Labile soil organic carbon and microbial activity in three subtropical plantations

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The effects of tree species on soil properties have been attracted much attention, but the specific responses of labile soil organic carbon (SOC) and microbial activity to changes in tree species of subtropical forest ecosystems remain unknown. In this study, we investigated the labile fractions of SOC from three different single species plantations, namely Pinus massoniana (PM), Cinnamomum camphora (CC) and Schima superba (SS) in subtropical China. Specifically, we analysed the soil microbial biomass C (MBC), dissolved organic C (DOC) and permanganate-oxidizable C (POC), soil respiration, and activities of six enzymes in surface mineral soil (0–20 cm). The MBC, POC and soil respiration, as well as the activities of urease, acid phosphatase and polyphenol oxidase significantly differed among the three plantations in the study. In contrast, changes in the DOC as well as the activities of invertase, catalase and cellulase were not significant. The CC soil exhibited the highest POC, DOC and urease activity. PM soil had the highest MBC, soil respiration and polyphenol oxidase activity, but also had the lowest POC, qCO₂, urease activity and acid phosphatase activity. DOC and MBC were significantly correlated with the soil respiration. Urease activity was significantly related to the SOC fractions, except for MBC. Invertase and polyphenol oxidase activities were correlated with MBC. The results suggested that the tree species had different effects on the labile SOC and microbial activity and the observed differences seemed not to be explained by the differences in the litter quality.

Introduction

Forest soil, at least its organic pool, is a finite and non-renewable system that plays a significant role in plant growth and C sequestration in terrestrial ecosystems (Huber et al. 2001; FAO 2006). However, most natural forests have been replaced by plantations because of the increasing demand for forest products. It is important for afforestation to choose suitable tree species. The effects of tree species on the soil properties in these plantations must be fully understood for sustainable soil productivity (France et al. 1989; Kulmatiski et al. 2008). Previous studies mainly focused on soil physicochemical properties (Augusto et al. 2002; Lovett et al. 2004). However, the effect on labile soil organic carbon (SOC) and microbial activity are not well understood.

Tree species affect SOC pools and stabilization (Wang and Wang 2007; Kiikkitil et al. 2011). However, the short-term total SOC changes are slow and difficult to detect because of the high background value of SOC. High turnover rates and lower residence times produce labile SOC fractions, including the microbial biomass C (MBC), dissolved organic C (DOC), and permanganate-oxidizable C (POC); these SOC fractions are more sensitive to changes in tree species or land use than the total SOC (Six et al. 2002; von Lützow et al. 2007). Inconsistent effects of tree species on the labile SOC fractions have been recorded (Yano et al. 2000; Strobel et al. 2001; Smolander et al. 2005; Xing et al. 2010; Smolander and Kitunen 2011; Wang and Wang 2011). In addition, most of these studies have been conducted in boreal or temperate forests, and few in subtropical forests. Therefore, more studies should be performed in subtropical forests to obtain better insight into the influence of tree species on labile SOC.

The soil microbial activity is often measured as soil respiration, which reflects the SOC decomposition (Waldrop et al. 2004; Zak et al. 2008). Previous studies have reported that the tree species can affect soil microbial activity. However, reports on the response of soil respiration to different tree species have contradictory results (Chodak and Niklinska 2010; Weand et al. 2010; Vesterdal et al. 2012). Soil extracellular enzymes produced by microbes are also vital to organic matter decomposition and nutrient cycling. The activities of soil enzymes follow inconsistent response patterns to the existing tree species (Sicardi et al. 2004; Acosta-Martínez et al. 2007; Ushio et al. 2010). Therefore, better understanding of the responses of soil microbes and enzymes to the existing tree species may improve our ability to accurately predict the influence of afforestation on soil C and N dynamics.
Table 1  Leaf litter quality of three species from the corresponding monoculture plantations (per cent)

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>Lignin</th>
<th>C/N</th>
<th>C/P</th>
<th>Lignin/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinus massoniana</td>
<td>49.7</td>
<td>0.50</td>
<td>0.034</td>
<td>0.083</td>
<td>0.44</td>
<td>0.125</td>
<td>29.4</td>
<td>100.3</td>
<td>1462</td>
<td>59.4</td>
</tr>
<tr>
<td>Schima superba</td>
<td>48.9</td>
<td>1.53</td>
<td>0.090</td>
<td>0.754</td>
<td>0.81</td>
<td>0.198</td>
<td>35.5</td>
<td>31.9</td>
<td>546</td>
<td>23.2</td>
</tr>
<tr>
<td>Cinnamomum camphora</td>
<td>50.3</td>
<td>1.98</td>
<td>0.131</td>
<td>0.508</td>
<td>1.00</td>
<td>0.218</td>
<td>29.4</td>
<td>25.4</td>
<td>384</td>
<td>14.8</td>
</tr>
</tbody>
</table>

Table 2  Main physiochemical characteristics (mean ± standard error) of soils from the three subtropical plantations

<table>
<thead>
<tr>
<th>Forest type</th>
<th>PM</th>
<th>CC</th>
<th>SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOC (g kg⁻¹)</td>
<td>20.3 ± 1.4</td>
<td>27.6 ± 0.7</td>
<td>23.9 ± 0.7</td>
</tr>
<tr>
<td>Total N (g kg⁻¹)</td>
<td>1.51 ± 0.08</td>
<td>2.14 ± 0.36</td>
<td>1.69 ± 0.18</td>
</tr>
<tr>
<td>Total P (g kg⁻¹)</td>
<td>0.24 ± 0.02</td>
<td>0.32 ± 0.03</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>Total K (g kg⁻¹)</td>
<td>35.4 ± 1.45</td>
<td>35.5 ± 0.4</td>
<td>31.9 ± 1.7</td>
</tr>
<tr>
<td>pH (H₂O)</td>
<td>4.27 ± 0.05</td>
<td>4.46 ± 0.05</td>
<td>4.38 ± 0.07</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>11.74 ± 1.90</td>
<td>8.27 ± 0.97</td>
<td>8.69 ± 1.13</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>43.2 ± 1.3</td>
<td>38.6 ± 1.3</td>
<td>39.4 ± 1.9</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>44.9 ± 2.8</td>
<td>53.1 ± 1.5</td>
<td>51.9 ± 1.3</td>
</tr>
<tr>
<td>Bulk density (g cm⁻³)</td>
<td>1.13 ± 0.07</td>
<td>1.20 ± 0.04</td>
<td>1.17 ± 0.11</td>
</tr>
</tbody>
</table>

PM, CC and SS denote Pinus massoniana, Cinnamomum camphora and Schima superba plantations, respectively.

In this study, six enzymes (i.e. invertase, urease, acid phosphatase, cellulase, catalase and polyphenol oxidase) were investigated. The effects of the tree species on the labile SOC pools, respiration and enzyme activity in three subtropical plantations were compared. We determined the soil MBC, DOC, POC, soil basal respiration and the activities of six enzymes in a coniferous plantation of Pinus massoniana (PM), and two broadleaf plantations, namely Cinnamomum camphora (CC) and Schima superba (SS), in subtropical China. Based on the obtained tree species-level differences in temperate or tropical forest ecosystems as well as the differences in leaf litter quality, we hypothesized that the PM plantation with nutrient-poor litter in subtropics had lower labile SOC fractions, soil respiration and enzyme activity than the CC and SS plantations with nutrient-rich litter.

Materials and methods

Site description

The study was conducted at the Huitong National Research Station of Forest Ecosystem (26°40' N to 27°09' N, 109°26' E to 110°08' E). The station lies at the transition zone from the Yunnan–Guizhou plateau to the lower mountains and hills on the southern side of the Yangtze River at 300 to 1000 m above sea level. This region has a humid mid-subtropical monsoon climate with a mean annual temperature of 16.5°C for 20 years. The mean annual precipitation is ~1200 mm.

Three single-species plantations were chosen, namely the conifer plantation PM and the broadleaf plantations CC and SS. These species were chosen based on the different qualities of their leaf litter (Table 1), as measured in 2011. These plantations were established in 1987 after the clearing of secondary broadleaf forests. The observed soil physicochemical properties are listed in Table 2. The three plantations had the same initial planting density of 2500 stem hm⁻², and all plantations were thinned once in the same year. The plantations were weeded annually during the first 3 years after planting, but were not fertilized. All three plantations occupied similar areas of ~1 ha at an altitude of 540 to 570 m above sea level, with a 18° to 20° slope.

Soil sampling and analysis

Soil sampling was performed in July 2011. Three plots each (20 m × 20 m) were randomly established for PM, CC and SS. The distance between plots was ~80 m in the same forest. After removing the litter layer, six soil cores were collected in each plot from the top mineral soil, with a depth of 0 to 20 cm; these cores were pooled into one composite soil sample. All soil samples were sieved through a 2 mm mesh. Selected samples were stored at 3°C and subsequently used within 7 days to determine the soil biological properties and DOC. The remaining samples were dried to determine the soil physicochemical properties and POC.

To measure P concentration, soil and litter samples were digested in triiodid mixture and the P concentration in digested samples was determined colorimetrically using the ammonium molybdate stannous chloride method. Lignin concentration was measured using acetyl bromide digestion (Jones and Case 1990). Contents of K, Ca and Mg in the digested solution were determined with a flame atomic absorption spectrophotometer following HClO₄ – HNO₃ digestion (Jones and Case 1990). Soil pH was measured using a pH meter in a 1:2.5 (weight:volume) mix of soil and deionized H₂O. The soil texture was analysed by pipette method and bulk density by soil core method.

The concentrations of total C and N in soil and litter samples were measured using an elemental analyser. The soil MBC was determined using the chloroform fumigation extraction method (Vance et al. 1987). The microbial quotient was calculated by dividing the soil MBC by the corresponding total SOC (Haynes 1999). The soil basal respiration was measured using the method described by Chen et al. (2000). The field-moist soils (50 g of oven-dried equivalent) were aerobically incubated at 28°C in a 500 mL sealed glass jar for 24 h. CO₂ evolved from the soil was trapped in 0.1 M NaOH and measured by titration with 0.05 M HCl. The evolved CO₂ was calculated from the difference between the normalities of the NaOH blanks and samples. The microbial metabolic quotient (qCO₂) was calculated by dividing the hourly basal respiration rate by the corresponding MBC.

The extraction of DOC was based on a previously described method (Liang et al. 1998). Approximately 10 g of fresh soil was suspended in 25 mL of ultrapure water in a centrifuge tube, incubated for 30 min on a reciprocal shaker and centrifuged at 6000 rpm for 15 min. The supernatant was filtered through a 0.45 μm glass fibre filter. The amount of C in the extract was measured using an Elementar High TOC analyser.

The amount of organic C oxidized by 333 mmol L⁻¹ KMnO₄ was defined as POC, which was determined based on a recommended procedure (Blair et al. 1995). Soil samples containing ~15 mg C were weighed and placed in 50 mL plastic screw-cap centrifuge tubes. Subsequently, 25 mL of 333 mmol L⁻¹ KMnO₄ was added to each vial. The centrifuge tubes were tightly sealed and incubated for 1 h at 12 rpm in a tumbler. The tubes were then centrifuged for 5 min at 2000 rpm, and the supernatants were diluted with deionized water. The absorbance of the diluted samples and standards were measured using a spectrophotometer at 565 nm.
Invertase activity was assayed using 5 g of soil incubated for 24 h at 37°C with 15 mL of 8 per cent sucrose, 5 mL of phosphate buffer (pH 5.5) and 0.1 mL of toluene (Guan 1986). The glucose released by invertase was allowed to react with 3,5-dinitrosalicylic acid and 3-amino-1-nitrosalicylic acid, and the absorbance of the reaction was measured at 508 nm. The results were expressed as the number of milligrams of glucose released by 1 g of soil per day. The soil urease activity was determined by a standard colorimetric assay; this value was expressed as the number milligrams of NH$_3$-N released by 1 kg of soil per hour at 37°C (Guan 1986).

The acid phosphatase activity was determined by adding 5 g of soil to 10 mL of disodiumphenyl phosphate solution (25 g L$^{-1}$), which was used as a substrate (Wu et al. 2006). The soil samples were incubated in acetate buffer (pH 5.0) for 12 h at 37°C. Approximately 5 mL of the buffer solution and 2 mL of the filtrate were transferred to a 50 mL volumetric flask and diluted with distilled water to 25 mL. After adding 0.5 mL of the 2,6-dibromoquinoine-chlorimide solution, the mixture was incubated for 20 min at room temperature and diluted to 50 mL. The absorbance of the released phenol was determined at 600 nm. The acid phosphatase activity was expressed as the number of milligrams of phenol hydrolyzed by 1 kg of soil per hour at 37°C.

Cellulase activity was assayed according to the modified method of Deng and Tabatabai (1994). Approximately 5 g of the soil sample was incubated for 4 h at 50°C with 20 mL of 50 mmol L$^{-1}$ acetate buffer (pH 5.5) and 2 per cent carboxymethyl cellulose. The mixture was centrifuged for 2 min at 12 000 rpm, and the supernatant was treated with the Somogyi–Nelson reagent. The solution was centrifuged once, as described above, before measuring the amount of reducing sugars based on the absorbance at 520 nm.

To measure the catalase activity, 2 g of soil was mixed with 40 mL of distilled water and 5 mL of 0.3 per cent H$_2$O$_2$ in centrifuge tubes (Guan 1986). The tubes were shaken for 20 min and the filtrate was titrated with 25 mmol L$^{-1}$ KMnO$_4$. The results were expressed as the number of millilitres that react with 25 mmol L$^{-1}$ KMnO$_4$ per 1 g of soil per hour.

The polyphenol oxidase activity was assayed based on a previously described method (Guan 1986). Approximately 5 g of soil was incubated for 2 min in a water bath at 30°C with 10 mL of distilled water, 6 mL of 0.1 percent ascorbic acid and 10 mL of 0.02 mol L$^{-1}$ catechol. Subsequently, 3 mL of 10 per cent phosphoric acid was added, and the filtrate was titrated with 0.005 mol L$^{-1}$ iodine. The results were expressed as the number of millilitres that react with 0.005 mol L$^{-1}$ I$_2$ per 1 g of soil per hour.

**Statistical analysis**

The data were expressed based on the oven-dry soil weight. We determined the effects of different tree species on the labile SOC, soil respiration and enzyme activities by one-way ANOVA after testing the data for normality. The more restrictive Tukey’s test was used for post hoc multiple comparisons. We used the Pearson’s linear correlation to assess the relationships of the soil respiration and the enzyme activities to the pooled SOC. Statistical analyses were performed using SPSS software (version 17.0 for Windows).

**Results**

**Labile fractions of SOC**

Significant differences in MBC and POC were observed among the different plantations (Figure 1). The highest DOC and POC were found in the CC plantation, with 173 mg kg$^{-1}$ and 6.01 g kg$^{-1}$, respectively. The highest MBC of 547 mg kg$^{-1}$ was recorded in the PM plantation. The lowest MBC and POC were observed in the CC and PM plantations, respectively. The proportions of DOC and POC to the total SOC did not differ among the different plantations. However, the microbial quotient (proportion of MBC to total SOC) was significantly different, with the highest value of 2.71 per cent in the PM forest.

**Soil respiration and enzyme activities**

The rate of soil respiration was affected by tree species. The PM plantation had the highest soil respiration (Figure 2). The soil qCO$_2$ was likewise significantly affected by the tree species; the highest value was observed in the CC plantation.
The activities of soil urease, acid phosphatase and polyphenol oxidase significantly differed among the tree species (Figure 3), but those of invertase, catalase and cellulase did not. This finding suggested that the enzymes had specific responses to each tree species. The urease activity ranged from 19.3 mg N H₃-N g⁻¹ h⁻¹ to 29.5 mg N H₃-N g⁻¹ h⁻¹; it was highest in the CC plantation and lowest in the PM plantation. The acid phosphatase activity ranged from 3.7 mg phenol kg⁻¹ h⁻¹ to 7.4 mg phenol kg⁻¹ h⁻¹, with the highest value in the SS plantation and the lowest in the PM plantation. The polyphenol oxidase activity varied from 3.48 mL of 0.005 mol L⁻¹ I₂ g⁻¹ h⁻¹ to 9.64 mL of 0.005 mol L⁻¹ I₂ g⁻¹ h⁻¹, with the largest in the PM plantation.

Soil respiration was significantly correlated to MBC and DOC (Table 3). The labile SOC fractions, except for MBC, had a significant positive correlation with the urease activity. The correlation coefficients between the urease activity and the different SOC fractions (i.e. DOC, POC and total SOC) increased with the increasing C stability. The acid phosphatase activity was significantly correlated with the total SOC and POC. The activities of invertase and polyphenol oxidase were both significantly correlated with MBC, but not to the other SOC fractions. In contrast, cellulase and catalase activities were not significantly correlated with any of the SOC fractions.

Discussion

Our results suggested that the labile fractions of SOC had different responses to the tree species in the plantations, which was partially in agreement with our original hypothesis (Figure 1). Although this result was based on a single date of sampling, and labile fractions of SOC are temporally sensitive, we noted that our results were consistent with those of previous studies in similar sites (Jiang et al. 2010; Wang and Wang 2011; Lu et al. 2012), but contrary to some studies in temperate forests (Yano et al. 2000; Strobel et al. 2001). Jiang et al. (2010) noted that the MBC and DOC were highest in the CC plantation and lowest in the PM plantation. The polyphenol oxidase activity varied from 3.48 mL of 0.005 mol L⁻¹ I₂ g⁻¹ h⁻¹ to 9.64 mL of 0.005 mol L⁻¹ I₂ g⁻¹ h⁻¹, with the largest in the PM plantation.

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Higher ratios of C/N, C/P and lignin/N than those from the CC and SS plantations (Table 1). Thus, the low-quality litter from the PM plantation suppresses the release of C and nutrients, thereby producing the lower DOC and POC, when compared with the other plantations. However, this potential mechanism could not explain why soils in the PM plantation had higher MBC than those in the CC plantation. The causes of the higher soil MB of the PM plantation require further study.

The PM plantation had higher soil respiration than the other plantations, which did not support our hypothesis that more nutrient-rich litter of broadleaved trees would lead to higher soil respiration. Coniferous forests had lower soil respiration than broadleaf forests at paired sites reviewed by Raich and Tufekcioglu (2000). However, Wang and Wang (2011) found that the CO₂ release was higher in secondary broadleaf forest soils than in Cunninghamia lanceolata plantation soils. In addition, the soil respiration in response to the tree species in temperate forest ecosystems was not consistent (Borken and Beese 2005; Ladegaard-Pedersen et al. 2005; Chodak and Niklinska 2010). The contradictory results suggest that the influence of tree species
on soil respiration is complex. Factors that simultaneously influence the production and consumption of organic matter are more important in controlling the overall rate of soil respiration than the tree species (Raich and Tufekcioglu 2000; Brechet et al. 2009; Vesterdal et al., 2012); these factors include the C availability, nutrient availability, soil temperature and soil moisture. The soils under the different tree species in the present study had varying pH, nutrient availability and texture (Table 2).

The tree species in the plantations influenced the urease, acid phosphatase and polyphenol oxidase activities in the soil, but these changes did not follow a similar pattern among the tree species (Figure 3). Although this result was based on a single date of sampling, we demonstrated that our results agreed with previous studies (Wang et al., 2008; Xing et al., 2010), where similar conditions were investigated. The soil acid phosphatase and polyphenol oxidase activities varied under different forest types. For example, the highest urease, acid phosphatase and polyphenol oxidase activities were observed in the CC, SS and PM forests, respectively. Similarly, Xing et al. (2010) reported that the urease activity was significantly higher in soils under broadleaf forests than in those under coniferous fir plantations. However, the acid phosphatase activity was not significantly different among tree species (Ushio et al., 2010). The varied results suggested that the soil enzyme activity depended on the specific tree species. The differences between the soil physicochemical environment and resource availability induced by the organic input of litter and root systems from different tree species greatly influenced the soil enzyme activity (Augusto et al., 2002; Chodak and Niklińska, 2010).

In conclusion, our study investigated the labile fractions of SOC, soil respiration and the activity levels of six enzymes in three subtropical tree species. The PM plantation was confirmed to have lower DOC, POC, urease and acid phosphatase activities. Contrary to our hypotheses, the nutrient-rich litter did not increase soil respiration. The different labile SOC fractions and microbial activity of different plantations suggested that the specific traits of these three tree species should have been considered when modelling of the long-term soil C storage dynamics.

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| Total SOC | 0.664* | 0.872* | ns | ns | ns | ns |
| POC | 0.633* | 0.857* | ns | ns | ns | ns |
| DOC | ns | 0.762* | ns | ns | ns | 0.748* |
| MBC | ns | ns | 0.627* | ns | 0.638* | 0.836* |

SOC, POC, DOC and MBC denote soil organic C, permanganate-oxidizable C, dissolved organic C and microbial biomass C, respectively. *P < 0.05; ‘ns’ indicates P > 0.05.

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