



Foliage application of nitrogen has less influence on soil microbial biomass and community composition than soil application of nitrogen

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Abstract

Purpose Many studies have shown the simulated effects of nitrogen (N) deposition on soil microbial community composition by adding N directly to the forest floor but have ignored the N retention process by the canopy. This study was conducted to compare the responses of soil microbial biomass and community composition between soil application of N (SAN) and foliage application of N (FAN).

Materials and methods A pot experiment was designed with (1) two N application methods (SAN and FAN), (2) three N application levels (5.6, 15.6 and 20.6 g N m⁻² year⁻¹), and (3) two tree species (*Schima superba* Gardn. et Champ. and *Pinus massoniana* Lamb.) following a nested factorial design. Soil microbial biomass and community composition were determined using phospholipid fatty acids (PLFAs) techniques after 1 and 1.5 years of treatments.

Results and discussion Nitrogen addition increased ($P < 0.05$) soil NH₄⁺-N content and soil NO₃⁻-N content and decreased ($P < 0.05$) soil pH and soil microbial (bacterial, fungal, and actinomycete) biomass for both N application methods. Compared with the SAN treatment, the FAN treatment had higher ($P < 0.05$) pH and lower ($P < 0.05$) contents of soil NH₄⁺-N and soil NO₃⁻-N. Soil microbial biomass and community composition were significantly different between the different N addition levels under the SAN treatment, but they showed no significant difference ($P < 0.05$) between the different N addition levels under the FAN treatment. The soil microbial biomass in the *S. superba* soil was higher ($P < 0.05$) than that in the *P. massoniana* soil for the FAN treatment, with the opposite trend observed under the SAN treatment. Moreover, redundancy analysis showed that soil microorganisms were significantly correlated with soil pH, soil water content, NH₄⁺-N, and NO₃⁻-N.

Conclusions The results showed that N addition affected soil properties, microbial biomass, and the composition of microbial communities; however, the FAN treatment had less influence on soil properties and soil microorganisms than did the SAN treatment over short time scales, and the extent of this effect was different between coniferous and broadleaf trees.

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Keywords Nitrogen application methods · Nitrogen deposition · Phospholipid fatty acid (PLFA) · Soil microbial biomass · Soil microbial community composition

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1 Introduction

Nitrogen (N), one of the major limiting nutrients for plant growth, plays a crucial role in substance circulation in terrestrial ecosystems (Xia and Wan 2008; Gao et al. 2014). N deposition has emerged as one of the greatest concerns in global change (Galloway et al. 2008; Pardo et al. 2011; Liu et al. 2013; Chen et al. 2015). Global deposition of reactive N

has increased approximately 4-fold over the last century (IPCC 2013), and it is predicted to continue increasing in the next few decades in China (Galloway et al. 2008; Liu et al. 2013). Numerous studies have shown that N deposition can reduce plant diversity (Zeng et al. 2016; Zhang et al. 2017), cause soil acidification (Gao et al. 2014; Lu et al. 2014; Tian and Niu 2015), and hence alter soil microbial biomass and community composition (Zhang et al. 2013; Zhang et al. 2017).

Soil microbes serve an important part in the material cycling and energy flow in soil ecosystem, which regulates soil carbon and N turnover rates (Wardle and Zackrisson 2005; Liang et al. 2011; Xiong et al. 2014). Empirical studies have shown that N addition increased soil NH_4^+ -N or NO_3^- -N contents, which satisfied the N demand for soil microbes, which further stimulated soil microbial activity (LeBauer and Treseder 2008; Fang et al. 2014; Zhang et al. 2014) and decreased the ratio of fungi to bacteria (Xiong et al. 2016). However, studies have also found that surplus N inhibited mycorrhizal fungi (Treseder et al. 2007; Zheng et al. 2014) and decreased soil microbial biomass and community composition (Frey et al. 2004; Treseder 2008; Zhang et al. 2013). The inconsistent results regarding the effect of N addition on soil microbial biomass and community compositions in recent decades (Demoling et al. 2008) were mainly a result of the quantity and quality of N applied to the soil (Fang et al. 2014).

Forest canopies could retain N from atmospheric deposition, which will change the quantity and quality of N compared with that deposited directly onto the forest floor soil, and it is believed to be a considerable process in N biogeochemical cycles (Sparks 2009; Wortman et al. 2012; Houle et al. 2015). In general, a majority of atmospheric N deposition first passes through the canopy layer before it reaches the soil floor (Gaige et al. 2007; Wortman et al. 2012; Zhang et al. 2015b). Part of this N can be retained by the forest canopy through foliar uptake and conversion, absorption into the woody plant surface, and chemical retention in or on the barks (Gaige et al. 2007; Cardelús et al. 2009; Sparks 2009). For example, Wortman et al. (2012) found that 20–25% of N deposition could be intercepted by the canopy layer in the Novaggio forest, while 52–59% N retention was reported in the Canadian boreal forest by Houle et al. (2015). The amount of canopy-intercepted N depends not only on forest type but also on the form of N. Fenn et al. (2013) have shown that there is a higher affinity of the canopy for NO_3^- -N than for NH_4^+ -N, whereas other studies have reported higher efficiency in NH_4^+ -N retention (Wortman et al. 2012; Houle et al. 2015). Therefore, canopy interception causes changes in the quantity and quality of natural N deposition before it is deposited on the forest floor soil (Adriaenssens et al. 2012; Wortman et al. 2012; Houle et al. 2015). Consequently, soil microorganisms responded differently with respect to biomass and composition to the N amount and forms within the microbial

communities (Merilä et al. 2010). However, most studies concerning the effects of simulated N deposition on soil microorganisms sprayed N directly on the soil surface, which might inaccurately estimate the effects of N deposition on soil microbial biomass and communities (Zhang et al. 2015b; Shi et al. 2016).

To explore the responses of soil microorganisms to different N application methods, a simulated N deposition experiment that included both N application on soil (SAN) and N application on foliage (FAN) at three N addition levels (5.6, 15.6 and 20.6 $\text{g N m}^{-2} \text{ year}^{-1}$) for two tree species (*Pinus massoniana* Lamb. and *Schima superba* Gardn. et Champ.) was conducted in a greenhouse. The hypothesis can be proposed that the effect of the FAN treatment on soil microbial biomass and community composition will be less than that for the SAN treatment, due to part of the N being intercepted by the canopy (Sparks 2009; Wortman et al. 2012; Houle et al. 2015). In addition, there may be differences in nutrient dynamics, growth, and root exudation between the tree species, with the coniferous species responded to N deposition more efficiently than the broadleaf species (Lovett et al. 2004; Grayston and Prescott 2005; Xia and Wan 2008; Weand et al. 2010b). Those differences may result in different responses of soil microbial community composition to N addition (Weand et al. 2010a). Therefore, the other hypothesis in this study is that the effects of N application methods (SAN vs. FAN) would be affected by tree species.

2 Materials and methods

2.1 Experimental design and sampling

The pretreatment soil was collected from the South China Botanical Garden (23° 20' N, 113° 30' E), which is located in Guangdong Province, southeast China. The lateritic red soils can be classified as mesic, Typic Kandiodox (Soil Survey Staff 2014). The soil was derived from sandstone, and the pH value of surface soil (0–20 cm) was 3.99 ± 0.1 , NH_4^+ -N was $6.01 \pm 0.69 \text{ mg kg}^{-1}$, NO_3^- -N was $17.39 \pm 1.34 \text{ mg kg}^{-1}$, and soil available phosphorus was $0.84 \pm 0.07 \text{ mg kg}^{-1}$. The study site is characterized by a subtropical monsoon climate, with mean annual temperature and mean annual precipitation 21.5 °C and 1700 mm, respectively, and over 80% of precipitation occurring in the warm growing season (April to September). The dominant plant species, evergreen broadleaf forest, at the experimental site included: *Schima superba* Gardn. et Champ., *Castanea henryi* (Skam) Rehd. et Wils., and *Cryptocarya concinna* Hance.

The experiment started in March 2012 and ended in October 2013. It was composed of a nested factorial design with three factors: (1) two N application methods (FAN and SAN), (2) three N addition levels ($5.6 \text{ g N m}^{-2} \text{ year}^{-1}$

(control), which is the background wet N deposition in this region (Liu et al. 2008); 15.6 g N m⁻² year⁻¹ (medium-N); and 20.6 g N m⁻² year⁻¹ (high-N)), and (3) two tree species (the coniferous species *Pinus massoniana* Lamb. (*P. massoniana*) and the broadleaf species *Schima superba* Gardn. et Champ. (*S. superba*)). Seedlings of the two species, which are native to southern China and the most widely distributed tree species in the area, that had experienced similar growing conditions (1-year-old nursery) were transplanted into plastic pots (ca. 40 cm in diameter and 30 cm in height) without damaging the roots in an open-air greenhouse. All treatments were replicated six times. A total of 72 pots were used. After 2 months of seedling growth, N additions with NH₄NO₃ were equally applied weekly, by spraying the N slowly and evenly to the soil or foliage. All pots received the same amount of tap water each time. No other fertilizer was used.

Soil samples were collected two times in the rainy season (RS, April 2013) and the dry season (DS, October 2013), respectively. In each pot, the litter layer was removed and surface soil at depth of 0–20 cm was collected. Two soil samples were collected randomly from the six replicates and were mixed thoroughly to form one composite sample. After the removal of gravel, these soil samples were sieved through 2-mm mesh and divided into two parts. One part was immediately cooled with ice blocks and stored at 4 °C for phospholipid fatty acid (PLFA) analysis, and the other part was used for soil physicochemical analyses.

2.2 Soil chemical analyses

Soil pH was measured in 1:2.5 (W/V) suspensions of soil in double distilled water. Soil water content (SWC) was measured by samples using oven dried to a constant weight at 105 °C. Total phosphorus (TP) was analyzed using the molybdenum blue colorimetric method with a UV/visible spectrophotometer after H₂SO₄-H₂O₂ digestion (UV-2550, Shimadzu, Kyoto, Japan). The concentration of available phosphorus (AP) was determined by the Mehlich III procedure (Mehlich 1984). Total nitrogen (TN) was measured using an Alpkem AutoAnalyzer (Kjeltec System 1026 Distilling Unit, Sweden) according to the Kjeldahl acid digestion method (Kirk 1950), and available nitrogen (AN) was analyzed using the alkali solution diffusion method. Soil nitrate nitrogen (NO₃⁻-N) and ammonium nitrogen (NH₄⁺-N) were determined and extracted with 2 M KCl solution and measured colorimetrically using a UV/visible spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan). The soil labile N was the sum of soil NO₃⁻-N and NH₄⁺-N concentration.

2.3 Soil microbial PLFA measurement

Soil samples equivalent to 8 g root-free soil dry weight were extracted, fractionated, and quantified for their PLFAs

following the detailed procedure according to Zhang et al. (2015a). The separated fatty acid methyl esters were identified by chromatographic retention time and mass spectral comparison using a standard qualitative mix ranging from C9 to C30 and a MIDI peak identification system (Microbial ID, Inc., Newark, DE). Methyl nonadecanoate (19:0) was added before the methylation step and used as an internal standard. The samples were analyzed using a gas chromatogram (6890GC, Agilent) equipped with a flame ionization detector (FID). The standard nomenclature was used to identify the individual fatty acids, and their abundance was determined as relative nanomoles per gram of dry soil. The total PLFAs of the soil concentration were calculated using all identified PLFAs (Frostegård and Bååth 1996). The sum of the following PLFAs was used as the measure of bacterial biomass: i15:0, a15:0, 15:0, i16:0, 16:1ω9c, 16:1ω5c, 15:0 3OH, 17:1ω8c, i17:0, a17:0, cy17:0, 17:0, 16:1 2OH, and 10Me17:0 (Bossio et al. 1998; Creamer et al. 2015; Frostegård and Bååth 1996). Fungi were considered to be represented by the PLFAs 18:3ω6c (6, 9, 12) and 18:1ω9c, and the fatty acid 10Me18:0 was used as the indicator of actinomycetes (Creamer et al. 2015). The fungi/bacteria (F/B) ratio, the lipid biomass of fungi divided by that of bacteria, was calculated to estimate the composition of soil microbial community (Frostegård and Bååth 1996; Cusack et al. 2011).

2.4 Statistical analysis

All statistical analyses were performed using SPSS 19.0 software, and statistical significance was set at *P* value = 0.05. Before analysis, all data were verified using the Shapiro-Wilk test for normality and using Levene's test for homogeneity of variances. Distributions that did not conform to homogeneity of variances or normality requirements were transformed (natural log, square, or rank). Three-way analysis of variance (ANOVA) with Duncan's test was applied to test the effects of N levels, sample time, and tree species on soil properties and soil PLFA concentrations. Data were further analyzed by Tukey's test when there was a significant effect of N addition treatment.

The PLFA data were expressed as mol % and subjected to principal component analysis (PCA). Soil microbial community composition and environment factors were compared using redundancy analysis (RDA) with a Monte Carlo permutation test by CANOCO 4.5. Before doing so, a detrended correspondence analysis was first run, and the estimate of the longest axis was less than 4, suggesting that the use of the linear ordination method is quite appropriate for determining the correlation between soil microbe community composition and environmental variables.

3 Results

3.1 Soil physicochemical properties

Nitrogen addition caused significant changes in soil pH, NH_4^+ -N content, NO_3^- -N content, and soil labile N (Table 1; Fig. 1). With an increase in the N addition rate, soil NH_4^+ -N, NO_3^- -N, and labile N contents significantly increased, while soil pH decreased ($P < 0.05$) in the two N application treatments. For example, soil NH_4^+ -N, NO_3^- -N, and labile N contents were significantly lower in the control than in the high-N treatment. Furthermore, soil NH_4^+ -N was higher than soil NO_3^- -N in all treatments (Table 1). Compared with the SAN treatment, the FAN treatment had higher ($P < 0.05$) pH and lower ($P < 0.05$) contents of soil NH_4^+ -N and NO_3^- -N, especially in the *S. superba* soil (Table 1). Between the two

species, the FAN treatment had higher labile N content than did the SAN treatment in the *P. massoniana* soil, while the opposite trend was observed in the *S. superba* soil (Fig. 1). Moreover, soil labile N was significantly lower in the *S. superba* soil than in the *P. massoniana* soil, except for the high-N level in the SAN treatment (Fig. 1). However, soil TP, AP, SWC, TN, and AN showed no significant changes in responses to the increasing N addition rates in the FAN and SAN treatments (Table S1, Electronic Supplementary Material).

3.2 Soil microbial biomass

The microbial functional groups included here, determined by the presence of specific PLFAs, include bacteria, fungi, and actinomycetes (Fig. 2). In the application of the SAN

Table 1 Effects of N levels (N), sample time (T), and N application methods (M) on soil pH, NH_4^+ -N, and NO_3^- -N in the *P. massoniana* soil and the *S. superba* soil

Sample time	N levels	pH		NH_4^+ -N (mg kg ⁻¹)		NO_3^- -N (mg kg ⁻¹)	
		SAN	FAN	SAN	FAN	SAN	FAN
<i>P. massoniana</i>							
Rainy season	Control	4.67 (0.05)a	4.59 (0.07)a	5.26 (2.06)b	5.05 (0.78)b	14.77 (1.04)c*	11.63 (0.78)c
	Medium-N	4.19 (0.19)b	4.25 (0.06)b	7.06 (0.42)b	6.33 (1.82)b	21.97 (6.17)b	39.56 (5.06)b*
	High-N	4.23 (0.04)b	4.20 (0.18)b	19.66 (0.85)a	38.69 (3.13)a*	40.11 (9.26)a	69.03 (2.25)a*
Dry season	Control	4.21 (0.11)a	4.44 (0.09)a*	1.87 (1.28)c	3.08 (1.97)b	20.29 (0.46)c*	10.47 (1.65)c
	Medium-N	3.92 (0.06)b	4.07 (0.06)b*	13.22 (5.46)b*	8.07 (1.47)b	24.50 (0.57)b	20.05 (0.52)b
	High-N	3.76 (0.09)b	3.96 (0.03)b*	25.61 (2.30)a	31.95 (4.34)a	29.32 (1.34)a	33.78 (2.51)a
Three-way ANOVA results (<i>F</i> and <i>P</i> values)							
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
N		69.342	< 0.001	477.843	< 0.001	168.06	< 0.001
T		82.012	< 0.001	1.319	0.262	54.364	< 0.001
M		7.605	0.011	17.377	< 0.001	16.925	< 0.001
<i>S. superba</i>							
Rainy season	Control	5.00 (0.29)a	4.49 (0.29)	3.20 (1.16)b	3.36 (1.90)	7.72 (0.33)c*	5.18 (0.02)c
	Medium-N	4.34 (0.13)b	4.44 (0.04)	7.57 (2.22)b	3.56 (1.36)	15.22 (0.56)b	15.99 (3.16)b
	High-N	4.25 (0.09)b	4.26 (0.06)	32.40 (7.22)a*	11.05 (7.88)	24.30 (0.78)a	26.71 (0.84)a
Dry season	Control	3.91 (0.01)ab	4.91 (0.12)a*	7.00 (3.37)b	5.24 (2.44)b	6.92 (0.06)a	1.78 (0.84)c
	Medium-N	3.99 (0.01)a	4.83 (0.06)a*	10.21 (5.35)ab*	4.75 (2.76)b	22.01 (0.23)c*	10.84 (2.85)b
	High-N	3.84 (0.08)b	4.54 (0.10)b*	17.28 (4.73)a	10.36 (1.78)a	51.99 (1.40)c*	14.40 (2.60)a
Three-way ANOVA results (<i>F</i> and <i>P</i> value)							
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
N		21.117	< 0.001	112.484	< 0.001	1480.599	< 0.001
T		53.822	< 0.001	2.898	0.102	334.439	< 0.001
M		9.033	0.006	98.708	< 0.001	43.737	< 0.001

Values are mean (SD), $n = 3$. Rainy season, April 2013; dry season, October 2013. Control, background N addition ($5.6 \text{ g N m}^{-2} \text{ year}^{-1}$); medium-N, intermediate N addition ($15.6 \text{ g N m}^{-2} \text{ year}^{-1}$); high-N, high N addition ($20.6 \text{ g N m}^{-2} \text{ year}^{-1}$). Different letters for a given variable indicate a significant difference ($P < 0.05$) in the same tree species, sample time, and N application method among different N levels. Repeated measures ANOVA results (*F* and *P* values) for N levels, sample times, and N application methods are shown in the table

SAN soil application of N, FAN foliage application of N

*A significant difference ($P < 0.05$) in the same tree species, sample time and N level between the different N application methods

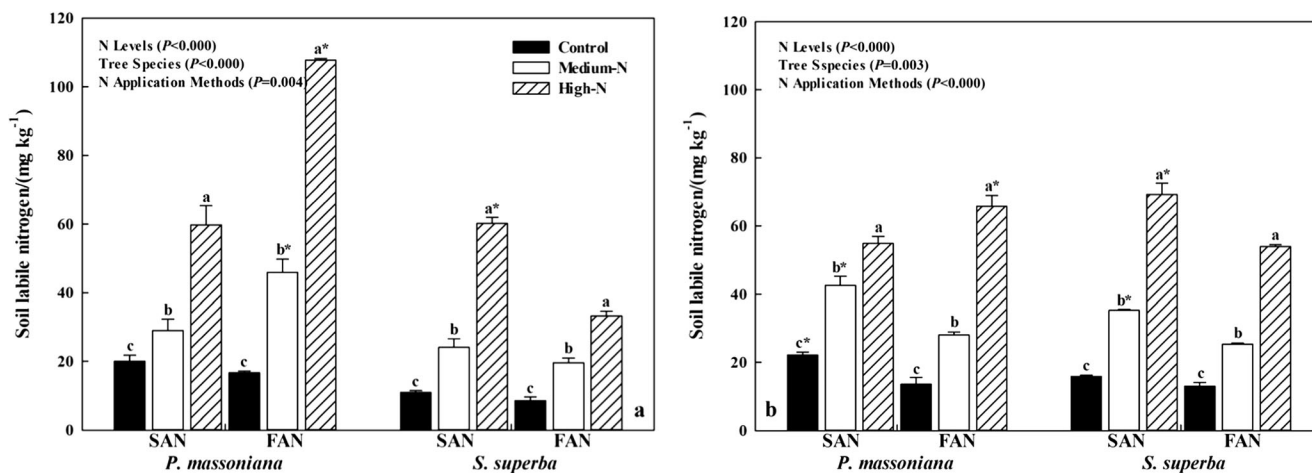


Fig. 1 Effects of N levels, tree species and N application methods on soil labile nitrogen (the sum of NH₄⁺-N and NO₃⁻-N concentration) in the rainy season (a, April 2013) and the dry season (b, October 2013). Values are mean ± SD (n = 3). Control, background N addition (5.6 g N m⁻² year⁻¹); medium-N, intermediate N addition (15.6 g N m⁻² year⁻¹); high-N, high N addition (20.6 g N m⁻² year⁻¹). SAN soil application of N, FAN foliage application of N. Different letters

for a given variable indicate a significant difference (P < 0.05) in the same tree species, sample time, and N application method among different N levels. Asterisk indicates a significant difference (P < 0.05) in the same tree species, sample time, and N level between different N application methods. Repeated measures ANOVA results (F and P values) of N levels, tree species, and N application methods are shown in the insets

treatment, soil total microbial (bacterial, fungal, and actinomycete) biomass significantly differed among the N levels (Fig. 2). With an increase in the N addition rate, soil microbial biomass decreased, except for in the medium-N treatment in the *S. superba* soil during the rainy season. Considering tree species, the high-N treatment decreased (P < 0.05) soil total PLFAs and bacterial biomass in the *P. massoniana* soil, while it significantly reduced soil actinomycete biomass in the *S. superba* soil. Additionally, the soil total PLFAs and bacterial and actinomycete biomass in the *S. superba* soil were lower (P < 0.05) than in the *P. massoniana* soil (Fig. 2a, c, g). Compared with the SAN treatment, soil total PLFAs and bacterial and fungal biomass did not show any obvious difference among the N levels and sample times in the FAN treatment (Fig. 2). However, considering tree species, soil total PLFAs and bacterial and fungal biomass were significantly higher in the *S. superba* soil than in the *P. massoniana* soil in the FAN treatment (Fig. 2).

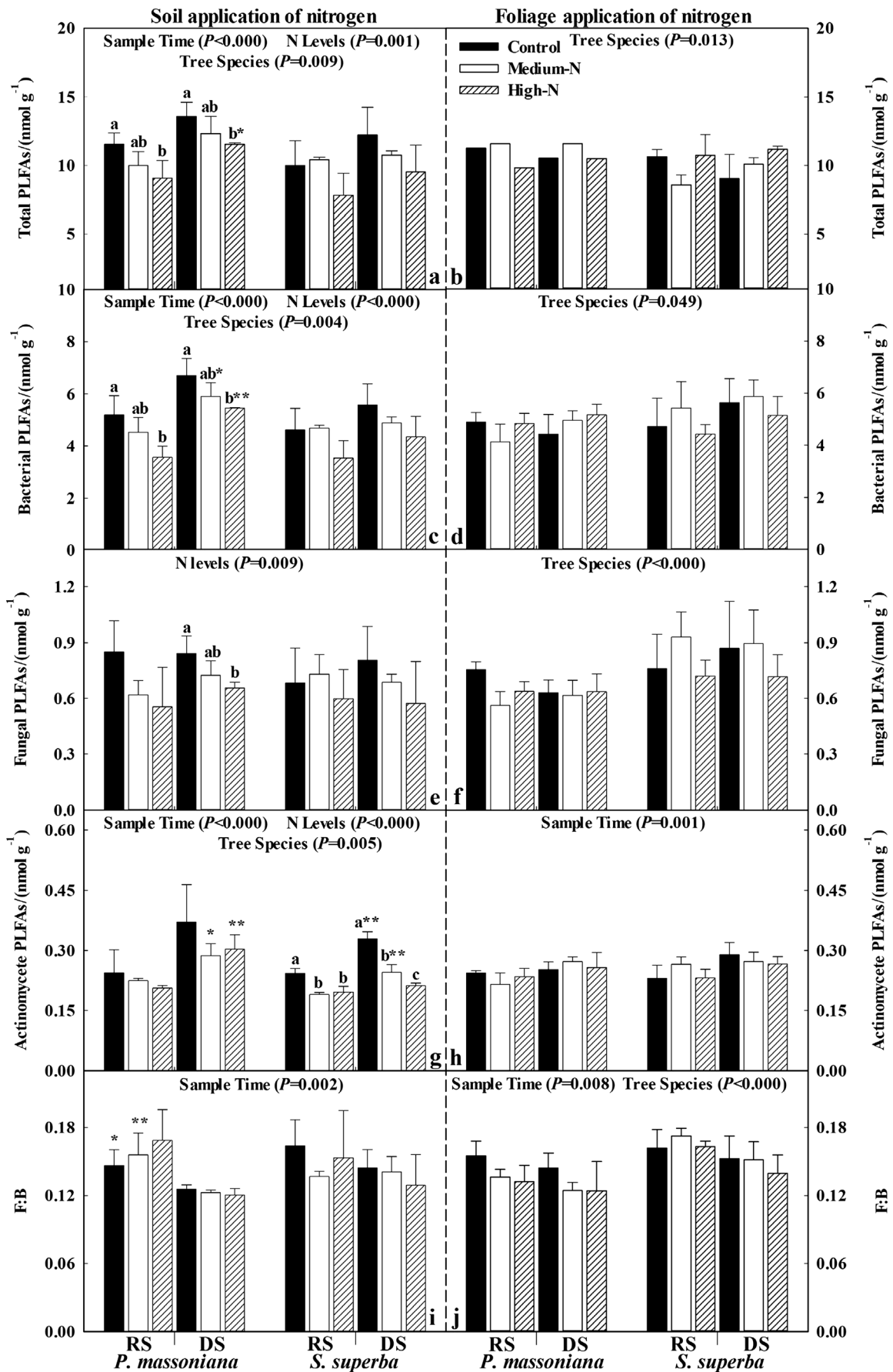
3.3 Soil microbial community composition

The mol % abundance of individual lipid biomarkers was subjected to PCA according to treatment. The PC1 and PC2 components together accounted for 54.5 and 46.4% of the total variation in the SAN and FAN treatments, respectively (Fig. 3). In the SAN treatment, the soil samples could be separated clearly into different colors (red, green, and blue; Figs. 3a and 5a), which indicate that the N addition levels had significant impacts on soil microbial community composition. In addition, soil bacterial and fungal abundance decreased (P

< 0.05) in the high-N treatment compared with the control-N treatment in the *P. massoniana* soil (Fig. 4a). Similarly, the PCA biplot distinguished between the *P. massoniana* and *S. superba* soil at the same N level in the FAN treatment (circle and square; Figs. 3b and 5b), which showed that the soil microbial community was significantly different between the tree species. However, the relative abundance of soil microbial biomass (bacterial, fungal, and actinomycetes) and the F/B ratio did not significantly change among the N levels under the FAN treatment (Figs. 2j and 4b).

3.4 Linking soil microbial community composition to environmental variables

In the RDA of microbial indices of PLFA profiles with soil properties as the explanatory variable, RDA1 (the x-axis) explained 26.4% of the variation in the dataset, with 18.3% of the variation accounted for by RDA2 (the y-axis) in the SAN treatment (Fig. 5a). Furthermore, the RDA1 and RDA2 explained 19.1 and 14.7%, respectively, of the variance in soil phospholipid fatty acid composition in the FAN treatment (Fig. 5b). Generally, the microbial indices showed inconsistent responses to soil properties. With an increase in the N addition rate, soil pH decreased, soil NH₄⁺-N and NO₃⁻-N increased, and the soil microorganisms were significantly correlated with soil pH, SWC, NH₄⁺-N, and NO₃⁻-N (Fig. 5). Besides, some of the PLFAs (16:1ω5c, 16:1ω9c, and i15:0) were located to the red color (control), showing that bacterial biomass was more abundant in the control treatment and was reduced by N addition.



◀ **Fig. 2** The PLFA concentrations (nmol g^{-1} dry soil) of soil microbial groups and the ratio of fungal to bacterial PLFAs (F/B) were affected by simulated nitrogen deposition on soil (**a, c, e, g, i**) and foliage (**b, d, f, h, j**). Values are mean \pm SD ($n = 3$). RS rainy season (April 2013), DS dry season (October 2013). Control, background N addition ($5.6 \text{ g N m}^{-2} \text{ year}^{-1}$); medium-N, intermediate N addition ($15.6 \text{ g N m}^{-2} \text{ year}^{-1}$); high-N, high N addition ($20.6 \text{ g N m}^{-2} \text{ year}^{-1}$). Different letters for a given variable indicate a significant difference ($P < 0.05$) in the same tree species, sample time, and N application method among different N levels. Single asterisk or double asterisks indicates a significant difference ($P < 0.01$ or $P < 0.05$, respectively) in the same tree species, sample time, and N level between different sample times. Repeated measures ANOVA results (P values) for N levels, tree species, and sample times are shown in the insets

4 Discussion

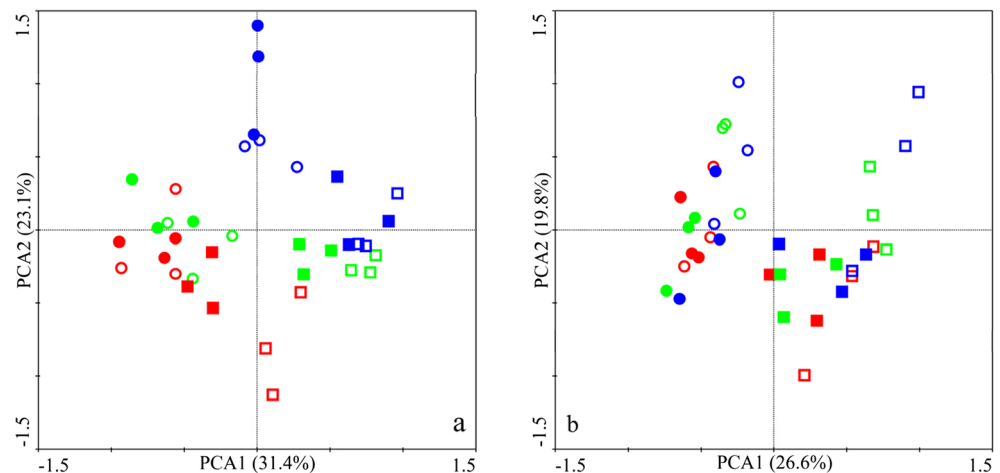
4.1 Different responses of the soil microbial community to the two N application methods

The main purpose of this study was to explore whether there are different responses of soil microbial communities to two N application methods. The SAN treatment had significant effects on soil bacterial, fungal, and actinomycete biomass and community composition under different N addition levels; however, the FAN treatment had no obvious effects on soil microbial biomass and community composition (Figs. 2, 3, 4, and 5). These results were consistent with the first hypothesis that the FAN treatment would have less influence on soil microbial biomass and community composition than the SAN treatment. The observed different effects of the two treatments on the biomass and composition of soil microbial communities in the present study have several explanations. The most important explanation is that less quantity of N was inputted into the soil (Bardgett and Watdle 2011; Wortman et al. 2012) in the foliage application of N treatment than in the soil application of N treatment (Table 1; Fig. 1). The nitrogen solution does not directly reach the soil but instead first contacts and passes through the leaf canopy (Gaiige et al. 2007; Wortman et

al. 2012; Zhang et al. 2015b), with the leaves and limbs retaining a substantial proportion of the N (Gaiige et al. 2007) or being absorbed by woody plant surfaces (Sparks 2009). It has been reported that the canopy layer can retain 20–25 and 52–59% of N deposition in the Novaggio forest and Canadian boreal forest, respectively (Wortman et al. 2012; Houle et al. 2015), which led to the soil microbial community being less affected by N addition (Blaško et al. 2013). A higher pH and lower soil NH_4^+ -N content and NO_3^- -N content ($P < 0.05$) were detected in the FAN treatment than in the SAN treatment (Table 1; Fig. 1), which resulted in N addition having no significant influence on soil microbial biomass and community composition in the FAN treatment (Figs. 2, 3b, 4, and 5b).

The variation of N forms after passing through the foliar layer is another explanation for the difference between the two N application treatments. Foliage had a higher affinity of NH_4^+ -N than NO_3^- -N in the present study (Table 1), which was consistent with the findings of Houle et al. (2015) and Adriaenssens et al. (2012). Besides, early studies proved that NH_4^+ -N and NO_3^- -N have absolutely different influences on soil ecosystem processes because of plant and soil microbe composition, plant preference for N uptake, and different N functions in the soil (Gavrachkova and Kuzyakov 2008; Fang et al. 2012). N intercepted by foliage could be taken up and/or transformed into other forms (Gaiige et al. 2007; Adriaenssens et al. 2012), which alters the quality of NH_4^+ -N and NO_3^- -N after N is deposited into the soil (Table 1). Additionally, soil NO_3^- -N is more mobile than NH_4^+ -N and could be absorbed by plants and soil microorganisms, while soil NH_4^+ -N will be immobilized into cation exchange sites in soil organic matter and clay minerals (Koba et al. 2003; Inselsbacher et al. 2010). Ultimately, the shift in different N forms mentioned above and its function in soils led to smaller changes in soil microbial biomass and community composition in the FAN treatment (Figs. 2, 3, 4, and 5).

Fig. 3 Principal component analysis (PCA) of soil microbial community compositions in the soil application of N treatment (**a**, SAN) and the foliage application of N treatment (**b**, FAN). Red, control (background N addition, $5.6 \text{ g N m}^{-2} \text{ year}^{-1}$); green, medium-N (intermediate N addition, $15.6 \text{ g N m}^{-2} \text{ year}^{-1}$); blue, high-N (high N addition, $20.6 \text{ g N m}^{-2} \text{ year}^{-1}$). Hollow, RS (rainy season, April 2013); solid, DS (dry season, October 2013). Circle, *P. massoniana*; square, *S. superba*



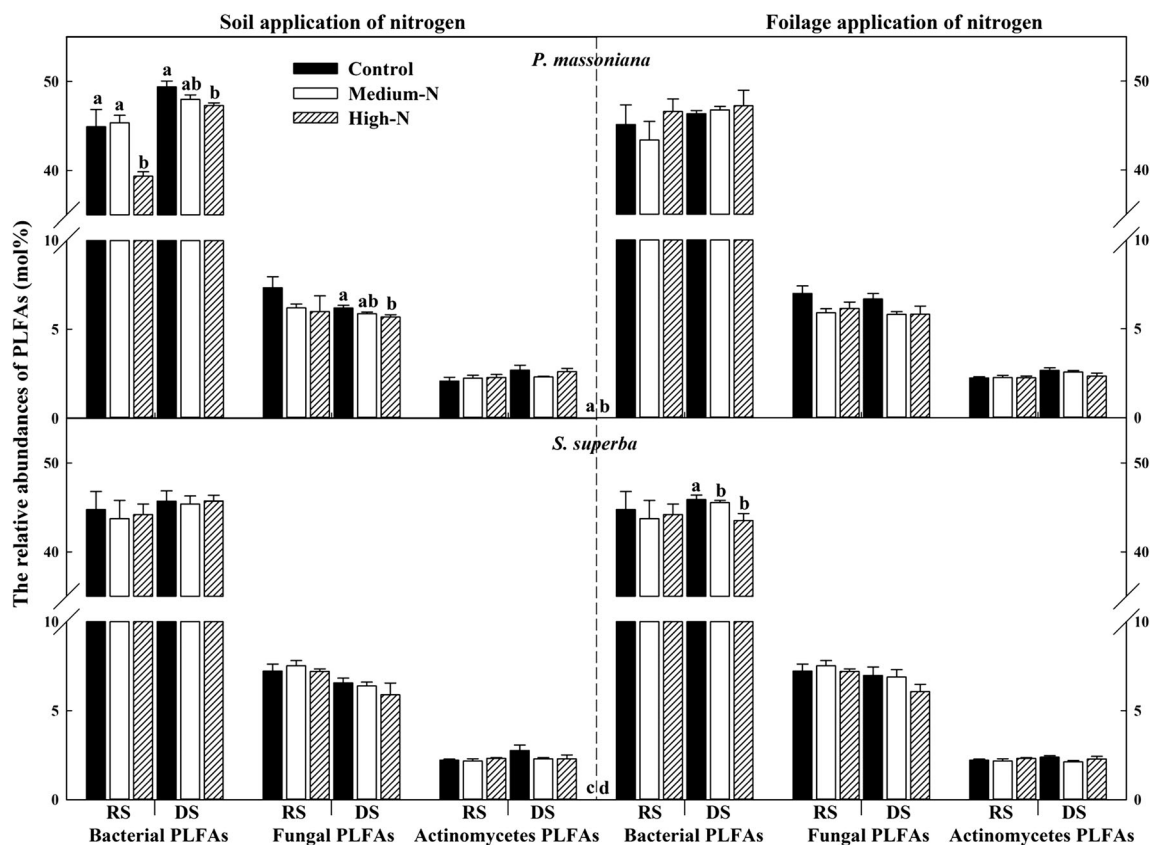


Fig. 4 The relative abundances of PLFAs of soil microbial groups in the soil application of N treatment (SAN) and foliage application of N treatment (FAN). Values are mean \pm SD ($n = 3$). RS rainy season (April 2013), DS dry season (October 2013). Control, background N addition ($5.6 \text{ g N m}^{-2} \text{ year}^{-1}$); medium-N, intermediate N addition

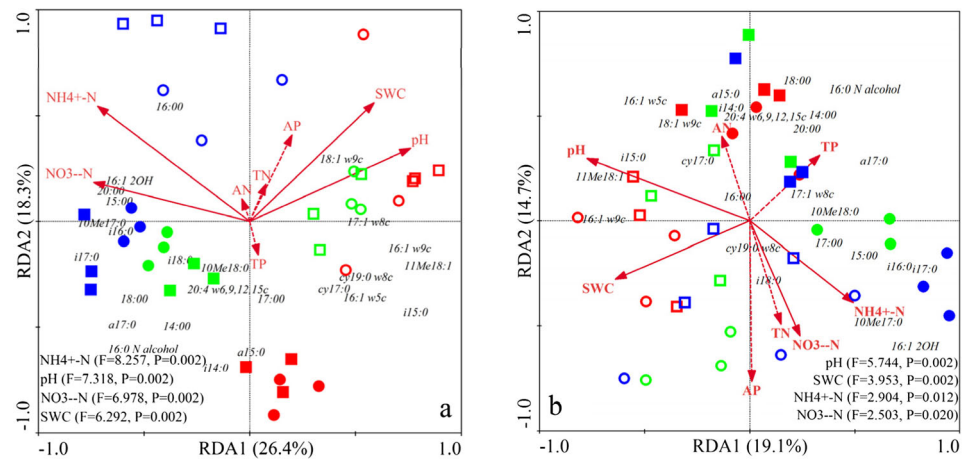
($15.6 \text{ g N m}^{-2} \text{ year}^{-1}$); high-N, high N addition ($20.6 \text{ g N m}^{-2} \text{ year}^{-1}$). Different letters for a given variable indicate a significant difference ($P < 0.05$) in the same tree species, sample time, and N application method among different N levels

The soil microbial community in the FAN and SAN treatments had different responses to the tree species (Fig. 2). Tree species is one of the most important factors that can significantly influence soil microbial community composition (Bardgett et al. 1999; DeForest et al. 2004), especially within a single ecosystem (Grayston and Prescott 2005). This study observed that tree species had significant influences on soil biomass and community composition, while N level had no influence ($P > 0.05$) on them in the FAN treatment. On the other hand, N level and tree species had significant effects on the biomass and composition of the soil microbial community in the SAN treatment (Figs. 2, 3, and 5). That is, N level had a smaller effect on soil microbes than tree species in the FAN treatment mainly due to amount of retained N by the foliar layer (Gaije et al. 2007; Sparks 2009). In correspondence with this study, Weand et al. (2010a) also found that tree species had stronger effects on the soil microbial community and enzyme activity than did N fertilization in the Catskill Mountains of southeastern New York.

Soil total PLFAs and bacterial and actinomycete biomass in the SAN treatment were higher ($P < 0.05$) in the *P. massoniana* soil than in the *S. superba* soil, whereas the

opposite responses were detected in the FAN treatment (Fig. 2), which confirmed the second hypothesis. Different tree species may have specific microbial groups (Priha et al. 2001; Grayston and Prescott 2005), root exudation, nutrient uptake rates, and influences on soil property (Lovett et al. 2004; Weand et al. 2010b), which cause tree species have different responses to N addition (Weand et al. 2010a), and ultimately changes the soil microbial community compositions (Merilä et al. 2010). Besides, the competition between plants and soil microbes for the same resources will result in a difference in plants growth status (e.g., N nutrient; Lovett et al. 2004). In a meta-analysis, Xia and Wan (2008) also pointed out that N deposition had more efficient effects on plant growth in coniferous species than in broad-leaf species. As a result, soil microbial biomass would be significantly different between the *P. massoniana* soil and the *S. superba* soil in the SAN and FAN treatments (Fig. 2). Furthermore, the relatively large-specific surface area in *S. superba* holds more N solution on the foliar layer and allows less N to be deposited into the soil (Table 1; Fig. 1). Last but not least, another explanation might be that growth stimulation in *S. superba* by N addition was significantly greater than that in *P. massoniana* (Liu et al.

Fig. 5 Redundancy analysis (RDA) of PLFA profiles for soil microbial PLFAs and environmental parameters in the soil application of N treatment (a, SAN) and the foliage application of N treatment (b, FAN). Red, control (background N addition, $5.6 \text{ g N m}^{-2} \text{ year}^{-1}$); green, medium-N (intermediate N addition, $15.6 \text{ g N m}^{-2} \text{ year}^{-1}$); blue, high-N (high N addition, $20.6 \text{ g N m}^{-2} \text{ year}^{-1}$). Hollow, RS (rainy season, April 2013); solid, DS (dry season, October 2013). Circle, *P. massoniana*; square, *S. superba*. TP total phosphorus, AP available phosphorus, SWC soil water content, TN total nitrogen, AN available nitrogen. The solid line indicates a significant difference ($P < 0.05$) between the soil microbial PLFAs and environmental variables. The dotted line indicates a nonsignificant difference ($P > 0.05$) between the soil microbial PLFAs and the environmental variables. Repeated measures ANOVA results (P values) of the environmental parameters are shown in the insets



2015). As mentioned above, the variation in soil N quantity and quality caused by foliar interception (Adriaenssens et al. 2012; Wortman et al. 2012; Houle et al. 2015) would lead to the different responses of soil microorganisms to the two N application methods (Figs. 2, 3, and 5).

4.2 Common responses of soil microbial communities to the two N application methods

Elevated N addition caused a reduction in microbial biomass in both the FAN and SAN treatments (Fig. 2), which was consistent with previous results from forest soils (DeForest et al. 2004; Demoling et al. 2008; Wang et al. 2015). There is adequate evidence that N deposition alters soil properties and that those influences and their turnover affect the soil nutrient pool and lead to associated changes in microenvironmental factors (Hoover et al. 2012; Xiong et al. 2016). Finally, all of these changes may lead to shifts in the biomass and composition of soil microbial communities (Xiong et al. 2014; Xiong et al. 2016). In this study, N addition significantly decreased soil pH and increased soil NH₄⁺-N content and NO₃⁻-N content directly in the two N application treatments (Table 1; Fig. 1), and these environmental factors had significant correlations with soil microbial community composition

(Fig. 5), which could support this interpretation. Another explanation for this decrease was that the accumulation of N aggravated soil acidification by ammonium nitrification and nitrate leaching (Wang et al. 2015), leading to increased Al³⁺ toxicity and inhibited soil microorganism activity (Zhang et al. 2011; Liu et al. 2013).

Our results also showed that there was no significant difference in the soil TP, AP, SWC, TN, and AN between the FAN and the SAN treatments (Table S1, Electronic Supplementary Material), which correspond with Shi et al. (2016) reporting that soil chemical properties (except for exchangeable manganese) did not significantly differ between the canopy application of N treatment and the soil application of N treatment. This might be due to the relatively short duration of this experiment, which suggests that a long-term foliar fertilization experiment is necessary (Bardgett and Watdle 2011; Shi et al. 2016). Moreover, with the high background N deposition rates (reaching up to $5.6 \text{ g N m}^{-2} \text{ year}^{-1}$) (Liu et al. 2008) and the high quantities of N (15.6 and $20.6 \text{ g N m}^{-2} \text{ year}^{-1}$) added in this experiment, perhaps there was a threshold for the effects of N on the soil parameters (Zhang et al. 2015b) in this experiment, such that the effects of the two N application methods on those soil properties were similar.

5 Conclusions

The soil microbial community response to simulated N addition and N application methods (adding N directly on soil or on foliage) was studied in two tree species (*P. massoniana* and *S. superba*) for 19 months. Elevated N deposition affected the soil properties for both the foliage application of N (FAN) and the soil application of N (SAN) method, including an increase in the soil NH_4^+ -N content and NO_3^- -N content and a decrease in the soil pH. Subsequently, those changes contributed to the decline in soil microbial biomass. Soil microbial biomass and community composition were significantly changed in the SAN treatment. However, they showed no obvious difference in the FAN treatment because part of the N was retained by the foliage. Consistent with the first hypothesis, the effects of the SAN treatment on soil microbial biomass and community composition were stronger than those of the FAN treatment, which suggested that previous studies that simulated N deposition by adding N directly to soil might have inaccurately estimated its effects on soil microorganisms. Furthermore, the soil microbes responded differently to the FAN and SAN treatments between *P. massoniana* and *S. superba*, which supported the second hypothesis that the influence of the N application methods (SAN vs. FAN) on soil microbes was affected by tree species. However, foliar retention is still not clear in this study. Therefore, further in-depth studies should be planned and focused on the quality and quantity of different N forms after foliage application of N and its long-term effects on soil microbes.

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