

# Response of nitrogen mineralization dynamics and biochemical properties to litter amendments to soils of a poplar plantation

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**Abstract** Understanding the impact of plant litters on soil nitrogen (N) dynamics could facilitate development of management strategies that promote plantation ecosystem function. Our objective was to evaluate the effects of different litter types on N mineralization and availability, microbial biomass, and activities of L-asparaginase and *o*-diphenol oxidase (*o*-DPO) in soils of a poplar (*Populus deltoides*) plantation through 24 weeks of incubation experiments. The tested litters included foliage (F), branch (B), or root (R) of poplar trees, and understory vegetation (U) or a mixture of F, B, and U (M). Litter amendments led to rapid N immobilization during the first 4 weeks of incubation, while net N mineralization was detected in all tested soils from 6 to 24 weeks of incubation, with zero-

order reaction rate constants (*k*) ranging from 7.7 to 9.6 mg N released kg<sup>-1</sup> soil wk<sup>-1</sup>. Moreover, litter addition led to increased microbial biomass carbon (C) 49–128% and increased MBC:MBN ratio by 5–92%, strengthened activities of L-asparaginase and *o*-DPO by 14–74%; Up to about 37 kg N ha<sup>-1</sup> net increase in mineralized N in litter added soils during 24 weeks of incubation suggests that adequate poplar and understory litter management could lead to reduced inputs while facilitate sustainable and economic viable plantation production.

**Keywords** Plant litter · Nitrogen mineralization · Soil microbial biomass · L-asparaginase · *o*-diphenol oxidase

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## Introduction

Wood resources from poplar plantations play an important role in maintaining regional ecological stability and economic vitality (Ge et al. 2015). To fulfill the increasing demand for timber production, poplar (*Populus* spp.) plantations are widely established in northern and central China because of their fast-growth and high timber yield. These plantations generate 5–14 t ha<sup>-1</sup> a<sup>-1</sup> of litter that serves as a vital source of soil organic matter and nutrients for sustaining productivity in plantation ecosystems (Dong et al. 2011; Meirsonne et al. 2007; Berthelot et al. 2000; Wan et al. 2009).

The types of litter include foliage, branches, and roots of aboveground and belowground parts of trees and understory vegetation. Tree foliage litter accounts for approximately 50–95% of total litter biomass in plantations, with its relative proportion decreases with increasing stand age while the proportion of branches increases (Lodhiyal et al. 1994; Wan et al. 2009; Dong et al. 2011). Past research

often focused on aboveground litter that originated from tree foliage (Zhang and Wang 2012; Xiong et al. 2014; Rinnan et al. 2007). Limited information is available on belowground or understory litters.

In poplar plantations, about 0.7–2.0 t ha<sup>-1</sup> a<sup>-1</sup> of litter is contributed by understory vegetation; of which about 80% is from annual herbaceous plants with relatively high nitrogen (N) content (Ge et al. 2015; Lodhiyal et al. 1994). The relatively high N content, high turnover rate, and heterogeneity of understory vegetation are drivers in promoting microbial diversity and activity that stimulate organic degradation and lead to nutrient mineralization and enrichment in soil and plantation ecosystems.

Nitrogen is one of the most limiting nutrients in soil for non-leguminous plants. Soil N availability is governed by mineralization and immobilization processes, which were mediated by the activities of microbes and enzymes in soil (Cayuela et al. 2009; Kabba and Aulakh 2004; Song et al. 2014). Litter degradation could be important in supplementing N nutrition in poplar plantations. Better understanding of N dynamics and the associated microbial and biochemical parameters in litter-amended soils could facilitate development of management strategies to maximize plantation production while maintaining sustainability.

Many factors affect litter degradation and nutrient release. Different types of litter possess different physico-chemical properties that impact the processes and interactions involved (Cayuela et al. 2009). Litter quality, such as C:N ratio and content of hemicellulose and lignin, was generally recognized to be crucial (Barzegar et al. 2002; Puttaso et al. 2011; Moreno-Cornejo et al. 2014). Mineralization rates of N are inversely related to C:N ratios of organic matter (Kabba and Aulakh 2004; Mondini et al. 2008). Litter quality differed among plant species and depended on growth conditions. The C:N ratio ranged from 28 to 73 for foliage litter of poplar in different plantation ecosystems (Zhang et al. 2004; Zhong and Gao 2003; Chen et al. 2012), and was lower than 20 for annual herbaceous plants (Ge et al. 2015; Lodhiyal et al. 1994).

Litter and organic residue amendments could affect microbial properties and degradation processes in soils (Henriksen and Breland 1999; Calderón et al. 2005; Kotrocó et al. 2014). Mixing of different litters increased diversity and heterogeneity, promote microbial diversity also, and strengthen litter degradation and N mineralization (Hossain and Sugiyama 2011; Handa et al. 2014). It is not clear, however, how and to what degree different litters affect such processes in poplar plantation ecosystems.

This study was conducted to assess the effects of different litter types, including foliage, branches, and roots of trees and understory vegetation, and litter mixing, on N dynamics and associated biochemical and microbiological

parameters in soils of a poplar plantation ecosystem. The intent was to facilitate development of management strategies to sustain productivity and maintain economic vitality of poplar plantations.

## Materials and methods

### Site description and sample collection

Our study site was a poplar (*Populus deltoides*) plantation located in Nanjing, Jiangsu, China (32°04'N, 118°37'E), under a subtropical monsoon climate with a mean annual air temperature of 15.4 °C and a mean annual rainfall of 1102 mm. The study site was paddy field before poplar plantation established. The initial planting density was 288 stems of poplar per hectare and the plantation was 13 years old at the time of sampling in 2013. The average height and diameter at breast height (DBH) of trees were 24.3 m and 25.8 cm, respectively. The understory vegetation was dominated by *Carpesium abrotanoides*, *Torilis japonica*, *Amaranthus retroflexus*, *Perilla frutescens*, *Morus alba*, and *Broussonetia papyrifera*.

Composite soil samples (0–10 cm) from 5 sampling sites were collected in a 40 × 40 m plot after removing organic layer. Immediately following sampling, soils were passed through a 2-mm sieve, mixed thoroughly, and divided into two portions. One portion was kept field-moist and stored in sealed containers at 4 °C for biological analysis and for use in the laboratory incubation experiment. The other portion was air-dried and stored in sealed containers at room temperature for analysis of soil chemical properties. Soil at the study site is a clay loam that contained 22.1 g kg<sup>-1</sup> organic C (TOC) and 1.0 g kg<sup>-1</sup> total nitrogen (TN). Soil pH was 7.9 and electrical conductivity (EC) was 26.1 μS cm<sup>-1</sup>.

Fresh foliage and branch litter, and fine roots (Ø < 2 mm) of poplar, as well as above-ground litter of understory vegetation were collected in November when poplar trees were defoliating. Litter samples were oven-dried at 65 °C for 72 h and then ground to pass through a 2-mm sieve. A portion of the samples was used to determine TOC and TN; the remaining samples were stored in sealed plastic bags for use in incubation experiments. Selected chemical properties of litter are shown in Table 1.

### Nitrogen mineralization

Nitrogen mineralization in soils following treatment with different litter amendments was determined in a laboratory incubation study based on changes in KCl-extractable N content over incubation periods up to 24 weeks. Five treatments and a control were used, viz. soil without litter

**Table 1** Selected basic properties of litter used in this study<sup>†</sup>

Litter source <sup>‡</sup>	TOC g kg <sup>-1</sup>	TN	C:N
F	408.5 (16.4)*	7.7 (0.1)	52.9 (2.4)
B	556.7 (92.1)	4.6 (0.3)	122.7 (27.3)
R	414.6 (20.9)	5.1 (0.2)	80.6 (1.2)
U	436.7 (5.1)	10.9 (0.2)	40.2 (0.2)
M	432.6 (23.1)	8.0 (0.6)	54.7 (6.6)

<sup>†</sup>TOC total organic carbon; TN total nitrogen; C:N ratio of TOC to TN

<sup>‡</sup>F, B, R, U and M refers to litters originated from foliage, branch, or fine root of poplar trees, understory litter, and a mixture of F, B, and U with mass ratio of 1:1:1, respectively

\*The numbers in parentheses represent the standard error of mean values

(CK), soil with poplar foliage (F), poplar branch (B), poplar root (R), understory vegetation (U), or a mixture of F, B, and U (M with mass ratio of 1:1:1). The incubation was conducted in a 180-mL cylindrical glass jar (5 cm of inner diameter and 9 cm of height), where 100 g of field-moist soil was mixed thoroughly with 1 g of plant litter (dried weight), equivalent to about 10 t ha<sup>-1</sup> of litter being added to forest land surface. Soils were adjusted to a moisture content of 70% water holding capacity, and incubated at 25 °C in the dark for 0, 1, 2, 3, 4, 6, 8, 10, 13, 16, 20 and 24 weeks. The jars were covered with plastic film with three pinholes for aeration. A total of 216 incubation jars were set up. Moisture loss during incubation was adjusted based on weight loss. Triplicates for each treatment were sampled at different incubation times. Activities of L-asparaginase and *o*-diphenol oxidase (*o*-DPO), and contents of microbial biomass carbon (MBC) and nitrogen (MBN) were determined at weeks 0, 3, 8, 16 and 24. Ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N), nitrate nitrogen (NO<sub>3</sub><sup>-</sup>-N), EC and pH value were determined at weeks 0, 1, 2, 3, 4, 6, 8, 10, 13, 16, 20 and 24.

The accumulation of KCl-extractable N in soil over time reflects net N mineralization. Incubation time versus extractable N fitted the linear regression Eq. (1).

$$N_t = a + kt \quad (1)$$

where  $N_t$  is the accumulation of KCl-extractable N (mg kg<sup>-1</sup>),  $k$  is zero-order reaction rate constant (mg N released kg<sup>-1</sup> soil wk<sup>-1</sup>), and  $t$  is the incubation time (week),  $a$  is a constant.

## Laboratory analysis

Soil pH was measured using a pH meter at a 1:2.5 (w/v) soil-to-water ratio. Soil electrical conductivity was determined using a conductivity meter at a 1:5 (w/v) soil-to-water ratio. To quantify total nitrogen (TN) in plant litter and soils, the samples were Kjeldahl-digested with concentrated perchloric and sulfuric acid, followed by determination using a continuous flow analyzer (Bran + Luebbe AA3, Germany). Total organic carbon (TOC) in these samples was determined using the potassium dichromate titrimetric method (Lu 1999). Soil extractable N (NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N) was determined by extraction with 2M KCl in 1:5 (w/v) soil-to-solution ratio, shaking for 1 h at 200 rpm, and followed by quantification using a continuous flow analyzer (Bran + Luebbe AA3, Germany).

Soil MBC and MBN were measured using the chloroform-fumigation extraction method (Vance et al. 1987). Briefly, 5 g of field-moist soil was weighed into a 50-mL beaker and fumigated for 24 h at 25 °C with chloroform (ethanol free) in the dark. Simultaneously another soil sample was treated similarly but without chloroform fumigation to serve as a control. After fumigation, we added to the soils 0.5M K<sub>2</sub>SO<sub>4</sub> at a 1:5 (w/v) soil-to-solution ratio and shaken for 30 min at 200 rpm and then filtered through a 0.45 µm membrane. The C content in the K<sub>2</sub>SO<sub>4</sub> extracts was determined using a Liquid TOC analyzer (Elementar, Germany), and the N content was determined spectrophotometrically at 570 nm using a method described by Joergensen and Brookes (1990). MBC and MBN contents were calculated based on their concentration differences in the fumigated and unfumigated samples using a conversion factor of 2.22 and 5.0, respectively (Vance et al. 1987; Joergensen and Brookes 1990).

L-asparaginase activity was determined using a method described by Frankenberger and Tabatabai (1991) with some modifications. Briefly, 5 g of field-moist soil was treated with 0.2 mL of toluene, and then incubated with 1 mL of 0.5M L-asparagine solution and 9 mL of 0.1M THAM (pH 10) at 25 °C for 2 h. Subsequently, the enzymatic reaction was stopped by adding 40 mL 2.5 M KCl-Ag<sub>2</sub>SO<sub>4</sub>. NH<sub>4</sub><sup>+</sup>-N released by L-asparaginase activity was determined using a continuous flow analyzer (Bran + Luebbe AA3, Germany) and expressed as mg NH<sub>4</sub><sup>+</sup>-N released 2 h<sup>-1</sup> g<sup>-1</sup> soil (dry weight).

*o*-DPO activity was determined using a method described by Perucci et al. (2000) and expressed as µmoles of catechol oxidized 10 min<sup>-1</sup> g<sup>-1</sup> soil (dry weight). Briefly, 1 g of field-moist soil was incubated with 3 mL of oxygenated reagent solution containing 0.2M catechol solution and 0.2M proline solution in phosphate buffer (0.1M, pH

6.5), and another 2 mL phosphate buffer. After incubating the mixture for 10 min at 30 °C, the enzymatic reaction was stopped by cooling on ice and adding 5 mL of ethanol. The mixture was then centrifuged for 5 min at 5000 g and 4 °C. The absorbance of the supernatant at 525 nm was determined using a spectrophotometer (Unico, 2100, China). *o*-DPO activity was calculated based on enzymatic oxidation of catechol under the above defined conditions having molar absorptivity of  $5 \times 10^3$  (Yamaguchi et al. 1970).

### Statistical analysis

All laboratory analyses were conducted in triplicate with means reported. Treatment effect was evaluated by one-way analysis of variance (ANOVA). Two-way ANOVA was used to examine the effects of litter treatment, incubation time and their interactions on soil parameters. Mean separation was determined according to least significant differences (LSD) at  $P < 0.10$ ,  $< 0.05$  or  $< 0.01$ . All statistical analyses were performed using SPSS 18.0.

### Results

Incubation time affected all of the tested soil parameters significantly, and litter amendments affected most of the tested soil parameters significantly, with the exception of L-asparaginase (Table 2). Significant interactions between incubation time and litter amendments were detected.

KCl-extractable N increased with increasing incubation time (Fig. 1). However, the trend of this increase varied among treatments. The increase in CK was detectable after week one of incubation, but appreciable increases in the litter-amended soils were not detectable until week eight of incubation. In fact, extractable N in amended soils decreased over the first 4 weeks of incubation and was significantly lower than in CK until week 16. By week 24, extractable N concentrations were in the order of  $F > CK > U > M > R > B$ , and the concentrations in CK and F were significantly higher than in the other treatments.

The varied patterns and contents of extractable N among treatments was due to variations in N mineralization and

immobilization rates during incubation. During the first 6 weeks of incubation, the net N mineralization rate constant ( $k$ ) for CK was about  $4 \text{ mg N kg}^{-1} \text{ soil per week}$ , while this value was near zero in the litter-amended soils (Fig. 2). From 6 to 24 weeks of incubation, N mineralization was highest in F- or U-treated soils ( $k = 9.57$ ), followed by M treatment ( $k = 8.80$ ), B or R treatments ( $k = 8.00$ ), and the lowest in CK ( $k = 7.66$ ). F and U treatments exhibited similar trends in N mineralization (Fig. 2B) as did B and R at a lower level of  $k$  (Fig. 2C).

The content of MBC in CK soils did not vary over the 24 weeks of incubation. MBN in these soils increased over the first 3 weeks of incubation, and then decreased to levels significantly lower than the initial levels (Fig. 3). In the litter-amended soils, MBC increased with increasing incubation time, with generally higher values at week three, and MBN increased significantly at week three but thereafter decreased to levels similar to or lower than those at the start of incubation. After 24 weeks of incubation, all litter-amended soils had higher MBC and MBN contents than did CK. Although MBN did not differ among the amended soils, MBC was significantly higher in R and M than in other treatments. The ratios of MBC to MBN generally increased with increasing incubation time in litter-amended soils. However, for CK this ratio remained similar throughout the incubation period. Following 24 weeks of incubation, MBC:MBN ratios were lowest in CK and F, highest in R, and were significantly higher than their initial levels in all litter-amended treatments.

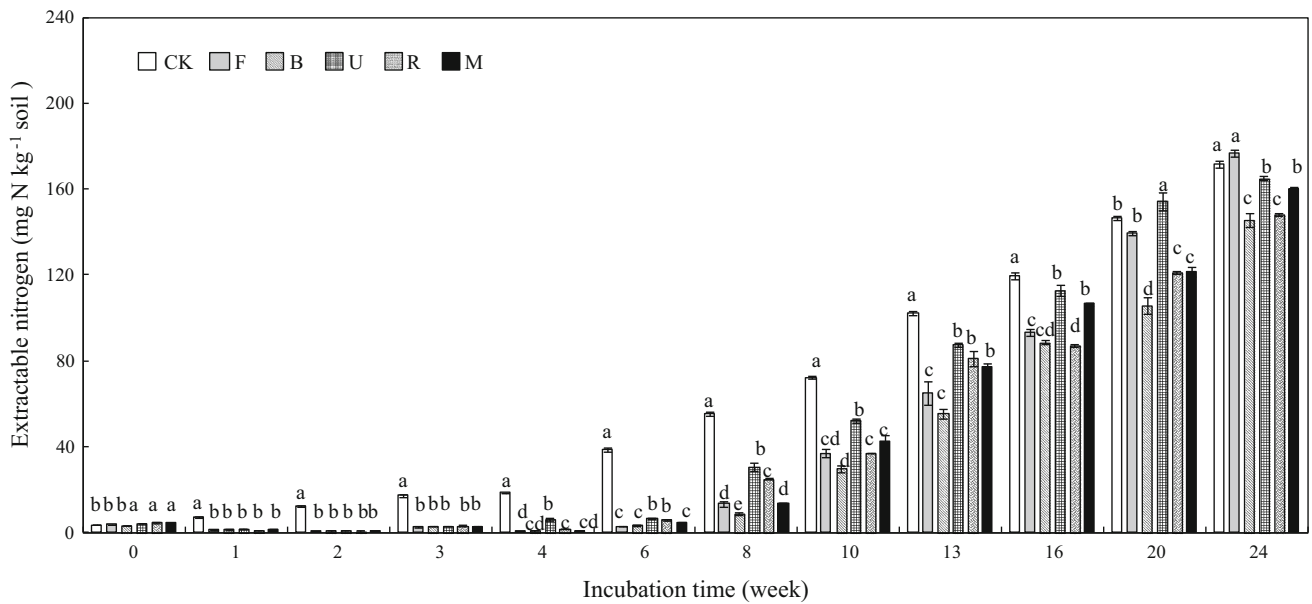
Compared to the initial levels, activities of L-asparaginase or *o*-DPO were generally higher at week 3 of the incubation, declined to lower levels at week 8, but returned to equivalent or higher than initial levels following subsequent incubation (Fig. 4). The highest activities of these two enzymes were at incubation week 3 or 24.

The impact of litter amendment on activities of these two enzymes varied during incubation (Fig. 4). At 24 weeks of incubation, activities of L-asparaginase were significantly higher than their initial levels in almost all litter-amended soils ( $P < 0.05$ ), except for B-treated soils. Trends for activities of *o*-DPO were different from those recorded for L-asparaginase. After equal durations of incubation, limited differences were observed among the

**Table 2** Variance analysis of the effects of litter amendment and incubation time on soil parameters<sup>†</sup>

Source of variance	D.f.	MBC	MBN	MBC:MBN	L-asparaginase	<i>o</i> -DPO	D.f.	Extractable N	pH	EC
Incubation Time (T)	4	$p < 0.001$	$< 0.001$	$< 0.001$	$< 0.001$	$< 0.001$	11	$< 0.001$	$< 0.001$	$< 0.001$
Litter Amendment (L)	4	$< 0.001$	0.002	$< 0.001$	0.671	0.012	4	$< 0.001$	$< 0.001$	$< 0.001$
T $\times$ L	16	$< 0.001$	0.012	$< 0.001$	0.541	0.008	44	$< 0.001$	$< 0.001$	$< 0.001$

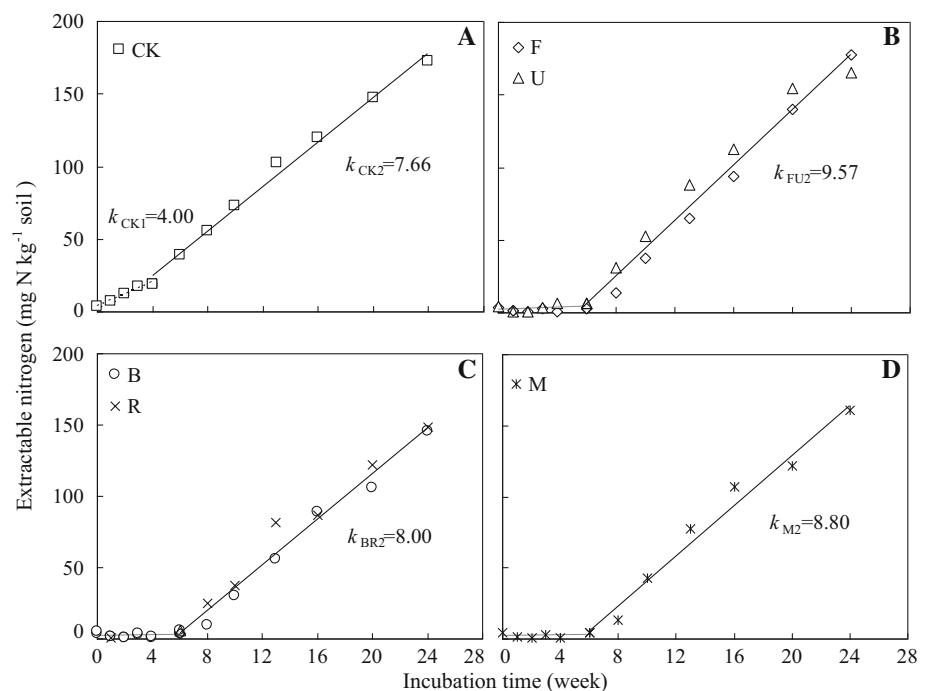
<sup>†</sup>Incubation times were 0–24 weeks, while litters included CK, F, B, R, U and M as defined in Table 1



**Fig. 1** Extractable-nitrogen (soil:2M KCl = 1:5) in the tested soils (Bars = SE). CK, untreated; F, foliage; B, branch; U, understory; R, root; and M, mixed litter of F, B and U with mass ratio of 1:1:1.

Different lower case letters indicate significantly different means among treatments of the same incubation time according to least significance test

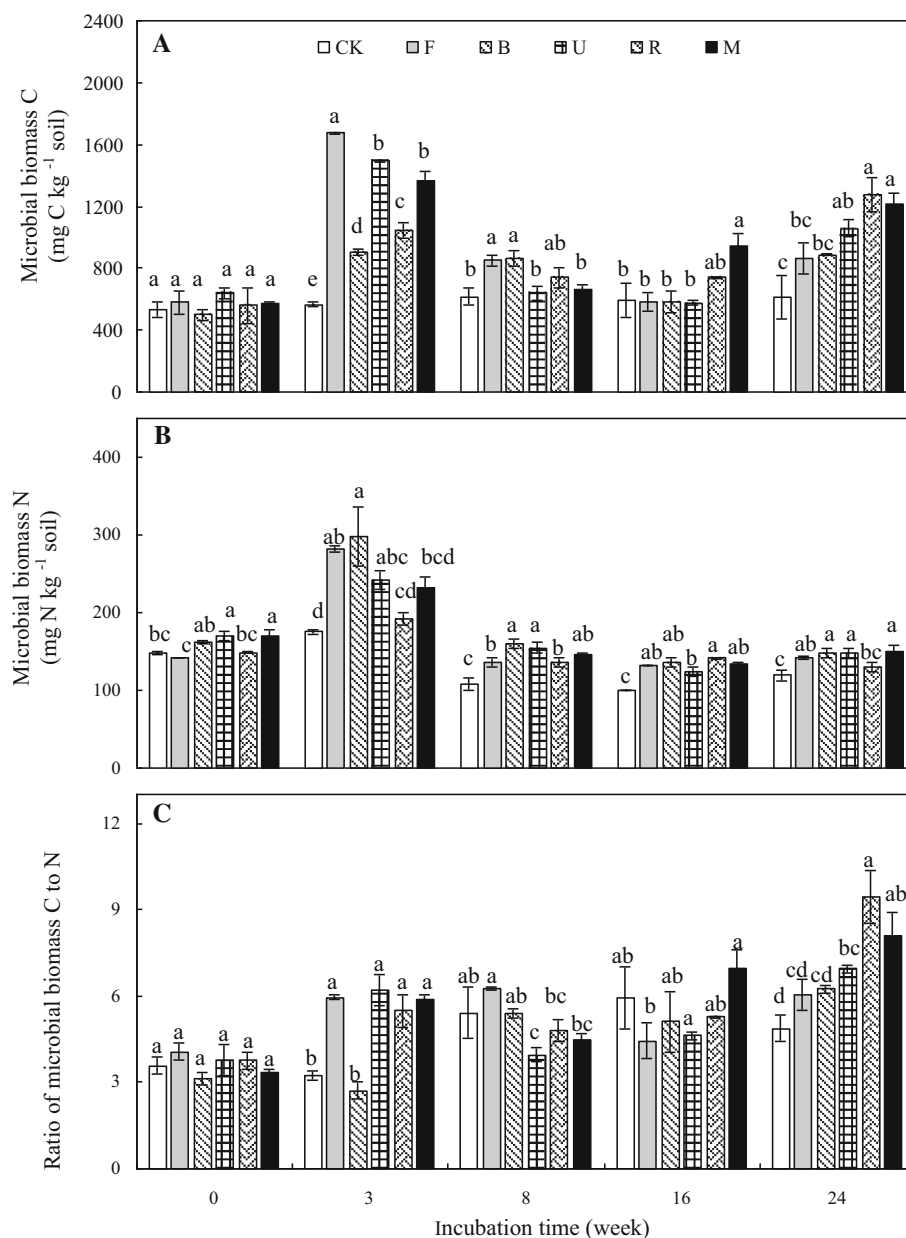
**Fig. 2** Net nitrogen mineralization in the tested soils (Bars = SE). CK, F, B, U, R and M were defined in Fig. 1;  $k_1$ , mineralization rate constant during incubation from 0 to 6 weeks;  $k_2$ , mineralization rate constant during incubation from 6 to 24 weeks



tested soils and the observed differences were often statistically insignificant. Similar to L-asparaginase, activities of *o*-DPO were highest for all treatments at week 3 of incubation. After 24 weeks of incubation, the activities of this enzyme in all tested soils, except for F, were significantly higher than their initial levels ( $P < 0.05$ ). The activity of *o*-DPO in M-treated soils was highest, and was significantly higher than in other tested soils ( $P < 0.05$ ).

Microbial biomass content was significantly and positively correlated with activities of L-asparaginase or *o*-DPO (Table 3). Although MBC was not significantly correlated with extractable N, pH or EC content, MBN was significantly and negatively correlated with extractable N and EC, but significantly and positively correlated with pH. The ratio of MBC to MBN was not significant correlated with activities of L-asparaginase or *o*-DPO, but was

**Fig. 3** Microbial biomass carbon (MBC) content (a) and nitrogen (MBN) content (b), and ratios of MBC to MBN (c) in the tested soils (Bars = SE). CK, F, B, U, R and M are defined in Fig. 1. Different lower case letters indicate significantly different means among treatments of the same incubation time according to least significance test

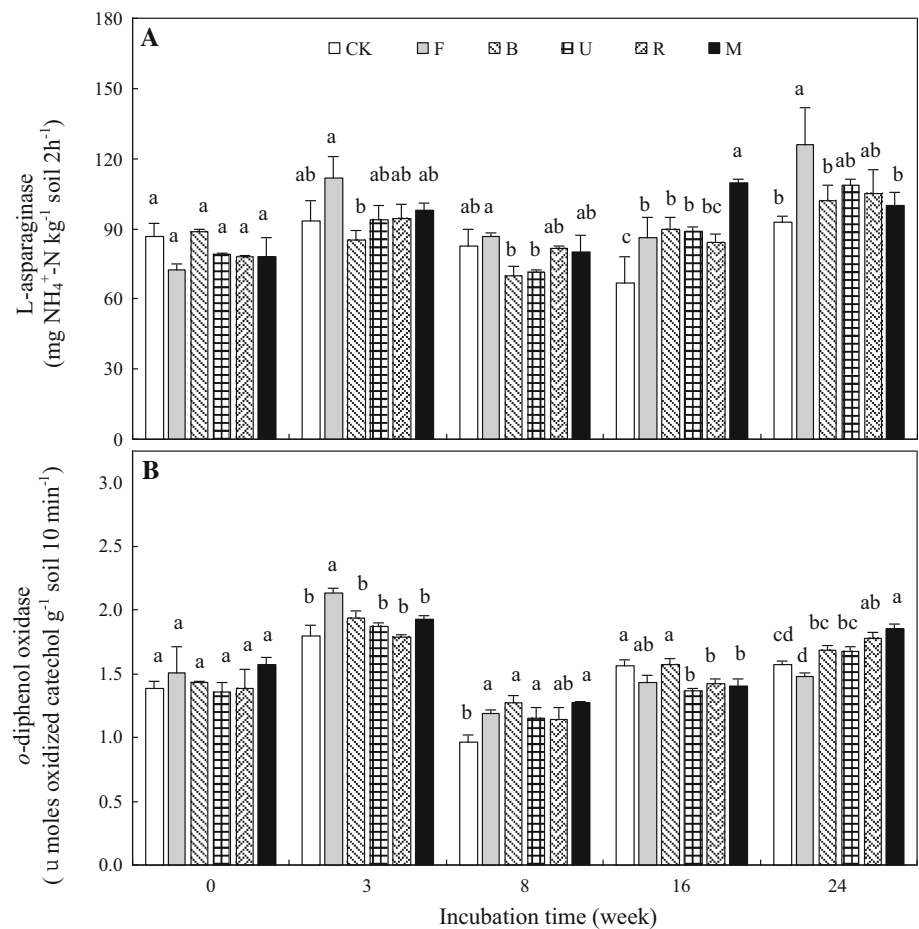


significantly and positively correlated with extractable N and EC, and significantly and negatively correlated with pH. However, activities of L-asparaginase were significantly and positively correlated with activities of *o*-DPO. Interestingly, activities of L-asparaginase were significantly correlated with extractable N, pH, and EC, but activities of *o*-DPO were not. Our results suggest that pH negatively influenced while extractable N and EC positively influenced activities of L-asparaginase.

## Discussion

The extractable N concentrations in litter-amended soils decreased during the first 4 weeks of incubation, suggesting enhanced N immobilization. During the first 16 weeks of incubation, extractable N concentrations were significantly lower in litter-amended soils than in the control. This showed the marked influence of N immobilization on extractable N content in these amended soils. The detected N immobilization was mainly due to microbial growth stimulated by the added litter (Tarafdar et al. 2001; He et al. 2014). We reach this conclusion because net N mineralization was observed in the untreated CK soil

**Fig. 4** Activities of L-asparaginase (**a**) and *o*-diphenol oxidase (*o*-DPO) (**b**) in the tested soils (Bars = SE). CK, F, B, U, R and M were defined in Fig. 1. Different lower case letters indicate significantly different means among treatments of the same incubation time according to least significance test



**Table 3** Correlation coefficient (*r*) between paired soil parameters (*n* = 216 for Extractable N, pH and EC; and *n* = 90 for MBC, MBN, MBC:MBN, L-asparaginase and *o*-DPO)<sup>†</sup>

	Extractable N	MBC	MBN	MBC:MBN	L-asparaginase	<i>o</i> -DPO	pH
MBC	0.05						
MBN	− 0.48***	0.45***					
MBC:MBN	0.40***	0.80***	− 0.14				
L-asparaginase	0.22**	0.26**	0.22**	0.12			
<i>o</i> -DPO	0.11	0.42***	0.55***	0.10	0.41***		
pH	− 0.66***	− 0.07	0.45***	− 0.37***	− 0.21**	− 0.06	
EC	0.94***	0.20*	− 0.38***	0.50***	0.31***	0.16	− 0.71***

<sup>†</sup>Extractable N, inorganic nitrogen extracted with 2 M KCl (soil:KCl = 1:5)

MBC microbial biomass carbon; MBN microbial biomass nitrogen; *o*-DPO *o*-diphenol oxidase; EC electrical conductivity

\*, \*\*, and \*\*\* indicate significant difference at *P* < 0.10, 0.05, and 0.01, respectively

throughout the incubation period and marked microbial growth was detected in the litter-amended soils.

Nevertheless, net N mineralization was detected from 6 to 24 weeks of incubation in all soils, suggesting the predominance of gross N mineralization over immobilization. Mineral N release rates are closely associated with litter

C:N ratios. Empirically, no net mineralization or immobilization in soil can be observed when C:N ratios of the added plant residues range from 20 to 25 (Probert et al. 2005; Myrold 2005). The soil used in this study had a C:N ratio of 21.6 which increased up to 25.7 following the addition of litter. The microbes prefer to utilize the easily

available litterfall-derived C source for their metabolism during the soil organic matter mineralization process (Potthast et al. 2010). Therefore, the observed net N mineralization in these soils might suggest that much of the C in the soil and litter occurred in recalcitrant C complexes that were not readily available for microbial growth.

Moreover, previous research has shown that bacterial C:N ratios averaged 4, while fungal C:N ratios were as high as 15, with a typical soil MBC:MBN ratio of 8 (Myrold 2005). Therefore, the ratio of MBC:MBN might indicate relative abundance of bacteria and fungi in the microbial community. Thus the shift in microbial community composition toward greater fungal dominance, which was speculated on the increase in soil MBC:MBN ratios with incubation time (Fig. 3c), would also result in the release of N from the excess N immobilized in the bacterial community. Wang et al. (2014) also reported that leaf-litter addition stimulated soil microbial activity and decreased the ratio of bacteria to fungi as a result of greater promotion on fungal growth.

The increased C:N ratios in litter-amended soils resulted in increased N immobilization and reduced extractable N content. The higher the C:N ratio of the litter was, the greater the demand for N to support microbial growth, and the higher N immobilization was expected. Of the litter types tested, B had the highest C:N ratio. This explains the low net N mineralization rates and the lowest extractable N concentration that were recorded for B-treated soils following 24 weeks of incubation. In general, the extractable N content was inversely related to C:N ratios. Mondini et al. (2008) and Kooijman and Martinez-Hernandez (2009) also found this relationship between soil N mineralization and the C:N ratio of organic matter amended in soils.

It has long been recognized that microorganisms are the driving force of organic matter decomposition and N mineralization. However, litter addition altered substrate quality, which in turn would impact microbial diversity and community structure as well as associated processes. In this study, litter addition resulted in significant increase in the MBC:MBN ratios, which suggested its favorable effect on fungal over bacterial growth. Of the litter types evaluated, roots most favored fungal growth, with MBC:MBN ratios increased from 3.5 to 9.4 in the 24 weeks of incubation. Aside from proliferation of the fungal community due to changes in organic matter quality and increasing proportions of recalcitrant substances during incubation, changes in soil pH from 7.9 to 7.3 (data not shown) might also be a key factor facilitating the shift, because the soil acidifying process probably resulted in the unfavorable conditions for bacteria and actinomycetes, while was favorable for fungal growth (Kaur et al. 2008; Kooijman and Martinez-Hernandez 2009; Stevenson et al. 2014).

Mineralization of N could also result from enzymatic processes. Some studies reported that soil enzyme activities were the chief factor dominating the mineralization process by enzymatic reactions (Deng et al. 2000; Yang et al. 2012). Our results suggest that activities of L-asparaginase, which plays a key role in N mineralization by catalyzing the hydrolysis of L-asparagine, contributed more to N mineralization than did activities of *o*-DPO, as evidenced by the significant positive correlations between extractable N and activities of L-asparaginase but not activities of *o*-DPO. On the other hand, activities of *o*-DPO have been shown to be involved in catalyzing recalcitrant and complex substances (Perucci et al. 2000). Of the litter types we tested, M had the most complex substrates and also exhibited significantly higher activity of *o*-DPO. Our results also suggest that mixed litters promoted growth of diverse microbe assemblages and stimulated synthesis of *o*-DPO. The limited differences in its recorded activities among soils at the same incubation times might reflect similarity in the source and stability of this enzyme in the tested soils.

It is generally accepted that most soil enzymes originate from microbes and that much of the detected enzymatic activity is contributed by accumulated enzymes that are free of microbial cells (Tabatabai 1994). The significant positive relationship between microbial biomass and activities of L-asparaginase or *o*-DPO demonstrated the close relationships between these two enzymes and microbial communities. Since fungi were favored by lower soil pH, the negative relationship between activity of L-asparaginase and pH might suggest that a greater proportion of L-asparaginase originated from fungi than bacteria.

Our results showed increases for both enzymatic activities for most litter-amended soils throughout 24 weeks of incubation (Fig. 4). This indicates that litter amendment favored a general increase in the soil capacity to transform and degrade organic matter and indirectly stimulated N mineralization.

Poplar tree and understory litters are important organic matter sources to soil in poplar plantation ecosystems. Our previous investigation showed that aboveground poplar litter biomass was approximately  $5 \text{ t ha}^{-1} \text{ a}^{-1}$ , and the biomass of understory litter was also up to  $4.35 \text{ t ha}^{-1}$  at our study site (data not published). Most poplar litter was produced during the non-growing stages of trees, while understory might be cut and covered in situ during the growing stages to reduce nutrient competition from understory assimilation. However, we found that the extractable N concentrations in litter-amended soils decreased during the first 4 weeks of incubation (Fig. 1). Therefore, such management of understory during the growing season may result in net N immobilization and lead to N limitation of tree growth. For these reasons, we

recommend not to cut understory but to retain it in situ during tree growth stages.

Soil mineral N was released or partly immobilized by microorganisms throughout laboratory incubation. The net increase (control subtracted) in total mineral N in litter-amended soils at the end of the incubation period was greatest for F-treated soils at  $28.5 \text{ mg kg}^{-1}$ , followed by U treatment with  $2.5 \text{ mg kg}^{-1}$ , suggesting that approximately 37 or  $3.3 \text{ kg N ha}^{-1}$ , respectively, could be released from net N mineralization in foliage or understory litter-amended soils during the 24 weeks of incubation period. B, R and M treatments showed net declines in total mineral N (control subtracted) following 24 weeks of incubation, suggesting that poplar foliage and understory litter additions could be more advantageous to stimulate soil N mineralization and net mineral N release. Retaining litter, especially poplar leaves and understory litters, could be an effective management strategy to enhance productivity of poplar plantations for greater economic benefit.

## Conclusions

Litter amendment impacted N dynamics and availability in soil. Net N mineralization was detected in all litter amended soils, signifying the predominance of mineralization over immobilization, especially, soil amended with poplar foliage and understory litters released more mineralization N, suggesting that poplar foliage and understory litter amendments were more favorable to soil organic matter mineralization, comparing with other litters in poplar plantation ecosystem. In general, the extractable N content and net N mineralization rate constants were inversely related to litter C:N ratios. Addition of litter to soils might lead to a shift of microbial groups toward greater fungal dominance. The activities of soil enzymes responded differently to the different plant litter types, but as a whole indicated an increased capacity of soil to mineralize N. In conclusion, different litter types promoted microbial growth and brought about temporal changes in N transformations and availability. This highlights the need for targeted efforts to better manage poplar tree and understory litters for sustainable and more profitable operation of poplar plantations. Continuous and intensive researches of N mineralization amended litters in poplar plantations are also necessary to understand processes and factors of N mineralization, and explore more management strategies to support sustainable production in the future.

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## Compliance with ethical standards

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

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