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Variations in the natural ^{13}C and ^{15}N abundance of plants and soils under long-term N addition and precipitation reduction: interpretation of C and N dynamics

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Abstract

Background: The nitrogen isotope natural abundance ($\delta^{15}N$) provides integrated information on ecosystem N dynamics, and carbon isotope natural abundance ($\delta^{13}C$) has been used to infer how water-using processes of plants change in terrestrial ecosystems. However, how $\delta^{13}C$ and $\delta^{15}N$ abundances in plant life and soils respond to N addition and water availability change is still unclear. Thus, $\delta^{13}C$ and $\delta^{15}N$ abundances in plant life and soils were used to investigate the effects of long-time (10 years) N addition (+ 50 kg N·ha^{-1}·yr^{-1}) and precipitation reduction (− 30% of throughfall) in forest C and N cycling traits in a temperate forest in northern China.

Results: We analyzed the $\delta^{13}C$ and $\delta^{15}N$ values of dominant plant foliage, litterfall, fungal sporophores, roots, and soils in the study. The results showed that $\delta^{15}N$ values of foliage, litterfall, and surface soil layer's (0–10 cm) total N were significantly increased by N addition, while $\delta^{15}N$ values of fine roots and coarse roots were considerably decreased. Nitrogen addition also significantly increased the $\delta^{13}C$ value of fine roots and total N concentration of the surface soil layer compared with the control. The C concentration, $\delta^{13}C$, and $\delta^{15}N$ values of foliage and $\delta^{15}N$ values of fine roots were significantly increased by precipitation reduction, while N concentration of foliage and litterfall significantly decreased. The combined effects of N addition and precipitation reduction significantly increased the $\delta^{13}C$ and $\delta^{15}N$ values of foliage as well as the $\delta^{15}N$ values of fine roots and $\delta^{13}C$ values of litterfall. Furthermore, foliar $\delta^{15}N$ values were significantly correlated with foliage $\delta^{13}C$ values, surface soil $\delta^{15}N$ values, surface soil C concentration, and N concentrations. Nitrogen concentrations and $\delta^{13}C$ values of foliage were significantly correlated with $\delta^{15}N$ values and N concentrations of fine roots.

Conclusions: This indicates that plants increasingly take up the heavier ^{15}N under N addition and the heavier ^{13}C and ^{15}N under precipitation reduction, suggesting that N addition and precipitation reduction may lead to more open forest ecosystem C and N cycling and affect plant nutrient acquisition strategies.

Keywords: $\delta^{13}C$, $\delta^{15}N$, N addition, Precipitation reduction, Nutrient acquisition strategies

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Background

Anthropogenic activities, such as fossil fuel combustion and fertilizer application, have greatly increased the amount of reactive nitrogen (N) deposited onto terrestrial ecosystems (Galloway et al. 2008; Liu et al. 2013). Increases in soil N availability can significantly alter the carbon (C) and N cycles by influencing the biochemical processes of plants and soils (Chen and Ruan 2018; Schulte-Uebbing and de Vries 2018). Similarly, precipitation reduction, which is expected to become more frequent as climate changes (Ciais et al. 2013), can directly or indirectly affect C and N cycling by altering the physical and biochemical processes of soil (Borken and Matzner 2009; Díaz-Guerra et al. 2018). Although the effects of N deposition and changes of precipitation pattern on ecosystem C and N dynamics have been the hotspot of ecological research for decades (Waldrop et al. 2004; Xiang et al. 2008; Thomas et al. 2010; Díaz-Guerra et al. 2018; Souza et al. 2019), how N deposition, precipitation reduction, and their interaction regulate the ecosystem C and N cycling is still unclear.

Based on previous studies, $\delta^{13}C$ and $\delta^{15}N$ provide a valuable approach exploring the C and N dynamics in different ecosystem components (Schlesinger 2009; Werth and Kuzyakov 2010). By analyzing $\delta^{15}N$ values in soils and plants, the fates and retention of deposited N in forest ecosystems can be better understood (Schlesinger 2009). Increased N availability caused by N deposition may lead to an increase in net nitrification, NO_3^- leaching and gaseous N loss in soil ecosystem (Wallenstein et al. 2006; Gao et al. 2015). Atmospheric N deposition and fertilizer applied are generally isotopically depleted sources in comparison to $\delta^{15}N$ values of soils and plants (Amundson et al. 2003). Processes associated with N loss under N deposition, e.g., mineralization, nitrification, denitrification, and N leaching discriminate against the heavier N isotope (^{15}N) in favor of the lighter N isotope (^{14}N) in soil ecosystems (Robinson 2001; Brookshire et al. 2012). A high loss of lighter ^{14}N causes overall enrichment of ^{15}N in soil ecosystems and indicates the openness of the N cycle (Watzka et al. 2006). Consequently, soil and plants have been expected to be more ^{15}N enriched because of the massive losses of the ^{15}N-depleted N compounds (Gurmesa et al. 2016). Furthermore, the plant absorbs N from soil mainly through fine roots and mycorrhizal fungi. The mycorrhizal fungi preferentially transfer ^{14}N and against ^{15}N to their host plants (Hobbie et al. 2000). Therefore, plant's $\delta^{15}N$ value under N deposition may reflect the forms and status of soil N utilized, mycorrhizal association type, and the subsequent fractionations in different plant organs (Conrad et al. 2018; Chen et al. 2019). Besides, the close coupling between plant leaf N concentration and C gain. The changes of N cycling under N deposition may alter the enrichment of plant-soil $\delta^{13}C$. Nevertheless, our knowledge of how the N deposition affects plant-soil $\delta^{13}C$ and its change mechanisms is limited.

In addition to N deposition, change of precipitation pattern also affects $\delta^{13}C$ and $\delta^{15}N$ values in plants and soils. Previous studies have suggested that plant and soil $\delta^{15}N$ values decreased with increasing precipitation intensity because the forms and the isotopic composition of the N left the ecosystems change (Schuur and Matson 2001). Studies have reported that plant-soil was much more enriched with $\delta^{15}N$ in drought regions than that in wet regions, which, as a result of N cycling, was likely to become more open as the precipitation was reduced (Swap et al. 2004; Cheng et al. 2009). However, it remains unclear how precipitation reduction causes the loss of ^{14}N and enrichment of ^{15}N in plants and soils. For $\delta^{13}C$ values, a strong correlation has been found between plant $\delta^{13}C$ values and soil water relations, which thus can be a powerful tool to evaluate plants water-efficient (Klaus et al. 2016). With precipitation reduction, plants absorb heavier ^{13}C due to closing stomata leading to less negative $\delta^{13}C$ values compared to plants with better water use efficiency (Farquhar et al. 1989; Mariotte et al. 2013). Therefore, the $\delta^{13}C$ values have been successfully used as an indicator of drought stress of plants (Mariotte et al. 2013). The effects of precipitation changes on $\delta^{13}C$ values have been mainly focused on sensitive grasslands in previous studies (Wrage et al. 2009; Mariotte et al. 2013), and it is still unclear how forest $\delta^{13}C$ values respond to precipitation reduction.

In general, plant-soil $\delta^{13}C$ and $\delta^{15}N$ values are related to the availability of soil N and water and are indicative of C and N cycling on different spatial scales (from soil to plant). Nitrogen rich soil system caused by N deposition tends to be ^{15}N enriched where the N cycle is more open to N loss. In such N rich soils, precipitation reduction may support low losses of N through the reducing leaching, indicating that precipitation patterns may potentially influence N deposition effects. Furthermore, increased soil N availability was found to potentially increase water use efficiency in plants, increasing their $\delta^{13}C$ enrichments (Ripullone et al. 2004). Thus, under the simultaneous scenarios of N deposition and precipitation reduction, it is necessary to understand how their interactions alter the $\delta^{13}C$ and $\delta^{15}N$ values of plant-soil for accurately grasping ecosystem C and N dynamics. However, little evidence is collected in the forest to support the authenticity of this interaction.

We identified some of the remaining questions needed to be answered in order to advance our understanding of plant-soil $\delta^{13}C$ and $\delta^{15}N$ values responding to N deposition and precipitation reduction and consequently the ecosystem's C and N cycle. Therefore, in this study, we examined the variation of $\delta^{13}C$ and $\delta^{15}N$ values in fresh plant foliage, litterfall, fine roots, coarse roots, fungal sporophores, and different soil layers in order to explore the effects of long-term stimulation of N deposition and precipitation reduction on the spatial variation of $\delta^{13}C$ and $\delta^{15}N$ values. We sought to determine whether the measurement of $\delta^{13}C$ and $\delta^{15}N$ values could be used as indicators of the relative sensitivity of soil C and N cycling across different tree species in response to N deposition and precipitation reduction. We did this by measuring the $\delta^{13}C$ and $\delta^{15}N$ values of three dominant species, including *Fraxinus mandshurica*, *Tilia amurensis* and *Pinus koraiensis.* We hypothesized that N deposition and precipitation reduction might lead to more open C and N cycling.

Methods

The study site was located at an old broad-leaved Korean pine mixed forest (over 300 years old) in the Changbai Mountains Natural Reserve, northeastern China (42°24′ N, 128°06′ E, 738 m above the sea level). The region is a typical temperate-continental climate, characterized by cold and long winters and warm and short summers. The mean annual precipitation is about 740 mm with more than 80% falling between May and October. The annual average temperature is approximately 3.6 °C with an average growing season (from May to October) temperature of 15 °C. The soil of the study site is classified as Eutric Cambisol (FAO classification) with 31.54% sand, 42.18% silt, 26.28% clay, and 25.42% organic matter in the topsoil in the 0–20 cm soil layer. Dominant tree species in this forest include *Pinus koraiensis*, *Fraxinus mandshurica* and *Tilia amurensis.* The tree density on the site was 432 trees·ha^{-1}. The main shrub species include *Euonymus alatus*, *Philadelphus schrenkii*, *Corylus mandshurica*, *Lonicera japonica*, and the main herbaceous species include *Anemone cathayensis*, *Anemone raddeana*, *Funaria officinalis*, *Cyperus microiria*, and *Filipendula palmate* (Zhou et al. 2019).

Experimental design

In May 2009, six 50 m × 50 m plots were randomly established in the forest, with at least 100 m buffer strips between each plot. Previous observation data showed that the precipitation was approximately 550 mm in drought years, which was almost 30% less than the long-term average annual precipitation of 740 mm in the last 30 years (Chinese Ecosystem Research Net data). To test the effect of precipitation reduction on ecosystem C and N cycling, three plots were treated with a 30% precipitation reduction and the other three were set as the control. The high light V-shaped panels, with high light transmittance, were installed to intercept through-fall. The V-shaped panels cover 30% of the plot area. The precipitation interception facility was approximately 1 m aboveground and was available to ensure normal air flow. The panels were removed in winter to avoid the disturbance of uneven snow reduction. To study the effect of N deposition on ecosystem C and N cycle, each plot was divided into two subplots (25 m × 50 m): one subplot was used to simulate N deposition and the other subplot was used as a control. The N addition level was 50 kg N·ha^{-1}·yr^{-1} and approximately two times the real N deposition rate in the study area (Lü and Tian 2007). Ammonium nitrate (NH_4NO_3) was used as a nitrogen source in the study. The solution of NH_4NO_3 was sprayed on the forest floor monthly using a backpack sprayer with six equal applications over the entire growing season (May to October). In every application, the NH_4NO_3 was weighed and mixed with 40 L of water and each control plot received 40 L water without N fertilizer.

Plant, soil and fungal sporophore sampling

The fresh foliage, litterfall, fine roots (< 2 mm), and coarse roots (> 2 mm) of *Pinus koraiensis* (PKS), *Tilia amurensis* (TAR) and *Fraxinus mandshurica* (FMR) were collected. In July 2017, all the plant samples from the three separate plants per species were collected in each subplot at the seasonal peak of aboveground biomass. Meanwhile, soil samples were collected from beneath each tree in four directions using a stainless soil corer (5-cm inner diameter), then divided into three soil layers (0–10, 10–20 and 20–30 cm soil layer). The six soil cores from each soil layer were mixed to form a composite sample in each subplot. All of the soil samples were put through a 2-mm soil sieve while the roots, stones, and other debris were removed. Six fungal sporophores were collected in the forest floor in each subplot.

In the laboratory, all samples for C concentration, ^{13}C, N concentration, and ^{15}N analysis were dried at 65 °C to a constant weight and then ground into fine powders using a Tecator sample mill (Subang, Shanghai, China). Subsequently, C%, $\delta^{13}C$, N% and $\delta^{15}N$ 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). When ^{13}C abundance of soil was measured, the soil needs acidification with 1 N HCL for 24 h at room temperature to remove any inorganic carbon. The soil is acid, pH range is 4.77 to 5.44 (Zhou et al. 2019). The reproducibility of the isotope determination was analyzed in multiple runs at several laboratories using an internal standard soil and plant sample from temperate forests. Natural abundance $\delta^{15}N$ and $\delta^{13}C$ in samples were reported in the conventional delta (δ) notation, with units of per mil (‰). The natural abundance of the N fertilizer (NH_4NO_3) in this study was 0.21% ± 0.43‰.

Statistical analysis

All data were tested with the Shapiro-Wilk test and Levene's tests for normality and homogeneity of variances respectively. Data were analyzed using the general linear mixed model (GLMM) for a split-plot design with Tukey's multiple comparisons. Nitrogen addition, precipitation reduction, and their interaction were considered fixed factors. Blocks, as random effects, were used to determine the effects of N addition, precipitation reduction, and their interaction on C concentration, δ^{13}C, δ^{15}N, and N concentration values of plant and soil samples. When a significant effect was detected, Tukey's honestly significant difference test was applied to elucidate treatment differences. Pearson correlation was used to test for correlation among C%, δ^{13}C, N%, and δ^{15}N values of fresh plant foliage, fine roots, and surface soil layer (0–10 cm). All statistical analyses were performed using the SPSS software (version 17.0).

Results

Simulated nitrogen deposition effects

There was a statistical significant effect of different treatments (N addition, precipitation reduction, and combined effect) ($p < 0.05$), tree species ($p < 0.05$), sample types ($P < 0.05$) and their interaction on C concentration, δ^{13}C, N concentration, and δ^{15}N of plant and soil (Table 1). The mean δ^{15}N values of fresh foliage and litterfall were significantly increased by N addition (Table 2, and Tables S1 and S2). When different plant species were considered, under N addition, δ^{15}N values of fresh foliage and litterfall were only significantly higher in *Tilia amurensis* and *Fraxinus mandshurica* ($p < 0.05$, Fig. 1). Contrary to foliage and litterfall, the δ^{15}N value of fine roots was significantly lower in N addition compared to that in control ($p = 0.006$, Table 2 and Table S3). There was also a trend for a lower δ^{15}N value of coarse roots in N addition compared to that in control, but this pattern was not statistically significant ($p = 0.963$) (Table 2 and Table S3). Similarly, roots δ^{15}N value of *Tilia amurensis* and *Fraxinus mandshurica*, including fine roots and coarse roots, was significantly decreased by N addition ($p < 0.05$, Fig. 1). The δ^{15}N value in the surface soil layer was increased with N addition among all plant species but did not increase in deeper soil layers (Fig. 1). For N concentration, N addition significantly increased the N concentration of litterfall and significantly decreased the N concentration of fine roots and coarse roots (Table 1, Fig. 1, and Tables S1–S7).

Nevertheless, N addition only significantly increased mean δ^{13}C values of fine root and significantly decreased C concentration of fine roots (Table 3). However, δ^{13}C values and C concentration of different plant species and organs showed different responses to N addition (Fig. 2). For *Pinus koraiensis*, N addition had a significantly positive effect on δ^{13}C values of fresh foliage and had a significant negative effect on δ^{13}C values of litterfall and subsurface soil layer (10–20 cm) (Fig. 2). Nitrogen addition significantly increased δ^{13}C values of litterfall and roots for *Tilia amurensis* ($p < 0.05$, Fig. 2). Moreover, a significant increase in C concentration of litterfall and δ^{13}C values of surface soil layer (0–10 cm) as well as a

Table 1 Effect of different treatments, plant species, sample types and their interaction on C concentration, N concentration, δ^{13}C and δ^{15}N plant and soil

		δ^{15}N value	N concentration	δ^{13}C value	C concentration
Treatments	*F* value	73.02	4.39	7.21	3.94
	P value	< 0.001	< 0.001	< 0.001	< 0.001
Plant species	*F* value	94.90	269.25	98.44	16.83
	P value	< 0.001	< 0.001	< 0.001	< 0.001
Sample types	*F* value	1992.63	843.83	493.79	2604.86
	P value	< 0.001	< 0.001	< 0.001	< 0.001
T × P	*F* value	7.94	8.97	5.02	9.12
	P value	< 0.001	< 0.001	< 0.001	< 0.001
T × S	*F* value	20.99	6.75	4.43	6.00
	P value	< 0.001	< 0.001	< 0.001	< 0.001
S × P	*F* value	29.95	66.54	22.62	9.00
	P value	< 0.001	< 0.001	< 0.001	< 0.001
T × P × S	*F* value	10.88	4.31	3.71	4.63
	P value	< 0.001	< 0.001	< 0.001	< 0.001

T × P represents interaction of treatments and plant species; T × S represents interaction of treatments and sample types; S × P represents interaction of sample types and plant species; T × P × S represents interaction of treatments, sample types and plant species

Table 2 The $\delta^{15}N$ values (‰) and N concentration (%) of trees and soils as affected by N addition, precipitation reduction and their combination (mean ± S.E)

		Control	N addition	Precipitation reduction	Combined effects
^{15}N (‰)	Foliage	−0.98 ± 0.03	− 0.43 ± 0.05	− 0.80 ± 0.01	0.73 ± 0.03
	Litterfall	−1.82 ± 0.07	−0.56 ± 0.02	−1.45 ± 0.04	− 0.10 ± 0.02
	Fine roots (< 2 mm)	− 0.46 ± 0.04	− 1.43 ± 0.04	− 0.32 ± 0.10	0.09 ± 0.05
	Coarse roots (> 2 mm)	− 0.76 ± 0.03	−1.72 ± 0.02	−1.09 ± 0.10	− 0.19 ± 0.09
	Soil layer (0–10 cm)	− 0.11 ± 0.02	1.79 ± 0.13	0.86 ± 0.01	1.23 ± 0.20
	Soil layer (10–20 cm)	3.37 ± 0.10	2.32 ± 0.16	3.35 ± 0.11	3.62 ± 0.22
	Soil layer (20–30 cm)	6.64 ± 0.09	6.14 ± 0.40	6.44 ± 0.11	6.46 ± 0.08
N (%)	Foliage	2.55 ± 0.01	2.45 ± 0.07	2.32 ± 0.02	2.35 ± 0.07
	Litterfall	1.11 ± 0.01	1.38 ± 0.03	1.01 ± 0.04	1.06 ± 0.02
	Fine roots (< 2 mm)	1.50 ± 0.01	1.35 ± 0.00	1.47 ± 0.01	1.46 ± 0.07
	Coarse roots (> 2 mm)	0.77 ± 0.00	0.61 ± 0.03	0.85 ± 0.08	0.79 ± 0.06
	Soil layer (0–10 cm)	1.71 ± 0.06	1.37 ± 0.01	1.71 ± 0.08	1.71 ± 0.02
	Soil layer (10–20 cm)	1.03 ± 0.07	1.19 ± 0.02	1.01 ± 0.05	1.18 ± 0.10
	Soil layer (20–30 cm)	0.18 ± 0.01	0.16 ± 0.01	0.29 ± 0.01	0.25 ± 0.08

significant decrease in $\delta^{13}C$ values of fresh foliage and C concentration of surface soil layer (0–10 cm) for *Fraxinus mandshurica* were found ($p < 0.05$, Fig. 2). Nitrogen addition also significantly decreased the $\delta^{13}C$ value, C and N concentration of fungal sporophore ($p < 0.05$), and mean N return proportion compared with control (Figs. 3 and 4 and Table S8).

Precipitation reduction effects

Our analysis shows that mean $\delta^{13}C$ and $\delta^{15}N$ values of foliage significantly increased with precipitation reduction (Tables 2 and 3 and Table S1). Precipitation reduction also significantly increased mean C concentration of foliage, the $\delta^{15}N$ value of fine roots, the $\delta^{15}N$ value of litterfall, and the C and N concentration of mineral soil layer (20–30 cm), but significantly decreased N concentration of foliage and litterfall, and $\delta^{13}C$ value of mineral soil layer (Tables 2 and 3 and Tables S1–S7).

Different plant species and different plant organs showed different responses to precipitation reduction (Figs. 1 and 2). The $\delta^{15}N$ value of litterfall, fine roots, coarse roots, and surface soil layer was significantly increased by precipitation reduction in *Pinus koraiensis.* Precipitation reduction significantly increased the $\delta^{15}N$ value of litterfall and surface soil layer but significantly decreased the $\delta^{15}N$ value of fine roots and coarse roots and N concentration of foliage and litterfall in *Tilia amurensis*. There was a significant increase in the $\delta^{15}N$ value of foliage and surface soil layer and N concentration of litterfall with precipitation reduction in *Fraxinus mandshurica.* Besides, precipitation reduction significantly increased the $\delta^{13}C$ value of foliage among all plant species. The C concentration of foliage and litterfall was significantly decreased by precipitation reduction in *Pinus koraiensis*, while the C concentration of foliage and roots was significantly increased by precipitation reduction in *Tilia amurensis.* For *Fraxinus mandshurica*, precipitation reduction had a positive effect on C concentration of foliage and a negative effect on C concentration of fine roots.

Interaction of simulated nitrogen deposition and precipitation reduction

Mean $\delta^{15}N$ value of foliage, litterfall, fine roots, and subsurface soil layer (10–20 cm) were significantly increased under the interaction of N addition and precipitation reduction (Tables 1 and 2 and Tables S1–S6). Moreover, the interaction of N addition and precipitation reduction significantly increased the $\delta^{13}C$ value of foliage but significantly decreased the $\delta^{13}C$ value of litterfall. Interaction of N addition and precipitation reduction had a significantly positive effect on the $\delta^{15}N$ value of foliage and litterfall among all plant species. Interaction of N addition and precipitation reduction had a positive effect on the $\delta^{15}N$ value of fine root and coarse roots in *Pinus koraiensis* and *Fraxinus mandshurica*, but a negative effect in *Tilia amurensis.* Furthermore, the interaction of N addition and precipitation reduction significantly increased the $\delta^{13}C$ value of foliage in *Pinus koraiensis*, the $\delta^{13}C$ value of coarse roots in *Tilia amurensis,* and the $\delta^{13}C$ value of fine roots in *Fraxinus mandshurica*, but significantly decreased the $\delta^{13}C$ value of foliage in *Pinus koraiensis.*

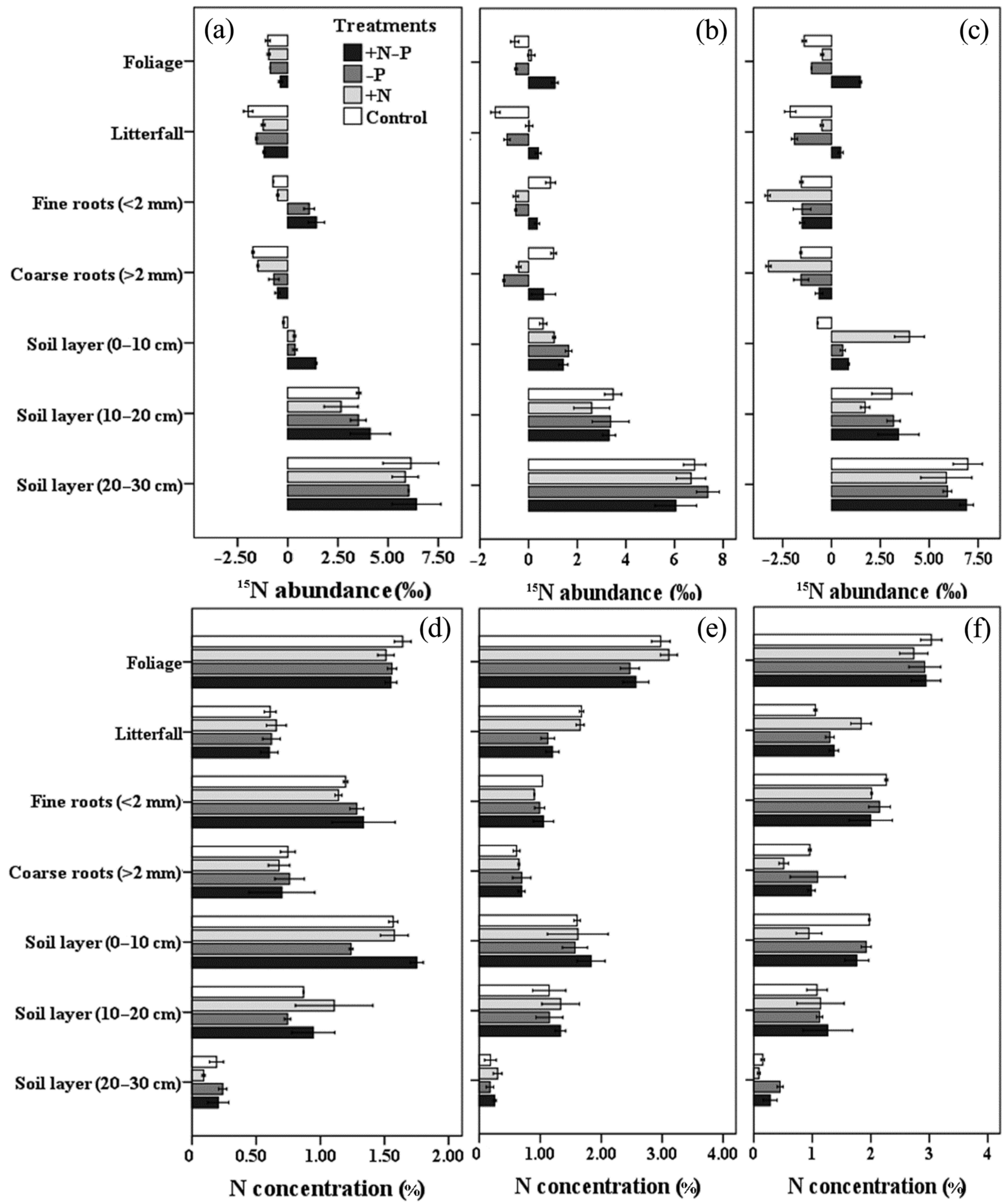

Fig. 1 Effects of N addition, precipitation reduction and their combination on $\delta^{15}N$ values and N concentration of trees and soils among different tree species. **a** and **d**: *Pinus koraiensis*; **b** and **e**: *Tilia amurensis*; and **c** and **f**: *Fraxinus mandshurica*. Error bars represent 1 SE of the mean

Correlation analysis

To elucidate the relationship between input and output of C and N values in the plant-soil system, Pearson correlation coefficients were calculated for C%, $\delta^{13}C$, $\delta^{15}N$, and N% in plants foliage, fine roots and surface soil layer (0–10 cm) (Table 4). This showed a strong

Table 3 The $\delta^{13}C$ values (‰) and C concentration (%) of plants and soils as affected by N addition, precipitation reduction and their combination (mean ± S.E)

		Control	N addition	Precipitation reduction	Combined effects
^{13}C (‰)	Foliage	−31.56 ± 0.11	−30.85 ± 0.03	−30.12 ± 0.12	−30.89 ± 0.10
	Litterfall	− 28.24 ± 0.06	−28.59 ± 0.07	−28.61 ± 0.02	−28.43 ± 0.07
	Fine roots (< 2 mm)	− 28.96 ± 0.00	−27.93 ± 0.03	− 28.16 ± 0.16	−28.01 ± 0.38
	Coarse roots (> 2 mm)	− 29.01 ± 0.07	−28.17 ± 0.06	−28.11 ± 0.20	−27.94 ± 0.33
	Soil layer (0–10 cm)	− 27.12 ± 0.04	−27.19 ± 0.47	−27.10 ± 0.04	−27.13 ± 0.07
	Soil layer (10–20 cm)	−26.07 ± 0.11	−26.55 ± 0.08	−26.02 ± 0.28	−26.26 ± 0.11
	Soil layer (20–30 cm)	−25.04 ± 0.04	−24.99 ± 0.08	−25.09 ± 0.04	−25.41 ± 0.20
C (%)	Foliage	45.49 ± 0.15	45.80 ± 0.13	46.25 ± 0.09	46.26 ± 0.14
	Litterfall	46.11 ± 0.14	46.68 ± 0.08	46.54 ± 0.33	46.46 ± 0.17
	Fine roots (< 2 mm)	47.89 ± 0.04	47.48 ± 0.05	48.21 ± 0.03	47.40 ± 0.33
	Coarse roots (> 2 mm)	47.55 ± 0.06	47.09 ± 0.10	47.91 ± 0.28	47.73 ± 0.05
	Soil layer (0–10 cm)	33.16 ± 0.60	22.77 ± 2.75	28.21 ± 1.36	28.51 ± 2.16
	Soil layer (10–20 cm)	12.58 ± 1.10	16.10 ± 1.19	14.78 ± 0.76	14.51 ± 1.45
	Soil layer (20–30 cm)	1.78 ± 0.11	1.62 ± 0.28	2.34 ± 0.05	2.76 ± 0.20

positive correlation between the $\delta^{15}N$ value of foliage and $\delta^{15}N$ value of the surface soil layer ($p = 0.001$) and $\delta^{13}C$ value of foliage ($p = 0.026$). The $\delta^{15}N$ value of foliage was also negatively correlated with the N concentration of the surface soil layer. A significant negative correlation was found between N concentration of foliage and $\delta^{15}N$ value and N concentration of fine roots ($p = 0.018$ and $p < 0.001$, respectively). The $\delta^{13}C$ value of foliage was positively correlated with the $\delta^{13}C$ value of fine roots and negatively correlated with the $\delta^{15}N$ value and N concentration of fine roots. Significant positive correlations were found between C concentration of foliage and $\delta^{15}N$ value and N concentration of fine roots. The $\delta^{15}N$ value of fine roots was negatively correlated with the $\delta^{15}N$ value of the surface soil layer, while the N concentration of fine roots was negatively correlated with C and N concentration of the surface soil layer.

Discussion

Effects of simulated nitrogen deposition on ecosystem C and N cycling

In this study, the N addition (0.21% ± 0.43‰) with a $\delta^{15}N$ abundance close to that of atmospheric N clearly affected the $\delta^{15}N$ values of both plants and soils (Table 2 and Fig. 1). We observed a significant increase in surface soil layer $\delta^{15}N$ value with N addition, which is consistent with a previous study (Liu et al. 2017). Increasing soil $\delta^{15}N$ value may be explained by (1) input of litterfall $\delta^{15}N$ value and (2) processes of N mineralization, immobilization, nitrification, denitrification leading to losses of ^{15}N-depleted mineral N with N addition (Watzka et al. 2006; Kleinebecker et al. 2014). In addition, plant N uptake could also cause isotope fractionating processes leading to N losses depleted in ^{15}N, enriching soil N with the heavier isotope (Evans 2001). The previous study in the experiment site showed that N addition significantly increased N_2O emission (Geng et al. 2017), which could partially explain the increase of soil $\delta^{15}N$ value.

Soil N availability is the source of plant N of non-N_2-fixing plants; thus the change of plant $\delta^{15}N$ value (foliage and root) was directly linked to the increase of soil $\delta^{15}N$ value (Table 4). However, the $\delta^{15}N$ value of foliage and root showed various responses to N addition in the study (Table 2 and Fig. 1). The foliage $\delta^{15}N$ value was significantly increased by N addition, indicating that the added N was indeed incorporated into the ecosystem N pools. The result is consistent with previous studies (Watzka et al. 2006; Högberg et al. 2014). The $\delta^{15}N$ value of plant foliage is dependent on (1) the source of soil N availability, (2) the depth to which fine roots access soil N, (3) the preference of plant N uptake (e.g. NH_4^+, NO_3^-, organic N), and (4) the fractionations by symbiotic mycorrhiza during N transfer (Robinson 2001; Conrad et al. 2018). In this study, the $\delta^{15}N$ value of foliage was positively correlated with the $\delta^{15}N$ value of the surface soil layer, suggesting that the increase of foliage $\delta^{15}N$ value may be caused by the increasing soil $\delta^{15}N$ value. Moreover, fine root $\delta^{15}N$ value was expected to be enriched because roots take up a more enriched $\delta^{15}N$ pool under N addition (Templer et al. 2007). Interestingly, we observed that the $\delta^{15}N$ value of fine root was significantly decreased by N addition and negatively correlated with the $\delta^{15}N$ value of the surface soil layer. The different responses between plant foliage and roots affirmed that different patterns of N assimilation

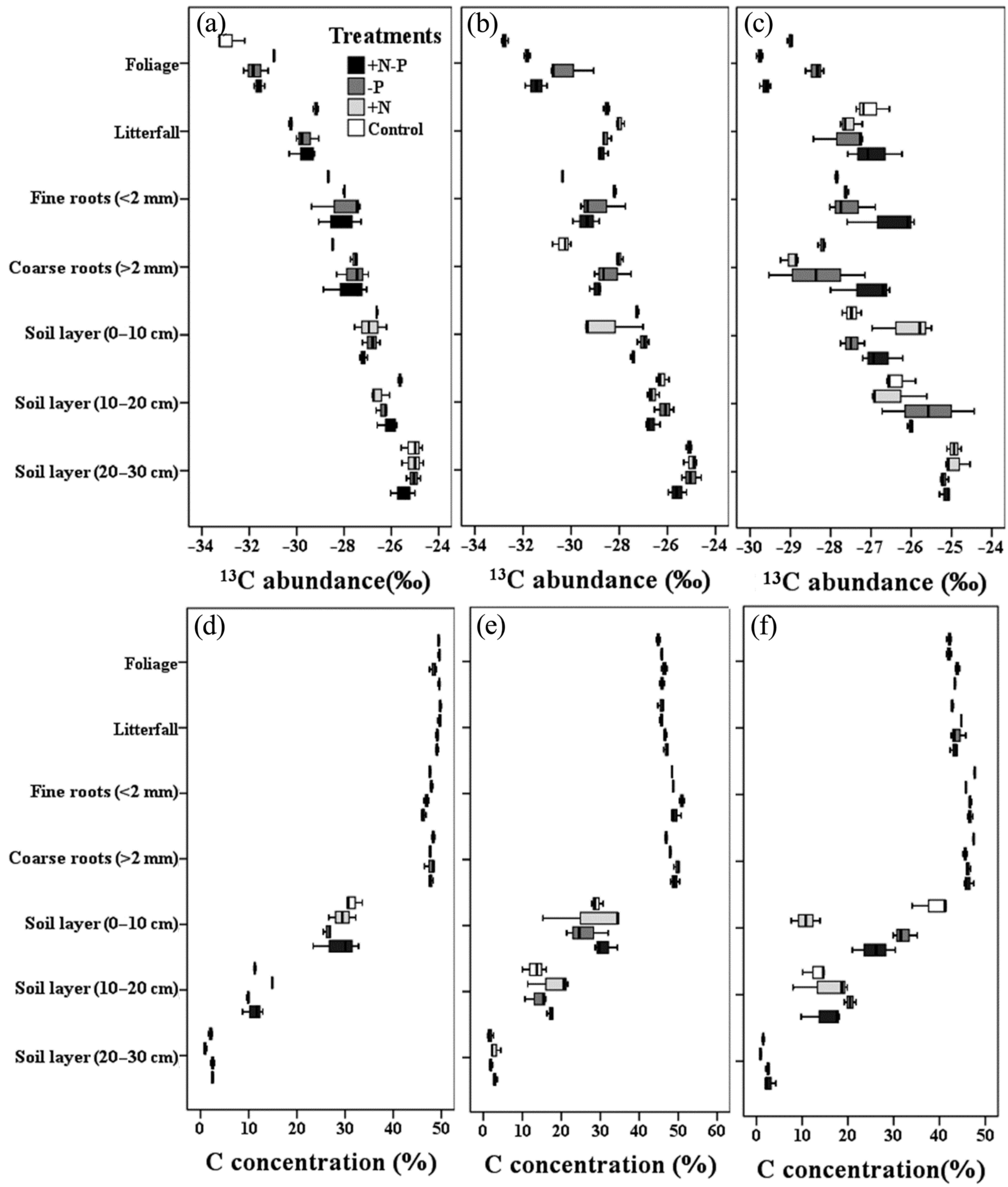

Fig. 2 Effects of N addition, precipitation reduction and their combination on $\delta^{13}C$ values and C concentration of trees and soils among different tree species. **a** and **d**: *Pinus koraiensis*; **b** and **e**: *Tilia amurensis*; and **c** and **f**: *Fraxinus mandshurica*. Error bars represent 1 SE of the mean

and reallocation of N can cause intra-plant variation in $\delta^{15}N$ (Evans 2001). The assimilation of NH_4^+ occurs in root and assimilation of NO_3^- occurs in shoot and root. Meanwhile, NO_3^- is commonly enriched in ^{15}N relative to that of NH_4^+ (Kendall 1998). Under N addition, the increase in foliage $\delta^{15}N$ and decrease in root $\delta^{15}N$ could be explained by the fact that the NO_3^- available for assimilation in foliage is more enriched relative to that in root because it

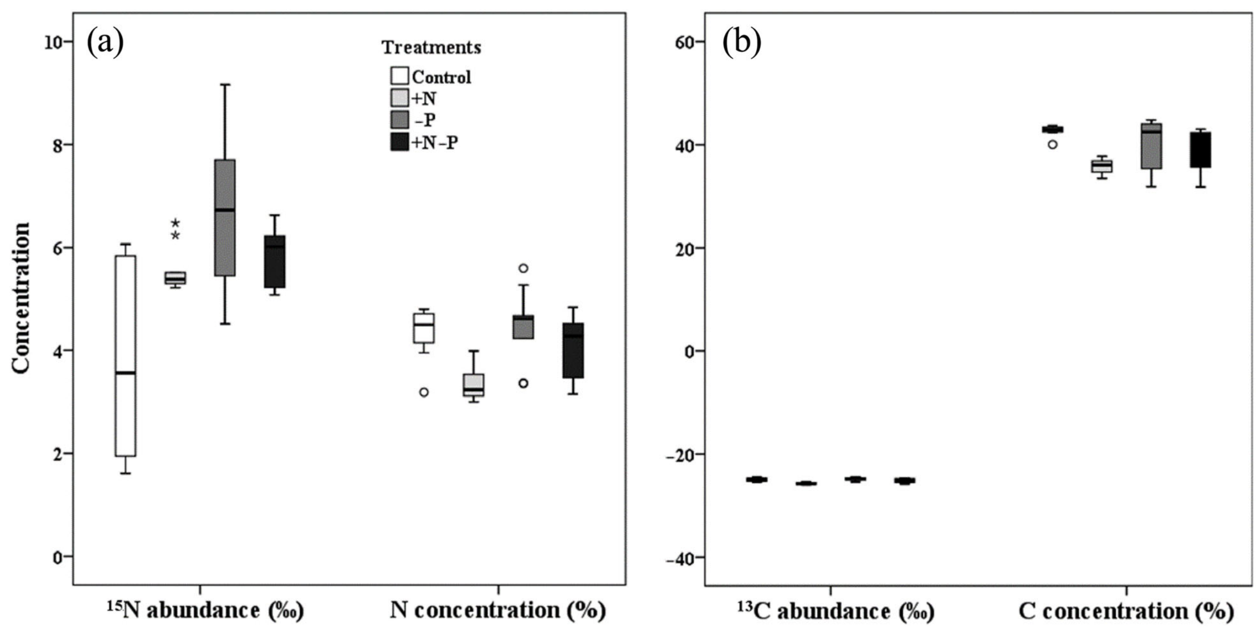

Fig. 3 Effects of N addition, precipitation reduction and their combination on $\delta^{15}N$, N concentration, $\delta^{13}C$ and C concentration of fungal sporophore

originates from a pool that has already been exposed to assimilation (Evans et al. 1996). The result indirectly indicates that NO_3^- has a higher $\delta^{15}N$ value than NH_4^+ in the study. Furthermore, our previous research in the experiment site suggests that plant roots increase uptake of NH_4^+ (Zhou et al. 2019), which could increase the assimilation of NH_4^+ in root and assimilation of NO_3^- in foliage and lead to the decrease of root $\delta^{15}N$ and increase of foliage $\delta^{15}N$.

Although N addition changed the $\delta^{15}N$ value of foliage and roots, it did not significantly change their total N concentration among all tree species, which indicated that N in plants had internal homeostasis. Nitrogen addition also significantly increased the mean N concentration of litterfall and decreased the mean N reabsorption of plant foliage, indicating that N addition increased available N in soil and altered plant N uptake strategies. For the $\delta^{13}C$ value of plant and soil, we found a positive correlation between foliar $\delta^{13}C$ values and foliar $\delta^{15}N$ values and N concentrations (Table 4), which indicated that N addition could alter C dynamic by changing N dynamic. However, compared with the change of N and $\delta^{15}N$ value, N addition had no or little effect on C and $\delta^{13}C$ value of plant and soil. The $\delta^{13}C$ value of the plant is relatively stable, and change in plant input would alter the $\delta^{13}C$ value of soil. However, the addition of N does not change the total N status in plant and soil, although it could change the form of N uptake by plants, and therefore has no less effect on the C status of plants. Moreover, different species often respond differently to increases in N inputs (Templer et al. 2005). In this study, *Pinus koraiensis* was less sensitive to N addition than others, suggesting C and N cycling in conifers may be less susceptible than broadleaved plants to N deposition in the temperate forest.

Effects of precipitation reduction on ecosystem C and N cycling

In addition to N deposition, change of precipitation also exerts strong controls on ^{15}N natural abundance on an ecosystem level (Cheng et al. 2009). Previous studies have shown that plants adapted to precipitation reduction environments are expected to have higher $\delta^{15}N$ value (Amundson et al. 2003; Swap et al. 2004; Cheng et al. 2009), which is consistent with our result that $\delta^{15}N$ value of foliage and fine root significantly increased with precipitation reduction. The soil is the main N source for plants; thus soil processes play a key role in plant $\delta^{15}N$ enrichment under precipitation reduction (Swap et al. 2004). In this study, the $\delta^{15}N$ value of the surface soil layer was also increased by precipitation reduction, which may lead to a positive effect on plant $\delta^{15}N$ values. Nevertheless, several possible mechanisms may explain this effect of precipitation reduction on the increase of plant-soil $\delta^{15}N$ value. Firstly, precipitation reduction may control ^{15}N abundance by influencing NH_3 volatilization (Cheng et al. 2009). Precipitation reduction limited leaching of base cations, and thus might raise surface soil pH value (Cheng et al. 2009). The higher pH values under precipitation reduction permit larger NH_3 volatilization than in control, thus increasing soil $\delta^{15}N$ value. Secondly, gross N mineralization reduced with precipitation reduction (Wu et al. 2011), which may have improved $\delta^{15}N$ values of plant-soil in this study. N

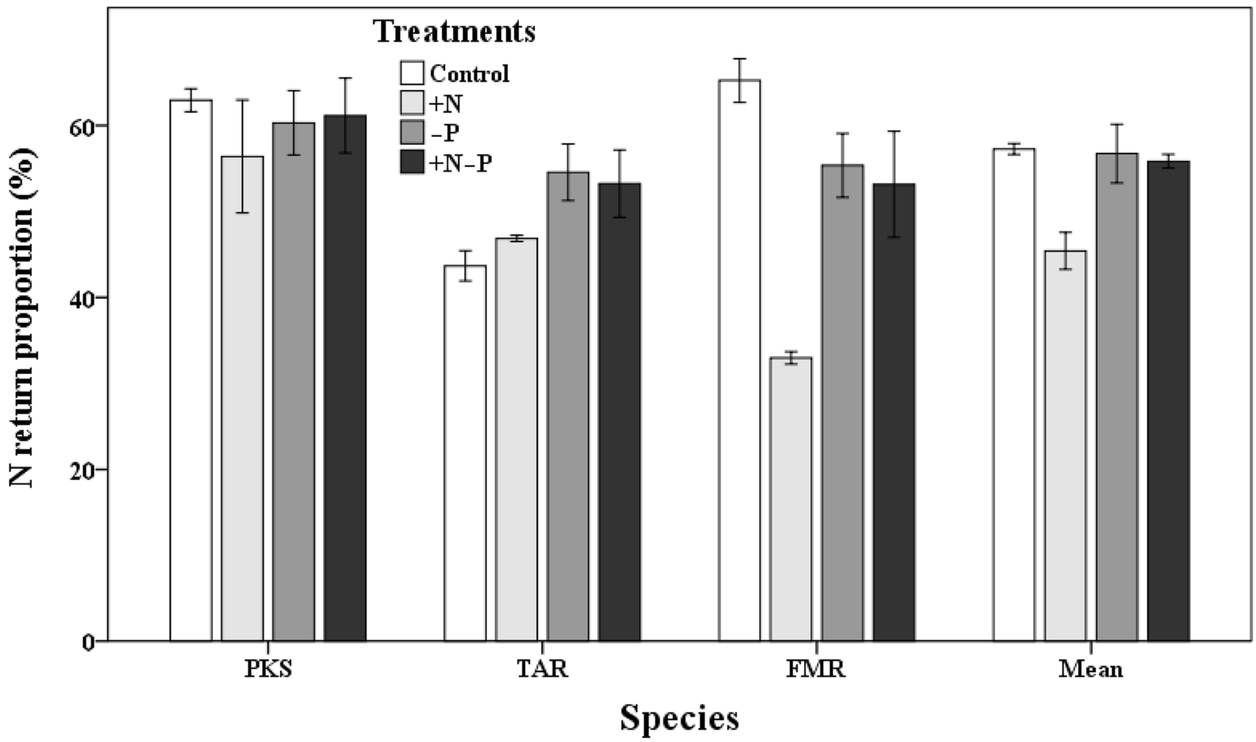

Fig. 4 Effects of N addition, precipitation reduction and their combination on N return proportion (%) in different tree species. PKS: *Pinus koraiensis*; TAR: *Tilia amurensis*; and FMR: *Fraxinus mandshurica*. Error bars represent 1 SE of the mean

mineralization discriminates against the heavier N isotope (^{15}N) (Brookshire et al. 2012), which may cause overall enrichment of ^{15}N in the soil ecosystem. Consequently, plants have been expected to be more ^{15}N enriched. The previous studies at the study site may indirectly confirm that precipitation reduction restrains mineralization by depressing litter decomposition (Zheng et al. 2017). Thus, the reduced N mineralization may be an essential process responsible for the enhancement of plant-soil $\delta^{15}N$ values in this study. Thirdly, under precipitation reduction, the increase of plant $\delta^{15}N$ values may be interpreted as the reduction in a shift in the N source for plants (Amundson et al. 2003). Precipitation reduction may decrease NO_3^- leaching (Nielsen and Ball 2015), and the enhancement of plant $\delta^{15}N$ values could be caused by increasing uptake and assimilation of NO_3^- in plants. However, the enhancement of plant-soil $\delta^{15}N$ values may be caused by

Table 4 Pearson correlation coefficients between $\delta^{15}N$, N, $\delta^{13}C$ and C in trees foliage, fine roots and surface soil layer (0–10 cm)

	Foliage $^{15}N^{+}$	Foliage $N^{\&}$	Foliage ^{13}C	Foliage $C^{\#}$	Fine root ^{15}N	Fine root N	Fine root $^{13}C^{\#}$	Fine root $C^{\#}$	Soil layer $^{15}N^{\#}$	Soil layer N^{+}	Soil layer ^{13}C
Foliage $^{15}N^{+}$	1										
Foliage $N^{\&}$	0.175										
Foliage ^{13}C	**−0.382***	**0.381***									
Foliage $C^{\#}$	0.268	**−0.779****	**−0.613****								
Fine root ^{15}N	−0.229	**−0.404***	**−0.623****	**0.661****							
Fine root N	−0.290	**−0.745****	**−0.430***	**0.603**	0.337						
Fine root $^{13}C^{\#}$	0.180	−0.077	**0.512****	−0.159	**− 0.481***	−0.034					
Fine root $C^{\#}$	−0.066	0.108	−0.157	0.106	0.233	**−0.399***	**−0.390***				
Soil layer $^{15}N^{\#}$	**−0.554****	0.061	0.143	−0.276	**−0.356**	0.170	0.069	−0.163			
Soil layer N^{+}	**0.418***	0.242	0.233	−0.082	0.154	**−0.452****	− 0.056	0.241	**− 0.502****		
Soil layer ^{13}C	0.275	−0.331	−0.117	0.097	−0.307	0.327	0.062	−0.274	**0.438****	**−0.678****	
Soil layer C^{+}	**0.496****	0.091	0.078	0.003	0.188	**−0.374***	− 0.054	0.187	**− 0.720****	**< 0.830****	**− 0.001**

To approximate normality, the response variables are Ln-transferred. # Log; + Square; & Exponent. * The level of significance of correlations is $p < 0.05$. ** The level of significance of correlations is $p < 0.01$

an N cycle that becomes more open as precipitation reduction.

According to our hypothesis, we also expected significant changes in $\delta^{13}C$ values of plant and soil due to the precipitation reduction. In this study, precipitation reduction significantly increased $\delta^{13}C$ values of foliage and fine root but did not significantly change the $\delta^{13}C$ values of the soil, which partly supported our hypothesis. Foliage $\delta^{13}C$ values vary across changes of precipitation, with the general pattern being that plants in precipitation reduction tend to have more enriched $\delta^{13}C$ in their foliage than those in moist environments (Werth and Kuzyakov 2010). Many previous studies reported that plants adapted to precipitation reduction environments are expected to have higher $\delta^{13}C$ values (Swap et al. 2004; Du et al. 2014; Blessing et al. 2016), which is consistent with our study. This result is mainly due to plant ^{13}C discrimination. According to a general notion, under precipitation reduction, plants tend to have low stomatal conductance, high water use efficiency, and low intercellular CO_2, which results in decreasing discrimination against $^{13}CO_2$ during photosynthesis leading to the increase of foliage $\delta^{13}C$ (Blessing et al. 2016). Compared to the increase of foliage $\delta^{13}C$ value, the increase in root $\delta^{13}C$ value was probably caused by processes occurring during the transport of photoassimilates from foliage to roots (Du et al. 2014). A recent study has also proved that plants maintain the transport of fixed photoassimilates into the roots under moderate drought stress (Hommel et al. 2016), which could lead to an increase in root $\delta^{13}C$ value. However, the change of soil $\delta^{13}C$ mainly derives from the plant litterfall. The foliage with high concentrations of sugars, starch, protein, organic acids, and cellulose displays high $\delta^{13}C$ values (Bowling et al. 2008). The increase of foliage $\delta^{13}C$ under precipitation reduction may be mainly stored in these organic matters. These organic matters with high $\delta^{13}C$ values may decrease with foliage senescence and consequently lead to depleted in litterfall $\delta^{13}C$, especially under the pressure of reduced precipitation. Thus, precipitation reduction did not significantly change in soil $\delta^{13}C$. Moreover, precipitation reduction may reduce the input of plant C into the soil, resulting in a slight decrease in soil C concentration, suggesting that reduced precipitation may lead to a more closed C cycle.

Effect of the interaction between simulated nitrogen deposition and precipitation reduction on ecosystem C and N cycling

In this study, our results directly showed that the interaction of N deposition and precipitation reduction might significantly alter the C and N cycle of plant-soil in a temperate forest. Firstly, in the study, $\delta^{15}N$ value of foliage, litterfall, fine root, coarse root and subsurface soil layer (10–20 cm) was higher in the interaction between N addition and precipitation reduction than that in others treatments, providing evidence that the interaction might increase the openness of N cycle compared with N addition alone. Precipitation reduction increased N addition effects mostly indirectly via changing the N status of soil and the physiology of the plants. Mechanistically it is evident that the reduction of precipitation inhibits mineralization and alters soil N status, causing plants to absorb more inorganic N from N addition. The N addition was enriched in ^{15}N relative to the control, and decreasing mineralization could also increase the ^{15}N enrichment of soil in the study, which may lead to increased plant $\delta^{15}N$ value. Furthermore, plants increase water uptake by regulating their osmotic pressure under precipitation reduction conditions, and plants may increase N uptake to regulate osmotic pressure under N addition, which could also increase plant $\delta^{15}N$ value. However, increasing plants $\delta^{15}N$ values under N addition are always associated with higher levels of water consumption causing plant water stress, being particularly pronounced in precipitation reduction. Previous studies found that N addition could induce an increase in water use efficiency, resulting in a decrease in plant $\delta^{13}C$ values (Gong et al. 2011). The result is consistent with the result that foliage $\delta^{13}C$ values in the interaction of N addition and precipitation reduction was a slightly lower than that in precipitation reduction alone but still significantly higher than that in the control, which indicated that N addition might increase water use efficiency but precipitation reduction had a stronger effect on $\delta^{13}C$ values than N addition, although we have found that interaction of N addition and precipitation reduction may affect ecosystem C and N dynamics.

Conclusions

Our study reveals that N addition, reduced precipitation, and their interaction altered C and N cycles in temperate forest ecosystems. Nitrogen addition and precipitation reduction increased plant-soil $\delta^{15}N$ values, suggesting that N cycling becomes more open as precipitation decreases and N deposition increases. Furthermore, precipitation reduction caused an increase in the $\delta^{13}C$ values of plants and soils as a result of improved water use efficiency, indicating that C-cycling could become tighter with decreased precipitation. Interaction of N addition and precipitation reduction showed that precipitation reduction might aggravate the effect of N addition on ecosystem N dynamics by regulating N status of soil and the physiology of the plants, while N addition may alleviate the effect of precipitation reduction on ecosystem C cycling by improving water use efficiency. These data give evidence that N deposition and reduced precipitation change the C and N cycles in forest ecosystems; however, the mechanisms that were responsible for these effects, and their intensity were not quantitatively assessed in this study.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s40663-020-00257-w.

Additional file 1: Table S1. Effects of N addition, precipitation reduction and their combination on $\delta^{15}N$ values (‰) and N concentration (%) of plants and soils among different plants. (HS): *Pinus koraiensis*; (ZD): *Tilia amurensis*; and (SQL): *Fraxinus mandshurica*. **Table S2.** Effects of N addition, precipitation reduction and their combination on $\delta^{13}C$ values (‰) and C concentration (%) of plants and soils among different plants. (HS): *Pinus koraiensis*; (ZD): *Tilia amurensis*; and (SQL): *Fraxinus mandshurica*. **Table S3.** Effects of N addition, precipitation reduction and their combination on mean $\delta^{15}N$ (‰), N. concentration (%), $\delta^{13}C$ (‰) and C concentration (%) of foliage. **Table S4.** Effects of N addition, precipitation reduction and their combination on mean $\delta^{15}N$ (‰), N concentration (%), $\delta^{13}C$ (‰) and C concentration (%) of litterfall. **Table S5.** Effects of N addition, precipitation reduction and their combination on mean $\delta^{15}N$ (‰), N concentration (%), $\delta^{13}C$ (‰) and C concentration (%) of fine roots. **Table S6.** Effects of N addition, precipitation reduction and their combination on mean $\delta^{15}N$ (‰), N concentration (%), $\delta^{13}C$ (‰) and C concentration (%) of coarse roots. **Table S7.** Effects of N addition, precipitation reduction and their combination on mean $\delta^{15}N$ (‰), N concentration (%), $\delta^{13}C$ (‰) and C concentration (%) of surface soil layer (0–10 cm). **Table S8.** Effects of N addition, precipitation reduction and their combination on mean $\delta^{15}N$ (‰), N concentration (%), $\delta^{13}C$ (‰) and C concentration (%) of subsurface soil layer (10–20 cm). **Table S9.** Effects of N addition, precipitation reduction and their combination on mean $\delta^{15}N$ (‰), N concentration (%), $\delta^{13}C$ (‰) and C concentration (%) of mineral soil layer (20–30 cm). **Table S10.** Effects of N addition, precipitation reduction and their combination on mean N return proportion (%).

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Authors' contributions

QW and YX designed the study, were awarded funding, supervised data collection and contributed to and edited manuscripts. QW, GY, YX, SH and MZ contributed the whole manuscript preparation and design and wrote the main manuscript text. QW, GY, YX, SH, MZ and WS prepared all figures, YX, HW, HS, BH, WS 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.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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