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Effects of single Chinese fir and mixed leaf litters on soil chemical, microbial properties and soil enzyme activities

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Abstract

The quality of leaf litter can control decomposition processes and affect the nutrient availability for plant uptake. In this study, we investigated the effect of single leaf litter (Chinese fir – *Cunninghamia lamcealata* (Lamb.) Hook) and mixed leaf litters (*C. lamcealata*, *Liquidamba formosana* Hance and *Alnus cremastogyne* Burk) on soil chemical properties, soil microbial properties and soil enzyme activities during 2 years decomposition. The results showed that soil microbial biomass C, the ratio of soil microbial biomass C to total soil organic C (soil microbial quotient, Cmic/Corg) and soil enzymes (urease, invertase, dehydrogenase) activities increased significantly in mixed leaf litters treatments whereas soil chemical properties remained unchanged. However, soil microbial metabolic quotient (qCO_2) values and soil polyphenol oxidase activity were higher in the single Chinese fir leaf litter treatment that had a higher C:N (carbon:nitrogen) ratio (79.53) compared with the mixed leaf litter (C:N ratios of 76.32, 56.90, 61.20, respectively). Our results demonstrated that the mixed leaf litter can improve forest soil quality, and that soil microbial properties and soil enzyme activities are more sensitive in response to litter quality change than soil chemical properties.

Introduction

In forest ecosystems, litter turnover provides a significant source of nutrients for plant and soil microbial uptake. Litter quality, such as nitrogen or lignin content, has been shown to be important in influencing decomposition rates, litter decomposers and soil C, N dynamics (Motavalli, 2006; Mungai and Wardle et al., 2006; Schwendener et al., 2005; Wardle et al., 2003).

Soil biotic activity is the driving force in the transformations of litter to soil organic matter, and development and maintenance of soil structure (Dick, 1992). Factors affecting soil microbial activity, such as temperature, moisture and litter quality, play essential roles in the maintenance of nutrient pools, especially through the formation and decomposition of soil organic matter (Wardle et al., 1999). Most studies about forest litter are about single leaf litter decomposition, and small number of studies used mixed leaf litter (Gartner and Cardon, 2004). Less understood are the effects of mixed litter on the activity of soil microbial biomass and soil enzymes during leaf litter decomposition processes (Christine and Dighton, 2000; Maithani et al., 1998).

Soil microbial biomass is a sensitive indicator of changes in the quantity and quality of soil organic matter (Jagadish et al., 2001; Lundquist et al., 1999). Microbial biomass and its contribution to nutrient concentration in forest soils were reported by Díaz-Raviña et al. (1993). The ratio

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of microbial biomass carbon to total organic carbon (Cmic/Corg) in soil can serve as a quantitative indicator of carbon dynamics in the soil (Insam et al., 1989). Vance and Chapin (2001) found that a lower Cmic/Corg value could imply that there is lower substrate availability in forest soils corresponding with their higher C:N. The metabolic quotient (qCO_2) has been applied to soil microbial biomass analyses in maintenance energy investigations, in studies on the effect of temperature, in comparisons of field managements, soil variables, in ecosystem successions and in studies on heavy metal stress (Anderson and Domsch, 1993).

Microorganisms produce enzymes that catalyze all biochemical reactions and this process is an integral part of nutrient cycling in the soil (Bandick and Dick, 1999). It has been proposed that measurement of changes in soil enzyme activities may provide a useful index of change in soil quality (Acosta-Martínez and Tabatabai, 2000). Soil enzymes have been suggested as potential indicators of soil quality because of their relationship to soil biology, ease of measurement, and rapid response to changes of soil management (Bandick and Dick, 1999). Enzymes activities may respond to changes of forest management more quickly than other soil variables and therefore might be useful as early indicators of biological changes.

Chinese fir (*Cunninghamia lamcealata* (Lamb.) Hook) is one of the most important plantation tree species in China in terms of area, yield and other forest resources uses. However, plantation productivity decline and soil degradation have negatively impacted monoculture Chinese fir stands (Chen et al., 1990). In order to preserve long-term productivity and restore soil fertility in degraded stands, many studies had been carried out in pure and mixed Chinese fir plantations (Huang et al., 2004; Wang et al., 1997; Yang, 1998; Zheng and Ding, 1998). It was reported that the possible advantage of mixed forests on tree growth and maintaining soil fertility lies in the great amount of litter from the broadleaf and coniferous trees, which can improve the nutrient status and the conditions for soil microorganisms (Zheng and Ding, 1998). The combinations of Chinese fir leaf litters with other broadleaf leaf litters can enhance litter decomposition and nutrient availability (Liao et al., 1997, 2000) and ammonium uptake for Chinese fir (Huang et al., 2002).

We hypothesized that diverse litter mixtures affect both the activity of the microbial biomass and their enzyme production. These changes may result from increased substrate availability afforded in the litter mixtures. Litter mixtures may affect substrate quality by providing a diverse array of substrates or higher N content not typically found in monotypic litters, especially of poorer quality litters such as Chinese fir. In this study, we examined the effect of litter quality in Chinese fir litter and mixtures of Chinese fir and broadleaf tree leaf litters on soil microbial biomass C, soil microbial quotient (Cmic/Corg), metabolic quotient (qCO_2) and the activity of four soil enzymes (urease, invertase, dehydrogenase, polyphenol oxidase) during 2 years decomposition.

Materials and methods

Site description and soil sampling procedure

This experimental site is located at Huitong Experimental Station of Forest Ecology, Chinese Academy of Sciences, in Hunan Province (109°36′ E, 26°51′ N), China. The site is 300–500 m in elevation with a mean annual temperature about 16.5 °C and annual rainfall of 1100–1300 mm. The clay loam soil (sand 29.35%, silt 45.53%, clay 25.12%) in this study is classified as a *paddy* soil and belongs to the *Anthrosols* series (Soil Database of China, http://www.soil.csdb.cn).

This experiment was carried out in croplands because it is difficult to choose a perfect plot in the Chinese fir plantation stand. Three near croplands were selected and each was divided into four subplots (each subplot was 1.5×14 m, with 1 m buffer zone between subplots). Leaf litter was collected from different plantation stands using litter traps in each month during 2002, and air-dried at room temperature before the experiment was carried out. The different litter treatments consisted of *Cunninghania lancceolata* (Fir), a mixture of 1/2 *C. lancceolata* and 1/2 *Liquidamba formosana* Hance (Mixed 1), a mixture of 1/2 *C. lancceolata* and 1/2 *Alnus cremastogyne* Burk (Mixed 2) and a mixture of 1/3 *C. lancceolata*, 1/3 *A. cremastogyne* and 1/3

L. formosana (Mixed 3). The leaf litter (540 g m^{-2}, according to the quantity of annual leaf litter fall in Chinese fir stand) was applied to soil surface of each subplot annually for a 2 year period on 10–15 May 2003, 2004. The litter C and N of four different tree species were determined before this study was carried out. Then we calculated the C and N composition and C:N ratios of the four different treatments.

After removal of the litter, 10 surface soil (0–10 cm) cores in each subplot were collected randomly with a stainless steel auger (5 cm diameter) on 28 April and 24 October 2004. Soils sampled from the same subplot were bulked and divided into two portions. One sub-sample was air-dried for 48 h at room temperature (22 °C) and ground to pass a 2-mm sieve for chemical analyses and enzyme activity analyses. The other sub-sample was sieved moist through a 2-mm sieve, and stored in plastic bags at 4 °C until soil microbial analyses were performed.

Analytical methods

Total organic carbon (TOC) was determined by Elementar High II TOC (Elementar, Germany). Total N was determined by semi-micro Kjeldahl digestion procedure and total P was determined by molybdenum blue colorimetry following H_2SO_4–$HClO_4$ digestion (GB7173-87, GB9837-88) as described by Liu (1996). Leaf litter C was determined by dichromate–sulphuric acid oxidation and litter N was determined by indophenol blues colorimetry following H_2SO_4–$HClO_4$ digestion (GB7888-87, GB7886-87) as described by Dong (1997). Soil microbial biomass C (Cmic) was determined by the chloroform fumigation-extraction method (Vance et al., 1987). Three replicate 25-g portions of soil were weighted into 100-mL beakers and fumigated with ethanol-free chloroform for 24 h at 25 °C. After fumigant removal the soil was extracted with 100 mL 0.5 M K_2SO_4 for 30 min. Additional unfumiganted soil was extracted similarly to determine background soluble C levels. The organic C in the soil extracts was measured by dichromate oxidation and the soil microbial biomass C was calculated by: $Cmic = EC/k_{EC}$, where EC is organic C extracted from fumigated soil minus organic C extracted from unfumigated soil, $k_{EC} = 0.38$ (Lu, 1999).

Soil basal respiration was determined by measuring CO_2 evolution (Xu and Zhen, 1986). Field-moisture soil samples (equivalent to 20 g oven-dry soil) were placed in gauze and incubated in 500-mL air-tight glass vessels at 28 °C for 48 h. The CO_2 evolved from the soil was absorbed in 15 mL 0.1 M NaOH and the unconsumed base titrated with 0.1 M HCl following addition of $BaCl_2$. The metabolic quotient (qCO_2) was calculated by dividing the hourly basal respiration rate by the corresponding Cmic. The microbial quotient was calculated by dividing Cmic by the corresponding soil total organic carbon (Corg).

The activities of the enzymes were assayed on the < 1 mm air-dried samples at their optimal pH values in duplicates and one control, and expressed on a moisture-free basis according to the methods given by Guan (1986). Urease activity (EC 3.5.1.5) was determined using urea (10% w/v) as the substrate, incubating the soil sample for 24 h at 37 °C and measuring the NH_3 released colorimetrically at 578 nm. Invertase activity (EC 3.2.1.26) was determined using sucrose as the substrate. Five gram of soil, 15 mL 8% sucrose and 5 mL phosphate buffer (pH 5.5) were added to a 50-mL flask, and then incubated for 24 h at 37 °C. After filtration, 1 mL of filtrate was added to a 50-mL flask and heated for 5 min with 3 mL 3,5-dinitrosalicylic acid (DNS). The color was measured by colorimetric assay at 508 nm. For dehydrogenase, 6 g soil was weighed into a 50-mL flask followed by 0.07 g $CaCO_3$, 1 mL of 1% 2,3,5-triphenyltetrazolium chloride (TTC) and 2.5 mL deionized water. After 24 h of incubation at 30 °C, triphenylforman was extracted with four successive portions of methanol totaling 100 mL. The color of the filtrate was determined at 460 nm using methanol as a blank. For polyphenol oxidase (EC 1.10.3.1), 1 g dry, sieved soil (< 0.25 mm) was added to a 50-mL flask followed by10 mL 1% pyrogallol solution. After incubated at 30 °C for 2 h, 4 mL citrate-phosphate buffer (pH 4.5) and 35 mL aether were added into the flask, and extracted for 30 min. The color was determined colorimetrically at 430 nm.

Statistical analyses

Unless otherwise stated, results are expressed on the basis of the oven-dry (105 °C, 48 h) weight

of the material. Means ($n = 3$) and standard errors of the means were calculated for total organic C, total N and so on. LSD analysis was also carried out, followed by Student t test at $P < 0.05$ in SPSS 10.0 software package.

Results

Leaf litter C, N and C:N ratios

Mixtures of Chinese fir leaf litter with the other broadleaf tree leaf litter changed the leaf litter C, N and C:N ratios of the four different treatments of Fir, Mixed 1, Mixed 2 and Mixed 3 (Table 1). The leaf litter C:N ratios of four treatments decreased in the following order: Mixed 2 < Mixed 3 < Mixed 1 < Fir.

Soil TOC, total N and total P

The results showed that there was no significant effect of different litter treatments on the soil total organic C and N in 0–10 cm soil as expected in a short-term study (Table 2). However, there was a trend of higher total soil N in the mixed litter treatments Mixed 1, Mixed 2 and Mixed 3 in the October sampling. The total soil P in mixed litters treatments was significantly higher in the Mixed 1 and Mixed 2 treatments compared to the Fir treatment.

Soil microbial properties

Microbial biomass carbon (MBC) in 0–10 cm soil was significantly higher in the mixed litter treat-

Table 1. Litter composition, C, N and C:N ratios of different leaf litter treatments

Treatments	Litter composition	Quantity of litter (g m^{-2})	C (g m^{-2})	N (g m^{-2})	C:N
Fir	(*C. lancceolata*)	540	242.1	3.04	79.53
Mixed 1	(*C. lancceolata)* + (*L. formosana*e)	270 + 270	230.82	3.02	76.32
Mixed 2	(*C lancceolata*) + (*A. cremastogyne*)	270 + 270	235.88	4.15	56.9
Mixed 3	(*C.lancceolata*) + (*A.cremastogyne*) + (*L. formosana*)	180 + 180 + 180	230.43	3.77	61.2

Table 2. Effects of mixed leaf litters on soil total organic carbon (TOC), total N and total P

Treatments	Total organic C (g kg^{-1})		Total N (g kg^{-1})		Total P (g kg^{-1})	
	Apr.	Oct.	Apr.	Oct.	Apr.	Oct.
Fir	16.31a	16.02a	1.68a	1.84a	0.31b	0.37b
Mixed 1	16.59a	15.84a	1.71a	1.82a	0.32ab	0.39a
Mixed 2	16.41a	15.73a	1.68a	1.86a	0.35a	0.37ab
Mixed 3	16.59a	16.26a	1.69a	1.85a	0.31b	0.37b

The different letter in the same column indicated that there was significant difference between different treatments ($P < 0.05$, $n = 3$). LSD is used in multiple comparisons.

Table 3. Effects of mixed leaf litters on soil microbial biomass C (MBC), basal respiration (BR), metabolic quotient (qCO_2) and microbial quotient (Cmic/Corg)

Treatments	MBC (mg kg^{-1})		BR (μg CO_2–C g^{-1} h^{-1})		qCO_2 (μg C mg $C_{bio}{}^{-1}$ h^{-1})		Cmic/Corg (%)	
	Apr.	Oct.	Apr.	Oct.	Apr.	Oct.	Apr.	Oct.
Fir	559.00c	558.27b	0.40a	0.44a	0.71a	0.80a	3.42c	3.47b
Mixed 1	714.81b	697.14ab	0.31bc	0.43a	0.43b	0.64b	4.32b	4.37ab
Mixed 2	917.406a	766.26ab	0.27c	0.47a	0.30c	0.61b	5.59a	4.87a
Mixed 3	919.03a	813.79a	0.33b	0.44a	0.36d	0.53b	5.42a	5.11a

ments compared to the Fir treatment (Table 3). The Cmic values increased in the following order: Mixed 3 > Mixed 2 > Mixed 1 > Fir. Soil basal respiration was significantly higher in the Fir than mixed litter treatments for the April sampling. There was no difference in soil basal respiration among treatments for the October sampling. The metabolic quotient (qCO_2) in mixed litter treatments was lower than the Fir treatment. The lowest qCO_2 values occurred in the Mixed 2 treatment. The microbial quotient (Cmic/Corg) exhibited the opposite trend with the mixed litters having higher values than the Fir treatment. The highest Cmic/Corg values occurred in the Mixed 2 treatment.

Soil enzyme activities

Soil urease, invertase and dehydrogenase activity were lowest in the Fir treatment compared to the mixed litter treatments (Table 4). In the mixed litters, these enzyme activities seemed to be the highest in the Mixed 2 treatment. Soil invertase activity was significantly lower in the April sampling compared to October. Soil dehydrogenase activity was significantly higher in April.

Soil polyphenol oxidase activity in the April sampling of the Fir treatment was significantly higher than the Mixed 3 treatment. There was no significant difference in polyphenol oxidase activity among treatments sampled in October. Overall, the polyphenol oxidase activity was higher in all treatments in October compared to the April sampling.

Discussion

The results in our study suggested that mixed leaf litter have no effect on soil organic C and soil N but have large effects on litter quality, soil microbial biomass C, microbial quotient (Cmic/Corg), metabolic quotient (qCO_2) and soil enzyme activity during 2 years decomposition. There was no statistically significant difference in total soil organic C and soil N during 2 years decomposition. This trend is consistent with results from Montagnini (2000), who found that there were no significant difference in soil organic carbon C and soil N between pure and mixed plantations. However, more recent comparative studies revealed that soil organic matter and other soil nutrients were enhanced when broadleaf trees were present in pure Chinese fir plantation stands (Wang et al., 1997; Yang, 1998; Zheng and Ding, 1998).

The effect of mixed leaf litters on soil microbial biomass C also reported by Bardgett and Shine (1999), who showed that the level of species diversity of litter significantly affected the size of the soil microbial biomass, accounting for 83% of the total variance in their study. Piao et al. (2006) also reported that soil microbial biomass C was significantly higher in the litter-amended soil than the controls. Although Maithani et al. (1998) didn't show any significant effects of mixed litter on soil microbial biomass C, they found that litter phenotype affected litter chemistry (such as C:N ratios, lignin, phenolic, condensed tannin) and litter chemistry accounted for 2.59% of the variation in microbial biomass C. In our study, we found that soil microbial

Table 4. Effects of mixed leaf litters on soil urease activity, invertase activity, dehydrogenase activity and polyphenol oxidase activities

Treatments	Urease (NH_3–N mg g^{-1}soil, 37 °C, 24 h)		Invertase (Glucose mg g^{-1}soil, 37 °C, 24 h)		Dehydrogenase (H^+ μL g^{-1}soil, 30 °C, 24 h)		Polyphenol oxidase (Gallic acidic mg g^{-1} soil, 30 °C, 2 h)	
	Apr.	Oct.	Apr.	Oct.	Apr.	Oct.	Apr.	Oct.
Fir	0.120c	0.110c	6.58c	8.98b	312.43c	115.92b	0.169a	0.235a
Mixed 1	0.125ab	0.132ab	7.64b	11.16a	340.69bc	141.76ab	0.162ab	0.254a
Mixed 2	0.128a	0.135a	7.99a	11.43a	377.94a	162.72a	0.155ab	0.217a
Mixed 3	0.121bc	0.129bc	7.61b	11.04a	352.76ab	154.25a	0.147b	0.207a

biomass C and Cmic/Corg increased but the qCO_2 decreased in mixed leaf litters treatments. This result was consistent with Bardgett and Shine (1999) who found that the metabolic quotient was significantly affected by change in litter diversity, being lower at higher levels of litter diversity. Anderson and Domsch (1993) also reported that qCO_2 value decreased in mixed (*Fagus–Quercus*) forest ecosystem compared with simple (*Fagus*, *Picea*) forest ecosystem.

The changes in soil enzyme activity demonstrated that leaf litter quality can affect soil enzyme activities during two years decomposition in our study. Many studies had reported that litter quality could influence enzyme activities in soil or litter during little decomposition (Christine and Dighton, 2000; Kourtev et al., 2000). Christine and Dighton (2000) investigated the effects of single and mixed leaf litter on ectomycorrhizal community structure and acid phosphatase activity, and found that there was a significant litter × mycorrhizal interaction for phosphatase activity. Kourtev et al. (2000) reported soil enzyme activities changed significantly after different litters (straw; straw plus 90 kg N ha^{-1}; straw plus pea vine; straw plus manure) were added into soils. Huang et al., 2004, data showed that soil urease, protease, catalase, acid phosphatase and dehydrogenase activities increased significantly in mixed plantation of Chinese fir with broadleaf trees whereas total organic matter remained unchanged.

The increased soil microbial biomass C, Cmic/Corg and soil enzyme activities (urease, invertase and dehydrogenase) of underlying soil in mixed leaf litter may have been caused by drop as diverse substrate availability as compared with single Chinese fir leaf litter. There are large effects of mixed leaf litter on patterns of mass loss, changes in nutrient concentration, and decomposer abundance and activity (Gartner and Cardon, 2004; Kaneko and Salamanca, 1999). Differences in resource quality between litter species have been postulated to explain why mixed litters may decompose at a different rate to that which would be predicted from single species litters (termed 'non-additive effects'). In particular, positive, non-additive effects of mixed litter on decomposition have been explained by differences in initial nitrogen concentration between litter species. This interpretation is confounded because litter species that differ in nitrogen content also differ by a number of other resource quality attributes (Smith and Bradford, 2003). The mixture of Chinese fir leaf litters with broadleaf tree can improve leaf litter quality of four different treatments (Table 1). *A. cremastogyne* (a N-fixing tree species) has the highest nitrogen concentration and lowest C:N ratio among four different tree species in our study. Mixed Chinese fir leaf litter with nutrient-rich litter can enhance litter decomposition rate and nutrient availability (Liao et al., 1997, 2000; Quested et al., 2005). The diverse substrate availability underlying mixed leaf litter may contribute to the increase of soil microbial biomass C and Cmic/Corg. Because soil enzymes are believed to be primarily microbial origin (Bandick and Dick, 1999), the increase of soil urease, invertase and dehydrogenase activities in mixed leaf litters of Chinese fir with broadleaf trees may be due to the higher soil microbial biomass in mixed leaf litters compared to single Chinese fir leaf litter.

The increase of soil qCO_2 value and soil polyphenol oxidase activity in Fir treatment may be due to the poor quality of Chinese fir leaf litter (highest C concentration and highest C:N ratio among four different tree species) and phenolics accumulated in the surface soil. Two possible mechanisms may explain the decrease of soil qCO_2 value in mixed leaf litter treatments. First, as broadleaf leaf litters were added, diverse substrates were incorporated into underlying soil and affected soil microbial community structure. Dilly and Munch (1996) proposed that the differences of qCO_2 value during the course of litter decomposition were due to the shift of litter decomposer. Another possible mechanism might be the adverse effects of polyphenolic materials produced during the process of Chinese fir litter decomposition (He et al., 2003; Huang et al., 2000). These phenolics might stimulate the synthesis of polyphenol oxidase in the Chinese fir stands. Castells and Peñuelas (2003) demonstrated the positive relationship between phenolic compound concentration and polyphenol oxidase activity. The lower C:N ratio in mixed leaf litters might limit synthesis of soil phenolic compounds and polyphenol oxidase in our study.

The changes of soil microbial properties and soil enzyme activities in mixed leaf litters treatments suggested that mixture of Chinese fir leaf

litter with broadleaf tree leaf litter can influence soil quality through supplying diversity substrates, promoting soil nutrient availability and improving soil conditions, although there was no significant difference in total soil organic C and soil N. During early stages of decomposition, soil microbial properties can change more sensitively than the quantity of soil organic matter. The results in our study implied that the change of leaf litter quality in mixed plantation stands may contribute to soil nutrient improvement, but this effect may need a relatively long time. Adding broadleaf tree species in Chinese fir plantation stand may help maintain and promote soil fertility and production in plantations.

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