Soil Biology & Biochemistry 47 (2012) 116–122

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Effects of NH_4^+ and NO_3^- on litter and soil organic carbon decomposition in a Chinese fir plantation forest in South China

Weidong Zhang [a,b], Silong Wang [a,b,*]

[a] Huitong Experimental Station of Forest Ecology, State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110164, PR China
[b] Huitong National Research Station of Forest Ecosystem, Huitong 418307, China

ARTICLE INFO

Article history:
Received 23 September 2011
Received in revised form
26 November 2011
Accepted 2 December 2011
Available online 3 January 2012

Keywords:
Inorganic N
^{13}C-labeled litter
Priming effect
Chinese fir
Soil organic carbon

ABSTRACT

Soil organic carbon (SOC) dynamics and nutrient availability determine the soil quality and fertility in a Chinese fir plantation forest in subtropical China. Uniformly ^{13}C-labeled Chinese fir (*Cunninghamia lanceolata*) and alder (*Alnus cremastogyne*) leaf litter with or without 100 mg NH_4^+ or NO_3^- were added to the soil. The purpose was to investigate the influence of N availability on the decomposition of the litter and native SOC. The production of CO_2, the natural abundance of ^{13}C–CO_2, and the inorganic N dynamics were monitored. The results showed that Chinese fir (with a high C:N ratio) and alder (with a low C:N ratio) leaf litter caused significant positive priming effects (PEs) of 24% and 42%, respectively, at the end of the experiment (235 d). The PE dynamics showed that positive PE can last for at least 87 d. However, the possible occurrence of a significant negative PE with a sufficient incubation period is difficult to confirm. The application of both NH_4^+ and NO_3^- was found to have a stimulating effect on the decomposition of Chinese fir and alder leaf litter in the early stage (0–15 d) of incubation, but an adverse effect in the late stage. Compared with NO_3^-, NH_4^+ caused a greater decrease in the PE induced by both Chinese fir and alder leaf litter. The effects of NH_4^+ and NO_3^- on the PE dynamics had different patterns for different incubation stages. This result may indicate that the stability or recalcitrance of SOC, especially in such plantation forest soils, strongly depends on available leaf litter and application of N to the soil.

1. Introduction

Soil organic carbon (SOC) is the most important factor in soil quality and fertility in subtropical forest ecosystems. Hence, the loss of SOC in soils, especially in plantation forests, has received increasing attention in recent years (Freibauer et al., 2004). The SOC level at a particular time is determined by the rate of decomposition and input of plant litter (Jenkinson et al., 1991).

Priming effects (PEs) are strong and generally short-term changes in the turnover of native SOC induced by organic substrates added to the soil. A positive PE can accelerate the decomposition of SOC, whereas a negative PE can retard it. The PE has recently gained growing interest because it commonly occurs in most plant–soil systems (Kuzyakov, 2010). A number of studies have shown that the PE could be induced by the addition of plant residues (Fontaine et al., 2004, 2007), simple sugars, amino acids (Hamer and Marschner, 2005), and root extracts (Mary et al., 1992). Previous studies have also suggested the mechanisms of PEs. One possible positive PE mechanism is the activation of microorganisms by easily available substrates (Hamer and Marschner, 2005). This activation refers to enhanced soil organic matter (SOM) degradation by microbial growth, and the accompanying increased in enzyme production (Kuzyakov et al., 2000). Possible negative priming mechanisms include the toxicity of the substrate to microorganisms, and the inhibition of enzyme activities or structural changes in organic matter (Gianfreda et al., 1993; Fierer et al., 2001). These previous studies have greatly elucidated native SOC decomposition induced by added organic materials in arable soils. In a forest ecosystem, litter not only serves the main source of SOC, but also alters the native SOC decomposition rate as added substrates. However, information on PEs in forest soils, especially in plantation forest soils, is relatively limited.

Chinese fir (*Cunninghamia Lanceolata*) is an important coniferous timber species that has been extensively grown in southern China for more than 1000 years (Chen and Wang, 2004). Since the 1980s, the yield and soil fertility of pure Chinese fir stands have declined

* Corresponding author. State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, 72 Wenhua Road, Shenyang, Liaoning 110016, China. Tel.: +86 24 83970470; fax: +86 24 83970300.
E-mail address: slwang@iae.ac.cn (S. Wang).

0038-0717/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2011.12.004

because of successive planting, short rotation times, whole-tree harvesting, and poor site preparation (Chen et al., 1990). To improve soil conservation, maintain, and increase the long-term productivity of Chinese fir plantations, a number of practices have been introduced. Such methods include the management of litter, forest fertilization, and planting of broadleaved tree species. Among these measures, fertilization with N is given more attention. Soils generally have low to moderate fertilities, and N is often unavailable to plants in terrestrial ecosystems (Vitousek and Howarth, 1991).

A number of published field and laboratory studies have tested the effects of N addition to belowground communities and processes (Waldrop et al., 2004a,b; Treseder, 2008; Janssens et al., 2010). N addition is found to commonly decrease microbial respiration (Thirukkumaran and Parkinson, 2000; Bowden et al., 2004; Craine et al., 2007; Treseder, 2008). However, microbial respiration and metabolic quotient are not affected by N addition in lodgepole pine forest soil (Thirukkumaran and Parkinson, 2000). Given that the forms of added N vary across studies, obtaining a general idea of the manner by which different N forms affect microbial processes is difficult.

Aside from using N fertilizers, keeping the litter in the stands is also a cheap and easy way of maintaining soil fertility as well as avoiding erosion in a Chinese fir plantation. Incorporating Chinese fir litter can increase SOM. Consequently, the decomposition products of Chinese fir litter need to be identified to predict nutrient availability. The chemical composition of the added litter, such as the C:N ratio, affects decomposition and nutrient release (Sundarapandian and Swamy, 1999; Polyakova and Billor, 2007). Chinese fir litter is characterized by a high C:N ratio. Hence, its decomposition may be limited by a lack of inorganic N (Liao et al., 2000). In the present study, the effect of inorganic N on the decomposition of Chinese fir in the soil of a second generation plantation was investigated. The findings were compared with the decomposition of alder (*Alnus cremastogyne*) litter, which was characterized by a lower C:N ratio. ^{13}C-labeled Chinese fir and alder litter with or without inorganic N (NH_4^+ or NO_3^-) were added to the soil. One objective was to investigate the effects of different forms of inorganic N on the decomposition of the added litter. Another aim objective was to examine the effects of the chemical composition of the added litter and the different forms of inorganic N on the dynamics of SOM, e.g., the PE.

2. Materials and methods

2.1. Soil used

The soil used was collected fm a 0–10 cm horizon in a second generation of Chinese fir plantation located at the Huitong Experimental Station of Forest Ecology, Chinese Academy of Sciences (latitude 26°40′–27°09′ N and longitude 109°26′–110°08′ E), Hunan Province, China. The soil bulk density was 1.4 g cm^{-3}. The texture was 29.35% clay, 45.53% silt, and 25.12% sand. The soil C and N contents were 12.61 and 1.18 g kg^{-1}, respectively, corresponding to a C:N ratio of 10.7. The soil pH was 4.3.

2.2. Labeled litter used

Homogeneously ^{13}C-labeled Chinese fir and alder leaf were obtained using ^{13}C-labeled carbon dioxide ($^{13}CO_2$)–C in a growth chamber. The details of the labeled materials are listed in Table 1.

2.3. Experimental design

The soil samples were taken to the laboratory and treated as follow. Approximately 12 kg of soil from a second-generation Chinese fir plantation was passed through a 2 mm sieve and adjusted to 40% water-holding capacity (WHC). The soil was pre-incubated for 15 d in a bucket containing a beaker with 100 ml of distilled H_2O to avoid desiccation, and a beaker with 100 ml of 1 M sodium hydroxide (NaOH) solution to trap the evolved CO_2. The experiment was set up for 7 treatments and the details are listed in Table 2.

Table 1
Characteristics of the ^{13}C-labeled materials.

Material	C (%)	N (%)	P (%)	C/N	C/P	$\delta^{13}C$
Chinese fir	46.51	0.81	0.099	62.5	512.25	243
Alder	47.33	1.63	0.118	31.55	435.0	246

For the incubation, 15 replicates of 145 g of dry soil (conversion according to water content) for each treatment were placed in 1000 ml incubation vessels. Then, 465 mg of ^{13}C-labeled litters (Chinese fir or Alder), 7.5 ml of 0.24 M ammonium sulfate solution (NH_4^+–N) and 15 ml of 0.24 M potassium nitrate solution (NO_3^-–N) were added in the soil, according to experiment design. Lastly, the water content in each treatment was adjusted by adding distilled water such that the same water content of 60% WHC was obtained, and the soil was thoroughly mixed. The 15 replicates for each treatment were divided into 3 groups. The first group contained 3 replicates, and was used to measure the CO_2 released from soil. The jars were incubated in the dark for 235 d at 16.5 °C (the average annual temperature). Three additional jars with a beaker of 10 ml of distilled H_2O and a beaker of 10 ml of 0.1 M NaOH were sealed, and served as controls to account for the CO_2 trapped from the air. To collect CO_2 from respiration, one glass vials containing 10 ml of 0.1 M NaOH solution were placed in the incubation jars and the jars were sealed. The NaOH traps were replaced periodically well before saturation occurred. In this way, the CO_2 released from the soil was measured daily for the first 15 d. The second group contained 3 replicates, and was used to analyze ^{13}C activity. Glass flasks were placed in 1000 ml glass jars containing a vessel with 10 ml of distilled H_2O, sealed, and then stored in the dark. After 15, 47, 87, 139, 200, and 235 d, the gas in each treatment was sampled for ^{13}C activity analysis. After sampling, all flasks were opened, aired for 10 min to avoid anaerobicity, sealed, and then stored in the dark. The third group contained 9 replicates. After 11, 70, and 235 d, 3 jars were randomly selected from each treatment and were opened. The soil samples were then used for inorganic N analysis.

2.4. Soil chemical analysis

The soil total organic C was measured by the dry combustion method (Nelson and Sommers, 1982). Total P was colorimetrically measured by the phosphomolybdic blue color method. Inorganic N was determined from 2 M KCl extractions (1:5 soil solution ratio.). NH_4^+–N was colorimetrically determined using a spectrophotometer.

Table 2
Amount of C and N amended within each treatment.

Treatments	Fir litter (g C kg^{-1} SOC)	Alder litter (g C kg^{-1} SOC)	NH_4^+–N (mg kg^{-1} dry soil)	NO_3^-–N (mg kg^{-1} dry soil)
CK	0	0	0	0
Fir	118	0	0	0
Fir + NH_4	118	0	100	0
Fir + NO_3	118	0	0	100
Alder	0	120	0	0
Alder + NH_4	0	120	100	0
Alder + NO_3	0	120	0	100

NO_3^-–N was determined by ion chromatography (Liu et al., 1996). Vials containing 10 ml of 0.1 M NaOH were placed inside the flasks. The evolved CO_2 was then determined by the titration of carbonates with 0.1 M HCl. The value of evolved CO_2 in the glass flask without soil was subtracted from the value of evolved CO_2 from the soil sample. The activities of ^{13}C in the gas samples were measured using stable isotope-ratio mass spectrometers (DELTA ^{plus} XP). The analytical precision of the $\delta^{13}C$ measurements was 0.15‰.

2.5. Calculation and quantification of the PE

When the ^{13}C-labeled litters were added in the soil, the CO_2 derived from litters ($\delta^{13}C = 243.0‰$ to 246.0‰) and soil organic carbon ($\delta^{13}C = -27.8‰$) would have different $\delta^{13}C$ values. Litter or soil organic carbon derived CO_2–C was estimated using the following equation:

$$C_L = C_t(\delta_t - \delta_S)/(\delta_L - \delta_S)$$

$$C_S = C_t(\delta_L - \delta_t)/(\delta_L - \delta_S)$$

In the equation, C_t is the total C from soil respiration ($C_t = C_L + C_S$) during the considered time interval, C_L is the amount of C derived from added litter, C_S is the amount of C derived from soil organic carbon, δ_t is the $\delta^{13}C$ value of C_t, δ_L is the $\delta^{13}C$ value of added litter, and δ_S is the $\delta^{13}C$ value of soil organic carbon.

The PE induced by the added litter was calculated by comparing the amount of CO_2 in the litter-containing samples with the amount of CO_2 in the control treatment (Hamer and Marschner, 2005). The PE intensity during the considered time interval (t) was calculated according to the following equation:

$$PE_{[t]} = 100^* \frac{CO_2 - C_{treatment_t} - CO_2 - C_{control_t}}{CO_2 - C_{control_t}},$$

where CO_2–$C_{treatment}$ is the accumulated amount of total evolved CO_2 minus the accumulated amount of CO_2 derived from the ^{13}C-labeled litter, assuming that the ^{13}C and ^{12}C litter were equally mineralized. The PE in each time interval was calculated only when the amount of CO_2–$C_{treatment}$ was significantly different from that of CO_2–$C_{control}$. The absolute amount of the additionally derived CO_2–C from SOC was obtained by subtracting CO_2–$C_{control}$ from CO_2–$C_{treatment}$.

2.6. Statistical analyses

All statistical analyses were performed using the SPSS software. ANOVA was conducted to analyze the effects of added litter and inorganic N on the extent of the PEs. The t-test was used to determine the PE significance at the $p < 0.05$ level.

3. Results

3.1. Litter decomposition

The form of inorganic N significantly influenced the decomposition rate dynamics of the added litter (Fig. 1). In the early phase (0–15 d), the decomposition rate of Chinese fir litter was 1.56% per day and was significantly enhanced to 1.88% and 2.21% per day ($p < 0.01$) because of NH_4^+ and NO_3^- addition, respectively. After 47 d of incubation, Chinese fir litter decomposition was restrained by the addition of NH_4^+ and NO_3^-. The effects of the inorganic N form on alder litter decomposition were slightly different from that on Chinese fir. In the early phase (0–15 d), the alder decomposition rate was enhanced by NO_3^- addition, but was not affected by NH_4^+.

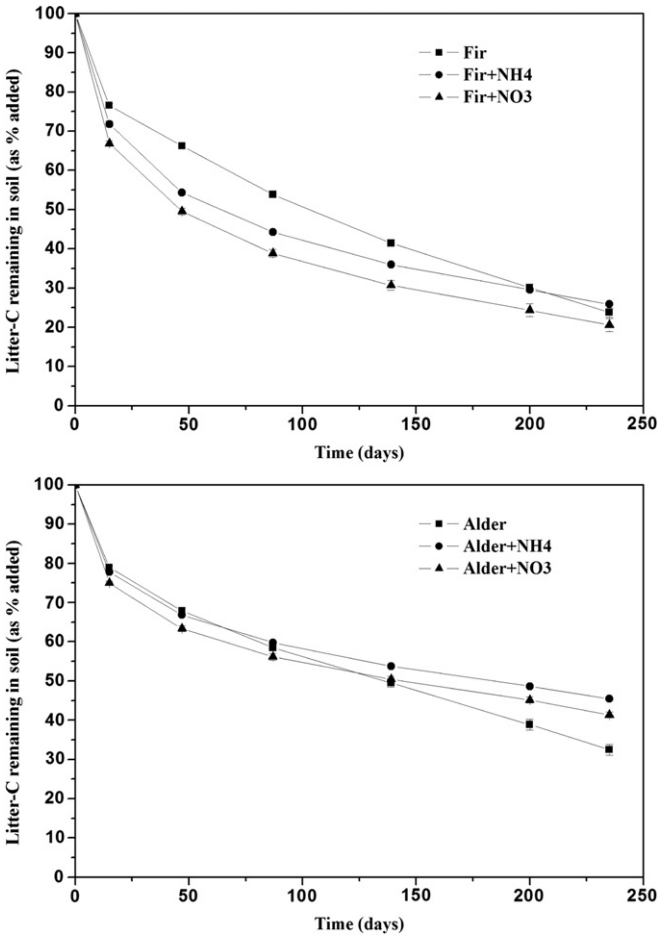

Fig. 1. Effects of NH_4^+ and NO_3^- addition on the mineralization rate of Chinese fir and alder leaf litter at different incubation intervals. Vertical bars are standard errors ($n = 3$).

Thereafter, the decomposition of alder litter was significantly restrained by both NH_4^+ and NO_3^- ($p < 0.05$).

Taking the total litter decomposition during incubation into consideration, litter decomposition was significantly influenced by litter type and inorganic N form (Fig. 2). After 235 d of incubation, about 76% of Chinese fir litter was mineralized. The percentage significantly increased to 79% ($p < 0.01$) when NO_3^- was added to the soil. No significant effect of NH_4^+ addition ($p > 0.05$) was observed. Only 68% of the added ^{13}C-labeled alder litter was mineralized after 235 d of incubation. This percentage significantly decreased to 55% and 59% ($p < 0.01$) due to NH_4^+ and NO_3^- addition, respectively.

3.2. PEs after 235 days of incubation

The addition of Chinese fir and alder litter as well as inorganic N to the soil sample influenced SOC decomposition to different extents (Fig. 3). CO_2 production from native soil was increased by 24% by the Fir treatment, as well as by −11% and 20% ($p < 0.05$) by the Fir + NH_4^+ and Fir + NO_3^- treatments, respectively. CO_2 production from native soil was increased by 42% by the alder treatment, as well as by 12% and 34% ($p < 0.05$) by the Alder + NH_4^+ and Alder + NO_3^- treatments, respectively. There was a significant interaction between the litter and inorganic N on native SOC decomposition ($p = 0.019$).

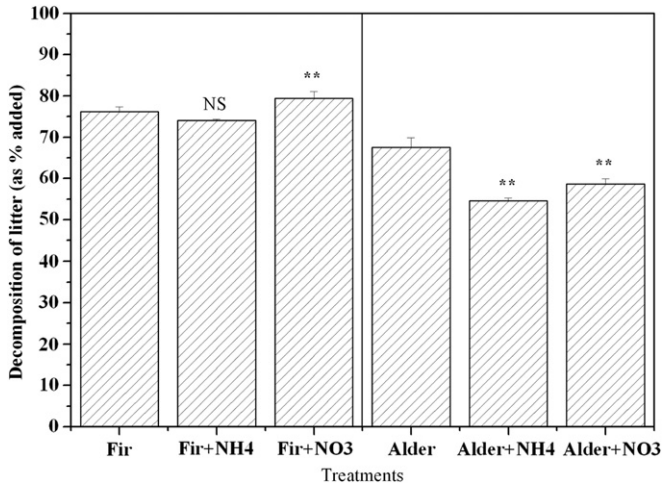

Fig. 2. Effects of NH_4^+ and NO_3^- addition on the mineralization of added ^{13}C-labeled Chinese fir and alder leaf litter after 235 d of incubation. Vertical bars are standard errors ($n = 3$). Asterisks denote significant effects of NH_4^+ or NO_3^- on leaf litter mineralization (t-test, $p < 0.01$).

3.3. Temporal dynamics of PEs

Fig. 4 shows that the direction and extension of PEs in the soil caused by litter and inorganic N addition also depended on the incubation stages. In all samples, the highest positive PEs occurred during the first 15 d of incubation. The PE caused by alder litter was significantly higher than that caused by fir litter. In the early stage of incubation (0–87 d), both fir and alder litter caused a significant positive PE ($p < 0.01$). A slight but insignificant negative PE then occurred. At the beginning of incubation (0–47 d), the positive PEs in both Fir and Alder treatments were weakened by NH_4^+ addition and enhanced by NO_3^- addition. However, in the later stage of incubation (140–235 d), the negative PEs induced by Chinese fir and alder were enhanced by both NH_4^+ and NO_3^-.

3.4. C balance

To evaluate the effects of the observed PEs on the C storage in the soil, C balances were calculated for each treatment by 100

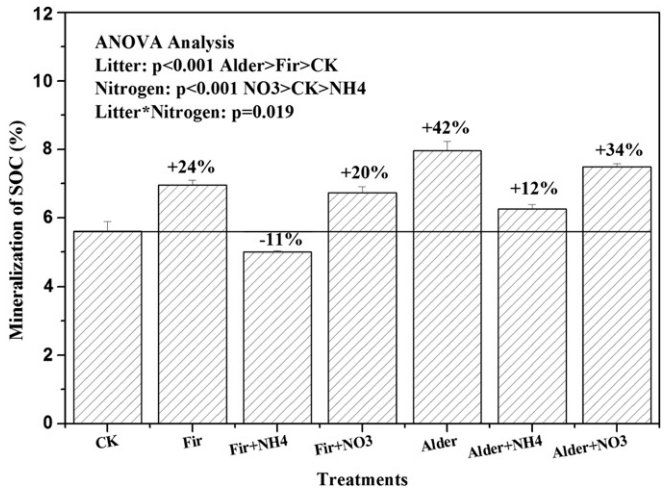

Fig. 3. Effects of inorganic N (NH_4^+ and NO_3^-) and leaf litter (Chinese fir and alder) addition on the mineralization of native SOC after 235 d of incubation. Vertical bars are standard errors ($n = 3$).

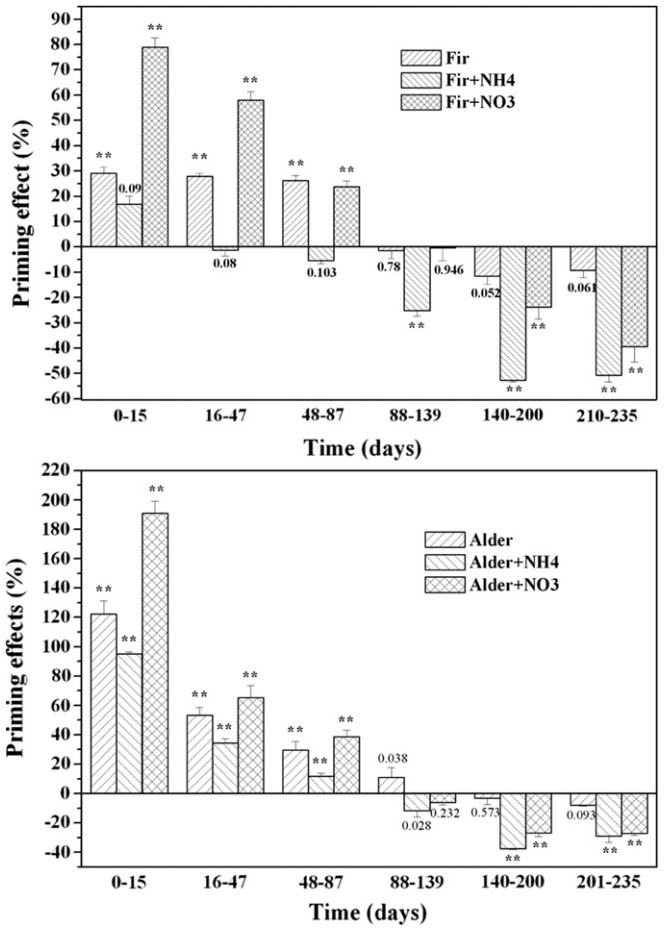

Fig. 4. Dynamics of the priming effect in different treatments. Vertical bars are standard errors ($n = 3$). The priming effect in each time interval was calculated only when the amount of CO_2–C in each treatment significantly differed from that of the control treatment. Significant differences are denoted by asterisks ($p < 0.001$).

$(SOC_{treatment} - SOC_{control})/SOC_{control}$ at the end of the incubation. As shown in Table 3, the C balance was generally positive even in the treatments with positive PEs; the amount of leaf litter that remained in the soil was still larger than that in the primed SOM-borne C–CO_2. The influence of inorganic N on the C balance was also significant.

3.5. Temporal dynamics of inorganic N

The concentration of NH_4^+–N in the control decreased from 21.3 mg kg^{-1} on the 11th day to 12.8 mg kg^{-1} at the end of the experiment (235th day). In contrast, the concentration of NO_3^-–N in the control increased from 5.7 mg kg^{-1} in the early stage of

Table 3
Effects of leaf litter and inorganic N addition on the net C balance of the soil samples and priming C relative to the control treatment after 235 d of incubation. Values are means and standard errors of 3 replicates per treatment. Significant differences between the mean values of the treatments in each row are indicated by different lower-case letters ($p < 0.05$).

Treatments	Fir	Fir + NH_4^+	Fir + NO_3^-	Alder	Alder + NH_4^+	Alder + NO_3^-
Carbon balance (% of C_{org})	0.022e (0.002)	0.025d (0.001)	0.02e (0.002)	0.031c (0.003)	0.049a (0.001)	0.045b (0.002)
Primed C (% of C_{org})	0.013c (0.001)	−0.008e (0.001)	0.011c (0.002)	0.023a (0.003)	0.007d (0.001)	0.019b (0.001)

incubation (11th day) to 30.1 mg kg^{-1} at the end of the experiment. The pattern of NH_4^+–N and NO_3^-–N dynamics were significantly affected by the addition of inorganic N and litter. NH_4^+–N concentration decreased at the beginning of incubation, and increased in the later stage due to NO_3^- addition. On the other hand, NO_3^-–N concentration increased for the entire incubation duration due to NH_4^+ addition. Litter addition caused a significantly decreased concentration of both NH_4^+–N and NO_3^-–N (Fig. 5).

4. Discussion

Generally speaking, the decomposition rate of the litter was controlled by the litter quality, such as the initial N content and C:N ratio (Singh et al., 1999; Tateno et al., 2007). In the present study, the amount of the labeled Chinese fir litter (C:N = 62.5) decomposition was higher than that of the alder litter (C:N = 31.5) after 235 d of incubation. This result seemed contradictory to our previous study comparing Chinese fir and broadleaved tree litter. Chinese fir litter, with its high C:N ratio and lignin content as well as low initial N content, had a lower decomposition rate than the broadleaved tree litter (Wang et al., 2008). The discrepancy may be related to "home-field advantage" (Ayres et al., 2009). In our study, the soil used was derived from a second generation of Chinese fir plantation and the microorganisms in the soil were adapted to decompose Chinese fir litter to gain nutrients and energy. When alder litter decomposed in Chinese fir forest soil, the decomposition rate decreased compared to in alder forest soil. Besides, the ground of added litters also enhanced the home-field advantage effect.

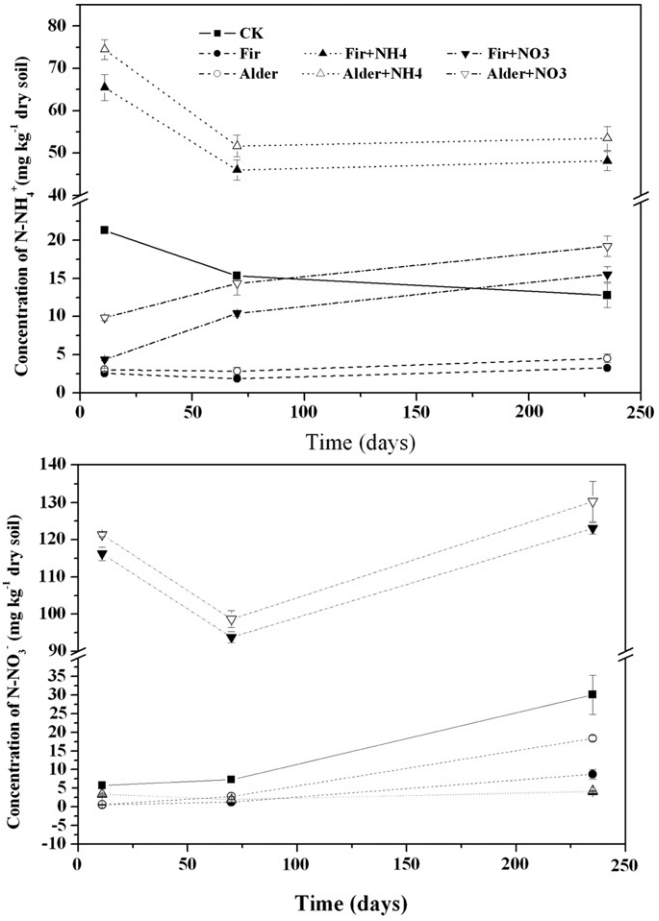

Fig. 5. Dynamics of NH_4^+–N and NO_3^-–N concentrations (mg N kg^{-1} dry soil) in the soil during incubation at 16.5 °C. Vertical bars are standard errors ($n = 3$).

In our study, approximate 21% to 34% of litter-C was lost as CO_2 in the first 15 d, which seems to be much higher than normally observed. For example, Wang et al. (2008) reported that only about 20% of *Cuninghamia lanceolata* and *Michelia macclurei* leaf litter decomposed in 2 months with litter-bag technique. This phenomenon may be due to different experimental techniques in different studies. The decomposition rates of added litters after ground in this study were enhanced compared to litter-bag technique. Conde et al. (2005) also found that more than 20% of added ^{14}C-labeled maize litter was mineralized in 14 d of incubation, which was consistent with our study.

Litter decomposition is well known to depend on the nutrient status of the soil (Fog, 1988; Swift et al., 1979). In the current study, the response of litter decomposition to inorganic N addition varied with the litter type, and showed no common pattern. After 235 d of incubation, the decomposition of Chinese fir litter was accelerated by the addition of NO_3^-, and was not affected by the addition of NH_4^+–N. This result was consistent with a previous study (Liao et al., 2000). The decomposition of alder was retarded by the addition of both NH_4^+ and NO_3^-. Other previous studies have reported that litter decomposition may increase (Micks et al., 2004), decrease (Bowden et al., 2004), or not change (McDowell et al., 2004) after N addition. Consequently, a general conclusion on the effects of N on litter decomposition cannot be drawn with ease, partly because of the wide ranging availability and chemical composition of plant litter.

The effects of inorganic N on the litter decomposition rate also depended on the decomposition stages and forms of inorganic N. Some studies have shown that the decomposition rate of litter was much faster in more nutrient-rich stands (Swift et al., 1979). The dynamics of litter decomposition may provide more information on the effects of inorganic N. In the current study, the decomposition of Chinese fir litter was accelerated by the addition of inorganic N in the early stage of incubation, especially in the first 0–15 d. However, the decomposition of alder litter was only accelerated by addition of NO_3^-–N in this stage. Then, the decomposition was retarded in the later stage of incubation. This phenomenon is assumed to be coincident with the C quality of the substrate in the litter in different decomposition stages. Generally speaking, the compounds in the litter could be roughly categorized as labile metabolic compounds (e.g., sugars and amino acids), and recalcitrant structural materials (e.g., lignin and cutin) (Chapin and Matson, 2002). The promotion effects of inorganic N on litter decomposition in the early stage conformed with a previous study showing that the decomposition rate of high-quality litter (i.e., with low lignin content and C:N ratio) is stimulated by elevated N deposition (Waldrop et al., 2004a,b). Several studies have also demonstrated that the addition of N increased the production of extracellular enzymes responsible for the decomposition of substrates, such as acid phosphatase activities (Saiya-Cork et al., 2002; Graham and Haynes, 2005; Allison et al., 2006) and phenol oxidase activity (Lucas et al., 2007). The added litter in the soil became more resistant in the later stage, and was retarded by the addition of inorganic N. This result conformed with a previous study showing that the substrate with the lower quality was retarded by the addition of N (Knorr et al., 2005).

The decomposition of native SOC changed with litter addition, thereby causing the PE (Kuzyakov et al., 2000). Different kinds of organic materials can induce PEs to different degrees (Hamer and Marschner, 2002; Shen and Bartha, 1996). The higher-quality alder litter caused a greater positive PE after 235 d of incubation than Chinese fir. This result was consistent with that of Conde et al. (2005), who reported that the PE was larger with the addition of glucose (with more microbial availability) than with maize addition to saline alkaline soil. The magnitude of the PE also depended on

the inorganic N status. At the end of incubation, the magnitude of the PE decreased due to the addition of inorganic N. Even in the Fir + NH_4^+ treatments, the PE became negative from a positive one.

Although PEs are defined as strong short-term changes in the turnover of native SOM induced by a comparatively moderate treatment of the soil, the duration of the PE is still unconfirmed (Kuzyakov, 2010). In the present study, the dynamics of the PE during incubation was monitored because it was much more informative than only the cumulative CO_2 recorded at the end of the experiment. As reported, the magnitude and direction of the PE can change with time (Sallih and Bottner, 1988). The significant positive PEs in early stage (0–15 d) may be related to the rapid decomposition of added litters. Fig. 1 shows that more than 20% of added litters were mineralized. Thereafter, the decomposition rates of the litter in the Fir and Alder treatments decreased to a constant value in less than 50 d. However, the positive PEs in these two treatments were still significant ($p < 0.01$) after 87 d of incubation. This finding may be attributed to the fact that some microorganisms remained active, and the extracellular enzymes produced during this period of high activity remained in the soil as well as contributed to SOM decomposition. Thereafter, a weak but insignificant ($p > 0.05$) negative PE occurred. However, confirming the possible significance of this weak negative PE after a sufficient incubation period is difficult.

In the later stage of incubation, the significant negative PE induced by the additional inorganic N occurred. There were three possible mechanisms for this negative effect of N on SOC decomposition. First, the added N changed the composition of the decomposer community through competition. Xue et al. (2007) has reported that the addition of N results in increased bacteria and decreased fungi in Dinghushan subtropical forests. Second, the addition of inorganic N suppressed the production of enzymes required for the degradation of lignin and other recalcitrant compounds. DeForest et al. (2005) has suggested that increased NO_3^- concentration could promote the output of water-soluble organic C by suppressing enzymes, which are responsible for lignin degradation. Third and last, the added inorganic N reacted with organic matter and caused a more rapid formation of recalcitrant material (Ågren et al., 2001).

The effects of inorganic N on PEs also depended on the form of inorganic N. Fig. 4 shows that the positive PEs in the early stage was enhanced by NO_3^- addition and lowered by NH_4^+ addition. Two possible mechanisms may explain the different effect of NO_3^- and NH_4^+ on the direction and magnitude of priming effect. The first one is direct effect. Microbes preferentially take up inorganic nitrogen as NH_4^+–N (Lavelle and Spain 2003) and cost more energy to uptake NO_3^-–N, hence the positive priming effect in NO_3^- addition treatments increased. The second one is indirect effect. The decreased priming effect in NH_4^+ addition treatment may be related to the soil acidity due to nitrification. However, it is hard to ascertain which effect is dominant. Further studies are needed to determine the possible relationship of the effect of inorganic N form on PEs with the soil type, nutrient status, and added materials.

It seems that positive PEs may cause a decrease in soil carbon storage, since the soil organic carbon decomposition is enhanced. However, the carbon balance calculated at the end of the experiment showed that net increases of carbon storage occurred in all treatments in this study. This may be due to the following reason. The soil organic carbon loss due to positive PEs in the early stage can be counteracted by litter-C remaining in the soil and negative PEs in later stage. In fact, Hamer and Marschner (2005) also reported that a net increase in carbon storage occurred after fructose, alanine, oxalic acid and catechol addition in most cases after short-term incubation (26 d) despite positive PEs. Besides, the soil carbon storage may further increased by NH_4^+–N addition.

5. Conclusions

PEs induced by Chinese fir and alder leaf litter were observed in the current study. The magnitude of PEs was altered by the addition of NH_4^+ and NO_3^-. Hence, the stability or recalcitrance of SOC, especially in plantation forest soils, strongly depended on the input of available leaf litter and the application of N to the soil. Further studies focusing on the activity and dynamics of microorganisms are warranted. The mechanisms of the effects of different inorganic forms on PEs in different incubation stages need to be explored.

Acknowledgements

We thank Zhang Xiuyong and Xu Guangbiao for assistance in collecting samples in the field, and two anonymous reviewers for helpful comments on revision of this manuscript. This work was financially supported by the National Natural Science Foundation of China (41030533 and 31070436).

References

Ågren, G.I., Bosatta, E., Magill, A.H., 2001. Combining theory and experiment to understand effects of inorganic nitrogen on litter decomposition. Oecologia 128, 94–98.
Allison, S.D., Nielsen, C., Hughes, R.F., 2006. Elevated enzyme activities in soils under the invasive nitrogen-fixing tree *Falcataria moluccana*. Soil Biology & Biochemistry 3, 1537–1544.
Ayres, E., Steltzer, H., Simmons, B.L., Simpson, R.T., Steinweg, J.M., Wallenstein, M.D., Mellor, N., Parton, W.J., Moore, J.C., Wall, D.H., 2009. Home-field advantage accelerates leaf litter decomposition in forests. Soil Biology & Biochemistry 41, 606–610.
Bowden, R.D., Davidson, E., Savage, K., Arabia, C., Steudler, P., 2004. Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest. Forest Ecology and Management 196, 43–56.
Chapin, F.S., Matson, P.A., 2002. Principles of Terrestrial Ecosystem Ecology. Springer-Verlag Inc, New York, p. 164.
Chen, C., Wang, S., 2004. Ecology of Mixed Plantation Forest. Science Press, Beijing, p. 3.
Chen, C., Zhang, J., Zhou, C., Zheng, H., 1990. Researches on improving the quality of forest land and the productivity of artificial *Cunninghamia lanceolata* stands. Chinese Journal of Applied Ecology, 97–106.
Conde, E., Cardenas, M., Ponce-Mendoza, A., Luna-Guido, M.L., Cruz-Mondragón, C., Dendooven, L., 2005. The impacts of inorganic nitrogen application on mineralization of ^{14}C-labelled maize and glucose, and on priming effect in saline alkaline soil. Soil Biology & Biochemistry 37, 681–691.
Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. Ecology 88, 2105–2113.
DeForest, J.L., Zak, D.R., Pregitzer, K.S., Burton, A.J., 2005. Atmospheric nitrate deposition and enhanced dissolved organic carbon leaching: test of a potential mechanism. Soil Science Society of America Journal 69, 1233–1237.
Fierer, N., Schimel, J.P., Cates, R.G., Zou, J., 2001. Influence of balsam poplar tannin fractions on carbon and nitrogen dynamics in Alaskan taiga floodplain soils. Soil Biology & Biochemistry 33, 1827–1839.
Fog, K., 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biological Reviews 63, 433–462.
Fontaine, S., Bardoux, G., Abbadie, L., Marìotti, A., 2004. Carbon input to soil may decrease soil carbon content. Ecology Letters 7, 314–320.
Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450, 277–280.
Freibauer, A., Rounsevell, M.D.A., Smith, P., Verhagen, J., 2004. Carbon sequestration in the agricultural soils of Europe. Geoderma 122, 1–23.
Gianfreda, L., Rao, M.A., Violante, A., 1993. Interactions of invertase with tannic acid, hydroxy-aluminium (OH-Al) species or montmorillonite. Soil Biology & Biochemistry 25, 671–677.
Graham, M.H., Haynes, R.J., 2005. Organic matter accumulation and fertilizer-induced acidification interact to affect soil microbial and enzyme activity on a long-term sugarcane management experiment. Biology and Fertility of Soils 41, 249–256.
Hamer, U., Marschner, B., 2002. Priming effects of sugars, amino acids, organic acids and catechol on the mineralization of lignin and peat. Journal of Plant Nutrition and Soil Science 165, 261–268.
Hamer, U., Marschner, B., 2005. Priming effects in different soil types induced by fructose, alanine, oxalic acid and catechol additions. Soil Biology & Biochemistry 37, 445–454.
Janssens, I.A., Dieleman, W., Luyssaert, S., Subke, J.A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A.J., Grace, J., Matteucci, G., Papale, D., Piao, S.L., Schulze, E.D.,

Tang, J., Law, B.E., 2010. Reduction of forest soil respiration in response to nitrogen deposition. Nature Geoscience 3, 315–322.
Jenkinson, D.S., Adams, D.E., Wild, A., 1991. Model estimates of CO_2 emissions from soil in response to global warming. Nature 351, 304–306.
Knorr, M., Frey, S.D., Curtio, P.S., 2005. Nitrogen additions and litter decomposition: a meta-analysis. Ecology 86, 3252–3253.
Kuzyakov, Y., 2010. Priming effects: interactions between living and dead organic matter. Soil Biology & Biochemistry 42, 1363–1371.
Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. Soil Biology & Biochemistry 32, 1485–1498.
Lavelle, P., Spain, A.V., 2003. Soil Ecology. Kluwer Scientific, Amsterdam. 95p.
Liao, L., Gao, H., Wang, S., 2000. The effect of nitrogen addition on soil nutrient leaching and the decomposition of Chinese fir leaf litter. Acta Phytoecologica Sinica 24, 34–39.
Liu, G.S., Jiang, N.H., Zhang, L.D., 1996. Standard Methods for Observation and Analysis in Chinese Ecosystem Research Network: Soil Physical and Chemical Analysis & Description of Soil Profiles. Standards Press of China, Beijing, China.
Lucas, R.W., Casper, B.B., Jackson, J.K., Balser, T.C., 2007. Soil microbial communities and extracellular enzyme activity in the New Jersey Pinelands. Soil Biology & Biochemistry 39, 2508–2519.
Mary, B., Mariotti, A., Morel, J.L., 1992. Use of ^{13}C variations at natural abundance for studying the biodegradation of root mucilage, roots and glucose in soil. Soil Biology & Biochemistry 24, 1065–1072.
McDowell, W.H., Magill, A.H., Aitkenhead-Peterson, J.A., Aber, J.D., Merriam, J.L., Kaushal, S.S., 2004. Effects of chronic nitrogen amendment on dissolved organic matter and inorganic nitrogen in soil solution. Forest Ecology and Management 196, 29–41.
Micks, P., Aber, J.D., Boone, R.D., Davidson, E.A., 2004. Short-term soil respiration and nitrogen immobilization response to nitrogen applications in control and nitrogen-enriched temperate forests. Forest Ecology and Management 196, 57–70.
Nelson, D.W., Sommers, L.E., 1982. Total carbon, OC, and organic matter. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis, Part 2. Agronomy Society of America and Soil Science Society of America, Madison, WI, pp. 539–577.
Polyakova, O., Billor, N., 2007. Impact of deciduous tree species on litterfall quality, decomposition rates and nutrient circulation in pine stands. Forest Ecology and Management 253, 11–18.
Saiya-Cork, K.R., Sinsabaugh, R.L., Zak, D.R., 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. Soil Biology & Biochemistry 34, 1309–1315.
Sallih, Z., Bottner, P., 1988. Effect of wheat *Tritium aestivum* roots on mineralization rates of soil organic matter. Biology and Fertility of Soils 7, 67–70.
Shen, J., Bartha, R., 1996. Priming effect of substrate addition in soil based biodegradation tests. Applied Environmental Microbiology 62, 1428–1430.
Singh, K.P., Singh, P.K., Tripathi, S.K., 1999. Litterall, litter decomposition and nutrient release patterns in four native tree species raised on coal mine spoilat Singrauli, India. Biology and Fertility of Soils 29, 371–378.
Sundarapandian, S.M., Swamy, P.S., 1999. Litter production and leaf-litter decomposition of selected tree species in tropical forests at Kodayar in the Western Ghats, India. Forest Ecology and Management 123, 231–244.
Swift, M.J., Heal, O.W., Anderson, J.M., 1979. Decomposition in Terrestrial Ecosystems. Blackwell Scientific Publications, Oxford.
Tateno, R., Tokuchi, N., Yamanaka, N., Du, S., Otsuki, K., Shimamura, T., Xue, Z., Wang, S., Hou, Q., 2007. Comparison of litterfall production and leaflitter decomposition between an exotic black locust plantation and anindigenous oak forest near Yan'an on the Loess Plateau, China. Forest Ecology and Management 241, 84–90.
Thirukkumaran, C.M., Parkinson, D., 2000. Microbial respiration, biomass, metabolic quotient and litter decomposition in a lodgepole pine forest floor amended with nitrogen and phosphorous fertilizers. Soil Biology & Biochemistry 32, 59–66.
Treseder, K., 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecology Letters 11, 1111–1120.
Vitousek, P.M., Howarth, R.W., 1991. Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry 13, 87–115.
Waldrop, M., Zak, D., Sinsabaugh, R., 2004a. Microbial community response to nitrogen deposition in northern forest ecosystems. Soil Biology & Biochemistry 36, 1443–1451.
Waldrop, M.P., Zak, D.R., Singsabaugh, R.L., Gallo, M., Lauber, C., 2004b. Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. Ecological Applications 14, 1172–1177.
Wang, Q., Wang, S., Huang, Y., 2008. Comparison of litterfall, litter decomposition and nutrient return in a monoculture *Cunninghamia lanceolata* and a mixed stand in southern China. Forest Ecology and Management 255, 1210–1218.
Xue, J.H., Mo, J.M., Li, J., Li, D.J., 2007. The short-term response of soil microorganism number to simulated nitrogen deposition. Guihaia 27, 174–179.