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Effect of N and P addition on soil organic C potential mineralization in forest soils in South China

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Abstract

Atmospheric nitrogen deposition is at a high level in some forests of South China. The effects of addition of exogenous N and P on soil organic carbon mineralization were studied to address: (1) if the atmospheric N deposition promotes soil C storage through decreasing mineralization; (2) if the soil available P is a limitation to organic carbon mineralization. Soils (0–10 cm) was sampled from monsoon evergreen broad-leaved forest (MEBF), coniferous and broad-leaved mixed forest (CBMF), and *Pinus massoniana* forest (PMF) in Dinghushan Biosphere Reserve (located in Guangdong Province, China). The soils were incubated at 25°C for 45 weeks, with addition of N (NH_4NO_3 solution) or P (KH_2PO_4 solution). CO_2-C emission and the inorganic N (NH_4^+-N and NO_3^--N) of the soils were determined during the incubation. The results showed that CO_2-C emission decreased with the N addition. The addition of P led to a short-term sharp increase in CO_2 emission after P application, and the responses of CO_2-C evolution to P addition in the later period of incubation related to forest types. Strong P inhibition to CO_2 emission occurred in both PMF and CBMF soils in the later incubation. The two-pool kinetic model was fitted well to the data for C turnover in this experiment. The model analysis demonstrated that the addition of N and P changed the distribution of soil organic C between the labile and recalcitrant pool, as well as their mineralization rates. In our experiment, soil pH can not completely explain the negative effect of N addition on CO_2-C emission. The changes of soil inorganic N during incubation seemed to support the hypothesis that the polymerization of added nitrogen with soil organic compound by abiotic reactions during incubation made the added nitrogen retard the soil organic carbon mineralization. We conclude that atmospheric N deposition contributes to soil C accretion in the three subtropical forest ecosystems, however, the shortage of soil available P in CBMF and PMF may also retard soil organic C mineralization.

Key words: addition; inorganic N; available P; mineralization; soil organic C; South China

Introduction

Atmospheric deposition of nitrogen remains elevated in industrial regions of the world and is accelerating in many developing regions (Galloway *et al*., 2003; Matson *et al*., 1999, 2002). Most of these exogenous N compounds which entered natural ecosystem were retained (Baba *et al*., 2001; Goodale *et al*., 2000; Gundersen and Rasmussen, 1995; Hagedorn *et al*., 2001; Wright and Tietema, 1995), and the mineral soil generally contained the largest fraction (Baron *et al*., 2000; Fang *et al*., 2006; Krupa, 2003; Magill *et al*., 1996, 2000; Seely and Lajtha, 1997). It has been reported that the addition of exogenous N considerably affects decomposition of soil organic matter. Aerts and Toet (1997) reported all treatments of N to *Carex*-dominated peat soils reduced CO_2 emission. Entry (2000) found that the addition of N slowed organic matter degradation and nutrient mineralization in blackwater and redwater forested wetland soils. Long-term N additions also caused decreased mineralization rates of C in mor humus from two N fertilized stands of Norway spruce (Sjöberg *et al.*, 2003). The mechanisms leading to retarding soil organic carbon mineralization include: (1) the addition of exogenous N decreased soil pH (Aerts and Toet, 1997) or aggravated C-limitation for microbial degradation (Berg *et al*., 1998), which reduced microbial activity; (2) N was incorporated in soil organic matter by microbial N assimilation (Sjöberg and Persson, 1998), which made the old and humified soil organic matter preserved (Hagedorn *et al.*, 2003) or stabilized soil organic matter in later stages of decomposition (Michel and Matzner, 2002; Neff *et al*., 2002). Long-term elevated N even stabilized organic matter in whole soil and soil fraction (Swanston *et al*., 2004). However, Hart *et al.* (1994) found that there was not direct relationship between soil organic carbon mineralization and available nitrogen concentration. The CO_2-C evolution during the incubation was almost constant and was not affected by N application rate (Sjöberg and Persson, 1998), or the

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addition of N had no effect on the soil microbial activity in the dry season (Galicia and García-Oliva, 2004). In contrast, Månsson and Falkengren-Grerup (2003) found that the soil respiration was somewhat high in soils subjected to high nitrogen deposition. However, most studies of the effects of nitrogen addition on soil organic carbon mineralization have been performed in temperate forest ecosystems. Little information is available about the responses to atmospheric nitrogen deposition in subtropical and tropical forests (Matson *et al*., 1999), especially in the forests of South China. And the effects of nitrogen deposition on ecosystems in temperate zone were different from tropical zone (Matson *et al*., 1999). Carbon cycling as affected by increasing N deposition in natural ecosystems still needs to be concerned in future research (Adams, 2003).

In Asia, the emission of reactive N increased from 14 $\times$ 10^{12} gN/a in 1961 to 68 $\times$ 10^{12} gN/a in 2000 and is excepted to reach 105 $\times$ 10^{12} gN/a in 2030 (Zheng *et al*., 2002). This has led to high atmospheric nitrogen deposition in some forests of South China. For example, the amount of nitrogen deposition from rainfall in a pine forest of Guangzhou, China was 60 kg/(hm^2·a) (Ma, 1989). In the forests of Dinghushan Biosphere Reserve in southern China, N deposition from rainfall was above 35 kg/(hm^2·a) both in 1989 and 1999 (Huang *et al*., 1994; Zhou and Yan, 2001). Close to the Pacific Ocean and the Indian Ocean, South China has a subtropical monsoon climate with an abundance of heat, light, and water resources (Ding *et al*., 2001). Because of its unique climate regime, moist subtropical forests dominate in southern China (Kong *et al*., 1993). Soil organic carbon is a donimating component of global carbon storage, and 73% of which stores in forest soil (Post *et al*., 1982). The effects of nitrogen deposition on soil organic carbon mineralization would change soil carbon accumulation rate and the regional carbon balance. Therefore, it is of great significance to study the effect to accurately evaluate the contribution of N deposition to regional carbon balance. However, direct measurements of change in total soil C were inconclusive due to the little change of soil organic carbon pool caused by N deposition when compared with the huge amount of soil organic carbon pool, and due to the high spatial variation (Conant and Paustian, 2002; Magill *et al*., 2000). Large spatial variability might conceal the effects of small N inputs (Vestgarden and Kjønaas, 2003) or of short-term treatment.

The objective of this study was to determine the effects of addition of exogenous N on soil organic carbon mineralization. We hypothesized the atmospheric N deposition would promote soil C storage by decreasing mineralization, and the available P shortage would limit the soil organic C mineralization. The results could provide experimental evidence for understanding the relationship between soil organic carbon accumulation (Zhou *et al*., 2006a, 2006b) and atmospheric N deposition in forest in South China or similar climatic areas.

1 Materials and methods

1.1 Sampling site

This study was conducted in the UNESCO/MAB Dinghushan Biosphere Reserve (DHSBR), in the central Guangdong Province (23°10′N and 112°34′E), China. The reserve has a monsoon climate, distinct seasonal pattern, and is located in the subtropical moist forest zone. The average annual rainfall is 1,927 mm, 75% of which happens from March to August and only 6% from December to February. Annual average relative humidity is 80%. Average annual temperature is 21.0°C, with the highest monthly temperature of 28.0°C in July and the lowest monthly temperature of 12.6°C in January (Kong *et al*., 1993).

Monsoon evergreen broadleaved forest (MEBF) has been protected from human impacts for more than 400 years by monks in the temple, and has a complicated community structure. The tree in this forest has heights ranging from 4 to 30 m and diameters from 5 to 163 cm (Wang *et al*., 1982). The biomass of this community is about 38 kg/m^2 (Peng and Zhang, 1994a). The soil of this forest is hydration lateritic soil about 80 cm deep, developed from sandy shale.

The coniferous and broad-leaved mixed forest (CBMF), which originated from artificial or natural *Pinus massoniana* forest after invasion by broad-leaved trees, is the representative forest type at the mid-successional stage, transiting to monsoon evergreen broad-leaved forest rapidly. The biomass of this community is about 28 kg/m^2 (Peng and Zhang, 1994b). The soil of this forest is hydration lateritic soil about 30–60 cm deep, developed from sandy shale.

Pinus massoniana forest (PMF), which is dominated by *P. massoniana*, is the representative forest type at the early-successional stage, and is advancing to coniferous and broad-leaved mixed forest rapidly. Pine stand density range from 100 to 1,000 trees/hm^2, with diameter ranging from 4 to 32 cm, heights from 3 to 11 m, and ages from 12 to 69 years (Brown *et al*., 1995). The biomass of this community is about 12.2 kg/m^2 (Peng and Zhang, 1994a). The soil under this forest is lateritic soil about 30 cm deep, developed from sandy shale. Selected soil properties are listed in Table 1.

1.2 Sampling, treatments and incubation

The soil samples were collected in December 2004 from the three forest types. A 7-cm diameter soil core was used for sampling. In each forest, nine soil samples to a depth of 10 cm from different sites were collected, and each sample was mixed by five or seven cores soils. Upon collection, soil samples were sieved through a 3-mm sieve to remove coarse roots and stones immediately, and mixed thoroughly. The sieved soil was divided into two portions, one was air dried for measuring soil properties, and the other was immediately treated for incubation or measured for ammonium (NH_4^+-N) and nitrate (NO_3^--N).

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Table 1 Soil properties for three forests (0–10 cm depth)

Forest type	MEBF	CBMF	PMF
pH_{H_2O}	3.83±0.06 b	3.91±0.11 ab	4.06±0.17 a
pH_{KCl}	3.18±0.07 a	3.23±0.04 a	3.23±0.09 a
Organic carbon (mg/g)	32.95±4.98 a	31.81±3.52 a	17.92±4.89 b
Total N (mg/kg)	1,093.97±87.68 a	720.15±60.26 b	700.09±100.60 b
Total P (mg/kg)	262.64±48.10 a	177.17±19.02 b	152.83±25.84 b
C:N	30.4±5.9 b	44.3±5.2 a	25.9±7.4 b
C:P	130.5±35.3 b	180.2±17.1 a	116.7±23.0 b
N:P	4.3±0.9 a	4.1±0.3 a	4.7±1.1 a
Available P (mg/kg)	2.02±0.72 a	2.08±1.02 a	2.75±0.79 a
Ammonium (NH_4^+-N) (mg/kg)	0.36±0.13 b	0.49±0.20 ab	0.55±0.17 a
Nitrite (NO_3^--N) (mg/kg)	3.53±1.12 a	1.43±0.19 b	0.63±0.34 c
Inorganic N (mg/kg)	4.00±1.01 a	1.92±0.32 b	1.17±0.37 c
Soil moisture (%)	20.97±1.55 a	19.21±1.93 b	8.22±1.17 c

Value in the same row (within forest types) followed by different letters are significantly different (LSD test or Games-Howell multiple range test, $P < 0.05$); data expressed as mean ± standard deviation. MEBF, CBMF, and PMF refer to monsoon evergreen broad-leaved forest, coniferous and broad-leaved mixed forest and *Pinus massoniana* forest, respectively.

Moist soil was weighed in 100 ml (equivalent to 40 g oven-dried soil) and 500 ml (equivalent to 280 g oven-dried soil) polypropylene beaker, respectively, and adjusted to 60% water holding capacity (WHC) after treatment with NH_4NO_3 or KH_2PO_4 solution. Six addition treatments were performed. The added N, P contents were 40, 200, 1,000 mgN/kg dry soil, 100 mgP/kg dry soil respectively. N and P mixure was 200 mgN/kg dry soil and 100 mgP/kg dry soil, and added distilled water as control. These concentrations were 4, 20, and 100 times of soil inorganic N concentration (10 mgN/kg dry soil), and about 20 times of soil available P concentration (5 mgP/kg dry soil). The 100 ml beakers containing soil samples were placed in 1,200-ml PVC containers with another 100 ml beaker containing 10 ml distilled water to maintain air humidity in the incubation chamber. The PVC containers were sealed with polyethylene film and placed in a dark room at 25°C. Eight blank PVC containers (no soil) were used to estimate background CO_2 concentration. The headspace of PVC containers was sampled for CO_2 concentration at week 1, 2, 4, 6, 9, 12, 15, 21, 28, 35, and 45 using medical gastight syringes. The film of the PVC container and the window of the room were opened and ventilated for approximately 2 h at each sampling interval to prevent anoxia in the container. After incubation, the soils in 100 ml beaker were air dried for measuring soil pH value. Every twenty-seven 500 ml beakers containing soil samples were placed in a box (66 cm × 50 cm × 37 cm, length × width × height). Three 500 ml beakers containing 50 ml distilled water were placed in the incubation chamber to maintain air humidity. The box were covered with polyethylene film and placed in a dark room at 25°C. The soil sample in the 500 ml beakers was used to measure ammonium (NH_4^+-N) and nitrate (NO_3^--N) at week 1, 2, 4, 8, 16 and 36. During the incubation, water content was maintained by adding distilled water. Because MEBF was more different from PMF than CBMF in community composition, and the time limitation of measuring sample, only four addition treatments were used for CBMF soil.

1.3 Analytical methods

Soil pH was determined in a 1:2.5 soil-to-KCl (1 mol/L) or soil-to-water suspension. Organic C and total N were measured (in duplicate) using the potassium dichromate heating method and semi-micro Kjeldahl method, respectively (Liu, 1996). The ammonium (NH_4^+-N) and nitrate (NO_3^--N) was extracted with 50 ml 2 mol/L KCl (soil:extractant, 1:5 (*m/V*)) on an end-and-end shaker at 160 r/min for 1 h and filtered through a qualitative filter paper. NH_4^+-N was determined by the Indophenol Blue method and followed by colorimetric, and NO_3^--N was determined after cadmium reduction to NO_2^--N followed by sulfanilamide-NAD reaction (Liu, 1996). The inorganic nitrogen was equal to the sum of NH_4^+-N and NO_3^--N. Total and available phosphorus were determined (in duplicate) by colorimetric after dissolved by acid and extracted with HCl-H_2SO_4 (0.05–0.025 mol/L), respectively (Liu, 1996). The CO_2 concentration in each syringe was determined immediately by using gas chromatography (HP4890D, Agilent, USA) with pure N_2 as a carrier gas. The measured CO_2 (μl/L) concentration for each sampling was used to calculate the amount of evolved CO_2-C (mg/kg dry soil) based on the PVC container headspace volume and the soil dry weight in the beaker. The factor of converting CO_2-C (μl) to CO_2-C (μg) were 1.80, assuming a mol volume of 22.4 for CO_2 at 25°C and under pressure of 1.013×10^3 Pa. All concentrations for soil are expressed as at 105°C oven-dry basis.

1.4 Kinetic models

The C mineralization kinetics was fit with a two-compartment model (Updegraff *et al.*, 1995):

$$C_t = C_0 \times (1 - e^{-kt}) + (T_c - C_0) \times (1 - e^{-ht}) \quad (1)$$

where, C_t (mg C mineralized/kg dry soil) is the cumulative amount of C released at time t; C_0 (mg C mineralized/kg dry soil) is the amount of potentially mineralizable C with an instantaneous release rate k (d^{-1}); C_0 and k refer to the pool size and mineralization rate of a labile organic fraction, respectively. T_c is total soil C, and (T_c–C_0) is the amount in a recalcitrant pool with instantaneous release rate h (d^{-1}). The model was fit to the data using a nonlinear estimation procedure in Origin 7.0 produced by OriginLab Corporation, USA.

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1.5 Data analyses

Differences in soil properties and cumulative gas production between treatments were tested with a one-way analysis of variance (ANOVA). The LSD or Games-Howell multiple range test was used at the 95% probability limit ($P < 0.05$) to assess the differences at each time when the treatment effects were found to be significant ($P < 0.05$). All the analyses were performed using SPSS software (version 11.0). Differences for all tests were considered to be significant at $P < 0.05$.

2 Results

For the PMF and CBMF, CO_2-C evolution from N-addition soils was lower than from control soils during all the incubation periods, and their differences were significant ($P < 0.05$) except for the first week. For the MEBF, CO_2-C evolution from 40 and 200 mgN-addition soils was lower than from control soils during all the incubation periods; however, CO_2-C evolution from 1,000 mgN-addition soils was higher than that from control soils during the first four weeks of incubation, thereafter, it was lower. This indicated that addition of NH_4NO_3-N decreased the C mineralization. The cumulative mineralized amounts of CO_2-C from 40, 200, and 1,000 mgN-addition soils after 45 weeks of incubation were (89.2 ± 11.2)%, (85.9 ± 13.1)%, and (83.2 ± 9.2)% ($P < 0.046$) of that from MEBF control soil, and (76.6 ± 11.6)%, (63.6 ± 11.0)% ($P < 0.002$), and (54.9 ±6.5)% ($P < 0.002$) of that from PMF control soil, respectively (Fig.1). The cumulative mineralized amount of CO_2-C from 200 mgN-addition soils was (79.7 ± 10.6)% ($P < 0.006$) of that from CBMF control soil. The percentages indicated that CO_2-C evolution decreased with the increasing N applied rates.

For the MEBF, CO_2-C evolution from 100 mgP-addition soil was always high as compared with the control soil for all incubation periods, but their differences were no significant. For the PMF and CBMF, CO_2-C evolution from 100 mgP-addition soil was slightly higher than that from control soil during the first week incubation, thereafter, it was lower. Furthermore, there were significant differences ($P < 0.05$) between 100 mgP-addition and control soil for CBMF from 12 to 45 weeks of incubation, and for PMF from 2 to 45 weeks of incubation (Fig.1). The cumulative mineralized amounts of CO_2-C from 100 mgP-addition soil after the 45 weeks incubation were (100.3 ± 10.0)%, (88.0 ± 18.1)% ($P < 0.05$), and (84.8 ± 9.7)% ($P < 0.05$) of that from MEBF, CBMF, and PMF control soils, respectively.

The CO_2-C evolution from NP-addition soil was always high as compared with the 200 mgN-addition soil during all incubation periods for the three forests, but no significant differences were found. The cumulative mineralized amounts of CO_2-C from NP-addition soil after the 45-week incubation were (111.34 ± 14.28)%, (104.22 ± 8.32)%, and (104.62 ± 13.89)% of that from MEBF, CBMF, and PMF 200 mgN-addition soils, respectively. For the MEBF, CO_2-C evolution from NP-addition soil was slightly higher both than that from control soil during the first four weeks incubation and than that from 100 mgP-addition soil in the first week, thereafter, it was lower. However, for the PMF and CBMF, CO_2-C evolution from NP-addition soil was lower than that from control soil, and from 100 mgP-addition soils during all the incubation periods (Fig.1). There were also significant differences ($P < 0.05$) between NP-addition soil and control soil for MPBF in the period from 6 to 45 weeks of incubation, as well as for PMF in

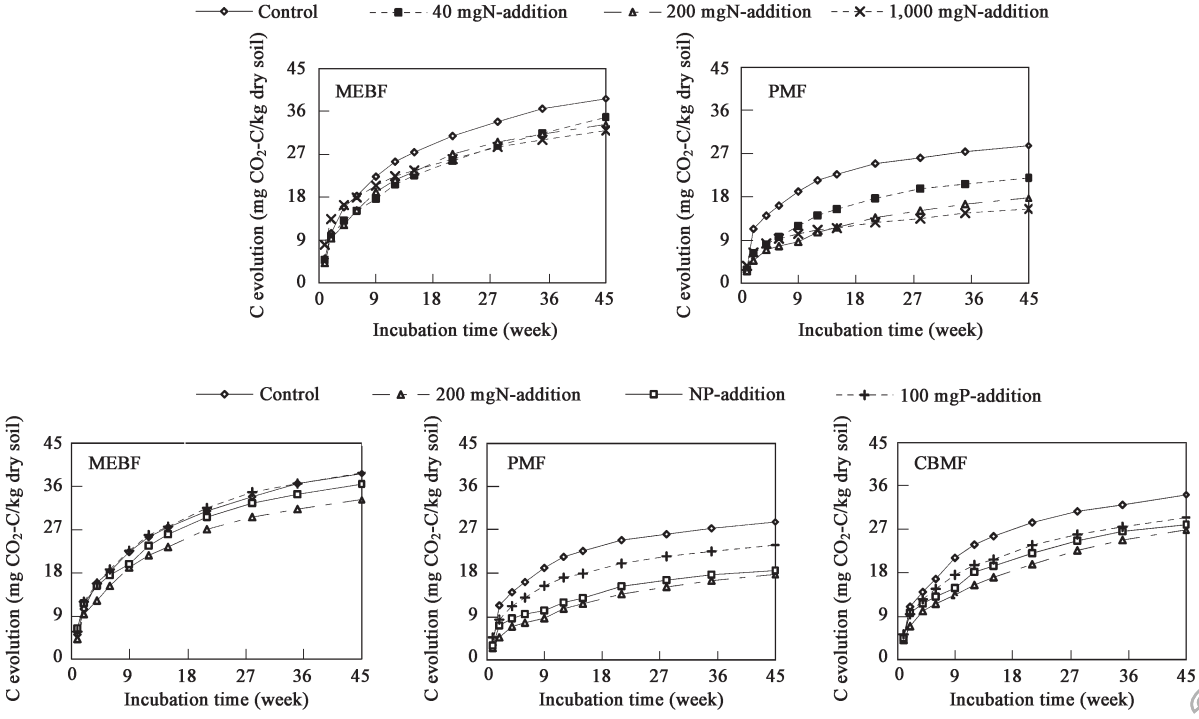


Fig. 1 Effects of additional N and P on cumulative amount CO_2-C release from soil microbial respiration during the incubation at 25°C ($n = 9$).

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the period from 2 to 45 weeks of incubation. For the PMF, there were significant differences ($P < 0.05$) between NP-addition and 100 mgP-addition soil in the period from 4 to 45 weeks of incubations. The cumulative mineralized amounts of CO_2-C from NP-addition soil after 45 weeks incubation were (94.7 ± 13.1)%, (82.7 ± 10.0)% ($P < 0.05$), and (65.5 ± 6.1)% ($P < 0.05$) of that from MEBF, CBMF, and PMF control soils, respectively.

The two-pool kinetic model (Eq.(1)) was significantly fit to the data for C turnover in this experiment (Table 2). All additions of N and P decreased the labile C pool size (C_0). Addition of P increased the mineralization rates of the labile and recalcitrant soil C. Addition of 40 mgN increased the mineralization rates of the labile and recalcitrant soil C from MEBF, but decreased from PMF. Addition of 200 mgN increased the mineralization rates of the labile and recalcitrant soil C from CBMF and PMF, but decreased from MEBF. Addition of 1,000 mgN increased the mineralization rate of the labile soil C, but decreased those of the recalcitrant soil C from MEBF and PMF.

3 Discussion

Our experimental results provided further evidence for the hypothesis that the addition of N inhibit soil organic C mineralization (Berg *et al*., 1998; Hagedorn *et al*., 2003; Michel and Matzner, 2002; Sjöberg *et al*., 2003; Swanston *et al*., 2004). Most of former research ascribed the negative effects of N addition on microbial activity and on CO_2 emission from soils to soil pH value. In general, ammonium addition leads to a reduction of soil pH value due to nitrification of the supplied ammonium. In our experiment, indeed, the soil pH value reduced after incubation (Tables 1 and 3). Moreover the pH value from the highest-N treated soil was lower than that from the control. However, the pH value from the low-N treated soil seemed to be close to or higher than that from the control soil after incubation (Table 3). This indicated that soil pH might not completely explain the negative effect of N addition in present work.

Fog (1998) reviewed more than 60 publications that reported negative or no effect of nitrogen added to decomposing organic matter on microbial activity, and his analysis showed that the negative effect of nitrogen was found mainly with decay-resistant organic matter with high C/N ratio (over 60). Although the C/N ratio in soils used in this experiment was preponderating over 25 (Table 1), we can not be certain of its effect in our experiment.

The N addition to forest or agricultural soils results in stabilized forms of soil organic matter (Kaye *et al*., 2002; Seely and Lajtha, 1997; Sjöberg and Persson, 1998), or the formation of stable polymers by abiotic reactions of ammonia, ammonium, or amino acids with phenols, quinines, and carbohydrates (Nommik and Vahtras, 1982), making soil organic carbon more recalcitrant (Hagedorn *et al.*, 2003). In our experiment, the active soil carbon pool was reduced by N addition, and the high-N addition reduced the mineralization rates of the recalcitrant soil C, although there was no obvious decreasing trend within N treatments (Table 2). Furthermore, the measurements of inorganic nitrogen during incubation seemed also to support the abiotic reaction hypothesis (Nommik and Vahtras, 1982). For the 200 and 1,000 mgN-addition treatments, above 50% (range from 56.75% to 67.94%) of the added N was immobilized into soil after one week (Table 4). For the 40 mgN-addition treatment, about 19.76% and 39.10% of the added N was immobilized into MEBF soil after week 1 and 2, respectively; and about 52.25%

Table 2 Estimated kinetic parameters for C mineralization based on Eq.(1) with different N, P addition treatment

Forest type	Parameter	Control	40 mgN	200 mgN	1,000 mgN	NP	100 mgP
MEBF	C_0 (mgC/kg dry soil)	22.68±4.17 a	16.01±3.55 c	20.20±6.17 ab	18.14±2.39 b	19.67±3.68 abc	22.89±3.97 a
	$k \times 10^{-2}$ (d^{-1})	3.47±0.72 cd	4.80±1.62 b	2.98±0.55 d	7.72±1.17 a	4.44±0.94 bc	3.67±1.11 cd
	$h \times 10^{-6}$ (d^{-1})	1.62±0.23 abc	1.86±0.29 a	1.33±0.25 c	1.44±0.27 bc	1.75±0.36 a	1.63±0.44 ab
	R^2	0.99±0.00*	0.99±0.00*	0.99±0.01*	0.99±0.00*	0.98±0.00*	0.99±0.00*
CBMF	C_0 (mgC/kg dry soil)	22.52±4.17 a	–	11.84±2.23 c	–	14.67±2.74 bc	16.52±2.62 b
	$k \times 10^{-2}$ (d^{-1})	3.25±0.75 c	–	5.14±1.67 a	–	5.41±2.19 a	4.47±1.04 ab
	$h \times 10^{-6}$ (d^{-1})	1.22±0.25 b	–	1.60±0.32 a	–	1.44±0.33 ab	1.37±0.27 ab
	R^2	0.99±0.01*	–	0.99±0.00*	–	0.98±0.01*	0.99±0.00*
PMF	C_0 (mgC/kg dry soil)	20.00±4.96 a	13.15±3.87 b	8.46±1.88 c	9.34±2.28 c	9.49±2.36 c	15.02±3.36 b
	$k \times 10^{-2}$ (d^{-1})	4.06±0.88 bc	2.95±0.53 c	4.62±1.67 b	6.93±0.98 a	6.72±1.92 a	4.58±0.84 b
	$h \times 10^{-6}$ (d^{-1})	1.70±0.34 a	1.69±0.45 a	1.90±0.62 a	1.22±0.33 b	1.87±0.52 a	1.78±0.48 a
	R^2	0.98±0.01*	0.99±0.01*	0.99±0.01*	0.99±0.01*	0.97±0.01*	0.99±0.01*

C_0 and k are pool size and mineralization rate of a labile fraction, respectively, h is mineralization rate of the recalcitrant soil C. R^2 refers to the correlation index that the data fit to the model. The same superscript letter in the same row indicated no significant differences among treatments at 0.05 level. *$P < 0.01$. "–" means no data available. For each treatment $n = 9$.

Table 3 Soil pH value determined in water extracts after 45 week incubation with different N, P addition treatment

Forest type	pH value with different N and P addition					
	Control	40 mgN	200 mgN	1,000 mgN	NP	100 mgP
MEBF	3.53±0.06 a	3.52±0.04 a	3.56±0.04 a	3.46±0.06 b	3.53±0.04 a	3.57±0.05 a
CBMF	3.55±0.04 c	–	3.69±0.06 a	–	3.71±0.06 a	3.61±0.05 b
PMF	3.55±0.08 cd	3.56±0.08 c	3.71±0.13 a	3.46±0.12 d	3.69±0.12 ab	3.61±0.09 bc

The same superscript letter in the same row indicated no significant differences among treatments at 0.05 level. Data expressed as mean ± SD. "–" means no data available.

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was immobilized into PMF soil after 2 weeks. Thereafter, the net production of inorganic N from N-addition soil fluctuated and tended to increase with the incubation time (Table 4). It was indicated that N immobilization happened rapidly after N application, and the pattern of immobilization and re-mineralization appeared during the rest period of incubation. These results seemed to show indirectly that the polymerization of added nitrogen with soil organic compound by abiotic reactions during incubation made the added nitrogen retard the soil organic carbon mineralization in present experiment.

Kaye *et al*. (2000) and Resh *et al*. (2002) found that forest with N-fixing trees typically accumulated more carbon in soils than similar forests without N-fixing trees. And about 55% of the greater total soil organic carbon sequestration under N-fixers resulted from greater retention of old soil organic carbon. Soil N accretion under the N-fixers explained 62% of the variability of the greater retention of old soil organic carbon under the N-fixers (Resh *et al*., 2002). N inputs may increase soil C storage by decreasing decomposition (Kaye *et al*., 2000). Across nineteen case studies, which were available in the literature with information the pool sizes of N and C under an N-fixing species and a paired non-N-fixing species, an increase of 1 gN was associated with an increase of 12 to 15 gC (Binkley, 2005). Similarly, Zhou *et al*. (2006a; 2006b) found that soil organic carbon increased annually between 1978 and 2002 in MEBF, CBMF, and PMF by 383 ± 97, 193 ± 85, and 213 ± 86 g/(m^2·a), respectively. This research site has experienced more than 3.5 gN/(m^2·a) of N deposition (not including dry deposition) during the last decade (Huang *et al*., 1994; Zhou and Yan, 2001). Based on our finding of added N retarding soil organic carbon mineralization, we hypothesize that atmospheric N deposition may increase soil C storage by decreasing mineralization.

If 60% of the real deposition (3.5 gN/(m^2·a)) from rainfall was retained in soil after leaching of rain water, and the soil accumulated 12 gC for every gram of N accumulated, the magnitude of soil organic carbon accretion by atmospheric N deposition would be about 25.2 g/(m^2·a). It means that atmospheric N deposition may contribute from 6.5% to 13.0% of the annual increase of soil organic carbon in current research forests. However, because N influences many soil C processes, including rates of C inputs, C transformation to stabilized forms (humus), and C losses, and the 12 gC accumulated in soil for every gram of N accumulated could not be completely ascribed to the N retarding. Atmospheric N deposition increasing soil C storage by decreasing mineralization would have much less contribution to soil C accretion be found.

In this experiments, the data indicated that the addition of P may lead to a rapid short-term increase on CO_2 emission after P application. A slight positive effect of P addition on CO_2 emissions in the initial incubation period was found, which is similar with the results of other literature (Cleveland *et al*., 2002; Ilstedt and Singh, 2005). Cleveland *et al*. (2002) ascribed the positive effect of P addition on CO_2 emission to available P limitation. Galicia and García-Oliva (2004) found that microbial activity was influenced by P addition in the rainy season soil due to decreasing the amount of nutrients uptake by plants and soil leaching, but it was not influenced in the dry season soil because of increasing the amount of nutrients from

Table 4 Changes of net production of inorganic N and the percentage of immobilized nitrogen among added nitrogen during incubation with different N, P addition treatment

Forest type	Treatment	Net production of inorganic N (mg/kg dry soil)					
		Week 1	Week 2	Week 4	Week 8	Week 16	Week 36
MEBF	Control	4.36±1.46	11.17±1.27	17.40±4.75	38.77±5.71	9.78±4.04	54.60±18.16
	40 mgN-addition	–7.91±3.23	–15.64±1.63	20.52±9.78	10.34±5.48	–15.38±4.21	46.62±16.83
	200 mgN-addition	–119.20±6.40	–123.44±6.50	–144.23±5.53	–79.71±10.98	–80.56±17.76	–48.52±13.43
	1,000 mgN-addition	–679.38±46.2	–615.19±22.1	–856.81±8.11	–582.00±23.2	–660.40±65.3	–682.39±18.4
	NP-addition	–133.35±3.61	–107.97±2.58	–147.23±3.67	–75.69±8.15	–103.43±10.2	–79.42±14.00
CBMF	Control	4.45±1.47	8.12±1.90	13.64±4.71	23.58±4.11	14.06±2.30	86.28±12.69
	200 mgN-addition	–120.94±17.9	–131.62±6.29	–126.18±7.78	–90.27±11.42	–94.93±6.71	–60.49±11.29
	NP-addition	–130.92±6.75	–120.99±9.18	–145.64±13.0	–88.05±20.71	–129.49±11.9	–113.73±18.6
PMF	Control	3.00±0.87	5.68±1.69	8.64±2.54	15.83±3.98	8.14±2.38	63.04±16.73
	40 mgN-addition	3.13±2.56	–20.90±2.21	10.15±2.68	–7.71±2.44	–7.86±2.77	12.79±6.97
	200 mgN-addition	–113.49±6.33	–144.18±7.98	–145.04±3.34	–87.53±5.71	–104.01±15.3	–54.07±9.79
	1,000 mgN-addition	–672.82±13.6	–680.02±60.9	–505.03±24.2	–666.01±41.6	–774.69±34.7	–605.10±83.6
	NP-addition	–118.75±12.7	–104.87±11.2	–142.95±4.78	–82.61±18.58	–96.19±17.25	–86.59±19.11
Forest type	Treatment	Percentage of immobilized nitrogen among added nitrogen (%)					
		Week 1	Week 2	Week 4	Week 8	Week 16	Week 36
MEBF	40 mgN-addition	19.76	39.10	–51.31	–25.85	38.44	–116.55
	200 mgN-addition	59.60	61.72	72.11	39.86	40.28	24.26
	1,000 mgN-addition	67.94	61.52	85.68	58.20	66.04	68.24
	NP-addition	66.67	53.98	73.61	37.85	51.71	39.71
CBMF	200 mgN-addition	58.84	65.81	63.09	46.63	47.29	29.99
	NP-addition	65.46	60.49	72.82	44.02	64.74	56.87
PMF	40 mgN-addition	–7.83	52.25	–25.37	19.28	19.65	–31.99
	200 mgN-addition	56.75	72.09	72.52	43.77	52.01	27.03
	1,000 mgN-addition	67.28	68.00	50.50	66.60	77.47	60.51
	NP-addition	59.38	52.43	71.47	41.30	48.10	43.29

Data expressed as mean ± SD.

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new inputs through litterfall and death-root biomass. The concentration of available P in soils used by Cleveland *et al*. (2002) was 21.3 ± 3.4 mg/kg dry soil, which was much higher than that used by us (2.02–2.75 mg/kg dry soil). The relatively low available P concentration in our soils might explain why only a slight positive effect of P addition be found.

Furthermore, the responses of CO_2-C evolution to P addition in the later incubation period were related to forest types, with strong inhibition by P addition more likely for both PMF and CBMF soil. We found a negative effect of P addition on CO_2 emission from CBMF and PMF soil in the later incubation period in this experiment. To our knowledge, there is little information in the literature about the long-term effects of P addition on soil microbial activity. In general, available PO_4^{3-} could be rapidly and, in some cases, nearly irreversibly sorbed to iron and aluminum oxide minerals that dominate soils (Wada, 1985), which reduces P available. When a pulse of available P that exceeds immediate biological demand enters the soil solution, what is not consumed by the biota is quickly sorbed to soil minerals (Olander and Vitousek, 2005). During the incubation, microbial activity weakened with time, and more available P may have been sorbed to soil minerals. The decreasing available P concentration during incubation could explain why the positive effects of P addition disappeared. The negative effect of P addition on CO_2 emissions in the later incubation period is still a puzzle for us.

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