0.198), respectively. Similarly, no significant increase of N concentration was observed in the L (P = 0.142), LD (P = 0.719), O (P = 0.208) and MS (P = 0.189) under nitrogen addition. And the C/N ratio was not influenced by nitrogen addition in the L (P = 0.136), LD (P = 0.512), O (P = 0.785) and MS (P = 0.449).

After six years of nitrogen application, no effect of nitrogen addition on carbon and nitrogen concentration was observed in litter and mineral soil layers (0-15 cm). The results of this study confirm the results of previous studies (Thomas *et al.,* 2013; Zhang *et al.,* 2013). The effect of nitrogen addition on the forest cover is likely to play an increasing role, and may also have a reduced effect or no significant change (Knorr *et al.,* 2005; Liu *et al.,* 2016; Asthir *et al.,* 2017). In addition, previous studies have shown that nitrogen addition increases the carbon concentration and provides support for the hypothesis that atmospheric nitrogen is one of the controlling factors for carbon accumulation in terrestrial ecosystems (Pregitzer *et al.,* 2008; Liu and Greaver, 2010; Cheng *et al.,* 2017; Guo *et al.,* 2017; Knapp *et al.,* 2017). The hypothesis is that the increase in nitrogen can cause a

Table 1 : Concentration of carbon (C) and nitrogen (N), and ratio of C to N (C/N) in litter (L), decomposed litter (LD), O horizon (O) and mineral soil (MS) of control and N addition plots

	Control	N addition	P
Carbon (%)			
L	46.6 ± 0.26	45.9 ± 0.34	0.157
LD	42.8 ± 0.70	43.7 ± 0.14	0.212
O	18.3 ± 1.24	20.2 ± 0.27	0.170
MS	2.0 ± 0.23	2.4 ± 0.13	0.198
Nitrogen (%)			
L	1.03 ± 0.02	1.24 ± 0.12	0.142
LD	1.70 ± 0.06	1.67 ± 0.06	0.719
O	1.31 ± 0.08	1.44 ± 0.04	0.208
MS	0.18 ± 0.02	0.22 ± 0.02	0.189
C/N ratio			
L	45.2 ± 1.19	38.2 ± 3.91	0.136
LD	25.3 ± 1.19	26.4 ± 1.07	0.512
O	13.9 ± 0.39	14.0 ± 0.22	0.785
MS	10.7 ± 0.08	11.0 ± 0.26	0.449

Values are mean ± one standard error for four plots per treatment

Table 2 : Values of d13C and d15N in litter (L), decomposed litter (LD), O horizon (O) and mineral soil (MS) of control and N addition plots

	Control	N addition	P
$\delta^{13}C$ (‰)			
L	-27.4 ± 0.17	-27.6 ± 0.14	0.440
LD	-27.7 ± 0.09	-27.7 ± 0.07	1.000
O	-26.9 ± 0.07	-26.9 ± 0.05	1.000
MS	-25.3 ± 0.03	-25.4 ± 0.07	0.437
$\delta^{15}N$ (‰)			
L	-1.1 ± 0.13	-1.2 ± 0.12	0.895
LD	-1.6 ± 0.16	-1.0 ± 0.10	0.039**
O	1.6 ± 0.16	1.5 ± 0.18	0.688
MS	5.5 ± 0.14	5.2 ± 0.13	0.149

Values are mean ± one standard error for four plots per treatment. ** represents significant differences between N addition and control samples at P≤ 0.050 level

decrease in the degradation of litter by microorganisms, which has an effect on the microbial oxidase activity (Frey *et al.,* 2004; Waldrop *et al.,* 2004; Song *et al.,* 2017). It is noteworthy that an increase in nitrogen deposition can result in increased plant biomass. Plant contains a lower C/N, which indicates a higher rate of decomposition on the basis of increasing the quality of litter (Oren *et al.,* 2001; Li *et al.,* 2017).

$\delta^{13}C$ and $\delta^{15}N$ are often used to indicate the source of the substance (Bai *et al.,* 2013; Virgil *et al.,* 2018; Wu *et al.,* 2018). In this study, the range of $\delta^{13}C$ was -27.6 ~ -25.3‰ (Table 2). The $\delta^{13}C$ values were about -27‰ in the L, LD and O layers, which significantly increased to -25.3‰ in MS. $\delta^{13}C$ values were not significantly affected by the addition of nitrogen in the four layers of L (P = 0.440), LD (P = 1.000), O (P = 1.000) and MS (P = 0.437).

In the forest cover layer and mineral soil, the range of $\delta^{15}N$ was -1.6 ~ 5.5‰ (Table 2), increasing with soil depth. Among them, $\delta^{15}N$ values were negative (-1‰ or so) in L and LD layers, and 1.5‰ in the O layer, and 5.5‰ in the MS layer. The $\delta^{15}N$ was not significantly influenced by nitrogen addition in the L (P = 0.895). While it was significantly enriched in LD (P = 0.039). The effect of nitrogen addition on $\delta^{15}N$ was not significant in O (P = 0.688) and MS (P = 0.149), respectively. The enrichment of $\delta^{15}N$ at LD layer suggested that more of the ^{15}N was remaining and more ^{14}N was released under the addition of nitrogen. Isotope fractionation and isotope mixing would induce to variation of $\delta^{15}N$ value among different materials in terrestrial ecosystem (Kramer *et al.,* 2017). In biogeochemical process, heavy isotope of ^{15}N usually remains in the substrate, and light isotope of ^{14}N is usually used for the product (Bai *et al.,* 2013). $\delta^{15}N$ value can be changed with input process and output process for an ecosystem N pool. In general, $\delta^{15}N$ values of soil and plant are higher than that of atmospheric N deposition and biological N fixation. Therefore, the increase of N input rates would decrease $\delta^{15}N$ values of soil and plant under the constant N output process (Liu *et al.,* 2017). However, N output process such as denitrification and leaching process are usually deficit in ^{15}N due to strong isotope fractionation effect. The rates of these output process may vary with increase in N, thus the response of plant and soil $\delta^{15}N$ values would be more complex. $\delta^{15}N$ would be higher with faster nitrogen turnover and circulation process caused by isotope fractionation (Liu *et al.,* 2017). The results of this study indicate that nitrogen addition can promote organic matter degradation in LD layer. Although, the carbon content did not show significant change, nitrogen addition can promote organic matter decomposition implied by enrichment of $\delta^{15}N$ in the LD layer.

In the whole forest cover and mineral soil layer, the short-term nitrogen deposition simulation of 6 yrs did not cause significant change in total carbon and nitrogen. C/N and carbon stable isotopes were also not significantly affected by N addition. The nitrogen stable isotope increased significantly in the LD layer, suggesting that nitrogen addition could promote organic matter mineralization. $\delta^{15}N$ was not significantly affected in the O layer and MS layer under nitrogen addition.

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