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Effects of nitrogen deposition on soil organic carbon fractions in the subtropical forest ecosystems of S China

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Abstract

Experiments were conducted between 2003 and 2008 to examine how N additions influence soil organic C (SOC) and its fractions in forests at different succession stages in the subtropical China. The succession stages included pine forest, pine and broadleaf mixed forest, and old-growth monsoon evergreen broadleaf forest. Three levels of N (NH_4NO_3)-addition treatments comprising control, low-N (50 kg N ha^{-1} y^{-1}), and medium-N (100 kg N ha^{-1} y^{-1}) were established. An additional treatment of high-N (150 kg N ha^{-1} y^{-1}) was established in the broadleaf mixed forest. Soil samples were obtained in July 2008 for analysis. Total organic C (TOC), particulate organic C (POC, > 53 μm), readily oxidizable organic C (ROC), nonreadily oxidizable organic C (NROC), microbial biomass C (MBC), and soil properties were analyzed. Nitrogen addition affected the TOC and its fractions significantly. Labile organic-C fractions (POC and ROC) in the topsoil (0–10 cm) increased in all the three forests in response to the N-addition treatments. NROC within the topsoil was higher in the medium-N and high-N treatments than in the controls. In the topsoil profiles of the broadleaf forest, N addition decreased MBC and increased TOC, while no significant effect on MBC and TOC occurred in the pine and mixed forests. Overall, elevated N deposition increased the availability of labile organic C (POC and ROC) and the accumulation of NROC within the topsoil irrespective of the forest succession stage, and might enhance the C-storage capacity of the forest soils.

Key words: China / forest succession stages / N deposition / soil organic carbon accumulation / subtropical forest

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1 Introduction

Anthropogenic N creation globally has increased from 15 Tg N y^{-1} in 1860s to 156 Tg N y^{-1} in the early 1990s, and is expected to be 270 Tg N y^{-1} in 2050 (*Galloway* et al., 2004). Studies show that N deposition is one of the obvious global changes with significant influence on C storage in forests. Nitrogen addition to soils accelerate decomposition of recent soil C and retard decomposition of older soil organic (SOC), resulting in a net long-term enhancement of C storage (*Neff* et al., 2002; *Hagedorn* et al., 2003; *Swanston* et al., 2004). Subtropical and tropical forest ecosystems store 46% and 11% of the world's living terrestrial and soil C pools, respectively (*Brown* and *Lugo*, 1982). Most of these forests are also prone to N deposition, arising from the rapid industrialization of the countries in these regions. In S China, for example, the current N-deposition rate in some of the forests is estimated at 30–73 kg ha^{-1} y^{-1} (*Ren* et al., 2000; *Zhou* and *Yan,* 2001), compared to 25–30 kg ha^{-1} y^{-1} in many forests in Europe which are partly subject to N saturation (*Aber* et al., 1998; *MacDonald* et al., 2002). Nonetheless, little is known regarding how the tropical and subtropical forests respond to the increasing N-deposition rates. Thus, there is an urgent need to understand how N deposition influences C dynamics in the tropical and subtropical forests in order to predict how rising atmospheric N levels will affect their role in the global C cycle.

The characteristics of SOC vary with forest structure and age and are also modified by the level of soil N concentration (*Hagedorn* et al., 2003). Most studies on the effect of N deposition on soil C dynamic, however, have been restricted to temperate forests which are often N-limited (*Pregitzer* et al., 2008). Those studies reveal that elevated N deposition modifies C dynamics in these forests by modulating the soil microbial enzymatic activity (*Waldrop* et al., 2004) and litter biochemical characteristics (*Gallo* et al., 2005). Preliminary results from subtropical forests show that N addition influences SOC by decreasing the microbial biomass C and increasing water-soluble organic C in the surface soils (*Fang* et al., 2009). Reduction in respiration after N addition in the broadleaf forests have also been reported for these forests (*Mo* et al., 2008). According to *Fang* et al. (2007), N addition significantly depresses litter decomposition but the magnitude varies with forest type. How natural subtropical forest ecosystems respond to N addition is still not well understood

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and calls for more investigations in order to define the future role of these forests in C storage.

Soil organic C is a conglomeration of C-containing fractions at different cycling rates (*Ågren* and *Bosatta*, 2002; *Knorr* et al., 2005; *Bradford* et al., 2008). Different SOC fractions, therefore, respond differently to the physical and biological environmental changes (*Lützow* et al., 2007, 2008). In the past, numerous fractionation methods including physical and chemical fractions have been developed to separate SOC fractions (*Lützow* et al., 2007). Finding the appropriate methods for separating the SOC fractions is a key to studying how the individual SOC fractions respond to environmental changes. Particulate organic C (POC) associated with sandy soils (> 53 μm) is a rich source of biologically available C and energy for soil microorganisms and has been recommended by *Yang* et al. (2009) for SOM investigations in the subtropical forests of China. Readily oxidizable organic C (ROC) has relatively high turnover, and is responsive to management practices (*Datta* et al., 2010). Soil microbial biomass C (MBC) can indirectly indicate soil microbial activity (*Fang* et al., 2009). Examining the changes in these labile C fractions is a more sensitive tool to measure the changes in SOC dynamics in response to environmental changes, but the long-term C storage is critically dependent on the stable SOC fractions (*Neff* et al., 2002). For example, nonreadily oxidizable organic C (NROC) shows a relatively weak response to environmental changes (*Blair* et al., 1995) and can be used to monitor the SOC stable over time. Therefore, we study the SOC dynamic in response to N deposition through combination of labile and stable C.

In this study, we investigated the SOC dynamics in response to N deposition in the subtropical forests of S China through combination of labile and stable C. Experimental N additions were set in forests at different succession stages namely: pine forest (PF), pine and broadleaf mixed forest (MF), and old-growth monsoon evergreen broadleaf forest (BF) in the Dinghushan Biosphere reserve. Total organic C (TOC), POC, ROC, NROC, MBC, and soil properties were analyzed. We hypothesized that (1) elevated N deposition increases TOC through accumulated labile organic C (POC and ROC) and retarded mineralization of nonreadily oxidizable organic C and (2) stimulation of TOC accumulation in subtropical forest soils as a result of N deposition is dependent on the age of the forest.

2 Materials and methods

2.1 Site descriptions

The study was carried out in Dinghushan Biosphere Reserve (Guangdong Province, subtropical S China: 112°10′ E, 23°10′ N). The reserve occupies an area of 1200 ha and experiences a typical monsoon and subtropical humid climate, with a mean annual temperature and relative humidity of 21.5°C and 80%, respectively. The mean annual rainfall is 1956 mm and has a distinct seasonality, with 80% of it falling from April to September (*Yang* et al., 2010). The reserve comprises three typical forest ecosystem types at different succession stages, namely: pine forest, mixed pine and broadleaf forest (hereafter mixed forest), and an old-growth monsoon evergreen broadleaf forest (hereafter broadleaf forest), representing early, middle, and advanced succession stages, respectively (*Peng* and *Wang*, 1993). The dominant species in each forest are given in Tab. 1. The density of pine decreases with increasing forest age so that in the broadleaf forest, pine is naturally replaced by broadleaf species. The pine forest originated from a 1950s clear-cutting and subsequent pine-plantation establishment. It represents forest at the early-succession stages. The mixed forest consists of pine and broadleaf trees, which originated from planted or natural pine forests, after self-seeding and invasion by broadleaf trees. It represents the mid-succession stages. The broadleaf forest represents a climax stage of succession.

All the sites occur at similar elevation (100–250 m asl) and have similar slope conditions (18°–25°, S-facing aspect). Soils at all the three forest sites are shallow Ultisols (USDA Soil Taxonomy) overlying sandstone and shale bedrocks, with a depth down to 40–60 cm. Characteristics of the three forest types are summarized in Tab. 1. The annual litter fall (g m^{-2} y^{-1}) was 981.8, 702.5, and 587.5, with initial average leaf-litter N concentration (mg g^{-1}) of 15.7, 14.2, and 12.2 for broadleaf, mixed, and pine forests, respectively (*cf. Mo* et al., 2006).

2.2 Experimental design and treatments

The experiments were conducted between 2003 and 2008. Three levels of N (NH_4NO_3)-addition treatments, comprising (1) control, (2) low-N addition (50 kg N ha^{-1} y^{-1}), and (3) medium-N addition (100 kg N ha^{-1} y^{-1}), were established in the pine, mixed, and broadleaf forest sites, while an additional treatment of high-N (150 kg N ha^{-1} y^{-1}) was established in

Table 1: Site characteristics of the three forests under study.

Forest type	**Forest coverage** / %	**Forest canopy density** / %	**Stand age** / y	**Litterfall** / g m^{-2} y^{-1}	**Dominant species**
Pine forest	70–80	79.3 ± 10.7	60–70	587.6 ± 115.8	*Pinus massoni*ana, *Rhodomyrtus tomentosa, Ficus variolosa, Baecra frutescens*
Mixed forest	80–90	89.1 ± 6.1	ca. 110	702.5 ± 79.3	*P. massoniana, Schima superba, Castanopsis chinensis, Psychotria rubra, F.variolosa, Evodia lepta, Gahnia tristis, Adiantum capillus-veneris*
Broadleaf forest	80–90	93.0 ± 3.1	ca. 400	891.8 ± 120	*C. chinensis, S. superba, Cryptocara concinna, Machilus chinensis, Cryptocarya chinensis, Blastus cochinchinensis, Psychotria rubra, Hemigramma decurrens*

the broadleaf-forest site. In each forest type, three replicate plots, each measuring 10 m × 20 m, surrounded by a 10 m wide buffer strip, were randomly selected for each level of N treatment. A total of 30 plots were established (9 in the pine, 9 in the mixed, and 12 in the broadleaf forests). A total area of 0.7 ha was demarcated for each forest type. The low-N treatment (50 kg N ha^{-1} y^{-1}) corresponded to a value higher than the current atmospheric N deposition (30–73 kg N ha^{-1} y^{-1}), but was lower than the projected N deposition (45–113 kg N ha^{-1} y^{-1}) for S China in 2030 (*Zheng* et al., 2002). Decisions on the other levels of N addition (100 kg N ha^{-1} y^{-1} and 150 kg N ha^{-1} y^{-1}) were based on these levels. The available N and total N of broadleaf-forest soils were twice as high as those of pine and mixed forests (Tab. 2), as reported previously by *Mo* et al. (2006). For each fertilization treatment, solid fertilizer (NH_4NO_3) was weighed, mixed with 20 L of water, and evenly applied onto the plots, below the canopy and close to the soil surface, using a backpack sprayer. The solution was sprayed as 12 equal monthly applications over the entire year, beginning July 2003 and continuing throughout the study period.

2.2.1 Soil sampling

Soil samples from two depths, 0–10 and 10–20 cm, respectively, were obtained from each of the replicate plots between 21 and 22 July 2008, using a soil corer (∅ 2.5 cm). From each depth in every plot, we collected five cores randomly and mixed them into one sample. At any sampling session, a total of 60 samples were obtained for analysis. The fresh soil samples were passed through a 2 mm sieve to remove rocks and plant roots. Subsamples were air-dried and ground for TOC- and SOC-fractions measurements.

2.2.2 SOC fractions

Particulate organic C (POC 53–2000 μm) was measured using the method described by *Cambardella* and *Elliot* (1992). Readily oxidizable organic C (ROC) and non-readily oxidizable organic C (NROC) were determined with 333 mmol L^{-1}–$KMnO_4$ oxidation (*Blair* et al., 1995) and MBC with the chloroform fumigation-extraction method (*Joergensen* and *Brookes*, 1990).

2.2.3 Soil measurements

Soil characteristics including soil pH (glass electrode), texture (*Page* et al., 1982), soil water content, total N, and aboveground litter input were measured. Soil water content (SWC) was determined gravimetrically. Total N was measured using the Kjeldahl method (*Liu*, 1996). In the study period 2003 to 2008, MBC in the three forests were investigated every 2 y.

2.4 Statistical analysis

Differences in soil TOC concentration and organic-C fractions among the three different treatments in the three forests were assessed with two-way analysis of variance (ANOVA). One-way ANOVA with LSD tests was performed to assess the effects of N addition within forest types. We applied multivariate linear mixed-effects models in order to correct for the repeated measures in the different plots. *p* values were calculated using likelihood-ratio tests based on changes in deviance when each term was dropped from the full model. Generalized Liner Model (GLM) was employed in order to check for interactions between factors. We checked the normality of the model residuals visually by normal probability plots, and we assured the homogeneity of variances and goodness of fit of the models by plotting residuals *vs.* fitted values. In some cases, regression analysis was employed to compare parameters. All the tests were performed using SPSS software (version 11.5). Differences for all tests were considered to be significant at $p < 0.05$.

3 Results

3.1 Soil properties

In the control plots, soil pH decreased with forest age, *i.e.,* pine > mixed > broadleaf forests (Figs. 1 A–C). Soil pH within the shallow soil profile of the three forest types subjected to medium-N and high-N treatments were lower than in the same profile in the control plots (Fig. 1). SWC at the pine and mixed forests were lower than that in the broadleaf forest, especially during winter. Mean TN in the control broadleaf forest was higher than in the mixed and pine forests (Tab. 2). Monthly soil MBC dynamics between 2003 and 2008 in the three forest sites showed that the highest MBC levels occurred in May and June. MBC was highest in broadleaf forest compared to the pine and mixed forests.

3.2 Spatial pattern of soil TOC response to N-addition treatment

Numbers of TOC within the shallow (0–10, 10–20 cm) soil profiles of the control plots were significantly (Tab. 3;

Table 2: Topsoil (0–10 cm) properties of the three forest types under study (Values are mean ± standard deviation).

Soil properties	Pine forest	Mixed forest	Broadleaf forest
Soil order	Ultisol	Ultisol	Ultisol
pH	4.12 ± 0.08	4.12 ± 0.06	4.10 ± 0.07
Soil water content / %	25.1 ± 5.2	26.0 ± 2.9	38.8 ± 3.0
SOC / g kg^{-1}	20.5 ± 0.9	19.7 ± 3.7	25.4 ± 3.4
Total available N (0–10 cm) / mg kg^{-1}	6.5 ± 0.4	7.7 ± 0.68	13.8 ± 1.5
Total N / g kg^{-1}	1.3 ± 0.1	1.2 ± 0.1	2.5 ± 0.2

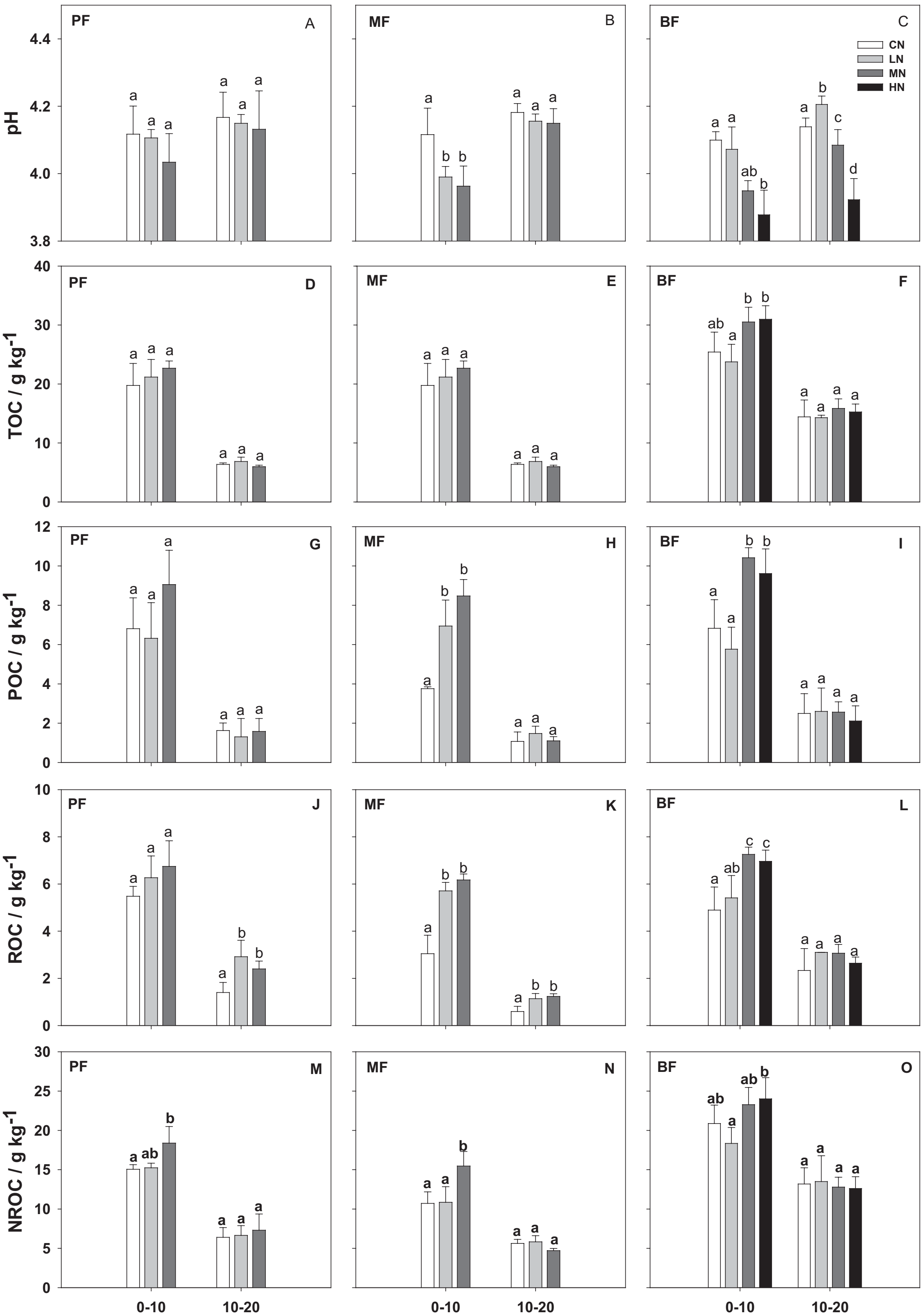

Figure 1: Effects of N addition on soil pH (upper panel), total organic C (TOC) (second panel), particulate organic C (POC) (third panel), readily oxidizable organic C (ROC) (fourth panel), and nonreadily oxidizable organic C (NROC) (bottom panel) in different soil profiles in the tree forests under study. LN: low-N treatment; MN: medium-N treatment; HN: high-N treatment; CN: control treatment. Pine forest, mixed forest, and broadleaf forest, abbreviated for all figures as PF, MF, and BF, respectively. Error bars are standard errors (n = 3).

Table 3: Analyses of variance on the effects of forest type, N-addition treatment, and their interactions on soil pH, total organic C (TOC), particulate organic C (POC), readily oxidizable organic C (ROC), nonreadily oxidizable organic C (NROC). Numbers are F values. Stars indicated the level of significance (*: $p < 0.05$; **: $p < 0.01$).

Soil depth / cm		**pH**	**TOC** / g kg^{-1}	**POC** / g kg^{-1}	**ROC** / g kg^{-1}	**NROC** / g kg^{-1}
0–10	forest	1.98	11.11**	1.69	4.16*	44.20**
	treatment	11.74**	4.62*	12.30**	13.98**	12.51**
	forest × treatment	104 525.2**	1155.61**	612.82**	1051.37**	1995.49**
10–20	forest	0.21	47.03**	8.97**	27.30**	40.18**
	treatment	1.47	0.68	0.16	7.92**	0.09
	forest × treatment	112 887.8**	596.84**	146.22**	377.80**	500.15**

$p = 0.006$ and 0.015 for 0–10 and 10–20 cm, respectively) higher in the broadleaf forest than in the pine and mixed forests (Figs. 1 D–F). No significant difference occurred between pine and mixed forests.

In the broadleaf-forest plots, TOC in the top 0–10 cm profiles followed the order: high ([30.97 ± 2.30] g kg^{-1}) > medium ([30.53 ± 2.50] g kg^{-1}) > control ([25.42 ± 3.38] g kg^{-1}) > low ([23.76 ± 2.98] g kg^{-1}) N applications. Differences among treatments were significant in the broadleaf forest, but not in the other two forest types (Figs. 1 D–E). No significant differences in TOC concentrations occurred in the deeper (10–20 cm) soil layers in all the three forest types (Figs. 1 D–F).

3.3 POC response to N-addition treatment

Concentrations of POC significantly increased within the top 10 cm soil profile in both the mixed and broadleaf forests in response to medium- and high-N additions (Figs. 1 H–I). Average POC concentrations within this profile in the pine forest were not significantly different (Fig. 1 G). Equally, POC concentrations within the 10–20 cm soil layers in the broadleaf, mixed, and pine forests were not significantly affected by N treatments (Tab. 3).

3.4 ROC and NROC variation with soil layer in different treatments

There was a significant increase in the concentrations of ROC within the 0–10 and 10–20 cm soil profiles of the three forest types in response to N addition (Figs. 1 J–L). In the broadleaf forest, ROC concentrations within the topsoil (0–10 cm) of medium- and high-N-addition plots were significantly higher than in the control treatments (Fig. 1 L). For mixed forests, ROC concentrations at the 0–20 cm depth in the low- and medium-N treatments were significantly higher than in the controls (Fig. 1 K).

Concentrations of NROC in the top 10 cm soil layers of control plots in the broadleaf forest were significantly higher than in the pine and mixed forests (Tab. 3; Figs. 1 M–O). No significant difference occurred between pine and mixed forests. NROC concentrations within the top 0–10 cm soil profile responded differently to N addition in each forest type (Figs. 1 M–O). For the old-growth monsoon evergreen broadleaf forest, high-N treatments increased nonoxidizable organic-C concentrations within the top 0–10 cm soil profile (Fig. 1 O). For mixed forests, nonoxidizable organic-C concentrations in medium-N treatments were significantly higher than in low-N and control treatments (Fig. 1 N). In the 10–20 cm layer, there were no significant differences resulting from N treatments in any of the forests (Tab. 3).

3.5 Response of MBC to N treatments

There was no significant difference in MBC among the three treatments in pine and mixed forests. For broadleaf forest, high-N treatment decreased the MBC significantly (Fig. 2).

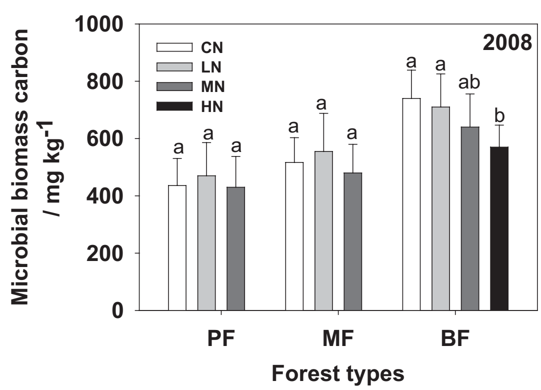

Figure 2: Microbial biomass C (MBC) at the 0–10 cm soil layer under the three N-addition treatments and controls. Different letters denote significant differences ($p < 0.05$) between treatment plots within a forest. Error bars are standard errors ($n = 3$).

4 Discussion

4.1 Effects of N addition on labile organic-C fractions in the topsoil

We observed an increase in POC and ROC within the top 10 cm soil layer in all the three forest types treated with medium- and high-N addition. POC and ROC account for the largest fraction of litter-altered plant-derived C, especially lignin derivatives (*Tirol-Padre* and *Ladha,* 2004; *Grandy* et al., 2008). Their increase may be due to the lower decomposition of litter under medium- and high-N additions (*Mo* et al., 2008). According to *Carreiro* et al. (2000) and *Sinsabaugh* et al. (2002), N saturation inhibits the activities of extracellular ligninolytic enzymes, resulting in a reduction in the rate of litter decomposition. A previous study (*Mo* et al., 2008) in the broadleaf forest showed depressed soil respiration rates as a result of medium- and high-N additions, an indication that these levels of N inhibit decomposition, most likely as a result of N saturation. Thus, medium- and high-N treatments lead to excess soil N availability and lowering N demand by the litter decomposers (*Mo* et al., 2008). The high-N treatment was also associated with increased soil acidity (Fig. 1), which may also inhibit microbial activity (*Waldrop* et al., 2004), potentially lowering the microbial biomass (Fig. 2) and resulting in POC and ROC accumulation in the topsoil.

4.2 Effects of N addition on nonreadily oxidizable organic C

Nitrogen addition promoted an accumulation of NROC in the topsoil as demonstrated by its higher concentration in the surface layer (0–10 cm) in the broadleaf mixed and pine forests subjected to the medium- or high-N treatment, compared to the controls (Fig. 1). Preferential substrate use could be one explanation for these observations (*Hagedorn* et al., 2003; *DeForest* et al., 2004). Availability of labile organic C (POC and ROC) was enhanced under elevated N deposition, which could lead to a decreased decomposition of nonlabile organic C. In our results, the labile fractions were significantly and positively correlated with the nonlabile fraction (NROC) ($p < 0.001$). Microbes in soil can first use the active C fraction, decreasing their demand on recalcitrant organic C. The additional N (medium-N and high-N treatments) also can be used by microorganisms, reducing their dependency on older SOM decomposition for N. This, therefore, indirectly slows down the microbial activities on SOM, inhibiting SOC decomposition.

Our results reveal that N addition increased soil acidity (Fig. 1), and NROC and soil pH were negatively correlated ($p < 0.001$). This may be one of the reasons for the accumulation of NROC. Soil acidification not only reduces microbial activity (*Berg* et al., 1999), but also helps soil humic colloids to form aggregates (*Goh* et al., 1987). SOC in the aggregate is protected from microbial decomposition, thus improving the stability of the nonlabile organic C.

4.3 Effects of N addition on soil TOC

Elevated N deposition (medium-N and high-N) increased the availability of the labile organic C and enhanced the accumulation of nonlabile organic C in the top 10 cm soil profile, which might be a process to lead to increased TOC accumulation in the long term (*Hagedorn* et al., 2003). Significant differences were observed among the forest types, with TOC concentration in the surface layers (0–10 cm) in the broadleaf forest being higher in the medium-N and high-N treatments than in the control (Fig. 1), but no influence of these treatments in the pine and mixed forest. The different responses can be attributed to differences in soil N available in the three forest ecosystems. Compared to the other two forest types, the broadleaf-forest soils were already N-rich at the beginning of the experiments (*Mo* et al., 2008) and further medium- and high-N additions for 5 years significantly lowered decomposition of litter, soil respiration (*Mo* et al., 2006, 2008), and soil microbial biomass C, thus favoring C storage. The pine and mixed forests, on the other hand, were still in early and middle stages, and their soil N availability was lower than that of the broadleaf forest (Tab. 2). This resulted in no significant effect on SOC storage in pine and mixed forests under N-addition treatments. Previous studies had shown that more than 6 y of N addition may significantly affect MBC in soils of temperate forests that are N-deficient (*Mo* et al., 2008). TOC and its fractions (POC, ROC, NROC) were all positively correlated ($p < 0.001$), indicating that the effects of N deposition on TOC may be investigated through the fraction variations. However, predicting the effect of N deposition on SOC dynamic needs to consider the long-term measurement.

5 Conclusions

Elevated N deposition increased the availability of labile organic carbon (POC and ROC) and stimulated the accumulation of nonreadily oxidizable organic C (NROC) in the topsoil of the three forest ecosystems at different succession stages. The 5-year N-addition experiment indicated that the accumulation of NROC may be a process associated with an increase in soil TOC in the forest ecosystems in subtropical S China. The old-growth forests demonstrated a higher capacity for SOC accumulation. Although our results indicate that increasing atmospheric N deposition may promote SOC-storage capacity of mature forest ecosystems of subtropical S China, more long-term investigations are needed in order to validate the role of nitrogen deposition on soil C dynamics.

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