

Research paper

## Low-molecular-weight Organic Acids and Metal Speciation in Rhizosphere and Bulk Soils of a Temperate Rain Forest in Chitou, Taiwan

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### 【 Summary 】

In this study, we investigated the basic soil physicochemical properties, low molecular weight organic acids (LMWOAs), and the associated speciation and sequential extractability of selected metals in rhizosphere and bulk soils of 3 trees, namely *Chamaecyparis formosensis*, *Cryptomeria japonica*, and *Phyllostachys pubescens*, of montane forests in Taiwan. LMWOAs were identified by gas chromatography, and the basic physicochemical properties of the soils were determined by conventional methods. Various fractions of metals in rhizosphere and bulk soils were extracted by a multiple-step extraction method. The pH of rhizosphere soils was found to be lower than that of bulk soils. The mean cation-exchange-capacity (CEC), organic carbon, nitrogen, and clay contents of rhizosphere soils as well as oxalic, malonic, fumaric, and succinic acids were greater than those in bulk soils ( $p < 0.05$ ). Metal speciation fractionated by multiple-step extractions showed that Al, Fe, Mn, Cu, and Zn were distributed in different exchangeable, carbonate-bound, organic complex-bound, amorphous mineral colloid-bound, and residual fractions. No significant differences were observed among the tree species in physicochemical properties or metal fractionation except for LMWOAs. Concentrations of extractable Al and Fe showed high correlation with oxalic acid and total LMWOAs ( $p < 0.01$ ). However, the extractable Mn, Cu, and Zn in the rhizosphere soils showed poor correlations with LMWOAs.

**Key words:** bulk soils, extractable metals, low molecular weight organic acids, multiple-step extraction, rhizosphere soils.

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## 研究報告

## 溪頭溫帶森林根圈及非根圈土壤中低分子量有機酸 與重金屬物種之研究

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### 摘 要

本研究之目的在於調查在中海拔紅檜、柳杉及孟宗竹根圈與非根圈土壤之基本土壤物理化學性質和低分子量有機酸以及可萃取土壤金屬之相關性。本研究使用傳統方法分析土壤物理化學性質，而在根圈及非根圈土壤之金屬劃分則使用連續萃取法，以氣體色層分析儀測定土壤之低分子量有機酸含量。一般而言，根圈土壤之酸鹼值低於非根圈土壤，根圈土壤之平均陽離子交換容量、有機碳、有機氮及黏土含量高於非根圈土壤( $p < 0.05$ )。另外，根圈土壤之草酸、丙二酸、反-丁烯二酸及琥珀酸含量也大於非根圈土壤。經連續萃取法所萃取之鋁、鐵、錳、銅及鋅分佈在可交換、碳酸根、有機錯合、無定型-膠體-鍵結以及殘留部份。可抽出之鋁及鐵的濃度與草酸及低分子量有機酸呈現相當高度相關性( $p < 0.01$ )。但在根圈土壤中可萃取出的錳、銅及鋅的含量與低分子量有機酸之含量的相關性不佳。

關鍵詞：非根圈土壤、可萃取金屬、低分子量有機酸、連續萃取法、根圈土壤。

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## INTRODUCTION

Plant survival and crop productivity are strictly dependent on the capability of plants to adapt to different environments. For such adaptation, the plant rhizosphere provides a direct interface from which plants can grow and interact with the soil. Rhizosphere soil is defined as the narrow zone of soil subject to the influence of living plant roots. Metabolic processes performed by roots create a physiochemical and biochemical environment within the rhizosphere, which greatly differs from that in the surrounding bulk soils (Curl and True 1986, Lynch 1990, Marschner 1998, McLaughlin et al. 1998, Pinton et al. 2001). Plant roots release a multitude of compounds, including sugars, polysaccharides, amino acids, organic acids, fatty acids, sterols, growth factors, enzymes, flavonones, and nucleotides, into the rhizosphere (Uren 2001). Low mo-

lecular-weight organic acids (LMWOAs) are among the important root exudates and are widely present in the rhizosphere. They are more mobile and are more readily assimilated by a wider range of microorganisms than are high-molecular-weight compounds (Szmigielska et al. 1995, 1996, Robert and Berthelin 1986, Fox and Comerford 1990, Satoh et al. 1990). Because of intense biological activity, the concentration of LMWOAs in rhizosphere soils is much higher than that in bulk soils (Robert and Berthelin 1986, Stevenson 1994). Therefore, the enhanced weathering of soil minerals, intense transformations of Al and Fe, and associated changes in speciation of other metals such as Mn, Cu, and Zn are expected. Furthermore, the LMWOAs of root exudates in the tree rhizosphere should vary with tree species (Chen et al. 2001). The

speciation, mobility, and extractability of these metals are influenced by the nature of the tree rhizosphere, and the impacts of rhizosphere chemistry on forest ecosystem merit further study.

To a large degree, the biological activities of microorganisms in the rhizosphere mediate the solubility and hence influence the extractability of metals at root surfaces. These highly sophisticated biological systems are worth further investigation in order to understand the complex interactions among the soil, microorganisms, and plants (Jones and Darrah 1994, Manthey et al. 1994, Huang et al. 1995a, b, Chiu et al. 2002). LMWOAs influence transformations of Al and Fe in soil environments (Kwong and Huang 1981, Schwertmann et al. 1986, Huang 1988). Different structures and fractions of LMWOAs can modify the crystallization and subsequent transformation of Al and Fe oxides, hydroxides, and oxyhydroxides. Research data indicate that LMWOAs can influence the surface reactivities of these metal oxides, hydroxides, and oxyhydroxides through modification of the structural configuration during their formation (Xue and Huang 1995). These LMWOAs differ in their abilities to mobilize nutrients and toxic metals, thus facilitating their transport in soil profiles and to groundwater, and stream, river, and lake waters. Even on a global scale, to date, little is known about the nature and physiochemical properties of Al and Fe oxides, hydroxides, and oxyhydroxides and associated soil colloids in the rhizosphere of tree species as a function of distance from the root surface and in various slope positions in forest soil extracts at different elevations (Satoh et al. 1990, Huang et al. 1995a, b). The impact of rhizospheric processes on the speciation and extractability of metals is even less well understood. Forests cover over 2/3 of Taiwan, and it is important to utilize and

preserve forest resources through scientific management. *Chamaecyparis formosensis*, *Cryptomeria japonica*, and *Phyllostachys pubescens* are 3 major tree species widely distributed in the mountainous areas of Taiwan. However, their rhizosphere chemistry has not been fully investigated. Thus, in this study, we examined the basic soil physiochemical properties, LMWOAs, and the associated speciation and sequential extractability of selected metals in the rhizosphere of the above 3 tree species.

## MATERIALS AND METHODS

### Study site and soil sampling

Three blocks of the study site were located within areas containing (A) *Cha. formosensis*, (B) *Cr. japonica*, and (C) *Phy. pubescens*. The study site was located in Chitou, central Taiwan (23°40'N and 120°47'E), with an annual precipitation of about 2,057 mm and average annual temperature of 17°C. Rhizosphere and bulk soil samples were collected from the *Cha. formosensis* (1310~1315 m in elevation), *Cry. japonica* (1194~1208 m in elevation), and *Phy. pubescens* (1178~1198 m in elevation) blocks. These blocks were adjacent to one another at a distance of about 50 m. Rhizosphere and bulk soils of the A horizon (0~30 cm) under the 3 tree species were collected. Sample collections were made in a completely random design (CRD) in triplicate for each block ( $n = 9$ ). The soil that adhered to the roots after gentle shaking was sampled as rhizosphere soils according to an operational definition (Lynch 1990, Chen et al. 2001). Soils were classified as sandy loam, mixed, mesic, or humic Dystrudepts (Soil Survey Staff 2003).

Rhizosphere soils primarily contain fine roots near the tips, which are usually closely spaced in the A soil horizon (Marschner et al.

1987, Lynch 1990, Marschner 1991, 1998). Bulk soils were sampled from each of the tree locations about 15 cm in distance from the main roots. The soil samples were immediately preserved with dry ice. Soils were passed through a sieve (< 2 mm), and then stored in a freezer at -24°C.

#### Soil physical and chemical analyses

Soil pH was measured in distilled water and a 0.1 M KCl solution (with a soil to solution ratio of 1: 5). Concentrations of total carbon and nitrogen in the soils were determined using a Carlo Erba CHN analyzer (Carlo Erba Instrument, Milan, Italy). The cation-exchange capacity (CEC) of the soils was determined by the NH<sub>4</sub>OAc method (Gillman 1979). Exchangeable cations of the soils were extracted by 1 M ammonium acetate (pH 7.0) solutions. Concentrations of exchangeable Na and K were determined using a Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (Perkin Elmer Optima 2000DV, Wellesley, USA), Mg and Ca were determined by atomic absorption (AA) spectrometry (Hitachi AAS-180-30, Tokyo, Japan) (Jackson 1979).

#### Determination of low molecular weight organic acids (LMWOAs)

Gas chromatographic (GC) analysis of methylated dicarboxylic acids was employed with a Agilent 6850 series GC system (New York, USA), equipped with a flame ionization detector and as a J & W scientific GC column (0.25 mm × 30 m, 0.1 mm, i.d., New York, USA). 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<sup>-1</sup>. The chromatograms were integrated using GC chemostatic Rev. A. 08103 software (New York, USA).

Standards for the LMWOAs (oxalic,

malonic, succinic, fumaric, and maleic acids) were obtained from Sigma (St. Louis Mo, USA), while methanol and chloroform used for sample preparation were analytical grade reagents. Methylmalonic acid, not found in soil extracts of *Cha. formosensis*, *Cry. japonica*, and *Phy. pubescens*, was used as an internal standard and added to both standard mixtures and to samples before methylation. Fifteen grams of rhizosphere and bulk soils was extracted in a 250 ml centrifuge tube for extractions with 20 ml 0.5 M HCl in methanol (MeOH) at a 1:1 ratio after being shaken for 1 h, and then centrifuged at 10,000 g. The detailed methylation, separation, injection into the GC column, and recovery test of samples were described by Chen et al. (2001). The significant differences between various treatments were determined by Duncan's tests.

#### Extractability of metals

The extractability of metals in the soils was determined by the ammonium acetate-acetic-acid-ethylene-diaminetetracetic acid (AAA-EDTA) method (Lakanen and Ervio 1971). Three grams of soils was added to 30 ml of the AAA-EDTA solutions, and the suspensions were shaken for 1 h and filtered through Whatman no. 42 (Maidstone, England) filter paper. Concentrations of extractable Al, Fe, Mn, Cu, and Zn were determined by AA.

#### Fractionations of metal species

Speciation of selected metals, namely Al, Fe, Mn, Cu, and Zn of the freeze-dried soils (< 2 mm), was determined by the analytical scheme described by Krishnamurti et al. (1995). The multi-step sequential extraction scheme of the metals included the following forms: (1) exchangeable, (2) carbonate-bound, (3) metal organic complex-bound, (4) easily reducible metal oxide-bound, (5) H<sub>2</sub>O<sub>2</sub>

extractable organic-bound, (6) amorphous mineral colloid-bound, (7) crystalline Fe oxide-bound, and (8) residual fractions (i.e., occluded forms).

## RESULTS AND DISCUSSION

### Soil physical and chemical properties

The pH values of bulk soils were higher than those of rhizosphere soils of *Cha. formosensis*, *Cry. japonica*, and *Phy. Pubescens*. (Table 1). The roots of forest exudates of LMWOAs cause lower pH in the rhizosphere than for of bulk soil (Marschner and Romheld 1983). On the other hand, higher carbon and nitrogen concentrations are present near rhizosphere soils; these organic carbon resources come of the exudates and dead cells from roots (Cushman 1982, Rovira et al. 1983, Lynch and Whipps 1990). However, the pH and exchangeable cations of the 3 tree species showed no significant differences in the rhizosphere or bulk soils. All soil sample textures were classified as silty loam. The cation-exchange capacity (CEC), and sum of exchangeable cations showed the following trend: *Phy. pubescens* rhizosphere soils (PRS) > *Cry. japonica* rhizosphere soils (JRS)

> *Cha. formosensis* rhizosphere soils (FRS) > bulk soils. These results suggest that these plants had profound effects on soils especially on the chemical indicators. However, no obvious differences among plant species were observed. Thus, similar physiological properties of these plant species on soils can be expected.

### Low molecular-weight organic acids (LMWOAs) in rhizosphere and bulk soils

The contents of LMWOAs in samples showed the following trend: oxalic acid > succinic > malonic acid > fumaric acid. The content of oxalic acid accounted from more than 60% of the total LMWOAs in the collected samples. Contents of LMWOAs in the rhizosphere soils were obviously higher than those in the bulk soils (Table 2). Although PRS had higher LMWOA contents than those of FRS and JRS, there was no significant difference in LMWOA contents between FRS and JRS ( $p < 0.05$ ). The content of total LMWOA was highest in PRS that corresponded to its pH value in the rhizosphere. A high correlation coefficient was found between the LMWOAs and pH values, suggesting that the lower pH in the rhizosphere mainly resulted

**Table 1. Selected soil physical and chemical properties of rhizosphere and bulk soils**

Sample	pH		Total C (%)	Total N (%)	C-N ratio	CEC <sup>2</sup>	Exchangeable cation					BS <sup>3</sup> (%)	Sand (%)	Silt (%)	Clay (%)	Texture
	H <sub>2</sub> O	KCl					Ca	K	Mg	Na	Sum					
FRS <sup>1</sup>	4.48 <sup>b5</sup>	3.47 <sup>b</sup>	5.18 <sup>a</sup>	0.41 <sup>a</sup>	12.7 <sup>b</sup>	33.50 <sup>a</sup>	3.02 <sup>a</sup>	2.32 <sup>a</sup>	2.33 <sup>a</sup>	1.69 <sup>a</sup>	9.36 <sup>a</sup>	28.1 <sup>a</sup>	66.3	18.1	15.6	SL <sup>4</sup>
JRS	4.42 <sup>b</sup>	3.36 <sup>b</sup>	5.32 <sup>a</sup>	0.40 <sup>a</sup>	13.3 <sup>a</sup>	35.43 <sup>a</sup>	3.11 <sup>a</sup>	2.39 <sup>a</sup>	2.29 <sup>a</sup>	2.09 <sup>a</sup>	9.88 <sup>a</sup>	27.9 <sup>a</sup>	63.9	21.8	14.3	SL
PRS	4.26 <sup>b</sup>	3.16 <sup>b</sup>	5.66 <sup>a</sup>	0.46 <sup>a</sup>	12.3 <sup>b</sup>	39.56 <sup>a</sup>	3.34 <sup>a</sup>	2.52 <sup>a</sup>	2.36 <sup>a</sup>	2.75 <sup>a</sup>	10.97 <sup>a</sup>	27.8 <sup>a</sup>	62.5	22.9	14.6	SL
Bulk soil	4.80 <sup>a</sup>	3.64 <sup>a</sup>	2.55 <sup>b</sup>	0.19 <sup>b</sup>	13.2 <sup>a</sup>	24.54 <sup>b</sup>	1.68 <sup>b</sup>	1.87 <sup>b</sup>	0.77 <sup>b</sup>	1.30 <sup>b</sup>	5.62 <sup>b</sup>	23.0 <sup>b</sup>	55.1	30.4	14.5	SL

<sup>1</sup>) FRS, *Chamaecyparis formosensis* rhizosphere soil; JRS, *Cryptomeria japonica* rhizosphere soil; PRS, *Phyllostachys pubescens* rhizosphere soil.

<sup>2</sup>) CEC, Cation-exchange capacity.

<sup>3</sup>) BS, base saturation.

<sup>4</sup>) SL, sandy loam.

<sup>5</sup>) Numbers in a column followed by the same letter do not significantly differ ( $p < 0.05$ ) as determined by Duncan's test.

**Table 2. Low-molecular-weight organic acid concentrations in rhizosphere and bulk soils (mg kg<sup>-1</sup> dry weight of soil)**

Sample	Oxalic acid	Malonic acid	Fumaric acid	Succinic acid	Maleic acid	Total acid
FRS <sup>1</sup>	54.4 <sup>b3</sup>	7.80 <sup>a</sup>	1.90 <sup>b</sup>	18.6 <sup>b</sup>	<sup>2</sup> N.D.	82.7 <sup>a</sup>
JRS	44.5 <sup>b</sup>	5.90 <sup>a</sup>	1.40 <sup>b</sup>	14.6 <sup>b</sup>	N.D.	66.4 <sup>a</sup>
PRS	63.2 <sup>a</sup>	11.80 <sup>a</sup>	2.60 <sup>a</sup>	23.1 <sup>a</sup>	N.D.	10.1 <sup>a</sup>
Bulk soil	30.8 <sup>c</sup>	4.30 <sup>b</sup>	1.10 <sup>c</sup>	9.20 <sup>c</sup>	N.D.	45.4 <sup>b</sup>

<sup>1</sup>) FRS, *Chamaecyparis formosensis* rhizosphere soil; JRS, *Cryptomeria japonica* rhizosphere soil; PRS, *Phyllostachys pubescens* rhizosphere soil.

<sup>2</sup>) N.D., not detectable. The minimum detection limit (MDL) was 0.1 mg kg<sup>-1</sup>.

<sup>3</sup>) Numbers in a column followed by the same letter do not significantly differ ( $p < 0.05$ ) as determined by Duncan's test.

from the exudates released by plants roots. Such a change in rhizosphere soils could be expected to change the environments of plant nutrient uptake.

#### Extractability of metals in rhizosphere and bulk soils

The contents of extracted Al showed the following tendency: PRS = FRS = JRS > bulk soils, which is related to low soil pH that is beneficial for Al dissolution. In general, Al which has accumulated in plant roots cannot easily be exchanged with other ions (Foy et al. 1978, Gahoonia 1993, Chiu et al. 2002). Highly soluble Al<sup>3+</sup> ions in acidic soils can cause toxicity to plant growth (Foy 1974, Mench et al. 1988). According to the statistical analysis, the extractability of Al versus oxalic acid or total LMWOAs contents in rhizosphere soils showed high correlations ( $R^2 > 0.96$ ,  $n = 9$ ), suggesting that the low pH resulting from root exudates also influence Al activities in the rhizosphere.

The extractability of Fe was found to be in the following order: PRS = FRS = JRS > bulk soils. The extractability of Fe in rhizosphere soils was also higher than that in bulk soils. Small amounts of Fe and organic complexes formed in rhizosphere soils contributed to the extractability of Fe.

However, high-LMWOA exudates produced from root exudates complexed with iron enhanced the solubility of iron, suggesting that the effects of LMWOAs on iron mobility are dependent on plant species and soil types. According to the statistical analysis, values of the correlation coefficient,  $R^2$ , of oxalic acid and LMWOA contents versus extractability of Fe were higher than 0.88 ( $n = 9$ ).

Similarly, the extractability of Mn in rhizosphere soils was greater than that in bulk soils. The extractabilities of Cu and Zn showed a trend similar to that of Mn. The extractability of Cu showed the trend of FRS = JRS = PRS > bulk soils (Table 3). Rhizosphere soils contained more extractable Cu than bulk soils, which was proportional to the LMWOA contents (Graham 1981, Chen et al. 2001). The extractability of Zn followed the trend of PRS = JRS = FRS > bulk soils. Iron and Mn oxides can especially absorb Zn specially (Xue and Huang 1995). High Fe and Mn contents absorb Zn when high oxidation occurs in a soil, resulting in the low extractability of Zn (Shuman and Wang 1997).

The extractability of metals in the rhizosphere is influenced by the ionic species and contents, which are dependent on the pH and chemical compositions of root exudates, such as LMWOAs. The decrease in pH in the

**Table 3. Extractability of selected cation in rhizosphere and bulk soils (mg kg<sup>-1</sup> soil)**

Sample	Al	Fe	Mn	Cu	Zn
FRS <sup>1</sup>	875 <sup>a2</sup>	465 <sup>a</sup>	14.6 <sup>a</sup>	5.87 <sup>a</sup>	5.31 <sup>a</sup>
JRS	827 <sup>a</sup>	430 <sup>a</sup>	14.9 <sup>a</sup>	5.80 <sup>a</sup>	5.88 <sup>a</sup>
PRS	900 <sup>a</sup>	576 <sup>a</sup>	15.2 <sup>a</sup>	5.69 <sup>a</sup>	6.48 <sup>a</sup>
Bulk soil	676 <sup>b</sup>	350 <sup>b</sup>	11.3 <sup>b</sup>	4.20 <sup>b</sup>	3.79 <sup>b</sup>

<sup>1)</sup> FRS, *Chamaecyparis formosensis* rhizosphere soil; JRS, *Cryptomeria japonica* rhizosphere soil; PRS, *Phyllostachys pubescens* rhizosphere soil.

<sup>2)</sup> Numbers in a column followed by the same letter do not significantly differ ( $p < 0.05$ ) as determined by Duncan's test.

vicinity of roots tends to reduce the amounts of soluble Cu and Zn. In spite of the decrease in pH, the solubilities of Mn and Fe increase in the rhizosphere (Youssef and Chino 1989). The  $R^2$  values of total concentrations of LMWOAs with extractable Al and Fe showed high correlations of 0.89 and 0.97, respectively. However, the total concentrations of LMWOAs showed lower correlations with extractable Mn (0.74), Cu (0.64), and Zn (0.75).

In soils containing high amount of undecomposed organic materials, Mn tends to be present in unavailable and chelated forms (Mengel and Kirby 1978, 1980). As for the influence of the rhizosphere on the supply of Fe, Mn, Cu, and Zn nutrients to plants, Kothari et al. (1991) observed profound differences in the properties of microbial populations between mycorrhizal and nonmycorrhizal plants. These differences were reflected in the changes in the relative levels of Mn-reducing and -oxidizing microorganisms, which in turn, control the solubility of Mn at the root surface. Microorganisms in the rhizosphere also control the Mn supply to plants involving Mn oxidation. Some microorganisms produce low-molecular-weight ferric-specific ligands, such as siderophores, to transport iron into cells in environments with low amounts of available ions (Neilands 1981). Siderophors

play a critical role in the mobility and availability of cations in the rhizosphere. Phytosiderophores of iron-deficit grasses mobilize Fe as well as Mn, Cu, and Zn from calcareous soils (Treeby et al. 1989).

#### Fractionations of metal speciation

In the multiple-step extractions, of exchangeable, carbonate-bound, metal-organic complex-bound, easily reducible metal oxide-bound, H<sub>2</sub>O<sub>2</sub> extractable organic-bound and residual Al concentrations were similar to that of the Fe fraction. However, these fractions in the rhizosphere are higher than in bulk soils due to high organic acid concentrations in the form of metal-organic complexes in rhizosphere soils (Wang et al. 1983, 1993). Huang and Wang (2000) reported that the mobility of metal-organic complexes in soil profiles plays important roles in soil ecology.

Contents of Mn, Cu, and Zn in both rhizosphere and bulk soils showed low concentrations in multiple-step extractions. The residual and carbonate-bound fractions of Mn were greater than those of other fractions, and there were no significant differences between rhizosphere and bulk soils ( $p < 0.05$ ) (Table 4). Easily reducible metal oxide-bound, amorphous mineral colloid-bound, and residual Cu fractions were higher in the multiple-step extractions (Table 4). The residual fractions

**Table 4. Metal fractionations by multiple-step extractions in rhizosphere and bulk soils (mg kg<sup>-1</sup> soil)**

Metal	Sample	Step 1 <sup>3</sup>	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7	Step 8
Al	FRS <sup>1</sup>	5.13 <sup>a4</sup>	34.8 <sup>a</sup>	62.0 <sup>a</sup>	0.34 <sup>a</sup>	612 <sup>a</sup>	36.7 <sup>a</sup>	36.4 <sup>a</sup>	458.18 <sup>b</sup>
	JRS	5.11 <sup>a</sup>	47.5 <sup>a</sup>	61.2 <sup>a</sup>	0.31 <sup>a</sup>	621 <sup>a</sup>	34.8 <sup>a</sup>	36.7 <sup>a</sup>	429.61 <sup>b</sup>
	PRS	5.77 <sup>a</sup>	60.6 <sup>a</sup>	78.1 <sup>a</sup>	0.31 <sup>a</sup>	681 <sup>a</sup>	38.5 <sup>a</sup>	36.3 <sup>a</sup>	359.50 <sup>b</sup>
	Bulk soil	2.97 <sup>b</sup>	12.3 <sup>b</sup>	56.9 <sup>b</sup>	0.36 <sup>a</sup>	520 <sup>b</sup>	39.3 <sup>a</sup>	36.6 <sup>a</sup>	500.04 <sup>a</sup>
Fe	FRS	7.57 <sup>b</sup>	33.3 <sup>a</sup>	409 <sup>a</sup>	71.8 <sup>b</sup>	129 <sup>a</sup>	73.2 <sup>a</sup>	313 <sup>a</sup>	105.19 <sup>b</sup>
	JRS	7.65 <sup>b</sup>	31.3 <sup>a</sup>	327 <sup>a</sup>	64.8 <sup>b</sup>	131 <sup>a</sup>	75.4 <sup>a</sup>	324 <sup>a</sup>	109.83 <sup>b</sup>
	PRS	10.2 <sup>a</sup>	33.7 <sup>a</sup>	276 <sup>a</sup>	60.7 <sup>b</sup>	137 <sup>a</sup>	69.0 <sup>b</sup>	348 <sup>a</sup>	113.63 <sup>b</sup>
	Bulk soil	7.48 <sup>b</sup>	20.2 <sup>b</sup>	160 <sup>b</sup>	83.0 <sup>a</sup>	119 <sup>b</sup>	69.4 <sup>b</sup>	400 <sup>a</sup>	155.65 <sup>a</sup>
Mn	FRS	2.80 <sup>a</sup>	7.00 <sup>a</sup>	0.47 <sup>a</sup>	0.22 <sup>a</sup>	3.41 <sup>a</sup>	2.48 <sup>a</sup>	2.57 <sup>a</sup>	41.07 <sup>a</sup>
	JRS	2.88 <sup>a</sup>	7.84 <sup>a</sup>	0.52 <sup>a</sup>	0.23 <sup>a</sup>	3.71 <sup>a</sup>	2.60 <sup>a</sup>	2.58 <sup>a</sup>	39.75 <sup>a</sup>
	PRS	3.15 <sup>a</sup>	8.34 <sup>a</sup>	0.55 <sup>a</sup>	0.23 <sup>a</sup>	2.95 <sup>a</sup>	2.88 <sup>a</sup>	2.60 <sup>a</sup>	40.97 <sup>a</sup>
	Bulk soil	2.34 <sup>a</sup>	6.87 <sup>a</sup>	0.46 <sup>a</sup>	0.22 <sup>a</sup>	3.83 <sup>a</sup>	3.23 <sup>a</sup>	2.68 <sup>a</sup>	41.08 <sup>a</sup>
Cu	FRS	1.20 <sup>a</sup>	1.08 <sup>a</sup>	1.14 <sup>a</sup>	22.2 <sup>a</sup>	1.73 <sup>a</sup>	15.2 <sup>a</sup>	0.25 <sup>a</sup>	20.83 <sup>a</sup>
	JRS	1.20 <sup>a</sup>	1.11 <sup>a</sup>	1.15 <sup>a</sup>	22.5 <sup>a</sup>	1.82 <sup>a</sup>	15.3 <sup>a</sup>	0.27 <sup>a</sup>	20.92 <sup>a</sup>
	PRS	1.27 <sup>a</sup>	1.11 <sup>a</sup>	1.18 <sup>a</sup>	22.6 <sup>a</sup>	1.87 <sup>a</sup>	15.5 <sup>a</sup>	0.30 <sup>a</sup>	21.22 <sup>a</sup>
	Bulk soil	1.08 <sup>a</sup>	0.98 <sup>a</sup>	1.03 <sup>a</sup>	23.3 <sup>a</sup>	0.90 <sup>b</sup>	16.9 <sup>a</sup>	0.25 <sup>a</sup>	23.08 <sup>a</sup>
Zn	FRS	N.D. <sup>2</sup>	N.D.	N.D.	N.D.	5.45 <sup>a</sup>	1.63 <sup>a</sup>	1.55 <sup>a</sup>	57.27 <sup>a</sup>
	JRS	N.D.	N.D.	N.D.	N.D.	5.53 <sup>a</sup>	1.70 <sup>a</sup>	1.58 <sup>a</sup>	57.42 <sup>a</sup>
	PRS	N.D.	N.D.	N.D.	N.D.	4.98 <sup>a</sup>	1.58 <sup>a</sup>	1.52 <sup>a</sup>	56.75 <sup>a</sup>
	Bulk soil	N.D.	N.D.	N.D.	N.D.	4.98 <sup>a</sup>	1.58 <sup>a</sup>	1.52 <sup>a</sup>	56.75 <sup>a</sup>

<sup>1)</sup> FRS, *Chamaecyparis formosensis* rhizosphere soil; JRS, *Cryptomeria japonica* rhizosphere soil; PRS, *Phyllostachys pubescens* rhizosphere soil.

<sup>2)</sup> N.D., not detectable. The method detection limit (MDL) was 0.025 mg kg<sup>-1</sup> soil.

<sup>3)</sup> step 1, exchangeable, step 2, carbonate-bound, step 3, metal-organic complex-bound, step 4, easily reducible metal oxide-bound, step 5, H<sub>2</sub>O<sub>2</sub> extractable organic-bound, step 6, amorphous mineral colloid-bound, step 7, crystalline Fe oxide-bound, step 8, residual fractions (i.e., occluded).

<sup>4)</sup> Numbers in a column for each metal followed by the same letter is not significantly different ( $p < 0.05$ ) as determined by Duncan's test.

of Zn showed the highest amounts of Zn with multiple-step fractionations (Table 4). However, no detectable Zn fractions were shown in the exchangeable, carbonate-bound, metal-organic complex-bound, and easily reducible metal oxide-bound fractions. The hydrogen-peroxide extractable organic-bound, amorphous mineral colloid-bound, crystalline Fe oxide-bound and residual fractions of Zn showed no significant differences between rhizosphere and bulk soil fractions ( $p < 0.05$ ).

These 3 blocks of different soils showed large variations. Low contents of Mn and Zn were predominant in the types with residual, organic-bound, and amorphous sesquioxide fractions.

## CONCLUSIONS

Our results reveal significant differences in basic soil physiochemical properties between rhizosphere and bulk soils of the 3

studied tree species. The extractability and sequential extraction of different metals were dependent on the species of metal and the soil environment. Extractable Al and Fe showed high correlations with oxalic acid and total LMWOA contents in rhizosphere soils. LMWOAs play an important role in soil pH and metal extractability in the rhizosphere of tree plant species. This knowledge contributes to our understanding of the mobility, extractability, and toxicity of metals and of how to sustain the integrity of forested ecosystems.

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