Communications in Soil Science and Plant Analysis, 38: 77–91, 2007

ISSN 0010-3624 print/1532-2416 online
DOI: 10.1080/00103620601093686

Characterization of Low-Molecular-Weight Organic Acids and Organic Carbon of Taiwan Red Cypress, Peacock Pine, and Moso Bamboo in a Temperate Rain Forest

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Abstract: Low-molecular-weight organic acids (LMWOAs) derived from root exudates and complexed with available metals in the rhizosphere soils of Taiwan red cypress (*Chamaeyparis formosensis*, FRS), peacock pine (*Cryptomeria japonica*, JRS), and moso bamboo (*Phyllostachys pubescens*, PRS) were identified by gas chromatograph (GC). The fresh plants (i.e., leaves, stems, roots, and litters) and soil samples of those three vegetations were examined for their organic functional groups. This study focused on (1) assessing methods for processing LMWOAs in the rhizosphere soils and fresh plants by GC analysis and (2) determining the relative proportions of organic carbon (C) functional groups in the three vegetations and fresh plant materials with ^{13}C nuclear magnetic resonance (NMR) analysis. The proportion of LMWOAs contents followed the order of PRS > FRS > JRS > bulk soils. The recovery and spiking tests analyzed by GC showed good recovery (>83.6%) and reproducibility of LMWOAs. The proportion of organic functional groups in the rhizosphere

Received 26 March 2004, Accepted 12 May 2006
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and bulk soil quantified by ^{13}C NMR analysis followed the general order alkyl-C > aromatic-C > O-alkyl-C > N-alkyl-C > phenolic-C > acetal-C > carboxyl-C. The carbohydrates, hemicellulose, lignin, and cellulose contents obtained by ^{13}C NMR analysis suggested that *Phyllostachys pubescen* (*PB*) cannot easily be decomposed under the mountain forest soil environments.

Keywords: Bulk soils, ^{13}C nuclear magnetic resonance (NMR), gas chromatograph (GC), rhizosphere soils, root exudates

INTRODUCTION

Rhizosphere is defined as that volume of soil affected by the presence of the roots of growing plants. The vegetation change may be related to biological, physical, and chemical properties of soils. A multiple of compounds are released into the rhizosphere of plants grown in soil, most of which are normal plant constituents derived from photosynthesis and other plant processes (Uren 2001). The relative and absolute amounts of low-molecular-weight organic acids (LMWOAs) produced by plant roots in the form of exudates vary with the plant species, cultivar, age of plant, and environmental conditions including soil properties (Robert and Berthelin 1986).

The biological activities of microorganisms in the rhizosphere mediate the solubility to a large degree. The complexities of the interactions among soil, microorganisms, and plants are needed to study these highly sophisticated biological systems (Jones and Darrah 1994; Manthey et al. 1994; Chiu et al. 2002). The soil that remained adhered to the roots after gentle shaking was sampled as rhizosphere soils according to operational definition (Lynch 1990). The root exudation takes place primarily from fine roots near root tips, which are usually closely spaced in the A soil horizon. A bulk soil was sampled from a central location of about 50 cm in distance from the main root surface between these trees.

Conventional characterization of soil organic matter (SOM) previously used alkaline and acid solutions to extract SOM, which were tightly bound to soil inorganic colloids, resulting in soil aggregation (Stevenson 1994). In recent decades, advances in gas chromatograph (GC) as well as cross-polarization and magic angle spinning ^{13}C solid-state nuclear magnetic resonance (^{13}C NMR) have assisted in the study of LMWOAs and SOM components (Oades 1993). The cross-polarization and magic angle spinning (CPMAS) solid-state ^{13}C NMR is a nondestructive method that can help characterize SOM in mineral and organic soils (Preston et al. 1989, 1990, 1994). Both GC and ^{13}C NMR analytical methods are useful tools for studying root exudates of various tree species and transformation of SOM.

Taiwan red cypress (*Chamaeyparis formosensis*, *CF*), peacock pine (*Cryptomeria japonica*, *CJ*), and moso bamboo (*Phyllostachys pubescens*, *PB*) are major tree vegetations widely distributed in the middle or high elevation of Taiwan. To clarify root exudate productions, transformation of SOM in these vegetations are important for the sustainable managements of forestry. Therefore, this study focused on (1) assessing methods for processing LMWOAs by GC analysis and (2) determining the relative proportions of organic C functional groups in the three vegetations and fresh plant materials with ^{13}C NMR analysis.

MATERIALS AND METHODS

Study Sites

Three study blocks were located within (A) *CF*, (B) *CJ*, and (C) *PB* vegetations. The study sites were located on Si-tou, Forest Experimental Station of National Taiwan University, in central Taiwan, with an annual precipitation of about 2057 mm (23°40′ N, 120°47′ E). The average annual temperature is 17°C. There are three sites in each block of *CF*, *CJ*, and *PB* vegetations with elevation of 1150 m. Rhizosphere and bulk soils (0–15 cm) of three species of trees were sampled in a triplicates. Sample collections were made in completed random design (CRD) at each block. Fresh roots, stems, leaves, and litters of these trees were also collected as comparison. The samples were immediately preserved with dry ice. Soil and plant materials were ground, passed through a sieve (<2 mm), and then stored in a deep freezer at −24°C.

Soil Physical and Chemical Properties

Soil pH of the samples was measured in distilled water and 0.1 *M* KCl solution (soil–solution = 1:5). Concentrations of organic C and nitrogen (N) of the soils were determined by a Carlo Erba CHN analyzer. Cation-exchange capacity (CEC) of the soils was determined by the conventional NH_4OAc method (Gillman 1979). Exchangeable cations in supernatants were extracted by 1 *M* ammonium acetate (pH 7.0) solutions. Concentrations of exchangeable sodium (Na) and potassium (K) were determined by flame photometer. Magnesium (Mg) and calcium (Ca) were determined by atomic absorption (AA) spectrometry (Hitachi 180-30). The dispersed soils were separated into clay, silt, and sand fractions by sedimentation and centrifugation (Jackson 1979). Soils were classified as silty loam, mixed, mesic, humic, Dystrudepts (Soil Survey Staff 2003).

Determination of Low-Molecular-Weight Organic Acids in Root Exudates by Gas Chromatograph

Gas chromatography analysis of methylated dicarboxylic acids was employed using an Agilent 6850 series GC system, equipped with flame ionization detector aid as J&W Scientific GC column (0.25 mm × 30 m, 0.1 mm, i.d.). The injector, column, and detector temperatures were 200, 125, and 200°C, respectively. Helium was used as the carrier gas at a flow rate of 59.6 mL min^{-1}. The chromatograms were integrated by GC chemostatic Rev. A. 08103 software.

Standards for the LMWOAs (oxalic, malonic, succinic, fumaric, and maleic) were obtained from Sigma Chemical Co., whereas methanol and chloroform used for sample preparation were analytical-grade reagents. Methylmalonic acid, not found in root exudates of *CF*, *CJ*, and *PB*, was used as an internal standard and added both standard mixtures and samples before methylation.

Acid Extraction and Concentration from Roots, Stems, Leaves, and Litter Exudates, Rhizosphere and Bulk Soils

Fresh materials corresponding to 1 g of dry weight of ground roots, stems, leaves, and litters and 15 g of soils were extracted in a 250-mL centrifuge tube for extractions with 20 mL of 0.5 *M* HCl in methanol (MeOH) 1:1 ratio after being shaken for 1 h and then centrifuged at 10,000 × g. The detailed methylation, separation and injection into GC column, and recovery test of samples were described by Chen et al. (2001).

Sample Stability

LMWOAs of derived samples were quite stable when samples were stored under refrigeration and transported to the laboratory in dry ice. Samples analyzed after 1 month of storage in the freezer (−24°C) showed no signs of decomposition.

CPMAS ^{13}C Nuclear Magnetic Resonance Analysis

Organic functional groups of ground fresh roots, stems, leaves, litters, and soils were examined by CPMAS ^{13}C nuclear magnetic resonance (NMR) spectroscopy (Oades 1995) using a Bruker MSL-200 NMR instrument. Samples were spun at the magic angle in the boron nitride rotor with a Kel-F cap. Data acquisition conditions were spectrometer frequency, 50.33 MHz; spinning speed, 3500 Hz; contact time, 1 ms; and pulse delay

time, 1 s. The NMR spectra were divided into the following chemical shift regions: alkyl-C (0–45 ppm), N-alkyl-C (46–65 ppm), O-alkyl-C (65–90 ppm), acetal-C (90–110 ppm), aromatic-C (110–140 ppm), phenolic-C (140–160 ppm), and carboxyl-C (160–200 ppm) (Preston et al. 1990). Areas of the chemical shifts measured by cutting and weighting were expressed as percentages of total area (relative intensity) (Preston et al. 1990; Chen et al. 2001).

RESULTS AND DISCUSSION

The pH of bulk soils (FBS, JBS, and PBS) was higher than that of FRS, JRS, and PRS rhizosphere soils owing to organic acid exudates. The pH values of the three trees' rhizosphere soils showed no significant differences. All sample textures were silty loam. Cation-exchange capacity (CEC) and sum of exchangeable cations showed the trend as PRS > JRS > FRS > PBS > FBS > JBS (Table 1). Organic C and total N contents showed a similar trend as soil pH (Tu 2002).

Determination of Low-Molecular-Weight Organic Acids

The five LMWOAs investigated in this study required more than 10 min for elution, making GC monitoring of the organic acid contents in solutions very fast and efficient. Each sample was injected at a 15-min interval, and methylation of standard mixtures yielded clean chromatograms with no artifacts formed. The results were similar to those reported by Chen et al. (2001).

Recovery of all LMWOAs was more than 83.6% (Table 2). The fumaric and maleic acids showed higher recovery percentages. The recovery of acids from the extraction/concentration and methylation procedure was examined by spiking the FRS, JRS, and PRS with 100 μg of selected acids. Good recovery and reproducibility were obtained for the saturated dicarboxylic, oxalic, malonic, and succinic acids (Lilieholm et al. 1992). It has been reported that the methylation of unsaturated dicarboxylic acids may be accompanied by undesirable side reactions, and cis-trans isomerization (Moghimi et al. 1978). Methodology evaluation demonstrated that the method developed in this study was accurate and precise. The LMWOAs in various rhizosphere soils of FRS, JRS, and PRS are shown in Table 3. The proportion of oxalic acid contents followed the order PRS > FRS > JRS > bulk soils, with significant differences ($p < 0.05$). On the other hand, malonic, fumaric, and succinic acids showed the same trends as oxalic acid concentrations.

LMWOAs in the fresh *formosensis* roots (FFR); twigs and leaves (FFS); fresh *japonica* roots (FJR) and twigs and leaves (FJS); fresh *pubescens* roots

Table 1. Selected soil physical and chemical properties of *Chamaecyparis formosensis*, *Cyrptomeria japonica*, and *Phyllostacys pubescens* rhizosphere and bulk soils

Sample	pH		Organic C (%)	Total N (%)	C-N ratio	CEC[a]	Exchangeable cation (cmol (+)kg^{-1} soil)					BS[a] (%)	Sand (%)	Silt (%)	Clay (%)	Texture
	H_2O	KCl					Ca	K	Mg	Na	Sum					
FRS[b]	4.48[ac]	3.47[a]	5.18[a]	0.41[a]	12.70[b]	33.50[a]	3.02[a]	2.32[a]	2.33[a]	1.69[a]	9.36[a]	28.05[a]	66.3	18.1	15.6	SL[a]
FBS	4.94[b]	3.86[b]	2.44[b]	0.17[b]	14.46[a]	21.57[b]	1.42[b]	1.32[b]	0.76[b]	1.12[b]	4.62[b]	21.46[b]	60.9	25.0	14.1	SL
JRS	4.42[a]	3.36[a]	5.32[a]	0.40[a]	13.30[a]	35.43[a]	3.11[a]	2.39[a]	2.29[a]	2.09[a]	9.88[a]	27.92[a]	63.9	21.8	14.3	SL
JBS	4.85[b]	3.78[b]	2.51[b]	0.19[b]	13.24[a]	22.22[b]	1.52[b]	1.50[b]	0.77[b]	1.23[b]	5.02[d]	22.71[b]	57.5	26.1	16.4	SL
PRS	4.26[a]	3.16[a]	5.66[a]	0.46[a]	12.25[b]	39.56[a]	3.34[a]	2.52[a]	2.36[a]	2.75[a]	10.97[a]	27.82[a]	62.5	22.9	14.6	SL
PBS	4.80[b]	3.64[b]	2.55[b]	0.19[b]	13.20[a]	24.54[b]	1.68[b]	1.87[b]	0.77[b]	1.30[b]	5.62[b]	22.99[b]	55.1	30.4	14.5	SL

[a]CEC: cation exchangeable capacity; BS: base saturation; SL: sandy loams.

[b]FRS: *Chamaecyparis formosensis* rhizosphere soil; FBS: *Chamaecyparis formosensis* bulk soil; JRS: *Cryptomeria japonica* rhizosphere soil; JBS: *Cryptomeria japonica* bulk soil; PRS: *Phyllostachys pubescens* rhizosphere soil; PBS: *Phyllostachys pubescens* bulk soil.

[c]The number followed by the same letter is not significantly different ($p < 0.05$) as determined by Duncan's multiple range test.

Table 2. Recovery of added spike of low-molecular-weight dicarboxylic acids of rhizosphere soils by HCl/MeOH extraction as determined by GC

	Amount found in unspiked samples (μg)			Amount found in spiked samples (μg)			Recovery ± SD (%)		
Acid[a]	FRS	JRS	PRS	FRS	JRS	PRS	FRS	JRS	PRS
Oxalic	374 ± 1.6[ab]	340 ± 8.2[a]	422 ± 4.7[a]	458 ± 4.3[a]	423 ± 2.6[b]	510 ± 1.7[a]	84.0 ± 5.5	83.6 ± 5.8	88.2 ± 6.5
Malonic	46.1 ± 2.7[b]	37.6 ± 2.2[b]	92.3 ± 1.6[a]	134 ± 3.5[b]	128 ± 5.9[b]	183 ± 1.7[a]	88.1 ± 3.0	89.9 ± 3.2	91.0 ± 3.1
Fumaric	11.0 ± 1.7[a]	9.9 ± 1.8[a]	12.7 ± 8.1[a]	103 ± 6.8[a]	102 ± 2.3[a]	105 ± 4.7[a]	91.8 ± 1.3	92.2 ± 1.5	92.6 ± 4.0
Succinic	89.2 ± 4.7[a]	81.1 ± 6.0[a]	111 ± 5.2[a]	179 ± 7.2[a]	172 ± 2.7[a]	200 ± 5.4[a]	90.2 ± 3.6	91.3 ± 3.7	89.8 ± 3.8
Maleic	—[c]	—	—	95.5 ± 1.8[a]	93.2 ±1.2[a]	94.5 ± 3.7[a]	95.5 ± 2.9	93.2 ± 2.5	94.5 ± 2.4

[a]100 μg of each acid added to 1 g of soil (n = 3).

[b]Data are expressed as mean ± SD. Averages followed by the same letter are not significantly different ($p < 0.05$) as determined by least significant difference (LSD) test.

[c]Not detectable (MDL < 1 mg kg^{-1}).

Note: FRS: *Chamaecyparis formosensis* rhizosphere soil; JRS: *Cryptomeria japonica* rhizosphere soil; PRS: *Phyllostachys pubescens* rhizosphere soil.

Table 3. Low-molecular-weight organic acids in FRS, JRS, PRS, FBS, JBS, and PBS

Sample[a]	Organic acid concentration (mg/kg^{-1} dry weight of soil)				
	Oxalic acid	Malonic acid	Fumaric acid	Succinic acid	Maleic acid
FRS	54.4 ± 10.1[b*b*]	7.8 ± 1.4[a]	1.9 ± 0.6[b]	18.6 ± 5.9[b]	—[c]
FBS	30.2 ± 10.3[c]	2.7 ± 1.0[b]	0.8 ± 0.5[c]	8.0 ± 3.2[c]	—
JRS	44.5 ± 11.9[b]	5.9 ± 1.2[a]	1.4 ± 0.4[b]	14.6 ± 5.5[b]	—
JBS	20.7 ± 10.3[b]	2.3 ± 1.2[b]	0.5 ± 0.3[c]	7.2 ± 3.8[c]	—
PRS	63.2 ± 8.9[a]	11.8 ± 2.9[a]	2.6 ± 0.8[a]	23.1 ± 8.7[a]	—
PRS	30.8 ± 13.1[c]	4.3 ± 1.4[b]	1.1 ± 0.5[c]	9.2 ± 3.0[c]	—

[a]FRS: *Chamaecyparis formosensis* rhizosphere soil; FBS: *Chamaecyparis formosensis* bulk soil; JRS: *Cryptomeria japonica* rhizosphere soil; JBS: *Cryptomeria japonica* bulk soil; PRS: *Phyllostachys pubescens* rhizosphere soil; PBS: *Phyllostachys pubescens* bulk soil.

[b]Data are expressed as mean ± SD. Averages followed by the same letter are not significantly different ($p < 0.05$) as determined by least significant difference (LSD) test.

[c]Not detectable (MDL < 1 mg kg^{-1}).

(FPR) and twigs and leaves (FPS) were identified on the basis of retention time by GC analysis (Table 4). The reproducibility of retention time was good, as indicated by the recovery test of more than 83.6%. High oxalic acid contents were present in FFS (2400 ± 1.2 mg kg^{-1}), FJS (1610 ± 2.4 mg kg^{-1}), and FPS (2530 ± 1.1 mg kg^{-1}). High malonic acids were also present in FFS (1540 ± 4.4 mg kg^{-1}) and FJS (1060 ± 7.9 mg kg^{-1}) and showed significant differences between FFS and FJS. The succinic acid contents in FFS and FJS showed a trend similar to malonic acid. The amounts of LMWOAs in fresh twigs and leaves were greater than those in fresh roots. The amounts of malonic and succinic acids in FFS were greater than those in FJS and FPS. There were low concentrations of furmaric acid present in FPR (78.0 ± 1.0 mg kg^{-1}) and FPS (40.4 ± 3.2 mg kg^{-1}). No maleic acids were present in any rhizosphere soils or plant tissues (Tables 3 and 4), respectively.

Nature of Organic Matter

The ^{13}C NMR analysis of soil organic matter (SOM) and organic carbon in FRS, JRS, PRS, and PBS can be classified into seven groups (Figure 1A). The concentrations of these organic functional groups in the rhizosphere soils followed the general trend alkyl-C > aromatic-C > O-alkyl-C > N-alkyl-C > phenolic-C > acetal-C > carboxyl-C (Table 5). The N-alkyl-C, O-alkyl-C, and carboxyl-C contents in PBS were greater than these in FRS

Table 4. Low-molecular-weight organic acids in plants

Sample[a]	Organic acid concentration (mg kg^{-1} dry weight of plant)[b]					
	Oxalic acid	Malonic acid	Fumaric acid	Succinic acid	Maleic acid	Sum
FFR	[c]280.3 ± 1.3[a]	82.6 ± 8.1[d]	—[d]	60.8 ± 1.6[d]	—	423.7
FFS	2400 ± 1.2[d]	1540 ± 4.4[a]	—	1320 ± 1.0[a]	—	5260
FJR	188 ± 2.7[d]	56.6 ± 2.2[d]	—	41.2 ± 1.2[d]	—	285.8
FJS	1610 ± 2.4[b]	1060 ± 7.9[b]	—	898 ± 5.0[b]	—	3568
FPR	980 ± 2.3[c]	113 ± 1.2[c]	78.0 ± 1.0[a]	75.3 ± 1.1[d]	—	2796.3
FPS	2530 ± 1.1[a]	93.6 ± 6.6[d]	40.4 ± 3.2[b]	233 ± 4.7[c]	—	1347

[a]FFR: *Chamaecyparis formosensis* fresh root; FFS: *Chamaecyparis formosensis* fresh twig and leaf; JFR: *Cryptomeria japonica* fresh root; JFS: *Cryptomeria japonica* fresh twig and leaf; PFR: *Phyllostachys pubescens* fresh root; PFS: *Phyllostachys pubescens* fresh twig and leaf.

[b]Recovery (>83.6%) of low-molecular-weight dicarboxylic acids from HCl/MeOH extraction of plants as determined by GC after sample methylation.

[c]Data are expressed as mean ± SD. Averages followed by the same letter are not significantly different ($p < 0.05$) as determined by least significant difference (LSD) test.

[d]Not detectable (MDL < 1 mg kg^{-1}).

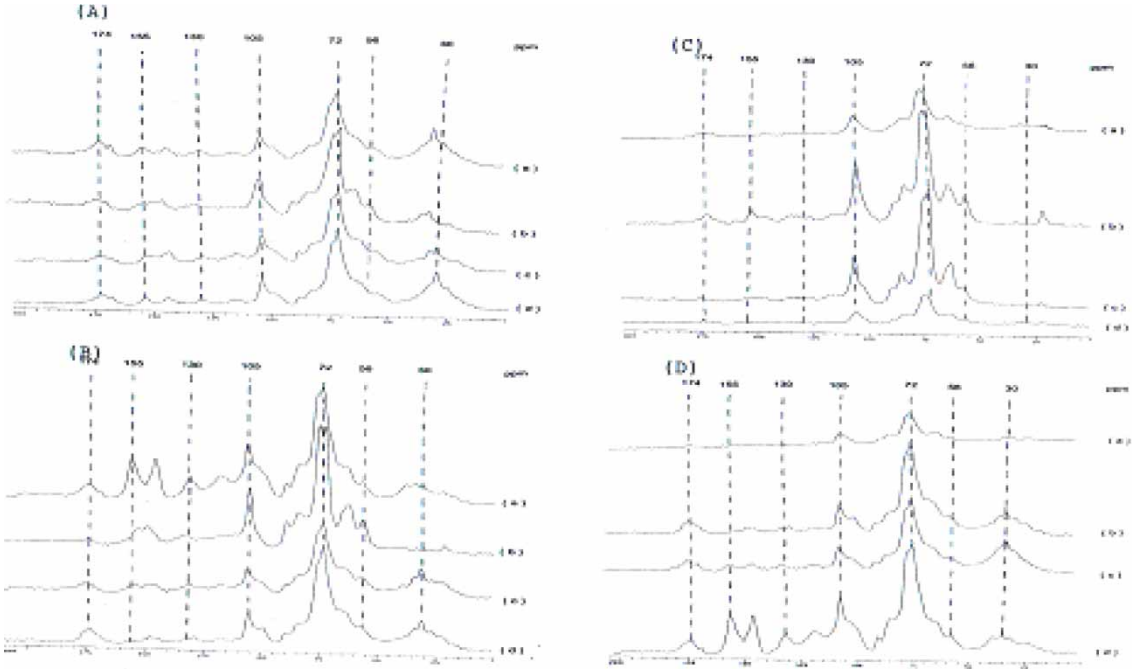

Figure 1. ^{13}C NMR analysis of soil organic matter (SOM) and organic C in (a) FRS, (b) JRS, (c) PRS, and (d) PBS can be classified into seven groups (1A). The distribution of organic functional groups in *Chamaecyparis formosensis* of (a) fresh leaves (FFL), (b) stems (FFS), (c) roots (FFR), and (d) litters (FL) (1B); *Crytomeria japonica* (a) fresh leaves (JFL), (b) stems (JFS), (c) roots (JFR), and (d) litters (JL) (1C); and *Phyllostachys pubescens* (a) fresh leaves (PFL), (b) stems (PFS), (c) roots (PFR), and (d) litters (PL) (1D).

Table 5. Distribution (%) of organic carbon functional groups in FRS, JRS, PRS, FBS, JBS, and PBS

	Functional group (%)						
Sample[a]	Alkyl-C	N-alkyl-C	O-alkyl-C	Acetal-C	Aromatic-C	Phenolic-C	Carboxyl-C
FRS	22.9 ± 9.0^{ab}	12.4 ± 1.8^{a}	13.7 ± 2.0^{a}	11.3 ± 2.1^{a}	18.3 ± 8.4^{a}	12.5 ± 2.7^{a}	8.9 ± 2.9^{b}
FBS	10.3 ± 6.1^{b}	6.3 ± 1.9^{b}	5.3 ± 1.2^{b}	7.5 ± 3.7^{a}	15.3 ± 7.3^{a}	10.5 ± 3.7^{a}	7.5 ± 3.6^{b}
JRS	24.5 ± 9.8^{a}	12.3 ± 1.8^{a}	13.7 ± 1.8^{a}	10.7 ± 1.9^{a}	18.5 ± 9.2^{a}	11.3 ± 3.4^{b}	9.0 ± 3.3^{b}
JBS	11.4 ± 3.7^{b}	5.2 ± 1.4^{b}	4.7 ± 1.3^{b}	6.3 ± 1.1^{a}	14.7 ± 2.7^{a}	8.3 ± 4.5^{b}	7.3 ± 4.5^{b}
PRS	23.6 ± 8.7^{a}	13.4 ± 2.1^{a}	16.4 ± 1.4^{a}	9.6 ± 1.1^{a}	16.3 ± 9.8^{a}	8.5 ± 1.1^{b}	12.2 ± 3.0^{b}
PBS	17.0 ± 8.3^{b}	6.4 ± 1.0^{b}	17.1 ± 1.3^{b}	7.2 ± 1.0^{a}	13.0 ± 8.8^{a}	7.3 ± 3.3^{b}	18.7 ± 3.5^{a}

[a]FRS: *Chamaecyparis formosensis* rhizosphere soil; FBS: *Chamaecyparis formosensis* bulk soil; JRS: *Cryptomeria japonica* rhizosphere soil; JBS: *Cryptomeria japonica* bulk soil; PRS: *Phyllostachys pubescens* rhizosphere soil; PBS: *Phyllostachys pubescens* bulk soil.

[b]Data are expressed as mean ±SD. Averages followed by the same letter are not significantly different ($p < 0.05$) as determined by east significant difference (LSD) test.

and JRS. High O-alkyl-C, aromatic-C, and phenolic-C and low N-alkly-C and acetal-C contents were present in bulk soils, indicating that the rate of SOM decomposition is slower than that of rhizosphere soils. The alkyl-C contents in rhizosphere soils were greater than those in bulk soils. The sources of alkyl-C were from root exudates and microbial resynthesis. Guggenberger et al. (1994, 1995) reported that monosaccharide is a major production of microbial resynthesis. Although the contents of organic C functional groups analyzed by NMR can be quantified from peak area with errors because of its peak overlap, it shows a reproducible trend (Malcolm 1989; Guggenberger et al. 1994, 1995; Oades 1995). Alkyl-C, N-alkyl-C, O-alkyl-C, and aromatic-C in rhizosphere soil samples of FRS, JRS, and PRS showed no significant differences, but phenolic-C and carboxyl-C did (Table 5).

The distribution of organic functional groups in *Chamaecyparis formosensis* fresh leaves (FFL), stems (FFS), roots (FFR), and litters (FL); *Crytomeria japonica* fresh leaves (JFL), stems (JFS), roots (JFR), and litters (JL); and *Phyllostachys pubescens* fresh leaves (PFL), stems (PFS), roots (PFR), and litters (PL) are shown in Table 6 and Figures 1(B), 1(C), and 1(D). The highest of O-alkyl-C (cellulose) contents were present in plant tissues of *Phyllostachys pubescens.* The amounts of O-alkyl-C and carboxyl-C in fresh leaves, stems, roots, and litters of the three tree species showed no significant differences. Lignin (aromatic-C and phenolic-C) contents of FL and JL are greater than those of *Chamaecyparis formosensis* and *Crytomeria japonica* plant tissues (Table 6). However, lignin concentrations in *Phyllostachys pubescens* litters (PL) showed no significant differences with PFL, PFS, and PFR. Hemicellulose (N-alkyl-C and acetal-C) showed the trend as follows: FL $>$ (FFL $=$ FFS $=$ FFR). On the other hand, cellulose contents in FL showed the reverse trend. Carbohydrates contents (alkyl-C) in the leaves, stems, roots, and litters of these trees showed random distribution, but in general, it can be summarized as the following tendency: *Chamaecyparis formosensis* $>$ *Crytomeria japonica* $>$ *Phyllostachys pubescens*. Carboxyl-C concentrations showed a trend similar to alkyl-C (carboxydrates) concentrations. After decomposition, the components of microsynthesis carboxydrate, hemicellulose, and lignin tended to increase (Hatcher 1987). *Chamaecyparis formosensis* decomposes more easily than *Crytomeria japonica* and *Phyllostachys pubescens*. The cellulose (O-alkyl-C) contents in *Phyllostachys pubescens* plant tissues were greater than those of *Crytomeria japonica* and *Chamaecyparis formosensis* (Table 6). On the other hand, the sum of carbohydrates (alkyl-C), hemicellulose (N-alkyl-C and acetal-C), lignin (aromatic-C and phenolic-C), and carboxyl-C are also show the trend as *Chamaecyparis formosensis* (FFL of 64.5%, FFS of 65.7%, FFR of 64.0%, and FL of 93.3%) $>$ *Crytomeria japonica* (JFL of 61.6%, JFS of 56.2%, JFR of 64.4% and JL of 65.1%) $>$ *Phyllostachys pubescens* (PFL of 57.9%, PFS of 53.2%, PFR of 49.7%, and PL of 62.1%). The microbial resynthesis production of carbohydrates is a major component in the temperate rain forest soil environment with high annual precipitation.

Table 6. Distribution (%) of organic carbon functional groups in the plants

Sample[a]	Functional group (%)						
	Alkyl-C	N-alkyl-C	O-alkyl-C	Acetal-C	Aromatic-C	Phenolic-C	Carboxyl-C
Chamaecyparis formosensis							
FFL	16.0 ± 2.7[b*b*]	15.9 ± 2.8[b]	35.5 ± 2.2[a]	11.3 ± 7.4[a]	7.9 ± 1.5[b]	5.9 ± 4.5[a]	7.5 ± 5.8[a]
FFS	23.6 ± 1.4[a]	14.9 ± 3.9[a]	34.3 ± 1.8[a]	10.7 ± 3.9[a]	6.8 ± 1.9[b]	4.4 ± 2.6[a]	5.3 ± 1.4[a]
FFR	15.7 ± 4.6[b]	15.1 ± 2.4[a]	36.0 ± 5.0[a]	12.7 ± 4.4[a]	9.9 ± 2.8[b]	6.2 ± 3.3[a]	4.4 ± 3.4[a]
FL	16.9 ± 5.2[a]	23.5 ± 3.2[b]	6.7 ± 4.5[b]	20.1 ± 5.8[a]	15.7 ± 2.2[a]	9.0 ± 5.7[a]	8.1 ± 4.3[a]
Cryptemeria japonica							
JFL	17.4 ± 7.3[a]	16.6 ± 4.6[a]	38.4 ± 1.1[a]	11.3 ± 2.1[a]	6.9 ± 1.8[b]	3.5 ± 2.2[a]	5.9 ± 1.8[a]
JFS	5.4 ± 2.3[b]	16.3 ± 5.4[a]	43.8 ± 8.4[a]	12.1 ± 4.2[a]	10.8 ± 1.7[a]	8.0 ± 3.2[a]	3.6 ± 2.0[a]
JFR	21.2 ± 5.6[a]	15.7 ± 2.8[a]	33.6 ± 5.8[a]	10.4 ± 4.8[a]	9.1 ± 1.3[a]	5.2 ± 3.0[b]	4.8 ± 2.2[a]
JL	10.8 ± 3.6[c]	10.6 ± 2.6[a]	34.9 ± 3.9[a]	14.6 ± 2.7[a]	12.0 ± 2.2[a]	12.0 ± 2.9[b]	5.1 ± 1.5[a]
Phyllostachys pubescens							
PFL	15.3 ± 2.2[a]	16.6 ± 3.7[a]	42.1 ± 7.8[a]	13.1 ± 1.2[a]	5.5 ± 1.7[b]	2.8 ± 1.0[b]	4.6 ± 2.3[a]
PFS	4.6 ± 1.5[a]	15.1 ± 4.3[a]	46.8 ± 6.5[a]	15.3 ± 2.2[a]	8.9 ± 1.0[a]	5.1 ± 1.2[a]	4.2 ± 2.0[a]
PFR	5.8 ± 1.0[b]	18.1 ± 4.5[a]	50.4 ± 8.3[a]	13.5 ± 1.7[a]	7.3 ± 1.1[a]	3.1 ± 1.1[a]	1.9 ± 1.0[a]
PL	16.1 ± 2.8[b]	15.4 ± 3.6[a]	37.9 ± 5.8[a]	13.0 ± 2.0[a]	8.2 ± 1.3[a]	4.4 ± 1.0[a]	5.0 ± 1.8[a]

[a]FFL: *Chamaecyparis formosensis* leaves; FFS: *Chamaecyparis formosensis* stems; FFR: *Chamaecyparis formosensis* roots; FL: *Chamaecyparis formosensis* litters; JFL: *Cryptemeria japonica* leaves; JFS: *Cryptemeria japonica* stems; JFR: *Cryptemeria japonica* roots; JL: *Cryptemeria japonica* litters; PFL: *Phyllostachys pubescens* leaves; PFS: *Phyllostachys pubescens* stems; PFR: *Phyllostachys pubescens* roots; PL: *Phyllostachys pubescens* litters.

[b]Data are expressed as mean ±SD. Averages followed by the same letter are not significantly different ($p < 0.05$) as determined by least significant difference (LSD) test.

In the temperate rain forest soil environments, the decomposition of *Phyllostachys pubescens* tissues is slower than that of *Crytomeria japonica* and *Chamaecyparis formosensis*. Hence, the cellulose contents (O-alkyl-C) in the PRS and PBS were greater than those in JRS and FRS (Table 5). However, the amounts of lignin (aromatic-C and phenolic-C) showed the reverse trend. The debris of the *Phyllostachys pubescens* is not easily decomposed under the mountain forest soil environment. Therefore, the PRS and PBS are the major organic C sources.

CONCLUSIONS

LMWOAs in rhizosphere soils analyzed by GC showed reproducible results and high recovery ($>83.6\%$). The ^{13}C NMR analysis could not accurately quantify its organic C functional groups owing to peak overlap. Quantitative organic C functional groups by ^{13}C NMR and the correlation among availability of metals, nutrients, and LMWOAs merit further study for plant growth.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support by the National Science Council, Taiwan, under projects NSC 85-2621-3002-017, 90-2313-B-002-279, 90-2621-B-002-001-A10, and 91-2623-7-002-004.

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