J Soils Sediments (2015) 15:1094–1103
DOI 10.1007/s11368-015-1118-2

SOILS, SEC 2 • GLOBAL CHANGE, ENVIRON RISK ASSESS, SUSTAINABLE LAND USE • RESEARCH ARTICLE

Soil microbial community composition rather than litter quality is linked with soil organic carbon chemical composition in plantations in subtropical China

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Received: 13 November 2014 / Accepted: 15 March 2015 / Published online: 2 April 2015

Abstract
Purpose Native broadleaf plantations are increasingly being developed as an alternative to coniferous plantations. This study examined the relationships among litter carbon (C) quality, soil microbial community composition, and soil organic C (SOC) chemical properties in plantations and how they were affected by tree species.
Materials and methods The solid-state ^{13}C nuclear magnetic resonance spectroscopy (NMR) technique was used to examine SOC chemical composition, and litter and fine root C quality in four plantations of native tree species (*Pinus massoniana*, *Castanopsis hystrix*, *Michelia macclurei*, and *Mytilaria laosensis*) in Pingxiang, Guangxi Zhuang Autonomous Region, in subtropical China. Soil microbial biomass C and nitrogen (N) were determined by the chloroform fumigation-extraction method and soil bacterial and fungal biomass were measured with the phospholipid fatty acid (PLFA) technique.
Results and discussion The proportions of O-alkyl C, alkyl C, aromatic C, and carbonyl C in SOC and the alkyl/O-alkyl C ratio (A/O-A) in litter and fine root samples, soil microbial C and N, microbial C/N ratios, and the amount of PLFAs were significantly different among the four plantations of different species. SOC in the 0–10-cm layer had 43–49 % O-alkyl C, 24–34 % alkyl C, 14–17 % aromatic C, and 9–11 % carbonyl C in SOC. The microbial C/N ratio, the amount of total PLFAs, and bacterial and Gram-positive bacterial population sizes were linked to the proportion of alkyl C in SOC and the A/O-A ratio in soil. The proportion of alkyl C in SOC was not related to the proportion of alkyl C in litter or fine root samples.
Conclusions The microbial community composition rather than plant litter or fine root quality was linked to chemical composition of SOC in the studied subtropical plantations. Future research should place more emphasis on the processes involved in the formation of SOC and their association with the microbial community.

Keywords Litter quality · Nuclear magnetic resonance spectroscopy (NMR) · Organic carbon chemical composition · Plantation · Soil microbial biomass and community

Responsible editor: Chengrong Chen

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1 Introduction

Soil organic matter (SOM) is a mixture of complex carbon (C)-rich organic molecules (Solomon et al. 2007; Crow et al. 2009). Those organic molecules have different degrees of stability against decomposition in the environment. For example, alkyl C chains in lipids and aromatic structures in aromatics and phenolics are more recalcitrant than carbohydrates

(Lorenz et al. 2007). Carbohydrates such as cellulose contain abundant O-alkyl groups (Baldock et al. 1992), and a higher alkyl C/O-alkyl C ratio (A/O-A) often indicates a loss of more labile C relative to poor-quality C compounds (Cusack et al. 2011). Recalcitrant lignin-derived aromatics become highly refractory during litter decay (Berg and Meentemeyer 2002), but they do not always appear as recalcitrant in soils as initially thought (Dignac and Rumpel 2006). Several studies showed that aliphatic compounds often accumulate in soil, thus contributing to increasingly stable soil organic C (SOC) pools (Mikutta et al. 2006; Lorenz et al. 2007).

The SOC chemical composition in forest soils is affected by the type of vegetation present at a site; for example, carbonyl C dominated in soils under oak (*Quercus*), O-alkyl C prevailed in soils under manzanita (*Arctostaphylos*), and alkyl C was prominent in soils under coniferous vegetation (Quideau et al. 2001). Conversion from natural forests to hoop pine (*Araucaria cunninghamii*) forests decreased the proportion of O-alkyl C and increased the proportion of alkyl C in SOC (Chen et al. 2004). The proportion of aromatic C in SOC was higher and the proportion of carbonyl C in SOC was lower in the forest floor (Oe + Oa horizon) in spruce (*Picea glauce* (Moench) Voss) than in aspen (*Populus tremuloides* Michx.) stands in boreal mixed wood forests in Alberta, Canada (Hannam et al. 2004).

Plant litter and soil microbial biomass are two of the major sources of SOM (Kögel-Knabner 2002), some of which are precursors of stable SOC fractions, formed through leaching, fragmentation, and chemical alteration (Chapin et al. 2002). The chemical composition of aboveground and belowground plant tissues can influence litter decomposability and the association of decomposition products with soil minerals (Kögel-Knabner 2002). Crow et al. (2009) showed that root-derived aliphatic compounds were a source of SOC with greater stability than leaf-derived C in soils of deciduous forests, whereas root-derived lignin and needle-derived aliphatic compounds were preferentially preserved in soils of coniferous forests, indicating that the dominant source of SOC can differ substantially between forest types.

Although most soil C is ultimately derived from plant material (Kögel-Knabner 2002), a substantial proportion of it is transformed from microbial biomass into SOM (Simpson et al. 2007; Liang et al. 2011; Miltner et al. 2012). Soil microbial biomass represents a significant source of SOC and is a biochemical precursor that contributes to the maintenance of SOM (Simpson et al. 2007). The cell wall compounds, metabolites and C use efficiencies all differ among microbial communities (Six et al. 2006). About 50 % of bacterial biomass-derived C was found to remain in the soil, mainly as a nonliving component of SOM in a 224-day soil incubation experiment studied with ^{13}C-labeled bacterial cells (Miltner et al. 2012). Fungal biomarkers indicate impaired degradation and preservation of fungal residues in late successional forests, and the dynamics of root-associated fungi is an important regulator of ecosystem C accumulation in boreal forests (Clemmensen et al. 2013). Soil microbial communities can be greatly affected by afforestation and reforestation as such activities change the litter type and the environment under a canopy (Liu et al. 2012). In particular, the SOC chemical composition can be influenced by vegetation through the organic compounds they produce and through their interaction with microbial communities (Lorenz et al. 2007).

Forest plantations are being established at an increasing rate throughout much of the world, primarily for the production of wood fiber (FAO 2001). There is growing recognition of the conservation value of plantations in reducing logging pressure on natural forests, in sequestering C, and in restoring degraded lands (Kelty 2006). In China, the total plantation area reached 6.2×10^7 ha, accounting for 31.8 % of the total forest area of the country, and was ranked first in the world in terms of the total plantation area established as documented in the 7th National Forest Inventory for 2004–2008 (SFA 2010). With an abundance of solar radiation and water resources, southern China accounts for 63 % of the plantation areas in the country (SFA 2007). Most of these plantations were planted with monocultural coniferous tree species (e.g., *Pinus massoniana* or *Cunninghamia lanceolata*) or exotic tree species such as *Eucalyptus* (SFA 2007), leading to reductions in biodiversity, ecosystem stability, and soil fertility (Peng et al. 2008). Plantations of native broadleaf species with high economic value can supply high-quality timber while enhancing biodiversity and ecosystem services (Carnevalea and Montagnini 2002). They are increasingly being developed as an alternative to coniferous plantations in many countries (Vesterdal et al. 2008). Few studies, however, have examined relationships among litter C quality, soil microbial community composition, and SOC chemical properties in forest plantations with different tree species.

In a previous study, we found that soils in several broadleaf plantations contained more decomposable C fractions as indicated by the lower proportion of alkyl C and higher proportion of O-alkyl C in SOC and the lower A/O-A ratio as compared with those in a pine plantation (Wang et al. 2010a). The factors controlling variations in SOC compositions among the plantations were not clearly understood. In this paper, we report the differences in litter C chemical composition, soil microbial biomass and microbial community composition among those same four planted forest types (*P. massoniana*, *Castanopsis hystrix*, *Michelia macclurei*, and *Mytilaria laosensis*). The purpose of this study was to explore the key factors affecting SOC chemical composition in different plantation types. Our hypotheses are as follows: (i) initial C chemical composition of litter, soil microbial biomass, and microbial community composition differ among the four plantations of different species; (ii) SOC chemical composition is linked to initial litter C quality and/or soil microbial biomass and microbial

community composition; and (iii) linkages between bacterial or fungal biomass and SOC chemical composition would be different among the four plantations of different species in subtropical China.

2 Materials and methods

2.1 Site description

The study area was located at the Experimental Center for Tropical Forestry of the Chinese Academy of Forestry (22° 10′ N, 106° 50′ E), in the outskirts of Pingxiang, Guangxi Zhuang Autonomous Region, in the subtropical region of the People's Republic of China. In the study area, the mean annual temperature was 22.5 °C and the annual rainfall was 1202.9 mm from May 2006 to April 2007, falling primarily from May through August (Lu et al. 2009). The sandy textured soil at the study site was formed from a granitic parent geological material and is classified as a Ferrosol in the Chinese system of soil classification, equivalent to an Oxisol in the USDA Soil Taxonomy (Liang and Wen 1992; State Soil Survey Service of China 1998; Soil Survey Staff of USDA 2006). Originally, the study site supported a subtropical evergreen broadleaf forest, and a *C. lanceolata* plantation was established in the 1950s after clear-cutting the natural forests. Four plantations were randomly established in 1983 as monoculture plantations after clear-cutting the *C. lanceolata* plantation.

Four plantations located at an elevation of 550 m were selected based on their similarity in topography, soil texture, stand age, and management history. These plantations included a coniferous plantation (*P. massoniana*) and three broadleaf plantations (*C. hystrix*, *M. macclurei*, and *M. laosensis*). The four tree species are the main native (non-N-fixing) species for afforestation and reforestation in the study area. In each type of plantation, four plots (each 20×20 m) were randomly delineated for sampling. The stand characteristics in this study were reported in Wang et al. (2010a).

2.2 Sample collection and analyses

This work was conducted based on the Forestry Standards "Observation Methodology for Long-term Forest Ecosystem Research" of the People's Republic of China (LY/T 1952-2011). Litterfall was collected monthly in the wet season from March through September 2008 using five litter traps (1×1 m, 1-mm mesh size) in each plot and sorted into leaf, small woody material, and miscellaneous material (everything other than leaf or small woody material) (Wang et al. 2013a). Those samples were oven dried at 65 °C to constant weight and weighed.

Fine roots (diameter ≤2 mm) of *P. massoniana*, *C. hystrix*, *M. macclurei*, and *M. laosensis* were collected from the top 10 cm of soil in their respective stands using an 8.7-cm diameter stainless steel corer in August 2008. A total of 12 soil cores were collected from each plot and bulked to form one composite sample to collect enough fine root samples for chemical analysis. Live root fragments were subsequently separated by visual inspection as described in Vogt and Persson (1991). We focused on fine roots because of their more rapid turnover rates compared with coarse roots; fine roots represent a substantial proportion of total tree root productivity (Gill and Jackson 2000; Guo et al. 2008). We opted to use fresh roots because they best represent roots that have not yet begun to decompose as described by Hobbie et al. (2010). Fine root samples were also oven dried at 65 °C to constant weight and weighed.

Mineral soil samples (0–10 cm) were collected in August 2008. A total of six soil cores were collected using an 8.7-cm diameter stainless steel cores and bulked to form one composite sample per plot. Soil samples were passed through a 2-mm sieve to remove plant, roots, and gravel carefully to minimize influence of the plant residues on chemical and microbial analysis (Zelles 1999). A subsample of the soil was air-dried at room temperature (25 °C) and was then ground with a mill to pass through a 0.25-mm sieve before physicochemical analysis. The samples used for microbial community analysis were immediately stored at −20 °C, without drying, for further analysis.

We used the solid-state ^{13}C nuclear magnetic resonance spectroscopy with cross-polarization and magic-angle spinning (CPMAS-NMR) technique to study the chemical composition of SOC. This technique has frequently been used to directly study the complex structure (at molecular level) of SOC in terrestrial ecosystems (Schnitzer 2001; Fontaine et al. 2007; Solomon et al. 2007). The solid state ^{13}C CPMA S NMR spectra of soil, litter, and fine root samples were obtained at a frequency of 100.64 MHz on a Bruker AVANCE 400 spectrometer. Soil samples were pretreated with 10 % (*v*/*v*) hydrofluoric acid (HF) before NMR spectroscopic analysis (Schmidt et al. 1997). For HF pretreatment, approximately 10*g* of ground sample was shaken with 50 mL HF for 2 h. After centrifugation (3000 rpm or 705*g*) for 10 min, the supernatant was removed. The procedure was repeated five times. The remaining sediment was washed with 50 mL deionized water five times to remove residual HF and freeze dried. The pretreatment removes a substantial amount of Fe and Mn in the soil, concentrates the organic C of the whole soil sample, and improves the signal/noise ratio of NMR spectroscopy (Schmidt et al. 1997).

For the NMR analysis, samples were packed into a ZrO_2 rotor (o.d. = 7 mm) and spun at 5 kHz at the magic angle. Single contact time of 1 ms was used with an acquisition time of 42 ms and a recycle delay of 1 s. Transients (20,000) were collected for all samples and a Lorentzian line broadening function of 50 Hz was applied to all spectra. Chemical shift values were referenced externally to glycine at 176.03 ppm, which is equivalent to tetramethylsilane at 0 ppm.

The ^{13}C CPMAS NMR spectra were divided into four chemical regions that are assigned to specific organic C groups (Kögel-Knabner 2002; Spielvogel et al. 2006; Wang et al. 2013b): 0–45 ppm, alkyl C (lipids, cutin, and suberin); 45–110 ppm, O-alkyl C (carbohydrates, cellulose, hemicellulose, and methoxyl C); 110–160 ppm, aromatic C (lignin, tannin, olefins, and aromatic compounds); and 160–220 ppm, carbonyl C (carboxylic acid, amide, and ketone groups). The corresponding areas under the curve of the four regions were quantified by integration. The A/O-A ratio, which has been used as an index of the extent of decomposition of SOM or substrate quality for microbes (Baldock and Preston 1995), was used as an indicator of SOC chemical stability (Chen et al. 2004; Huang et al. 2008).

Soil microbial biomass C (MBC) and nitrogen (MBN) were measured by the chloroform fumigation-extraction method (Brookes et al. 1985; Vance et al. 1987). Soil samples were also analyzed for phospholipid fatty acids (PLFAs) following Bossio and Scow (1998). The abundance of individual fatty acids was determined as nanomoles per gram of dry soil using standard nomenclature (Tunlid et al. 1989). In our study, the concentration of each PLFA was calculated based on the concentrations of the 19:0 internal standards. Bacteria were identified by the following PLFAs: i14:0, i15:0, a15:0, 15:0, i16:0, a17:0, i17:0, 15:0 3OH, 16:1 2OH, cy17:0, 17:0, 16:1ω7c, and 18:1ω7c (Frostergård and Bååth 1996; Zelles 1999). We calculated the sum of i14:0, i15:0, a15:0, 15:0, i16:0, a17:0, and i17:0 as the Gram-positive bacteria (Kourtev et al. 2002; Liu et al. 2012), and the sum of 15:0 3OH, 16:1 2OH, cy17:0, 17:0, 16:1ω7c, and 18:1ω7c as the Gram-negative bacteria (Zelles 1999). Fungi were identified by the PLFAs 18:2ω6, 9c, and 18:1ω9c (Cusack et al. 2011; Thoms and Gleixner 2013). Other PLFAs such as 16:0, 18:0, cy 19:0ω8c, 17:1ω8c, 16:1ω5c, 16:0 10methyl, and 17:0 10methyl phospholipid were used as general markers for the microbial community (Liu et al. 2012). Taken together, all of the PLFAs indicated above were considered to be representative of the total PLFAs of the soil microbial community.

2.3 Statistical analysis

The initial C chemical compositions (e.g., alkyl C, O-alkyl C, aromatic C, carbonyl C, and A/O-A ratio) of litter and fine roots were analyzed by one-way analysis of variance using plantation type as the main factor. Each forest type had four replicated samples. In the same way, soil microbial properties such as soil MBC, MBN, microbial C/N ratio, and soil total microbial, fungal, bacterial, Gram-positive bacterial, and Gram-negative bacterial PLFAs were also analyzed using one-way analysis of variance to determine differences among the four plantation types. Each forest type also had four replicated samples. Comparisons of means of the above variables among the four plantation types were made with Duncan's multiple-range test. We related the proportion of soil alkyl C and A/O-A ratio to soil microbial biomass, soil microbial PLFAs, and C chemical compositions (e.g., alkyl C and A/O-A ratio) of litter and fine roots in the four plantation types using bivariate linear regressions. Each regression had 16 samples. The relationships between primary indexes of SOC chemical composition and litter initial quality and soil microbial community composition were used to test our hypotheses. A logarithm transformation was performed on soil A/O-A ratio and Gram-negative bacterial PLFAs to meet the assumption of normal distribution prior to analysis. Statistically significant differences were set at $\alpha=0.05$. All analyses were performed using SPSS 19.0 for Windows.

3 Results

3.1 Litter C chemical composition of four tree species

The litter had 42–54 % O-alkyl C, 19–34 % alkyl C, 14–19 % aromatic C, and 8–9 % carbonyl C in SOC (Fig. 1). The fine root samples had 44–60 % O-alkyl C, 13–26 % alkyl C, 17–

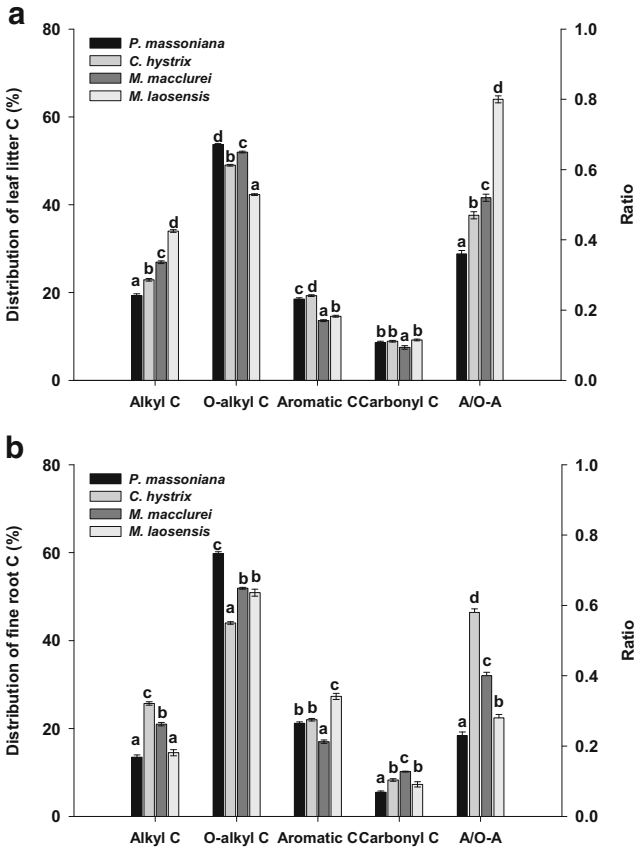

Fig. 1 Distribution of alkyl C, O-alkyl C, aromatic C and carbonyl C chemical compositions, and alkyl/O-alkyl C (A/O-A) ratio in litter C and fine root C in plantations in subtropical China. *Error bars* are standard errors ($n=4$). *Different lowercase letters* indicate significant differences among tree species at $p<0.05$

27 % aromatic C, and 6–10 % carbonyl C in SOC (Fig. 1). The proportions of O-alkyl C, alkyl C, aromatic C, and carbonyl C in SOC, and A/O-A ratio in litter and fine root samples were significantly different among the four plantations of different species (Fig. 1). The *P. massoniana* plantation had the lowest alkyl C and the highest O-alkyl C in both litter and fine roots (Fig. 1).

3.2 Soil microbial community composition

Soil MBC was lower in *C. hystrix* than in the other three plantations ($p<0.05$; Table 1). Soil MBN, however, was higher in *M. macclurei* and *M. laosensis* than in the other two plantations ($p<0.05$; Table 1), with differences between *M. macclurei* and *M. laosensis* or between *P. massoniana* and *C. hystrix* plantations not significant (Table 1). Soil microbial biomass C/N ratio was lower in *M. macclurei* than in the other three plantations ($p<0.05$; Table 1).

The amount of soil microbial PLFAs was significantly different among the four plantations (Table 1). Total PLFAs, bacterial PLFAs, and Gram-negative bacterial PLFAs were lower in *C. hystrix* than in the other plantations ($p<0.05$; Table 1). Fungal PLFAs were higher in *M. laosensis* than in the other plantations ($p<0.05$; Table 1). Gram-positive bacterial PLFAs were significantly higher in *P. massoniana* than in the other plantations ($p<0.05$; Table 1). Soil MBC was positively correlated with the amount of soil microbial total PLFAs ($R^2=0.39$, $p<0.05$).

3.3 Dominant factors affecting SOC chemical composition

Across the four planted forests, SOC had 43–49 % O-alkyl C, 24–34 % alkyl C, 14–17 % aromatic C, and 9–11 % carbonyl C in SOC (Table 2). The SOC chemical composition varied with the planted species type, with 34 % of the SOC found in the alkyl C fraction in the *P. massoniana* plantation compared with <29 % in the broadleaf plantations.

There was no significant relationship between the proportions of alkyl C in SOC in the soil and litter or fine roots across the four plantations. The soil A/O-A ratio was not correlated with the litter A/O-A ratio ($R^2=0.14$, $p=0.16$) but was negatively correlated with the fine root A/O-A ratio ($R^2=0.45$, $p<0.05$).

Total PLFAs of the soil microbial community, microbial biomass C/N ratio, bacterial PLFAs, and Gram-positive bacterial PLFAs were all positively correlated with the proportion of soil alkyl C in SOC ($p<0.05$; Table 3). Soil bacterial PLFAs and Gram-positive bacterial PLFAs were also positively correlated with soil A/O-A ratio ($p<0.05$; Table 3). Soil fungal PLFAs were not correlated with either the proportion of soil alkyl C in SOC or the A/O-A ratio (Table 3).

4 Discussion

4.1 Differences in litter quality and soil microbial properties among plantations

Litter is a raw material for SOC formation, and litter decomposition is a fundamental ecosystem process in SOC stabilization. First, our results verified the hypothesis (i) that initial C chemical composition of litter, soil microbial biomass, and microbial community composition differed among the four plantations of different species. The significant difference in initial C chemical composition of litter among the four tree species is the foundation for analyzing the role of chemical complexity of initial C input on SOC chemical composition. Previous work has shown that initial differences in litter chemistry could even persist while litter is incorporated into SOM (Angers and Mehuys 1990), and recent studies have shown that chemical differences in litter inputs are reflected in the

Table 1 Soil microbial biomass C and N, microbial C/N ratio, and PLFAs in four plantations in subtropical China (means with SE in brackets)

Soil microbial properties	*Pinus massoniana*	*Castanopsis hystrix*	*Michelia macclurei*	*Mytilaria laosensis*
Microbial biomass C (mg/kg)	342.9 (13.5) b	272.7 (23.3) a	392.6 (11.9) bc	438.8 (28.3) c
Microbial biomass N (mg/kg)	25.9 (0.8) a	21.8 (1.0) a	37.5 (1.1) b	34.4 (3.7) b
MBC/MBN	13.3 (0.4) b	12.5 (0.7) b	10.5 (0.6) a	12.9 (0.5) b
Total PLFAs (nmol/g)	45.1 (6.1) b	32.4 (2.6) a	39.3 (2.6) ab	42.6 (2.6) ab
Fungal PLFAs (nmol/g)	3.6 (0.4) ab	3.0 (0.3) a	3.8 (0.3) ab	4.5 (0.2) b
Bacterial PLFAs (nmol/g)	24.6 (0.4) b	16.1 (0.3) a	18.8 (0.3) a	18.9 (0.3) a
Gram positive bacterial PLFAs (nmol/g)	16.6 (1.2) b	10.4 (0.8) a	12.3 (0.9) a	12.1 (1.0) a
Gram negative bacterial PLFAs (nmol/g)	8.0 (0.6) b	5.7 (0.4) a	6.5 (0.6) ab	6.8 (0.7) ab

Different lowercase letters indicate significant differences among forest types at $p<0.05$ ($n=4$)

Table 2 Distribution of SOC among functional groups in plantations in subtropical China (means with SE in brackets)

Distribution of SOC	*Pinus massoniana*	*Castanopsis hystrix*	*Michelia macclurei*	*Mytilaria laosensis*
Alkyl C (%)	33.5 (1.0) c	24.9 (0.4) a	23.9 (1.7) a	28.3 (0.4) b
O-alkyl C (%)	42.9 (0.8) a	47.9 (0.9) b	48.8 (0.8) b	48.3 (0.4) b
Aromatic C (%)	15.0 (0.4) b	16.5 (0.7) b	16.6 (1.0) b	13.5 (0.3) a
Carbonyl C (%)	8.6 (1.0) a	10.7 (0.6) a	10.6 (1.6) a	9.9 (0.8) a
Alkyl/O-alkyl C	0.78 (0.03) c	0.52 (0.01) a	0.49 (0.04) a	0.59 (0.01) b

Different lowercase letters indicate significant differences among forest types at $p<0.05$ ($n=4$)

chemistry of both aggregated and nonaggregated SOM (Stewart et al. 2011).

Changes in environmental conditions (e.g., those associated with human activities) among the four plantation types could affect the function of the decomposer communities. Differences in fungi and bacteria, particularly Gram-positive and Gram-negative bacteria among the four plantations indicated changes in the soil decomposer community in the previous 25 years of forest management. These changes might give rise to (1) differences in enzyme activities that could result in selective decomposition of some compounds (Gallo et al. 2005; Grandy and Neff 2008), (2) variation in the metabolic capabilities of decomposers (Balser and Firestone 2005), and/or (3) differences in the chemical structure of microbial necromass (Kögel-Knabner 2002). We compared our soil MBC data and soil MBC/SOC ratio with those reported for subtropical forest soils in Dinghushan and Heshan in southern China (Chen et al. 2010; Liu et al. 2012). In Dinghushan, soil MBC and SOC were 320–566 mg kg^{-1} and 37–73 g kg^{-1}, respectively, that resulting in a MBC/SOC ratio of 0.8–0.9 % (Liu et al. 2012). In Heshan, soil MBC and SOC were 190–220 mg kg^{-1} and 7.5–13.5 g kg^{-1}, respectively, resulting in a MBC/SOC ratio of 1.6–2.5 % (Chen et al. 2010). In our study, soil MBC, SOC, and the MBC/SOC ratio were 270–438 mg kg^{-1}, 26–31 g kg^{-1}, and 0.9–1.4 %, respectively, within the range reported for sites with similar climatic conditions. We found that different plantation types with different tree species (e.g., varying litterfall input and site environmental conditions) influenced the chemical composition of SOC (Wang et al. 2010a). Next, we explored how C stabilization of forest soils varied among the plantations.

4.2 Relationships between chemical compositions of SOC and litter quality and soil microbial community composition

Recalcitrance can have several different meanings, depending on the context. For this study, we define chemical recalcitrance as an inherent chemical property of a molecule rendering it resistant to decomposition (Sollins et al. 1996). Alkyl C in clay size fractions has been proposed to be "recalcitrant" (Baldock and Skjemstad 2000). Organic matter in intimate contact with mineral surfaces is often depleted in recalcitrant aromatic structures, such as lignin and phenolic components (Guggenberger et al. 1994; Kiem and Koegel-Knabner 2003), and enriched in carbonyl and O-alkyl C, which are considered highly labile structures (Mahieu et al. 1999; Spielvogel et al. 2008). In this study, a significantly higher soil A/O-A ratio in *P. massoniana* than in the three broadleaf plantations indicated that a greater amount of relatively stable and recalcitrant C accumulated in soil of the *P. massoniana* plantation compared with the broadleaf plantations, as the A/O-A ratio is an index of the extent of SOM decomposition (Baldock and Preston 1995). Similar differences were reported among pine plantations and oak or natural forests in temperate regions (Quideau et al. 2001; Chen et al. 2004). The fact that the *P. massoniana* stand had the highest A/O-A ratio despite having litter and fine roots with the lowest proportion of alkyl C and highest O-alkyl C could be because of selective preservation of chemically complex plant-derived compounds in the SOM. Needle-derived aliphatic compounds were preferentially preserved in soils of coniferous forests (Crow et al. 2009). The litter and fine root decomposition rates of *P. massoniana* were obviously lower than those of broadleaved tree species because of the

Table 3 Coefficients of determination (R^2) and p values of regression between the proportion of soil alkyl C in total C, soil alkyl C/O-alkyl C ratio (A/O-A), and soil microbial biomass C/N ratio and PLFAs in plantations in subtropical China ($n=16$)

	MBC/MBN	Total PLFAs (nmol/g)	Bacterial PLFAs (nmol/g)	Gram positive bacterial PLFAs (nmol/g)	Fungal PLFAs (nmol/g)
Alkyl C (%)	$R^2=0.30$	$R^2=0.28$	$R^2=0.45$	$R^2=0.51$	$R^2=0.03$
	$p<0.05$	$p<0.05$	$p<0.01$	$p<0.01$	$p=0.54$
A/O-A			$R^2=0.46$	$R^2=0.52$	$R^2=0.01$
			$p<0.01$	$p<0.01$	$p=0.73$

higher C/N ratio in the *P. massoniana* litter and fine roots (Wang et al. 2010b). The selective preservation of alkyl C in the soil from coniferous than broadleaved litter and fine roots might have played a role in this study. Although the A/O-A ratio is generally used as an index of the degree of humification (Baldock et al. 1997), there is some uncertainty in the general applicability of the A/O-A ratio in different forest systems (Mathers et al. 2003, 2007). Other ratios (e.g., carbohydrate C-to-methoxyl C ratio) might be the useful indicators of humification (Mathers et al. 2003, 2007). Further research is required to examine the relationship between these ratios and soil C dynamics in different ecosystems. Soil organic C and total N stocks and effects of forest types on the composition of SOC in the 0–10-cm soil layer in the four plantations were reported in Wang et al. (2010a).

Plant litter contains diverse organic compounds including polysaccharides (e.g., cellulose), aromatics (e.g., lignin and tannins), and aliphatics (e.g., waxes, suberin, and cutin) (Crow et al. 2009). The amount of plant litter, its chemical composition, and properties are some of the key factors that affect the formation of SOM (such as the humification process) in terrestrial ecosystems (Scholes et al. 1997). The effects of vegetation type on SOC chemical composition could be attributed to the diversity in the C chemical fraction of litter materials and variations in the process of decomposition and humification (Quideau et al. 2001; Hannam et al. 2004). Previous studies suggested that the input and quality of litter are important regulators of C and N sequestration (Cornwell et al. 2008; Brovkin et al. 2012; Wardle et al. 2012). Recent studies demonstrated that SOM formation is an ecosystem property (Schmidt et al. 2011). The evergreen broadleaf forest and *C. lanceolata* plantation that existed before the current plantations were established would have an effect on the SOM. However, given that the area where the current four plantations are established had gone through the same evergreen broadleaf forest, *C. lanceolata* plantation rotation, the significant differences among the four plantations should reflect the current conditions. The fast turnover rate of SOM in the subtropical region would also mean that 25 years after the establishment of the current plantations, the SOM should more likely reflect the effect of the current tree species in this study. The lack of correlation between SOC chemical composition and the quality of litter or fine roots indicated that other processes were involved in SOC accumulation. This is supported by the findings from a 3-year litter manipulation experiment where the biochemical recalcitrance of the added litter had limited influence on the long-term stabilization of SOC (Gentile et al. 2011). It has been shown that aboveground plant litter dynamics on its own cannot explain the increasing rate of organic matter accumulation with time (Clemmensen et al. 2013). In this study, the significant differences in soil microbial biomass and the quantity of total PLFAs among the four plantations (Table 1), and the positive relationships between the proportion of soil alkyl C in SOC and total PLFAs and microbial biomass C/N ratio (Table 3), indicate that the high soil microbial biomass might linked to the accumulation of alkyl C. Therefore, our study suggests that the formation and stabilization of SOC are likely linked to soil microbial populations, rather than the initial litter C chemical composition. Tree species selection aiming to increase the input of recalcitrant litter might not be an effective practice to increase the proportion of stable SOC in total SOC. The above discussion illustrated our hypothesis (ii) that soil microbial community composition rather than initial litter quality was linked with SOC chemical composition in the four subtropical plantations of different species.

The presence/absence of certain groups of decomposer organisms as affected by litter chemistry and environmental conditions could influence SOC chemical composition under different vegetation types (Baldock and Preston 1995; Quideau et al. 2001; Hannam et al. 2004). Microbial residues in the soil are an important source for humus formation, and cell wall envelopes of bacteria and fungi are stabilized in the soil and contribute significantly to small-particulate SOM formation (Miltner et al. 2012). Soil microorganisms are regarded as catalysts for the transformation of plant residue, and these organisms use plant material as their C source, transforming it to CO_2, intermediate metabolites, and microbial biomass (Miltner et al. 2012). The high abundance of submicrometer structures is related to microorganisms found in the soil; these structures include fragments of hyphae, cells, cell wall fragments, and extracellular polysaccharides (Foster 1988; Tisdall and Oades 1982). Webster et al. (2000) also reported that the soil A/O-A ratio as well as microbial activity increased during a 28-day incubation. In contrast, lower soil alkyl C but higher soil microbial biomass have been found to exist under a natural forest compared with a first-rotation hoop pine plantation in Australia (Chen et al. 2004).

The contribution of soil microbial populations to the build-up of stable soil C is often affected by the biochemical fraction and macromolecular structure of microbial groups (Throckmorton et al. 2012). Both fungal and bacterial necromass can be stabilized in the soil (Miltner et al. 2012). The resistance of soil humic acids to microbial degradation has been shown to be related to differences in their chemical structure and microbial species (Yanagi et al. 2002). Melanin, waxes, terpenoids, and tetrapyrrole pigments produced by bacteria can be biochemically recalcitrant and resistant to biodegradation in the soil (Gleixner et al. 2001); the nonhydrolyzable melanin of fungi consists of proteins, carbohydrates, lipids, and phenolic polymers (Kögel-Knabner 2002). In most cases, the contribution of fungi to recalcitrant SOC forms is higher than that of bacteria (Six et al. 2006). In this study, soil fungal PLFAs were not correlated with the proportion of soil alkyl C in SOC or the A/O-A ratio (Table 3), indicating that the stable SOC chemical

composition was not linked to fungal abundance. The significant relationships among soil bacterial, gram-positive bacterial PLFAs, and the proportion of soil alkyl C in SOC and the A/O-A ratio in this study (Table 3) demonstrate that the high abundance of soil bacteria and Gram-positive bacteria was linked to the high proportion of stable C in total SOC. This is because bacteria contain more alkyl C but less O-alkyl C than fungi (Baldock et al. 1990), and unique to Gram-positive bacteria is the presence of teichoic acids (containing lipid components) in the cell wall (Madigan and Martinko 2006). It is likely that more aliphatic compounds of Gram-positive than Gram-negative bacteria could have been accumulated in soils in this study. Such linkages are supported by the finding of greater soil retention of Gram-positive compared with Gram-negative bacteria necromass at a California forest site (Throckmorton et al. 2012).

Our data indicate that the specific microbial communities played a key role in the formation of characteristic SOC chemical compositions in the different plantations, which supported our third hypothesis that linkages between bacterial or fungal biomass and SOC chemical composition would be different among the four plantations of different species in subtropical China.

5 Conclusions

There was no significant linkage between the chemical composition of SOC and the chemical composition of plant litter C in the four plantations of native tree species in subtropical China. The proportion of the chemical composition of SOC was linked to the microbial community composition rather than initial plant litter or fine root quality. We conclude that C input from litter did not directly contribute to the proportion of the chemical composition of SOC in the planted subtropical forests we studied; soil microbial community composition was a major factor affecting SOC chemical composition. Future research should place more emphasis on the processes involved in the formation of SOC and their association with the microbial community.

Acknowledgments We would like to thank the Guangxi Youyiguan Forest Ecosystem Research Station for assisting with research permits and providing logistical support; Jia Xu, Pizheng Hong, Jian Hao, Haolong Yu, Da Luo, Yi Wang, Riming He, Hai Chen, Angang Ming, Zhongguo Li, Lu Zheng, and Chaoying Li for assistance with field work and laboratory analysis; and Jiangming Mo and Yunting Fang for reviewing drafts of this manuscript. Grants from the Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry (CAFRIFEEP201104), the China National Natural Science Foundation (31100380, 31290223), the Ministry of Science and Technology (2012BAD22B01, 2015DFA31440), the Lecture and Study Program for Outstanding Scholars from Home and Abroad (CAFYBB2011007), and CFERN & GENE Award Funds on Ecological Paper supported this study.

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