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RESEARCH PAPER

Nitrogen addition method affects growth and nitrogen accumulation in seedlings of four subtropical tree species: *Schima superba* Gardner & Champ., *Pinus massoniana* Lamb., *Acacia mangium* Willd., and *Ormosia pinnata* Lour

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Abstract

· ***Key message*** **N addition (56, 156, and 206 kg N ha^{-1} yr^{-1}as dissolved NH_4NO_3) method (canopy vs soil) did not affect the biomass of N_2-fixers (*Acacia mangium* Willd. and *Ormosia pinnata* Lour.), but significantly affected the biomass of non-N_2-fixers (*Schima superba* Gardner & Champ., *Pinus massoniana* Lamb.). Coniferous species exposed to N addition on the canopy rather than the soil had higher N accumulation.**

· ***Context*** Previous experiments simulating nitrogen (N) addition in forests were conducted by adding N fertilizer directly to soils, which neglects the fact that N uptake can be done by canopy leaves.

· ***Aims*** The objective of this study is to examine how different N addition methods (canopy vs soil) affect growth and N accumulation of four subtropical tree seedlings.

· ***Methods*** An open-air greenhouse experiment was conducted to expose four tree species (*Schima superba* Gardner & Champ., *Pinus massoniana* Lamb., *Acacia mangium* Willd. and *Ormosia pinnata* Lour.) to different N addition methods (canopy or soil) and N levels (ambient, medium, or high).

· ***Results*** N addition method affected the biomass of non-N_2-fixers (*Schima superba* Gardner & Champ. and *Pinus massoniana* Lamb.), while N_2-fixers (*Acacia mangium* Willd. and *Ormosia pinnata* Lour.) were unaffected. N concentrations in the soils and leaves of all trees were significantly increased by the medium and high N additions, and soil N concentrations resulted from N addition via soil rather than the canopy. Although leaf N concentration was significantly affected by N addition method in all trees except for *Ormosia pinnata*, only N accumulation in *Pinus massoniana* was significantly affected by N addition method.

· ***Conclusion*** N addition method affected the biomass of non-N_2-fixers and N accumulation in coniferous species, while it did not affect the biomass of N_2-fixers and N accumulation in broadleaf species.

Keywords Soil N addition · Canopy N addition · N accumulation · N_2-fixers · Subtropical trees

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1 Introduction

An excess of 10 kg nitrogen (N) ha^{-1} $yr.^{-1}$ deposition has been detected in 11% of the world's natural vegetation (Pardo et al. 2011). China is a rapidly developing country in Asia that is also experiencing intensifying N deposition in its central and southeastern areas (Xu et al. 2015; Zhu et al. 2015). High N deposition within the range 30–73 kg N ha^{-1} $yr.^{-1}$ was observed in forests in Southern China (Mo et al. 2006; Zhao et al. 2017). N deposition below a critical threshold can increase the foliar N and photosynthetic capacity of tree species (Bauer et al. 2004; Fleischer et al. 2013); however, N deposition above the threshold directly damages leaf tissues (Liu et al. 2007) and inhibits tree growth (Lu et al. 2010, 2014). Excess N deposition can often increase soil acidity, which causes nutrient loss from the soil (Bowman et al. 2008; Lu et al. 2014, 2015) and a decrease in plant diversity (Hogberg et al. 2006; Bobbink et al. 2010).

Numerous studies have reported how N deposition affects plant growth (Lu et al. 2010; Liu et al. 2017). However, almost all the experiments simulating N deposition have been conducted by adding N solution and/or fertilizer directly onto the soil (forest floor). Conventional experiments with addition of N to the understory largely neglect the fact that nutrient uptake can be done directly by canopy leaves and/or twigs (Adriaenssens et al. 2012; Fernández and Brown 2013; Guerrieri et al. 2015; Chater and Garner 2018), and therefore may not realistically simulate atmospheric N deposition or generate reliable impacts on forest ecosystems (Zhang et al. 2015). It is well known that natural N deposition passes through the canopy layer before it reaches the forest floor. When N is deposited directly on the canopy, it becomes immediately available to plant leaves and subsequently stimulates photosynthesis and increases production (Wortman et al. 2012). However, when N solution and/or fertilizer is added directly onto the soil, it affects plant growth indirectly via soil acidification and nutrient loss (Bowman et al. 2008; Lu et al. 2014). Hence, the effects of N addition via plant leaves and soils on plant growth should be different. Therefore, it is necessary to understand the effects of different N addition methods on plant growth.

Responses of tree growth to N addition are often species-specific (Elvir et al. 2006; Liu et al. 2017; Mao et al. 2017). Elvir et al. (2006) found that *Acer saccharum* Marshall, but not *Fagus grandifolia* Ehrh. and *Pinus resinosa* Sol. ex Aiton, showed significantly higher photosynthetic rates after a decade of N inputs. Mao et al. (2017) found that the highly light-dependent species responded significantly to N addition, but medium-light and shade-tolerant species did not in a tropical forest. Mo et al. (2008) reported that net photosynthetic rate of two tree seedlings (*Schima superba* Gardner & Champ. and *Cryptocarya concinna* Hance) varied in response to simulated N deposition depending on the rate of N addition and species-specific N requirements. Recently, Liu et al. (2017) demonstrated that two N_2 fixers grew significantly faster than two non-N_2 fixers in response to N addition in subtropical China.

Here, we examined the N accumulation and plant growth of four tree species, including two N_2-fixers and two non-N_2-fixers, in a greenhouse in response to three N addition levels (ambient, medium, and high) and two addition methods (canopy and soil) in subtropical China. We hypothesized that: (1) the responses of tree growth in N-rich tropical areas to N addition are species-specific; (2) different N addition methods have different effects on plant growth and N accumulation in the four tree species, and (3) the stimulation of N accumulation is greater in canopy N addition than soil N addition as plant leaves can directly take-up the added N from the canopy.

2 Materials and methods

2.1 Experimental set-up

The study was conducted in an open-air greenhouse at the South China Botanical Garden in Guangzhou city, Guangdong Province, China (23°20′ N, 113°30′ E). The mean annual temperature, precipitation, and relative humidity were 21.5 °C, 1750 mm, and 77%, respectively. In March 2012, 144 plastic pots (40-cm diameter, 30-cm height) were prepared. Each pot was filled with Ultisol soil. The soil was collected from a nearby evergreen broad-leaved forest. The pH for the selected soil was 4.00 ± 0.10. Soil organic matter was 22.72 ± 1.20 g kg^{-1}. In the soil, the NO_3^-N and NH_4^+-N were 17.39 ± 1.34 mg kg^{-1} and 6.01 ± 0.69 mg kg^{-1}, respectively. One-year-old seedlings of four species were collected from a nursery and transplanted into the pots (one seedling per pot) avoiding root damage. Among the four species, *Ormosia pinnata* Lour. and *Acacia mangium* Willd. are N_2-fixers, while *Schima superba* and *Pinus massoniana* Lamb. are non-N_2-fixers. In the two non-N_2-fixers, *S. superba* is a broad-leaf species and *P. massoniana* is a needle species. These species are common tree species in southern China.

On 10 April 2012, the 144 pots were arranged in six randomized complete blocks. Each block was made of six different N treatments, with four plant species per treatment, for a total of 24 pots per block. The six N treatments were: S-CK (ambient N addition to the soil), S-MN (medium N addition to the soil), S-HN (high N addition to the soil), C-CK (ambient N addition to the canopy), C-MN (medium N addition to the canopy), and C-HN (high N addition to the canopy). As the ambient wet N deposition was about 56 kg ha^{-1} $yr.^{-1}$ in the South China Botanical Garden in 2006 (Liu et al. 2008), we set the three N levels at 56, 156, and 206 kg ha^{-1} $yr.^{-1}$, for the ambient, medium, and high levels, respectively. NH_4NO_3-N was dissolved in tap water and then slowly added to the seedling canopies by spraying or directly onto pot soil once a week. For the canopy addition, we first covered the soil in each pot with clingfilm, and then sprayed the N solution slowly from the top of the seedling using a mist sprinkler. We aimed to cover all branches. No other fertilizer was used. All pots received the same amount of water.

2.2 Sample collection and measurement

To measure initial soil parameters, soil samples were collected from the 0 to 20 cm layer in each pot before the seedlings were transplanted. In June and December 2012 and April, July, and November 2013, soil samples were collected from the 0 to 20 cm layer in each pot to measure the concentrations of NO_3^--N and NH_4^+-N in the soil. Each soil sample was a pool sampled from three soil cores (inner diameter: 2.5 cm), and was stored in a hard, plastic container for transport to the laboratory. Soil samples were air-dried and sieved using a 2-mm mesh. Dead roots and plant residues were removed. Soil pH was determined using a glass electrode in the supernatants by shaking for 2 h and sedimentation in a beaker for 24 h in deionized CO_2-free water. The soil to H_2O ratio was 1:2.5. Soil organic C was determined following the Walkley-Black wet digestion method (Nelson and Sommers 1982). The soil NO_3^-N was determined using a modified ultraviolet-visible (UV) spectrophotometry method from Norman et al. (1985). The soil NO_3^--N was extracted using a 1 M KCl solution with a 1:5 soil to solution ratio. Then, the mixture was shaken at 20 ± 2 °C for 1 h in an oscillator. After filtration through Whatman no. 1 filter paper, the leachate was then determined at 220 nm and 275 nm, respectively. The measurement principle of the method was based on the difference in the spectral adsorption properties between dissolved organic carbon (DOC) and nitrate. NO_3^--N has the strongest absorption of UV light at 220 nm wavelength and the weakest absorption at 275 nm. DOC absorbs light at all wavelengths between 200 and 300 nm. In this study, NO_3-N concentration was obtained by measuring the absorbance of the KCl extract at 220 nm and 275 nm wavelengths. More detailed information is given in Song et al. (2007). To measure soil NH_4^+-N, the soil was also extracted using a 1 M KCl solution with a 1:5 soil to solution ratio. However, in this case, the KCl extract was determined at 625 nm using the indophenol blue spectrophotometric method (Horn and Squire 1966).

To measure soil phospholipid fatty acid (PLFA), soil samples were also collected from each pot in April and October 2013. Three soil cores (2.5 cm in diameter) were randomly chosen from the 0 to 20 cm layer and homogenized into one sample per pot. Soil samples were put into sealed plastic bags and transported to the laboratory immediately. Roots, stones, and other materials were removed; each soil sample was screened through a 2-mm sieve and stored at 4 °C until analysis. Detailed information about soil PLFA measurements are given in Liu et al. (2017).

In March, June, and December 2012 and April, July, and November 2013, about 6–10 new fully expanded leaves from each seedling were randomly sampled to analyze N concentration. All leaves were carefully rinsed and air dried, and then dried at 70 °C in an oven for 72 h, then finely ground (0.25 mm). Foliar N concentration was measured using the Kjeldahl method (Bremner and Mulvaney 1982). To measure tree biomass, all seedlings were harvested by carefully digging them out of the soil in November 2013. The biomass (B, g dry matter) of fine roots (diameter ≤ 2 mm), coarse roots (diameter > 2 mm), stems, and leaves in each harvested seedling were obtained after oven-drying at 60 °C for 1 day. The accumulation of N was calculated as follows:

$$\begin{aligned}\text{N accumulation (g/plant)} &= \text{N concentration}_{\text{coarse root}}\\ &\times \text{biomass}_{\text{coarse root}} + \text{N concentration}_{\text{fine root}} \times \text{biomass}_{\text{fine root}}\\ &+ \text{N concentration}_{\text{stem}} \times \text{biomass}_{\text{stem}} + \text{N concentration}_{\text{leaf}}\\ &\times \text{biomass}_{\text{leaf}}.\end{aligned}$$

2.3 Statistical analysis

Shapiro-Wilk's test was used to test the normality and homogeneity of variance before statistical analysis. The effects of N addition level, N addition method, and their interaction on tree growth and N accumulation were analyzed using a linear mixed model. The effects of N addition, N addition method, sampling time, and their interactions on N concentrations in both soil and leaf samples were analyzed using repeated measures analysis of variance with a linear mixed model. Effects of N addition level and addition method on the microbial PLFAs were examined using a general linear model. When there was a significant effect of N addition level or method, data were further analyzed using Tukey's test. Differences were considered as statistically significant at $p < 0.05$. SAS software (SAS Institute Inc., Cary, NC, USA) was used for the data analysis.

3 Results

3.1 Biomass

N addition levels, addition methods, and their interaction all significantly affected tree root biomass, but the effect was species-specific (Fig. 1). High N addition via the plant canopy significantly increased ($p < 0.05$) fine root biomass of *A. mangium* and *P. massoniana*. However, only medium N addition via soil significantly increased ($p < 0.05$) fine root biomass of *A. mangium*. High N addition via soil significantly decreased ($p < 0.05$) fine root biomass of *O. pinnata* and *P. massoniana*.

Both medium and high N addition significantly increased ($p < 0.05$) the biomass of *A. mangium* compared with the ambient N addition, irrespective of N addition method (Fig. 1). High N addition did not affect the biomass of *O. pinnata*. Only medium N addition via soil significantly increased ($p < 0.05$) the biomass of *S. superba*.

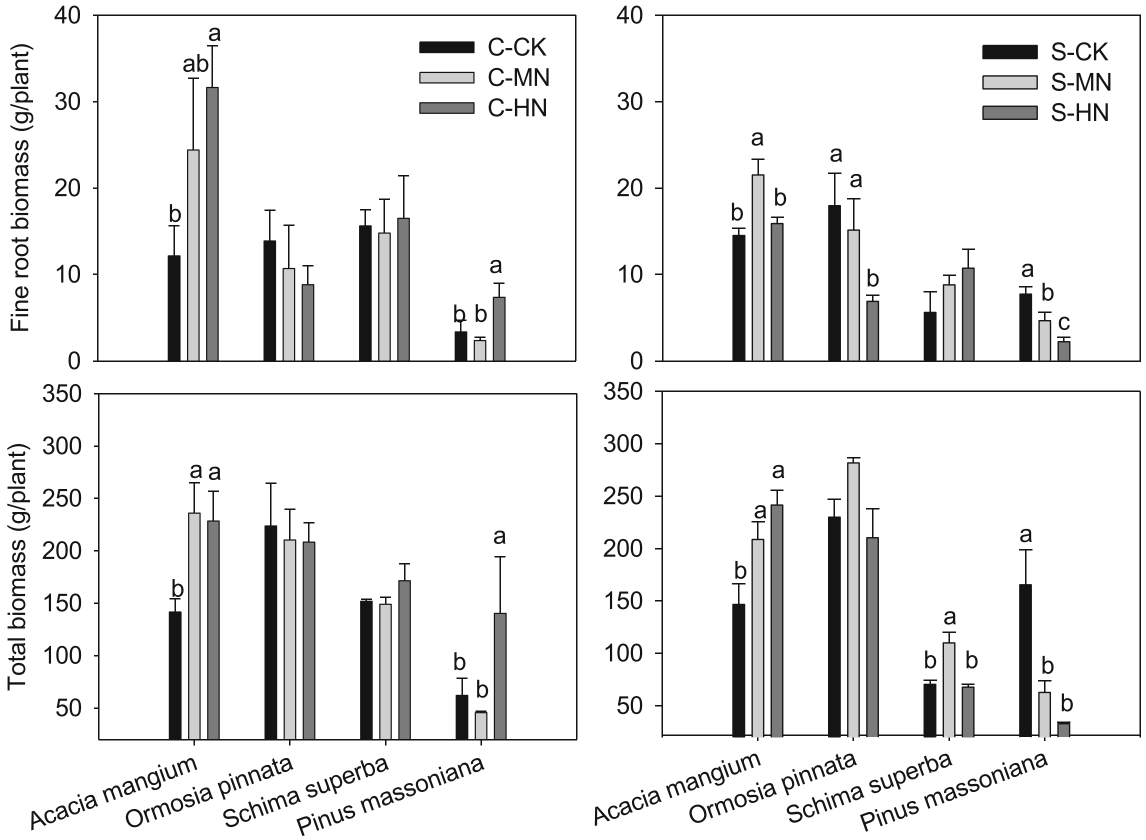

Fig. 1 Effects of N addition levels and methods on total and fine root biomass in four tree species (mean ± SD). Different lowercase letters above the error bars indicate significant differences among N addition level in each species. S-CK: ambient N addition to the soil, S-MN: medium N addition to the soil, S-HN: high N addition to the soil, C-CK: ambient N addition to the canopy, C-MN: medium N addition to the canopy, and C-HN: high N addition to the canopy. Data of soil N addition are from Liu et al. (2017)

High N addition via plant canopy significantly increased ($p < 0.01$) the biomass of *P. massoniana*, while both high and medium N addition via soil significantly decreased ($p < 0.01$) the biomass of *P. massoniana* (Fig. 1), which suggests that the N addition method significantly affected ($p < 0.05$) the biomass of this needle species. Overall, N addition method significantly affected ($p < 0.01$) the biomass of non-N_2-fixers (*S. superba* and *P. massoniana*), while N_2-fixers (*A. mangium* and *O. pinnata*) were unaffected.

3.2 Leaf stoichiometry

Leaf N concentrations in all species increased over time. Both high and medium N addition resulted in higher leaf N concentrations in all the species ($p < 0.005$) (Fig. 2). N addition method significantly affected ($p \leq 0.01$) leaf N concentration in all species, except *O. pinnata*. Higher leaf N concentrations were found in *A. mangium* when they received N addition via the soil, rather than the canopy. However, the opposite was observed in *P. massoniana*, as higher leaf N concentrations were shown in the pots that received N addition via the canopy ($p < 0.001$).

3.3 N accumulation

High N addition via the plant canopy significantly increased ($p < 0.01$) N accumulation in *A. mangium*, *S. superba*, and *P. massoniana* (Fig. 3). Both high and medium N addition via soil significantly increased ($p < 0.01$) N accumulation in the N_2-fixers (*A. mangium* and *O. pinnata*), while both high and medium N addition via soil significantly decreased ($p < 0.01$) N accumulation in *P. massoniana* (Fig. 3). Overall, N addition method did not affect N accumulation in any of the tree species, except for *P. massoniana*. Higher N accumulation was observed in *P. massoniana* exposed to canopy rather than soil N addition.

3.4 Soil N concentrations

Soil NH_4^+-N concentrations were lower than NO_3^--N in all species (Tables 1 and 2). High N addition significantly increased ($p < 0.05$) NH_4^+-N concentrations in all species, irrespective of N addition method. Higher NH_4^+-N concentrations were found in the pots that received N addition via soil. Sampling time significantly affected soil NH_4^+-N concentrations.

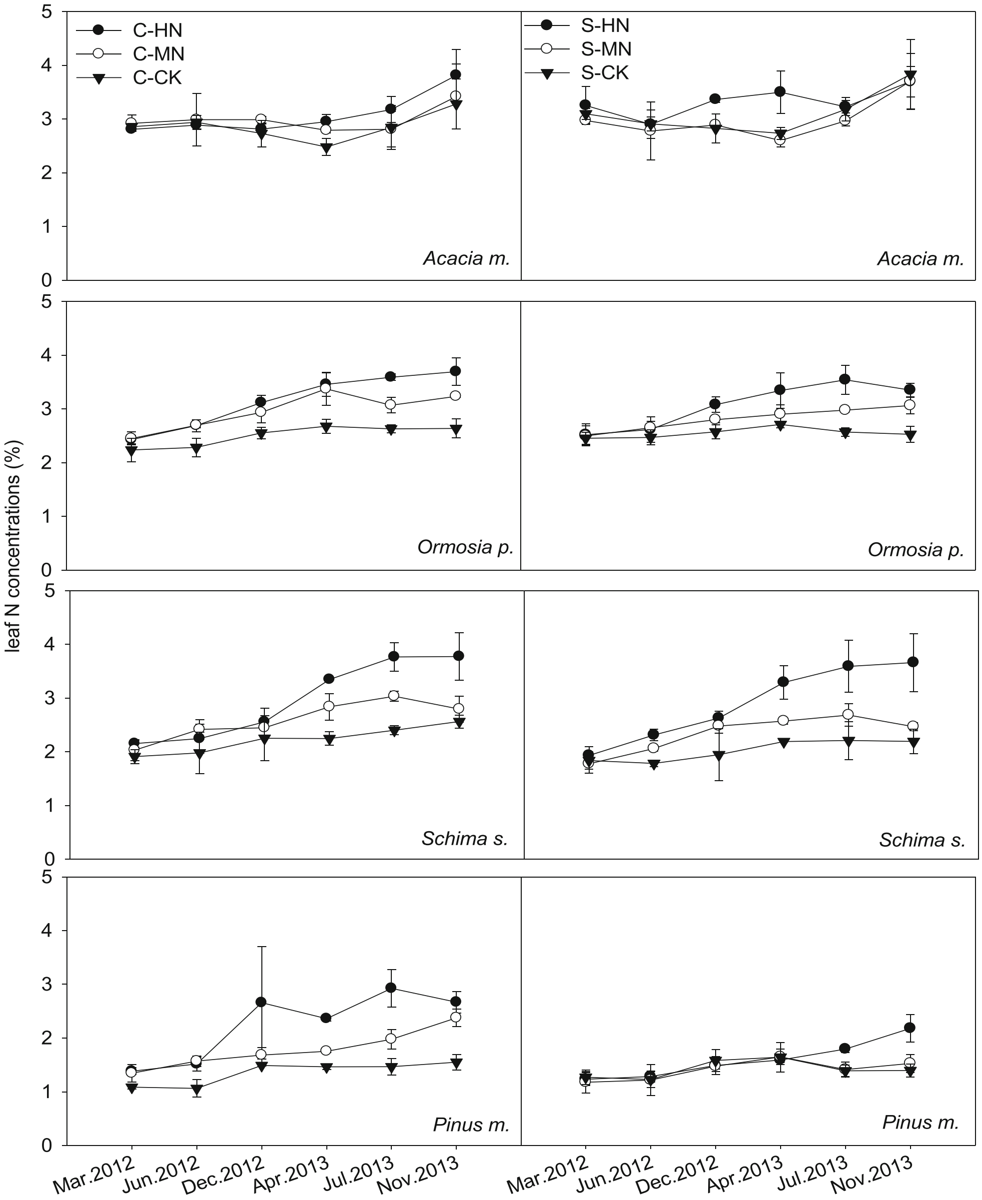

Fig. 2 Effects of N addition levels, methods, and time on leaf N concentrations in four tree species. S-CK: ambient N addition to the soil, S-MN: medium N addition to the soil, S-HN: high N addition to the soil, C-CK: ambient N addition to the canopy, C-MN: medium N addition to the canopy, and C-HN: high N addition to the canopy

Compared with the ambient N addition, high N addition significantly increased ($p < 0.05$) NO_3^--N concentration in the soil of pots of all species. N addition method significantly affected soil NO_3^--N concentration, with higher values in the pots that received N addition via soil. Among the four species, the soil in *P. massoniana* pots showed the highest NO_3^--N concentration, followed by the soil in the *A. mangium* and *S. superba* pots. The soil in *O. pinnata* pots had the lowest NO_3^--N concentration (Table 2).

3.5 Phospholipid fatty acids (PLFAs)

Higher N addition significantly decreased microbial PLFAs in the soil of all the species pots, irrespective of the N addition method. In April 2013, N addition significantly decreased ($p < 0.05$) Arbuscular mycorrhizal fungi (AMF) in the soil in all species, irrespective of the N addition method. However, in October 2013, N addition significantly decreased ($p < 0.05$) the PLFAs in all pots, except for those in the soil around *S. superba* (Table 3). Meanwhile, N addition via soil significantly decreased PLFAs in the soil around *O. pinnata* and *S. superba* in October 2013. In both sampling periods, N addition method did not affect soil microbial PLFAs in the pots with *A. mangium*, *O. pinnata*, or *P. massoniana*. However, for *S. superba*, higher soil microbial PLFAs were found in the pots that received canopy rather than soil N addition ($p < 0.05$).

4 Discussion

N_2-fixers show a significant increase in yield in response to N addition when N_2 fixation is unable to meet plant N demand

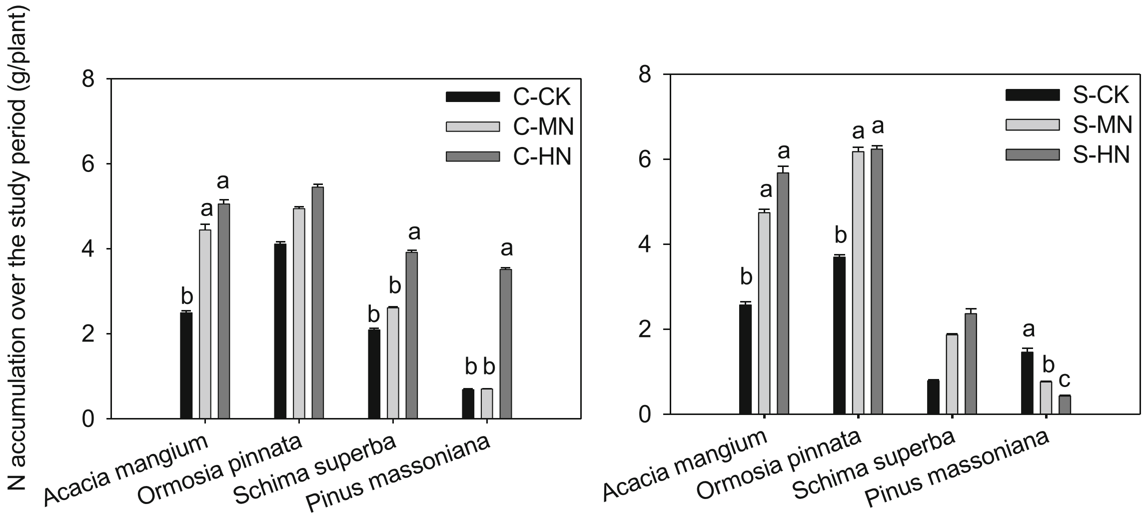

Fig. 3 Effects of N addition levels and methods on N accumulation in four tree species (mean ± SD). Different lowercase letters above the error bars indicate significant differences among N addition level in each species ($p < 0.05$). S-CK: ambient N addition to the soil, S-MN: medium N addition to the soil, S-HN: high N addition to the soil, C-CK: ambient N addition to the canopy, C-MN: medium N addition to the canopy, and C-HN: high N addition to the canopy

(Salvagiotti et al. 2008; Thies et al. 1995). The growth stimulation in N_2-fixers in response to N addition was also observed in our study. Medium and high N addition significantly increased the biomass of *A. mangium*. However, high N addition did not affect the total biomass of *O. pinnata*. Some previous studies also showed that N addition had no effect on the biomass production of N_2-fixers (Xia and Wan 2008; Zhang et al. 2011), as high N addition inhibited symbiotic nitrogenase activity in some

Table 1 Effects of N addition levels and methods on NH_4^+-N concentrations in soil planted with four tree species during the experimental period

Species	Sample times	Soil N addition			Canopy N addition		
		High N	Medium N	Ambient N	High N	Medium N	Ambient N
AM	Jun. 2012	8.0 ± 1.2ab	9.9 ± 1.8a	5.6 ± 1.3bc	4.9 ± 0.3c	4.4 ± 0.6c	4.1 ± 1.0c
	Dec. 2012	11.4 ± 0.8a	6.9 ± 0.8bc	5.7 ± 1.0bc	8.0 ± 0.3b	4.6 ± 0.9 cd	4.3 ± 1.3d
	Apr. 2013	25.6 ± 3.6a	14.8 ± 2.9bc	12.5 ± 1.0c	22.8 ± 4.3ab	14.6 ± 1.6c	14.0 ± 3.1c
	Jul. 2013	25.6 ± 4.4a	7.3 ± 0.5b	6.4 ± 1.2b	19.6 ± 4.7a	10.2 ± 1.5b	5.0 ± 0.1b
	Nov. 2013	37.9 ± 0.8a	23.0 ± 0.7bc	16.9 ± 4.0 cd	25.8 ± 6.1b	10.6 ± 1.0d	10.8 ± 2.0d
OP	Jun. 2012	4.3 ± 0.5	5.8 ± 2.2	6.4 ± 0.4	5.2 ± 0.3	5.3 ± 0.5	4.1 ± 0.6
	Dec. 2012	9.2 ± 2.4a	5.8 ± 0.4bc	7.3 ± 1.9ab	8.5 ± 1.6a	3.6 ± 0.7bc	3.2 ± 0.8c
	Apr. 2013	26.1 ± 3.7	17.5 ± 2.2	15.2 ± 1.3	27.1 ± 8.0	17.0 ± 3.5	18.3 ± 4.4
	Jul. 2013	19.7 ± 3.5a	8.7 ± 3.3ab	6.4 ± 1.9b	17.1 ± 7.4ab	10.9 ± 1.9ab	7.7 ± 3.9b
	Nov. 2013	26.9 ± 2.9a	19.6 ± 1.5a	9.9 ± 1.3bc	18.4 ± 6.0ab	10.3 ± 2.9bc	8.4 ± 1.6c
SS	Jun. 2012	7.1 ± 0.5a	4.7 ± 1.4b	4.6 ± 0.8b	5.4 ± 1.0ab	5.3 ± 0.8ab	4.6 ± 0.3b
	Dec. 2012	10.0 ± 2.4a	4.2 ± 1.5b	4.0 ± 0.5b	6.3 ± 0.7ab	3.7 ± 0.6b	4.8 ± 1.3b
	Apr. 2013	30.1 ± 0.4a	16.4 ± 1.9 cd	9.5 ± 1.4d	26.7 ± 2.2ab	19.6 ± 2.5bc	17.9 ± 4.9c
	Jul. 2013	21.4 ± 1.8ab	6.2 ± 1.7c	4.0 ± 0.8c	26.1 ± 8.7a	12.0 ± 5.7bc	9.4 ± 1.7bc
	Nov. 2013	30.1 ± 2.2a	16.0 ± 1.3bc	11.5 ± 1.4c	22.4 ± 4.3ab	13.6 ± 1.9bc	9.4 ± 1.5c
PM	Jun. 2012	6.4 ± 0.6	5.5 ± 0.9	4.7 ± 0.3	6.8 ± 0.4	6.8 ± 0.5	5.7 ± 1.6
	Dec. 2012	8.4 ± 3.6ab	4.1 ± 1.1b	4.0 ± 0.1b	11.9 ± 3.4a	5.2 ± 0.6b	5.4 ± 1.2b
	Apr. 2013	19.5 ± 6.7ab	15.3 ± 0.1b	19.5 ± 3.8ab	32.2 ± 1.8a	18.7 ± 4.3b	13.4 ± 4.0b
	Jul. 2013	27.3 ± 2.7a	14.2 ± 5.0b	7.1 ± 0.5b	25.9 ± 1.5a	6.9 ± 2.2b	5.7 ± 2.7b
	Nov. 2013	32.5 ± 4.9a	12.1 ± 1.8bc	7.9 ± 1.5c	28.4 ± 8.9a	20.7 ± 2.0ab	10.7 ± 1.9bc

AM Acacia mangium, *OP Ormosia pinnata*, *SS Schima superba*, *PM Pinus massoniana*

Unit: mg/kg. Treatments with different superscript letter are significantly different from each other ($p > 0.05$). Data of soil N addition are from Liu et al. (2017)

Table 2 Effects of N addition levels and methods on NO_3^--N concentrations in soil planted with four tree species during the experimental period

Species	Sample times	Soil N addition			Canopy N addition		
		High N	Medium N	Ambient N	High N	Medium N	Ambient N
AM	Jun.2012	30.3 ± 10.1a	22.7 ± 5.4ab	14.5 ± 3.2bc	11.7 ± 3.9bc	5.6 ± 1.3c	2.3 ± 1.5c
	Dec.2012	43.6 ± 2.2a	14.0 ± 3.7bc	3.5 ± 1.8d	19.0 ± 2.5b	5.8 ± 4.5 cd	3.0 ± 4.1d
	Apr.2013	106.2 ± 8.3a	12.9 ± 11.4b	7.1 ± 6.4b	69.1 ± 15.0a	5.2 ± 1.7b	4.9 ± 4.6b
	Jul.2013	139.2 ± 14.0a	23.4 ± 8.5b	7.3 ± 9.5b	34.3 ± 11.5b	10.0 ± 8.9b	16.4 ± 0.7b
	Nov.2013	130.2 ± 16.3a	59.0 ± 8.1b	20.8 ± 7.0b	55.5 ± 15.0b	17.8 ± 8.3b	18.7 ± 6.6b
OP	Jun.2012	49.4 ± 6.1a	44.9 ± 7.6ab	31.3 ± 3.1c	41.2 ± 4.4bc	52.2 ± 6.8a	28.3 ± 2.1c
	Dec.2012	38.0 ± 6.2a	16.7 ± 3.4bc	4.7 ± 1.5c	20.5 ± 10.4ab	14.9 ± 10.3bc	3.8 ± 1.3c
	Apr.2013	69.0 ± 17.5a	13.2 ± 2.6b	5.8 ± 3.4b	50.0 ± 6.3a	18.4 ± 5.3b	5.9 ± 3.3b
	Jul.2013	89.3 ± 7.4a	31.8 ± 10.7b	5.5 ± 0.4c	31.1 ± 7.9b	14.7 ± 9.2bc	3.6 ± 3.4c
	Nov.2013	87.5 ± 6.1a	24.1 ± 2.9b	2.8 ± 1.7d	27.2 ± 6.3b	18.1 ± 8.3bc	5.9 ± 2.1 cd
SS	Jun.2012	47.3 ± 9.1a	46.4 ± 4.4ab	20.9 ± 7.2c	37.4 ± 9.8abc	29.0 ± 2.8bc	24.3 ± 4.8bc
	Dec.2012	48.0 ± 6.7a	26.3 ± 5.0b	10.8 ± 3.9c	19.2 ± 5.4bc	15.0 ± 5.4bc	8.9 ± 2.2c
	Apr.2013	65.2 ± 7.5ab	37.3 ± 15.8bc	14.2 ± 1.3c	74.4 ± 19.8a	25.8 ± 12.4c	14.6 ± 3.9c
	Jul.2013	70.0 ± 17.2a	30.8 ± 3.1b	7.9 ± 0b	65.3 ± 14.6a	25.9 ± 6.5b	15.9 ± 9.7b
	Nov.2013	75.3 ± 10.3a	38.7 ± 4.2c	8.0 ± 2.0d	56.9 ± 4.6b	20.1 ± 2.8d	15.4 ± 3.0d
PM	Jun.2012	30.6 ± 9.5b	42.3 ± 10.3ab	35.8 ± 2.5ab	54.3 ± 1.2a	50.0 ± 5.8ab	34.7 ± 2.0ab
	Dec.2012	39.6 ± 5.0b	26.8 ± 5.1c	12.5 ± 5.1d	57.9 ± 3.6a	30.1 ± 1.0bc	15.3 ± 2.2d
	Apr.2013	59.4 ± 27.5ab	46.5 ± 15.8ab	31.7 ± 1.2ab	100.7 ± 6.5a	45.8 ± 8.6ab	25.2 ± 6.0b
	Jul.2013	86.5 ± 15.5a	73.7 ± 15.7a	17.1 ± 7.2b	95.3 ± 14.8a	73.5 ± 26.3a	51.0 ± 8.8ab
	Nov.2013	121.5 ± 13.7a	38.4 ± 1.9bc	18.5 ± 10.0c	112.5 ± 21.6a	71.3 ± 19.7b	40.7 ± 2.1bc

AM Acacia mangium, *OP Ormosia pinnata*, *SS Schima superba*, *PM Pinus massoniana*

Unit: mg/kg. Treatments with different superscript letter are significantly different from each other ($p > 0.05$). Data of soil N addition are from Liu et al. (2017)

legumes (Lee et al. 2003; Thomas et al. 2000; Zhang et al. 2011). Different responses of *A. mangium* and *O. pinnata* to N additions indicated that the effects of N addition on N_2-fixers were species-specific. N addition method did not affect the total biomass of N_2-fixers, but significantly affected the biomass of non-N_2-fixers (*S. superba* and *P. massoniana*). This might have been in part because N addition method did not affect soil microbial PLFAs of N_2-fixers in our study. N_2-fixers are able to fix N_2 to meet plant N demand, which probably explains the lack of growth response to the different N addition methods. Our results support our first hypothesis that the responses of tree growth in N-rich tropical areas to N addition method are species-specific.

In all species, high and medium additions of N improved the concentration of N in the leaves. This was a consequence of the increased input of N fertilizer with the treatments. N addition methods significantly affected leaf N concentrations in *A. mangium* and *P. massoniana*. Higher leaf N concentrations were found in *A. mangium* that received N addition via the soil rather than the canopy. However, the opposite effects were observed in *P. massoniana*. This indicated that responses of plant N accumulation to different N addition methods were species-specific. Fernández and Eichert (2009) also found that the response to foliar nutrient sprays was variable among species. Foliar spray uptake is determined by plant characteristics such as leaf shape and chemistry, and stomata and physical attributes including cuticle composition and surface wax architecture (Fernández et al. 2008; Fernández and Brown 2013). The structure and composition of the cuticle and cuticular waxes vary greatly between plant species (Heredia-Guerrero et al. 2008; Leide et al. 2007). The obvious difference in leaf shape and physical properties among the tree species in this study might explain the varied response to nutrient canopy sprays. We found that N addition method only affected the N accumulation ability of *P. massoniana*. Higher N accumulation was observed in *P. massoniana* exposed to canopy rather than soil N addition. Coniferous needles are covered by long-chain, aliphatic epicuticular waxes (Wytteenbach et al. 1987; Cape and Percy 1998), which might induce high needle N uptake. The occurrence of biological nitrification in coniferous needle and the high N retention by twigs of coniferous needle when the needle was exposed to high N deposition might also lead to high needle N uptake (Adriaenssens et al. 2012; Guerrieri et al. 2015). Compared to the other species with broad-leaves, the needles of *P. massoniana* can directly take-up more nutrients, which led to higher leaf N concentrations and greater biomass of this species after canopy N addition. Dail et al. (2009) also

Table 3 Effects of N addition levels and methods on the microbial phospholipid fatty acids of soil planted with four tree species

Species	Microbial PLFAs	Canopy N addition			Soil N addition		
		High N	Medium N	Ambient N	High N	Medium N	Ambient N
Soil samples were collected in Aril 2013							
AM	Total PLFAs	13.88 ± 1.46	14.54 ± 2.36	12.35 ± 1.04	12.87 ± 0.60	13.22 ± 0.90	14.84 ± 1.13
	Bacteria PLFAs	5.91 ± 0.60	6.18 ± 1.12	4.95 ± 0.98	5.46 ± 0.36	5.72 ± 0.53	6.56 ± 0.51
	Fungal	1.25 ± 0.14	1.24 ± 0.32	0.99 ± 0.06	1.01 ± 0.06	1.27 ± 0.06	1.34 ± 0.07
	AMF	0.21 ± 0.04b	0.27 ± 0.03ab	0.24 ± 0.02ab	0.21 ± 0.03b	0.23 ± 0.03b	0.32 ± 0.03a
OP	Total PLFAs	11.20 ± 0.80	12.98 ± 0.47	11.93 ± 1.18	11.42 ± 1.10	12.92 ± 1.41	11.64 ± 1.80
	Bacteria PLFAs	4.48 ± 0.35	5.33 ± 0.21	5.01 ± 0.51	4.63 ± 0.57	5.68 ± 0.80	5.66 ± 0.84
	Fungal	0.98 ± 0.15	1.03 ± 0.03	0.84 ± 0.12	0.79 ± 0.10	1.02 ± 0.04	0.98 ± 0.20
	AMF	0.28 ± 0.04ab	0.32 ± 0.02ab	0.33 ± 0.03ab	0.24 ± 0.05b	0.32 ± 0.02ab	0.36 ± 0.05a
SS	Total PLFAs	11.24 ± 0.70	14.01 ± 2.10	13.26 ± 1.10	9.40 ± 1.88	11.26 ± 0.44	11.19 ± 2.04
	Bacteria PLFAs	4.46 ± 0.33	5.66 ± 1.11	5.43 ± 0.47	3.62 ± 0.78	4.78 ± 0.20	4.79 ± 0.94
	Fungal	0.99 ± 0.14	1.25 ± 0.18	1.07 ± 0.32	0.80 ± 0.27	0.90 ± 0.14	0.80 ± 0.22
	AMF	0.22 ± 0.01ab	0.28 ± 0.05a	0.27 ± 0.01a	0.18 ± 0.03b	0.25 ± 0.02ab	0.29 ± 0.04a
PM	Total PLFAs	11.55 ± 0.60	10.33 ± 1.50	12.29 ± 0.60	9.36 ± 1.44	10.81 ± 1.35	12.26 ± 0.94
	Bacteria PLFAs	4.79 ± 0.34	4.17 ± 0.69	4.98 ± 0.49	3.61 ± 0.50	4.68 ± 0.68	5.03 ± 0.57
	Fungal	0.83 ± 0.06	0.76 ± 0.09	0.96 ± 0.13	0.71 ± 0.26	0.76 ± 0.08	1.05 ± 0.20
	AMF	0.20 ± 0.02bc	0.19 ± 0.03bc	0.26 ± 0.05 ab	0.16 ± 0.01c	0.22 ± 0.04bc	0.29 ± 0.05a
Soil samples were collected in October 2013							
AM	Total PLFAs	15.85 ± 0.52b	16.23 ± 1.56b	13.66 ± 2.35c	13.66 ± 0.53c	15.04 ± 0.73c	16.98 ± 0.59a
	Bacteria PLFAs	6.78 ± 0.22	7.22 ± 0.81	5.80 ± 1.13	5.88 ± 0.22b	6.45 ± 0.34b	7.39 ± 0.27a
	Fungal	1.35 ± 0.10a	1.19 ± 0.13ab	1.11 ± 0.20ab	0.96 ± 0.07b	1.17 ± 0.10ab	1.39 ± 0.09a
	AMF	0.21 ± 0.01c	0.28 ± 0.02ab	0.28 ± 0.05ab	0.21 ± 0.00c	0.24 ± 0.02bc	0.34 ± 0.02a
OP	Total PLFAs	12.93 ± 0.80b	13.45 ± 1.07b	15.92 ± 1.44ab	12.45 ± 1.19b	17.65 ± 0.84a	17.91 ± 2.47a
	Bacteria PLFAs	5.36 ± 0.33bc	5.73 ± 0.54ab	6.90 ± 0.67ab	4.99 ± 0.64c	7.39 ± 0.55a	7.51 ± 0.99a
	Fungal	1.03 ± 0.03bc	0.92 ± 0.06c	1.14 ± 0.12ab	0.82 ± 0.09c	1.38 ± 0.05ab	1.56 ± 0.35a
	AMF	0.28 ± 0.b	0.36 ± 0.02a	0.41 ± 0.01a	0.24 ± 0.03b	0.39 ± 0.01a	0.41 ± 0.05a
SS	Total PLFAs	13.57 ± 2.30	14.64 ± 1.62	14.16 ± 2.36	10.85 ± 2.25	12.02 ± 0.30	13.74 ± 2.26
	Bacteria PLFAs	5.25 ± 0.75	5.99 ± 0.72	5.89 ± 1.01	4.32 ± 0.80	5.12 ± 0.24	5.95 ± 0.88
	Fungal	1.42 ± 0.87	1.12 ± 0.29	1.08 ± 0.35	0.77 ± 0.37	0.89 ± 0.07	0.95 ± 0.22
	AMF	0.25 ± 0.02c	0.33 ± 0.03ab	0.34 ± 0.06ab	0.22 ± 0.05c	0.25 ± 0.00bc	0.39 ± 0.06a
PM	Total PLFAs	12.55 ± 0.32ab	11.96 ± 1.33b	10.99 ± 1.07b	12.81 ± 0.09ab	13.61 ± 1.29ab	15.01 ± 1.12a
	Bacteria PLFAs	5.15 ± 0.47b	5.00 ± 0.36b	4.66 ± 0.65b	5.54 ± 0.07b	6.00 ± 0.55ab	6.97 ± 0.70a
	Fungal	0.88 ± 0.18	0.76 ± 0.09	0.76 ± 0.08	0.89 ± 0.07	0.92 ± 0.09	1.03 ± 0.16
	AMF	0.19 ± 0.01c	0.21 ± 0.01bc	0.25 ± 0.04ab	0.26 ± 0.02ab	0.24 ± 0.04bc	0.31 ± 0.01a

AM Acacia mangium, *OP Ormosia pinnata*, *SS Schima superba*, *PM Pinus massoniana*. *Total PLFAs* total microorganisms (sum of all bacterial and fungal PLFAs), *Bacteria PLFAs* total bacteria, *AMF* arbuscular mycorrhizal fungi

Unit: nmol g^{-1} dry soil. Treatments with different superscript letter are significantly different from each other ($p > 0.05$). Data of soil N addition are from Liu et al. (2017)

found that coniferous species showed high canopy retention of N rather than soils when they added 18 kg N ha^{-1} yr.$^{-1}$ as dissolved NH_4NO_3 directly to the canopy of spruce-hemlock forest. We note that higher N accumulation was not shown in *P. massoniana* exposed to soil N addition in our study. Our results partly support our second hypothesis that different N addition methods have different effects on plant growth and N accumulation in the four tree species. N addition method did not affect N accumulation in *A. mangium*, *O. pinnata*, or *S. superba*, which was not consistent with our hypothesis that the stimulation of nutrient accumulation was greater after canopy N addition than soil N addition.

Soil microorganisms are essential for nutrient cycling in terrestrial ecosystems. Previous studies have shown that high N deposition has a negative influence on soil microbial communities (LeBauer and Treseder 2008; Treseder

2008; Wallenstein et al. 2006). Treseder (2008) conducted a meta-analysis and found that microbial biomass declined 15% on average due to N addition, and that the declines in abundances of microbes were more obvious with longer durations and higher total amounts of N addition. Our results are consistent with these findings. Compared to the control, high and medium N additions significantly reduced microbial PLFAs in the soil of all pots in our experiment, irrespective of N addition method. High N addition decreased microbial PLFAs more than medium N addition. During the experimental period, N addition method did not affect soil microbial PLFAs in *A. mangium*, *O. pinnata*, or *P. massoniana* pots. However, higher soil microbial PLFAs were found in the pots of *S. superba* that received N addition via the canopy rather than the soil. When the soil was treated with a high N level, soil microorganisms were directly affected, which resulted in lower soil microbial PLFAs in the pots planted with *S. superb*. As our experiment lasted only 1.5 years, we did not detect the effects of soil N addition on the soil microbial PLFAs of the other three species. We suspect that N addition via soil would have had stronger effects on microorganisms than N addition via the canopy in a long-term experiment of the other three species. N addition via soil did not affect soil microbial PLFAs for either of the N_2-fixers (*A. mangium* and *O. pinnata*) over the study period. This indicated that N_2-fixers have a higher ability to resist the negative influences of high N addition than non-N_2-fixers in tropical areas. Among the four species, the soil in *P. massoniana* pots showed the highest NO_3^--N concentration. A higher N accumulation was shown in *P. massoniana* exposed to canopy N addition than soil N addition, which suggested that needle species accumulate nutrients largely via the leaves.

Our knowledge of N deposition impacts on forest plants mainly derives from numerous field manipulation experiments. Almost all the previous experiments simulating N deposition have been conducted by adding N fertilizer directly onto the understory plants or forest soils. However, natural atmospheric N deposition often arrives on the canopy before the soil. Thus, conventional experiments neglect many components and ecological processes in the canopy that are critical for forest plants. Hence, further experiments simulating N deposition by adding N fertilizer to the canopy are necessary. In our study, we added N fertilizer via both canopy and soil and showed that the effects of different N addition methods on plant growth and N accumulation were species-specific. Therefore, N addition method should be accounted for in future N deposition experiments. As the pots used in our experiment were limited in volume and all seedlings grew very fast, our experiment only lasted about 1.5 years. N deposition over the short term often stimulates plant growth and nutrient uptake in tropical tree species (Liu et al. 2017; Mao et al. 2017); however, some studies also showed that plant growth does not generally respond to N fertilization as soils in many humid tropical forests are already N-rich (Cusack et al. 2011, 2016; Kaspari et al. 2008). As the effect of N addition on soil microorganisms over the short term is also different to that over the long term, long-term and continuous field experiments are necessary to further improve our understanding on the effects of different N addition methods on plant growth in tropical areas.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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