Plant Soil (2009) 320:243–254
DOI 10.1007/s11104-009-9890-7

REGULAR ARTICLE

^{13}C abundance, water-soluble and microbial biomass carbon as potential indicators of soil organic carbon dynamics in subtropical forests at different successional stages and subject to different nitrogen loads

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Received: 2 May 2008 / Accepted: 5 January 2009 / Published online: 20 January 2009

Abstract Chronic atmospheric nitrogen deposition affects the cycling of carbon (C) and nitrogen (N) in forest ecosystems, and thereby alters the stable C isotopic abundance of plant and soil. Three successional stages, disturbed, rehabilitated and mature forests were studied for their responses to different nitrogen input levels. N-addition manipulative experiments were conducted at low, medium and high N levels. To study the responses of C cycling to N addition, the C concentration and ^{13}C natural abundances for leaf, litter and soil were measured. Labile organic carbon fractions in mineral soils were measured to quantify the dynamics of soil organic C (SOC). Results showed that three-year continuous N addition did not significantly increase foliar C and N concentration, but decreased C/N ratio and enriched ^{13}C in N-rich forests. In addition, N addition significantly decreased microbial biomass C, and increased water soluble organic C in surface soils of N-rich forests. This study suggests that N addition enhances the water consumption per unit C assimilation of dominant plant species, restricts SOC turnover in N-poor forests at early and medium successional stages (thus favored SOC sequestration), and vice versa for N-rich mature forests.

Keywords N deposition · ^{13}C natural abundance · Labile C fractions · SOC turnover · Successional stages · Subtropical China

Introduction

Chronic atmospheric N deposition can increase C storage of N-limited forest ecosystems (Aber et al. 1989, 1998), which has been identified as explanation of missing C sink (IPCC 2007). Anthropogenic N input to terrestrial ecosystems has increased from 15 TgN y^{-1} (1Tg=10^{12}g) in 1860 to 165 TgN y^{-1} in 2000, and will reach 270 TgN y^{-1} in 2050 (Galloway et al. 2004). Neff et al. (2002) reported that global atmospheric N deposition varies from 25 to 40 TgN

Responsible Editor: Ute Skiba.

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y^{-1} and will be doubled in the next 25 years. The input of plant-available N promotes C sequestration of N-limited forest ecosystems by increasing net primary production, and delays, to a certain degree, the increase of atmospheric CO_2 concentration (Mäkipää et al. 1999; Turunen et al. 2004). However, other studies indicate that N saturation resulting from excessive N input will accelerate the plant mortality, and thus weaken potential C sequestration of forest ecosystems (Nadelhoffer et al. 1999; Stevens et al. 2004).

Nitrogen deposition can obviously affect foliar and soil C isotopic abundance ($\delta^{13}C$) through changing C cycling processes. N addition improves water acquisition of plants, affects foliar stomatal conductance and the ratio of intercellular ambient CO_2, and thus changes water use efficiency and $\delta^{13}C$ of plant tissues (Choi et al. 2005). Addition of N to soil may increase (Tessier and Raynal 2003; Micks et al. 2004), decrease (Bowden et al. 2004; Chantigny et al. 1999) or have no affect on (Prescott et al. 1999; McDowell et al. 2004) litter decomposition, SOC turnover and emissions of C-containing trace gases. Generally, coarse sized and low density fractions of soil organic matter (SOM) originate from recent plant residues and are more ^{13}C depleted, whereas SOM in fine sized and high density materials are inherited from old plant materials and are more ^{13}C enriched (Cadisch et al. 1996; Roscoe et al. 2001; Lynch et al. 2006). Moreover, the respired CO_2 is relatively ^{13}C depleted with respect to bulk soil and residual products of microbial decomposition (Wynn et al. 2006). Therefore, we assume that there may be obvious differences in plant and soil C isotopic abundance in N-limited and N-saturated forest ecosystems.

An important study in the Dinghushan forest reported that soil organic C content (0–20 cm depth) over two decades accumulated at the rate of about 54 g Cm^{-2} y^{-1} (Zhou et al. 2006). The reason for this accumulation was unclear, but one suggestion was that the elevated N deposition (>30 kgN ha^{-1} y^{-1}) over recent decades increased litter C input, decreased soil respiration or both (Mo et al. 2008). However, the net result of N addition on the soil C balance in subtropical forests remains unclear. So, more studies are needed to fully understand how the increasing N deposition affects C concentration and its stable isotopic abundance of leaf-litter-soil continuum at forest stands with different successional stages. Based on our previous studies, nitrogen addition both increased annual litterfall and litter decomposition, while reduced soil respiration in the N-limited rehabilitated forest (Mo et al. 2006; Fang et al. 2007). However, nitrogen addition did not change annual litterfall, but it significantly depressed litter decomposition and soil respiration in the N-saturated mature forest (Fang et al. 2007; Mo et al. 2008). Therefore, the objective of this paper is to test our hypothesis that N addition can restrain/promote SOC turnover in the rehabilitated/mature forest, respectively, by examining the effects of different levels of N deposition on contents of labile SOC fractions and ^{13}C aboundance of leaves, litters and soils in three subtropical forests in southern China.

Materials and methods

Site descriptions

The study area is located in the Dinghushan Natural Reserve, the middle part of Guangdong Province in southern China (112°30′39″–112°33′41″E, 23°09′21″–23°11′30″ N). The reserve occupies an area of 1,133 ha and is dominated by typical south subtropical monsoon climate. The mean annual precipitation is 1,927 mm, of which nearly 80% falls in hot-humid season (April–September). The annual average temperature is 21.4°C, with average temperature of 12.6°C and 28.0°C in the coldest and hottest month, respectively. Disturbed pine forest (hereafter referred to as disturbed forest), conifer and broadleaf mixed forest (hereafter referred to as rehabilitated forest), and evergreen broadleaf forest (hereafter referred to as mature forest) are three major forest types in the reserve. These three forests represent forests in early-, mid-, and advanced-successional stages, respectively (Peng and Wang 1995). The disturbed forest was originally planted in 1930s and has suffered long-term human disturbances in litter and understory harvest. This forest is dominated by *Pinus massoniana* in tree layer, *Rhodomyrtus tomentosa*, *Dicranopteris linearis* and *Baeckea frutescens* in shrub and herb layers. The rehabilitated forest has been free from human disturbance for about 70 years and developed from artificial disturbed forest with a gradual invasion of some pioneer broadleaf species through natural succession. Dominant species in the rehabilitated forest include

Pinus massoniana, *Schima superba*, *Castanopsis chinensis*, and *Craibiodendron kwangtungense*. The mature forest is the regional climax vegetation and has been well protected from human disturbance for more than 400 years by Buddhist monks. Dominant species in the mature forest include *Castanopsis chinensis*, *Cryptocarya chinensis*, *Cryptocarya concinna*, *Erythrophleum fordii*, and *Cyathea podophylla* (Kong et al. 1993). Soils in the above-mentioned three study sites are categorized as Lateritic Red Earth (Oxisol) formed from Devonian sandstone and shale. The soils are loamy-textured, acidic and with low base saturation (He et al. 1982). In the mature forest, soil depth is over 60 cm. In the rehabilitated forest, soil depth ranges from 30 to 60 cm. In the disturbed forest, soil depth is generally less than 40 cm to bedrock. The main characteristics of surface soils of the three forests are provided in Table 1.

Experimental design

Chronic N treatments, started in 2003, include an untreated control, low-N (5 g m^{-2} y^{-1}), medium-N (10 g m^{-2} y^{-1}) and high-N (15 g m^{-2} y^{-1}). The concentration of soil available N (NH_4^+-N and NO_3^-N) in the disturbed and rehabilitated forests is only about half of that in the mature forest (Table 1). High N input to poor-N environment could damage some plants and soil microorganisms resulting in early mortality of forests. In addition, the major objective was to accelerate ecosystem responses so they might become evidence during a practical experimental period (within 10 years). So, the high-N treatment was only employed in the N-rich mature forest in order to test hypotheses related to the concept of N saturation (Aber et al. 1998). Replicated plots (12 plots for the mature forest, 9 plots for the rehabilitated forest and 9 plots for the disturbed forest) were randomly laid out in three forest stands and surrounded by a 10 m wide buffer strip. Each plot has an area of 0.02 ha (20 m × 10 m). To simulate N deposition as that in rain, NH_4NO_3 solution was sprayed to the plots once a month as 12 equal applications over the entire year, and control plots received equivalent deionized water only (Mo et al. 2007). During each application, fertilizer was

Table 1 Stand characteristics and surface soil (0–20 cm) properties of three forests in Dinghushan Nature Reserve (mean, SE in parentheses, *n*=9)[a]

Forest Successional stage	Disturbed forest Early	Rehabilitated forest Medium	Broadleaf forest Advanced
Dominant species	*Pinus massoniana*	*Pinus massoniana, Schima superba*	*Castanopsis chinensis, Schima superba, Cryptocarya chinensis, Machilus chinensis, Syzygium rehderianum*
Mean tree height (m)	6.9	7.7	10.0
Mean diameter at breast height (cm)	17.5	14.2	18.5
Biomass (Mg C ha^{-1})	40.6	116.2	147.8
Microbial biomass (×10^6 g^{-1}dry soil)	1.2	1.4	2.1
Fine root biomass in top soil (Mg ha^{-1})	1.9 (1.1)	2.8 (1.1)	4.9 (3.0)
Litter input (Mg C ha^{-1} y^{-1})	1.8	4.3	4.2
Soil thickness (cm)	<40	30–60	>60
pH	4.02	3.92	3.8
Sand (2-0.05) (%)	39.2	36.8	24.8
Silt (0.05-0.002) (%)	26.5	29.4	34.7
Clay (<0.002) (%)	34.3	33.8	40.5
Soil moisture (%)	24.9(1.1)	25.97(0.91)	38.57(1.19)
SOC (Mg ha^{-1})	105.2	111.3	164.1
Total N (kgN ha^{-1})	0.9(0.1)	1.0 (0.1)	1.9(0.1)
Soil C/N ratio	25(1)	28(1)	24(1)
NO_3^-N (kg ha^{-1})	2.8 (0.7)	3.0 (0.5)	8.4 (1.3)
NH_4^+-N (kg ha^{-1})	3.6 (0.1)	2.7 (0.1)	2.1 (0.1)

[a] Data are from Tang et al. (2006) and Fang et al. (2006)

weighed (5.7, 11.4 and 16.1 kg for the low, medium, high treatment, respectively), mixed with 20 L of deionized water, and applied to each plot below the canopy using a backpack sprayer. Spraying objects included herbaceous layer and forest floor. Spraying height was about 50 cm above the ground. Each N application (30 plots) was conducted on the same day.

Foliar and soils sampling

In April 2006, one-year-old leaves of *Castanopsis chinensis*, *Cryptocarya chinensis*, *Schima superba* (in the mature forest), *Pinus massoniana* and *Schima superba* (in the rehabilitated forest), and *Pinus massoniana* (in the disturbed forest) were cut with a pole pruner from the topmost whirls (one tree per species per plot). Litter samples were randomly taken from the top O_i and the below O_e+O_a soil horizons with a trowel within 3 cm radius. Due to optimal temperature and moisture in the mature forest, rapid litter decay resulting from strong microbial activity led to a lack of O_a and O_e layers in soil profiles. Mineral soil samples were taken from three increasing soil depth intervals (0–10, 10–20, 20–40 cm) with a 5 cm-diameter auger. Eight sub-samples were pooled to one composite sample for each soil layer. Foliar and litter samples were dried to constant weight at 60°C. Mineral soils were air dried at room temperature and then sieved through a 2 mm sieve to remove roots, gravel and stones. All samples were ground into fine powder with a planetary mill and then oven dried at 70°C for 24 h before analysis.

Organic C and δ^{13}C analyses

Carbon concentration and δ^{13}C values were determined simultaneously with an automatic, online elemental analyzer (Flash EA1112, ThermoFinnigan, Milan, Italy) coupled to an isotope ratio mass spectrometer (Finnigan MAT 253, Thermo Electron, Bremen, Germany). Carbon isotope values were reported in per mil (‰) relative to the Pee Dee Belemnite standard. Standard deviation of 10 repeated samples was <0.4‰. Following Amundson and Baisden (2000), mineralization rather than other processes such as bioturbation and dissolved organic carbon leaching was the dominant mechanism influencing the vertical patterns of δ^{13}C in soil profiles. The slope of a linear regression relating isotopic composition to the logarithm of C concentration was used to describe the changes in δ^{13}C that accompany the transformation of fresh organic carbon into soil organic matter (Garten et al. 2000; Powers and Schlesinger 2002). The slope of the regression line (K) predicted the expected change in the δ^{13}C or isotopic discrimination, for every tenfold increase in C concentration. More negative slopes were indicative of faster soil C turnover rates via microbial mineralization (Garten et al. 2000). In this study, parameter K was used to indicate the effects of N addition on SOC turnover in forests with different successional stages.

Labile C fractions measurements

Microbial biomass C was determined with the chloroform fumigation -extraction method (Vance et al. 1987). Six 3 g (dry weight) subsamples from the upper 20 cm soil layer were weighed into 50 ml centrifuge tubes for each replicate. Three subsamples were extracted with 30 ml deionized water and were shaken for 1 h. After centrifugation at 3500 rev min^{-1} for 5 min, the extracts were filtered through pre-rinsed Whatman #1 filters (Sinopharm Chemical Reagent Beijing Co., Ltd). The extracts were acidified using HCl to pH 2.0. Cotton balls were placed into the vials containing the remaining three subsamples, and 3 ml of $CHCl_3$ were pippetted onto the cotton balls. The vials were capped, and stored in darkness at 25°C for 7 days before they were extracted following the same procedure as above. Dissolved organic carbon in the solutions was determined with a Shimadzu TOC analyzer whose lower detection limit was 50 μg l^{-1} (Shimadzu Corp. Kyoto, Japan). Microbial biomass C was calculated as the difference between fumigated and non-fumigated samples. The concentration of dissolved organic carbon in the non-fumigated samples was referred to as water soluble organic C.

Statistical analysis

The differences of foliar C, N concentrations and δ^{13}C, soil microbial biomass C and water soluble organic C, soil organic C and δ^{13}C among forest types and N addition treatments were tested with analysis of variance (ANOVA). Means comparisons were done with Tukey's HSD test. Regression analysis was used to test the relationship between foliar δ^{13}C and N concentration, as well as soil $\delta^{13}C$ and its log OC. All the analyses were conducted with SAS software package. Statistically significant difference was set as $p<0.05$ unless stated otherwise.

Results

Foliar C, N concentrations and ^{13}C abundances

Average foliar N concentration of *Pinus massoniana* in the control of disturbed and rehabilitated forests ranged from 1.41% to 1.53%, significantly less than the values (1.89%~2.12%) of three dominant species in the control of mature forest (Fig. 1a). In the rehabilitated forest, foliar N concentration of *Pinus massoniana* was significantly less than that of *Schima superba* (Fig. 1a). Average foliar C varied slightly among the three forests with a mean of 48.53% (Fig. 1b), while C/N ratios of needles ranged from 33.12 to 36.27 and were significantly greater than those of broadleaves that ranged from 22.28 to 24.91 (Fig. 1c). In addition, foliar $\delta^{13}C$ of three species in the mature forest (−30.35‰~−31.58‰) was less than those of *Pinus massoniana* in the disturbed (−28.39‰) and the rehabilitated forests (−28.22‰) (Fig. 1d).

Except for *Pinus massoniana* in the rehabilitated forest, foliar N concentration increased with increasing N addition, but it was not statistically significantly different among N treatments (Fig. 1a). Similarly, there was no significant difference in foliar C of all species among different N treatments (Fig. 1b). However, N addition decreased foliar C/N of the three forests (Fig. 1c). N addition did not influence the foliar $\delta^{13}C$ in the disturbed and rehabilitated forests, whereas low- and high-N treatments significantly increased the foliar $\delta^{13}C$ of the mature forest (Fig. 1d). If the N-rich mature forest was excluded, foliar $\delta^{13}C$ of the disturbed and rehabilitated forests poor in N was positively correlated to the foliar N concentration ($R^2=0.40$, $p<0.05$) (Fig. 2). The results show that foliar C isotopic discrimination (Δ) decreased with increasing N concentration.

Microbial biomass C and water soluble organic C in surface soils

In control plots, microbial biomass C of surface soils in the disturbed and rehabilitated forests were 435.8

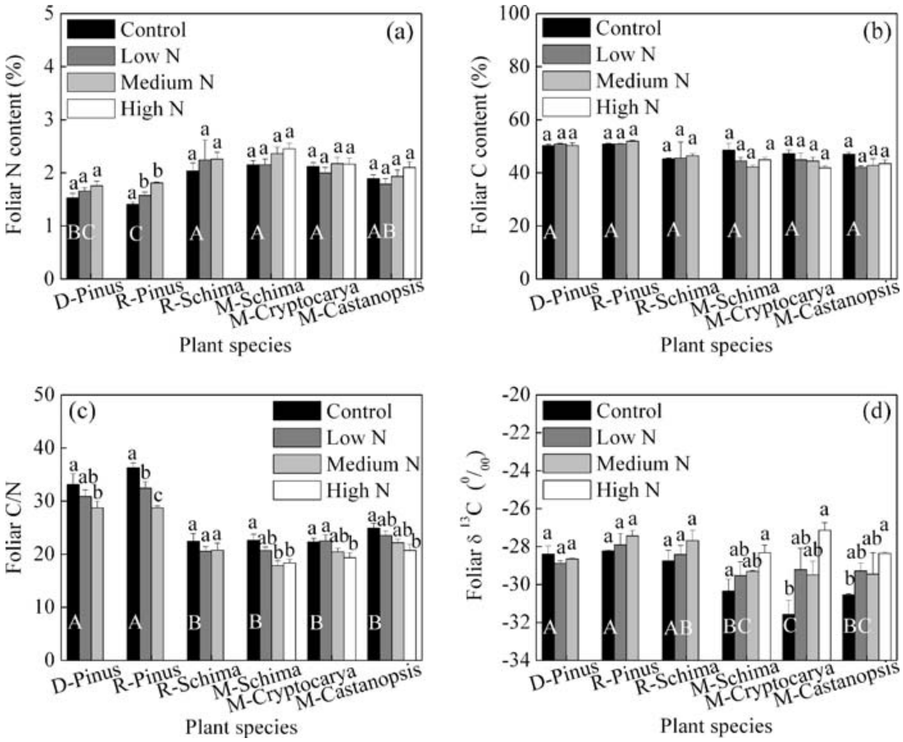

Fig. 1 Variation of foliar C, N concentrations and ^{13}C abundances among different **dominant** species and N treatments (D, R and M referred to the disturbed, rehabilitated and mature forests. The different capital and lowercase letters indicated significant differences among plant species and N treatments, respectively)

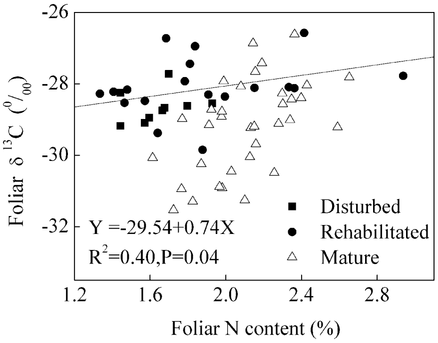

Fig. 2 Relationships between foliar N concentration and ^{13}C abundances

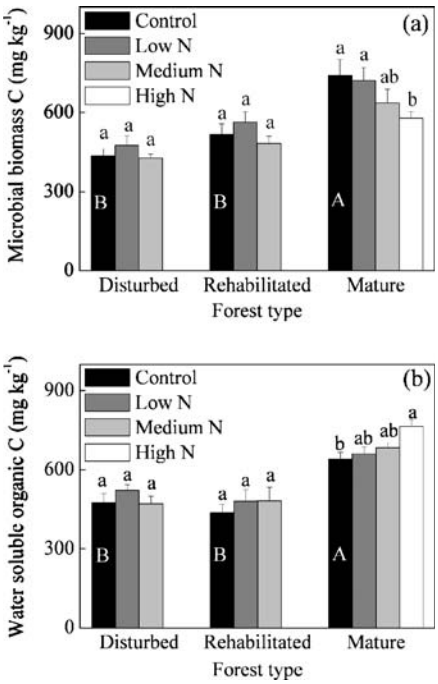

Fig. 3 Variation of soil microbial biomass C and water soluble organic C among different N treatments and forest stands (The different capital and lowercase letters indicated significant differences among forest types under natural conditions and N treatments, respectively)

and 516.6 mg C kg^{-1}soil, which was significantly lower than that in the mature forest with a mean of 740.8 mg C kg^{-1}soil (Fig. 3a). Similarly, water soluble organic C was 475.2 mg C kg^{-1}soil in the disturbed forest and 436.7 mg C kg^{-1}soil in the rehabilitated forest, which was significantly lower than the mean of the mature forest (640.2 mg C kg^{-1}soil) (Fig. 3b). For the disturbed and rehabilitated forests, there was no significant difference in microbial biomass C and water soluble organic C concentrations in surface soils among N treatments (Fig. 3). In contrast, added N significantly decreased microbial biomass C concentration, whereas the water soluble organic C concentration of in surface soils increased in the mature forest (Fig. 3).

At the control plots, SOC decreased with soil depth while soil δ^{13}C increased with soil depth (Fig. 4). The average SOC of O$_i$ layer in the disturbed and rehabilitated forests were 55.13% and 52.90%, respectively, and, hence, significantly higher than that

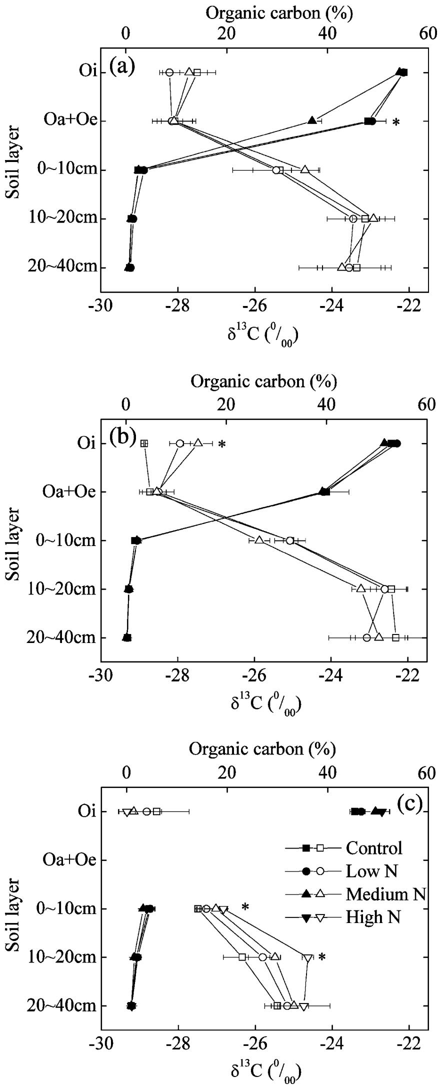

Fig. 4 Variation of SOC concentration and δ^{13}C with soil depth at different N treatment plots and forest stands. Asterisks (*) indicates significant differences among treatments. **(a=disturbed, b=rehabilitated, and c=mature forests)**

in the mature forest (45.38%) (p<0.001, Table 2). SOC content of the 0–10 cm layer was 2.44% in the disturbed forest and 1.77% in the rehabilitated forests, which was significantly lower than that of the mature forest (4.49%). Average δ^{13}C of the 0–20 cm layer was between −25.35‰ and −22.31‰ in the disturbed and rehabilitated forest, thus, significantly higher than that of mature forest (−27.49‰~−25.43‰) (Fig. 4 and Table 2). Except for O_i layer in the rehabilitated forest, N addition treatments did not change δ^{13}C of each soil layer in the disturbed and rehabilitated forest (Fig. 4a and Fig. 4b), but significantly increased δ^{13}C of the upper 20 cm soil layer in the mature forest with an increased range from 0.66‰ to 1.71‰ (Fig. 4c).

Relationship between δ^{13}C and and soil organic carbon content

Similar to the results reported by Garten et al. (2000) and Powers and Schlesinger (2002), soil δ^{13}C was negatively correlated with the logarithm of the soil organic carbon content for different N treatment plots in three successional stage forests (Fig. 5). In control plots, the order of parameter K was rehabilitated (−3.04), disturbed (−2.46) and mature forests (−1.79) (Table 3), implying that SOC turnover in the mature forest was less than that in early and medium successional stage forests. Compared to the control treatments, the absolute value of K decreased with the increasing level of N addition in the rehabilitated forest (Fig. 5b and Table 3), while it was opposite in the mature forest (Fig. 5c and Table 3). In the disturbed forest, the absolute value of K was lower in low-N treatment and higher in medium-N treatment than that in the control, respectively (Fig. 5a and Table 3). The results showed that plant-available N input restrained SOC turnover in the N-limited rehabilitated forest and was in favor of C sequestration. However, it promoted SOC turnover in the mature forest rich in N. In addition, N status at the plots with medium-N addition in the disturbed forest served as a turning point between the forests poor and rich in N.

Discussions

N addition did not significantly increase foliar N concentration of most plant species at three forests although an increasing trend occurred (Fig. 1a). This was not consistent with many results reported for temperate forests (Murthy et al. 1996; Maier et al. 2002; Albaugh et al. 2004; Choi et al. 2005). The possible reasons are as follows. Trees in subtropical forests in southern China with high N deposition (>30 kgN ha^{-1} r^{-1}) may have adapted to the continuous N enrichments by elevated atmospheric deposition. The absorption of plant roots to soil available N basically reached saturation point, thus N addition was not enough to change foliar N content in a short time. However, in the N-limited disturbed and rehabilitated forests where trees have a great

Table 2 Variance analysis for soil C concentration and δ^{13}C at control treatment plots

Soil layers	Forests	δ^{13}C			%C		
		Mean (Se)	F	p	Mean (Se)	F	p
	disturbed	−27.49 (0.26)			55.13 (0.25)		
O_i	mixed	−28.87 (0.02)	2.01	0.21	52.90 (1.13)	32.68	<0.001
	mature	−28.57 (0.84)			45.38 (1.03)		
0–10 cm	disturbed	−25.35 (0.70)			2.44 (0.73)		
	mixed	−25.05 (0.19)	9.61	0.01	1.77 (0.15)	16.2	0.01
	mature	−27.49 (0.05)			4.49 (1.15)		
	disturbed	−23.14 (0.52)			1.09 (0.11)		
10–20 cm	mixed	−22.43 (0.39)	19.74	0.002	0.54 (0.07)	7.1	0.03
	mature	−26.34 (0.49)			1.90 (0.43)		
	disturbed	−23.36 (0.89)			0.77 (0.05)		
20–40 cm	mixed	−22.31 (1.06)	3.74	0.09	0.29 (0.17)	9.09	0.02
	mature	−25.43 (0.33)			0.92 (0.06)		

demand for nitrogen (Mo et al. 2006), added N should promote the accumulation of foliar N. This was similar to many N-limited temperate forests (Brown et al. 1996; Aber et al. 1998; Nakaji et al. 2002). In addition, the decline of foliar C/N ratio should contribute to the acceleration of litter decomposition after the leaves fell. One of our previous studies also indicated that litter decomposition rates were stimulated by N addition in the disturbed and rehabilitated forests limited by N (Mo et al. 2006).

At the plant species level, the foliar C isotopic discrimination value(Δ)could reflect water use efficiency (WUE) during photosynthesis (Ripullone et al. 2004), and δ^{13}C of foliar tissues could represent integrated effects of exchange between plant and atmosphere during plant growing period of the year. The plant species studies were representative within their forests. The relative contribution of *Pinus massoniana* in the disturbed forest, *Pinus massoniana* and *Schima superba* in the rehabilitated forest and *Castanopsis chinensis*, *Cryptocarya chinensis* and *Schima superba* in the mature forest were 95.07%, 94.56% and 67.3%, respectively (Fang et al. 2006). In the N-unsaturated disturbed and rehabilitated forests, foliar C isotopic discrimination decreased with the increase of foliar N concentration (Fig. 2), which indicated that N availability had a positive effect on photosynthesis when foliar N was below the threshold (Sparks and Ehleringer 1997; Ripullone et al. 2004). Brown et al. (1996) reported that the photosynthetic rate of seedlings of *Picea sitchensis, Thuja plicata* and *Tsuga heterophylla* decreased when foliar N exceeded 21 mg g^{-1}. In Dinghushan reserve, Li et al (2004) also found that the net photosynthesis rate of

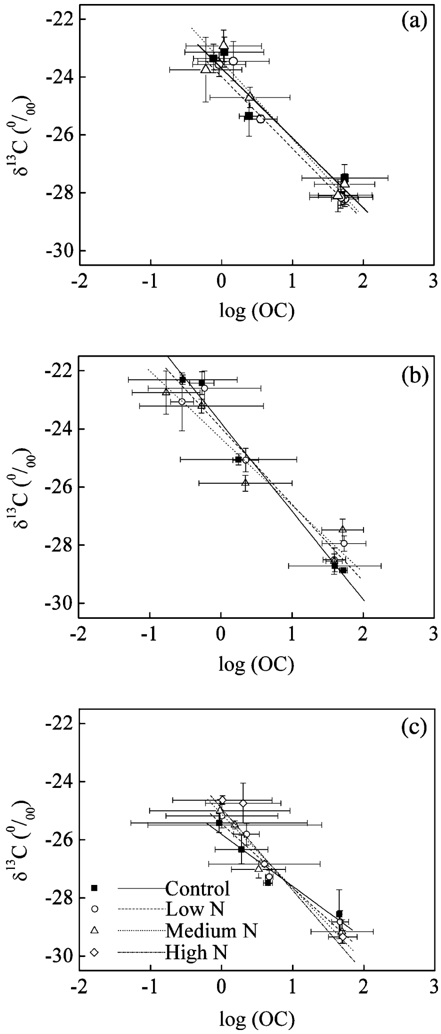

Fig. 5 Relationships between δ^{13}C and log **organic carbon** of different N treatments fitted with linear regressions. **(a=disturbed, b=rehabilitated, and c=mature forests)**

Table 3 Linear model for the relationship between the soil δ^{13}C and log organic carbon (δ^{13}C= a + b*log organic carbon)

Forest	Treatment	a	b	R^2	*P* value
Disturbed	Control	−23.65	−2.46	0.943	0.006
	Low N	−24.04	−2.44	0.984	<0.001
	Medium N	−23.45	−2.69	0.956	0.004
Mixed	Control	−23.79	−3.04	0.986	<0.001
	Low N	−23.96	−2.64	0.935	0.007
	Medium N	−24.31	−2.31	0.899	0.014
Mature	Control	−25.82	−1.79	0.927	0.037
	Low N	−24.45	−2.20	0.912	0.045
	Medium N	−25.11	−2.54	0.947	0.027
	High N	−24.92	−2.82	0.955	0.023

Schima superba, Castanopsis chinensis and *Cryptocarya concinna* increased in low-N treatment and decreased in high-N treatment, and the critical level of N addition was 10 g N m^{-2} y^{-1}. The decrease in net photosynthesis resulting from high N treatment was partially due to self-shedding of canopy (Brown et al. 1996; Nakaji et al. 2001) and nutrient imbalances (e.g. increasing N/base cations ratios and reducing Ca/Al ratios) (Nakaji et al. 2002).

Soil microbial biomass C decreased with the increasing levels of N addition only in the mature forest, (Fig. 3a). This is in agreement with results from three long-term experiments in New England (Harvard Forest MA; Mt. Ascutney VT; Bear Brook ME). For these Wallenstein et al. (2006) reported that N addition decreased microbial biomass C by an average of 40%~59% and that microbial biomass C had negative relationships with total N inputs in both mineral soils and organic soils. In Dinghushan subtropical forests, Xue et al. (2007) reported that N addition resulted in an increase of bacteria and a decrease of fungi, and had not significantly affected actinomycetes.

Therefore, microbial biomass and microbial biomass carbon could partially reflect the number of bacteria and fungi. Because N additions decreased the abundance of fungi faster than increase of bacteria (28.7% vs 4.4%, Xue et al. 2007), the reduction of microbial biomass and fungal/bacterial ratio could be mainly attributed to the decrease of fungal biomass. Other studies showed the same results in temperate forests (Wallenstein et al. 2006; Frey et al. 2004). Moreover, the decrease in soil C/N ratio and soil acidification could also have an important contribution to the decrease in microbial biomass (Table 1).

However, water soluble organic C exhibited an opposite pattern in response to N additions, with N additions significantly increasing water soluble organic C concentrations (Fig. 3b). The main sources of soil WSOC were plant root exudation and decomposition of litter and soil organic matter (Kalbitz et al. 2000). Because WSOC was an available C for soil microorganism to immobilize added inorganic N, most of WSOC would be depleted after N added to N-poor forests (McDowell et al. 2004). Therefore, WSOC content did not significantly increase even if microbial decomposition activity increased in the N-poor forests (Fig. 3a). However, N addition could restrain the decomposition of litter and SOM, and promote the release of soluble organic fractions in the N-saturated forests. De Forest et al. (2004, 2005) suggested that the increase of NO_3^- concentration could promote the output of water soluble organic C by suppressing enzymes which were responsible for lignin degradation. Therefore, loss of dissolved organic C might be an important pathway of SOC loss in the scenario of increasing nitrogen deposition (Findlay 2005; Waldrop and Zak 2006).

Based on the regression between log organic carbon and δ^{13}C, we found that SOC turnover was slower in the mature forest than in the disturbed and rehabilitated forests (Table 3), which was partially attributed to thicker soil and higher silt and clay content (Table 1). Plant roots were prone to extend downwards to absorb nutrients in the deeper soil layers, which can increase root biomass and decrease the decomposition of root residues (Jobbágy and Jackson 2000; Berger et al. 2002). Soil clay and silt had strong ability to conserve soil organic matter, thus SOC associated with fine soil particles was difficult to be decomposed by soil microorganism (Garten et al. 1999; Carter et al. 2003). In addition, N addition restricted SOC turnover in the low-N rehabilitated forest, while promoted SOC turnover in the N-rich mature forest (Fig. 5). This might relate to the following mechanisms. First, there was obvious less C input to soil through litterfall in N-limited than for N-saturated forests. N addition significantly increased C input through increasing litter input and decomposition rates in the rehabilitated forests, but decreased C input in the mature forest due to unchanged annual litterfall and restrained decomposition (Mo et al. 2006; Fang et al. 2007). Second, soil CO_2 emission in the two forests was both restrained by N addition through decreasing autotrophic respiration from plant roots and heterotrophic respiration from the microbial community (Mo et al. 2007, 2008). Lastly, the obvious loss of dissolved organic carbon occurred in the mature forest rather than the disturbed and rehabilitated forest. Therefore, in the rehabilitated forests, the increasing input and decreasing output of C would promote the accumulation of SOC by restraining SOC turnover to a certain degree. In the mature forest, both the C input from litterfall and decomposition and the C output from soil respiration decreased, but the dissolved organic carbon significantly lost through accelerating the SOC turnover.

Conclusions

Based on N addition manipulative experiments, this study addressed the effects of N addition on C dynamics of subtropical forests at different successional stages. Continuous 3-year N addition did not significantly increase foliar C, N concentration, but significantly decreased the foliar C/N ratio. In the N-rich mature forest, N addition significantly decreased and increased the microbial biomass C and water soluble organic C concentration, respectively. In subtropical forest soils, N addition could restrain SOC turnover in N-limited forest ecosystems and promoted C sequestration, but it accelerated C turnover in N-rich forest ecosystem through water soluble organic C loss. In the long-term, input of nitrogen deposition would go against carbon sequestration of mature forest ecosystem in southern China.

Acknowledgements This research was supported by National Natural Science Foundation of China (40601097, 30590381, 30600071), Knowledge Innovation Project of the Chinese Academy of Sciences (KZCX2-YW-432,O7V70080SZ),the President Fund of GUCAS(O85101PM03) and "Hundred Talents" Program (CXTD-Z2005-1). We gratefully acknowledge Dr. Yang Xueming and Dr. Zhuang Jie for revising the English writing of the manuscript.

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