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LARCH (*LARIX GMELINII*) BULK SOIL PHENOLIC ACIDS PROMOTE MANCHURIAN WALNUT (*JUGLANS MANSHURICA*) GROWTH AND SOILMICROORGANISM BIOMASS

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Abstract

The current study investigated the effect of seasonal variation on larch root exudates and the impact of some phenolic acids allelopathic on soil microbes and seedling growth. The soil microbial profiles were determined by phospholipid fatty acids (PLFA) digest method, whereas phenolic acids identification and their concentration in larch rhizosphere and bulk soil was determined by HPLC during spring, summer and autumn 2011. Combinations of four phenolic acids concentration (2, 4-2 hydroxy acid, 7-hydroxy coumarin, ferulic acid and abietic acids) were added to Manchurian walnut seedling pot soil and their PLFA profile and seedling biomass was measured after seedling harvest. Results indicated that seasonal variation of larch total root exudates was highest in soil in autumn and summer than spring . Bulk phenolic acids exerted stimulation effect on the microbial biomass and Manchurian seedling biomass. The rhizosphere soil phenolic acids had a negative impact on the microbial biomass and seedling growth due to high phenolic acids concentration. Principal component analysis explained 63.6% and 65.5% of total variation in PLFA composition in larch rhizosphere and bulk soil. Thus, bulk soil phenolic acid combination, particularly 7-hydroxyl coumarin + ferulic acid, has considerable stimulative impact on seedling and microbial biomass.

Key words: Allelopathic stimulation; Phenolic acids; Seedlings biomass; Soil microbes.

Introduction

Manchurian walnut (*Juglans mandshurica*) and Manchurian ash (*Fraxinus mandshurica*) are highly commercial value and precious broad leaves species of northeast China. Manchurian walnut forest area has stridently reduced due to excessive deforestation and its regeneration problems (Shi *et al.*, 1991). However, it is relatively difficult and non productive to establish pure Manchurian walnut forest because of its biological and ecological characteristics. Afforestation practice shows that walnut-larch mixed plantation and establishment has become a successful mixed plantation strategy for the productivity improvement (Yang, 2005). However, the principal interaction mechanism between the species and environmental factors are still poorly understood. Manchurian walnut-larch mixed plantation has some allelopathic interactions and functions. Larch roots and bark extract stimulates Manchurian walnut seedlings growth (Yang, 2006). Larch root exudates increases the chlorophyll b, soluble sugar content and seedlings biomass of Manchurian walnut (Yang *et al.*, 2007). Manchurian walnut-larch mixed plantation roots exudates improve the survival rate of Manchurian walnut (Yang *et al.*, 2010) because root exudates in Manchurian walnut pure plantation inhibits its seedlings survival, but seedlings survival is very well in Manchurian walnut-larch mixed plantation (Yang *et al.*, 2012).

Root exudates provide carbon and energy source, which plays an intermediary role in the interaction between plants and rhizosphere microorganisms. Plants can affect the metabolism of rhizosphere microorganism growth and development through root exudates (Inderjit *et al.*, 2009). Cotton root exudates affect the abundance of the bacteria, fungi and actinomycetes in soil.

Over the years, phenolic acids have been studied as allelochemicals because of the impact on growth and development of micro-organisms and plants. However, the concentration of phenolic acids determines the stimulation or inhibition of microbes in soil (Bertin, 2003). A wide variety of phenolic compounds were identified in the plant debris and root exudates of several plant species (Yu & Matsui, 1994; Grayston *et al.*, 1995; Zhou *et al.*, 2012a, 2012c). Larch exudates contain eleven kinds of different allelochemicals, including 2,4-2 hydroxy acid, 7-hydroxy coumarin, ferulic acid and abietic acids which are commercially used (Yang *et al.*, 2007). The information about the amount of root exudates and phenoilc contents release into soil from larch root and their relation with seasonal fluctuation is still unknown.

This study was conducted to i) determine the root exudates and phenolic acid contentin larch rhizophere and bulk soil during Spring (May), Summer (July) and Autumn (Sept) and ii) effect of four phenolic acids with different combinations on the soil microbial profile and Manchurian walnut seedling biomass.

Materials and methods

Experiment location: The study site is located at the National Maoershan Experimental Station of the Forest Ecosystem range of the Changbai Mountains, Heilongjiang Province, China (45°16′ N, 128°34′E). It has a continental temperate monsoon climate at altitude of 300 m above sea level and mean slope of 10-15°. Mean annual temperature and precipitation are 2.8°C and 723 mm. Mean annual evaporation is 1093 mm and frost-free period is 120-140 days. Soil type is typical dark brown with lighter texture (Yang *et al.*, 2006).

The Manchurian ash, larch and mixed-species plantations were established with their one year old seedlings in the experimental station in spring 1987. Row to row and plant to plant spaces were kept 1.5 ×1.5 m in all three plantations, but line-mixing (three rows of Manchurian ash × five rows of larch) was used in the mixed-species plantation (Yang *et al.*, 2010).

Phenolic acid determination: Soil samples were collected randomly in early spring (May), summer (July) and autumn (Sept) from larch rows in Manchurian walnut-larch mixed plantation during 2011. Rhizosphere soil samples were taken from 0-2mm distance from roots. Emphasis was made on the collection of fine roots (Uren, 2007). Bulk soil samples were taken about at 0-10 cm depth between the adjacent rows of larch with a drill. Soil was bulked and mixed separately. All soil samples were carried to laboratory, placed in sealed bags, and stored in freezer at a low temperature. Soil samples were stored at -60^{o}C for preservation to remove the moisture. Samples were crushed and sieved through 0.25 mm to remove stones and other impurities, and finally stored at -20^{o}C.

Quantification of phenolic acids concentration were done with an HPLC HP-1-100 equipped with a Zorbax SB-C18 reversed phase column (Hypersil 150 × 4.6 mm, 5 µm) fitted with an ultraviolet detector. For 2, 4-2 hydroxy benzoic acid, ferulic acid and 7-hydroxyl coumarin acid determination, the mobile phase was glacial acetic acid (3%) - methanol (75:25), flow rate was 1.0 ml/min, column temperature was 25^{o}C and wave length was 280 nm. For abietic acid determination, the mobile phase was glacial acetic acid (3%) - methanol (5:95), flow rate: 1.0 ml/min, column temperature, 25^{o}C and wave length, 241 nm (Li *et al.*, 2009; Peng *et al.*, 2012; Qin *et al.*, 2012; Xiao *et al.*, 2008).

All solvents and chemicals used were analytical grade. 2, 4-2 hydroxy benzoic acid, 7-hydroxyl coumarin, ferulic acid and abietic acid were purchased from Sigma Co. (USA), fatty acid standard (Methyl nonadecanoate) was from Accu Standard Inc. USA, and other chemicals were purchased from a local market. 16 phenolic acids combination were tested including control group and each treatment was replicated six times (Table. 1).

We used permutation and combination method and divided the rhizosphere soil and bulk soil into 15 combinations, separately. A1 (B1) to A4 (B4) is single phenolic acid, A5 (B5) to A10 (B10) is combination of two phenolic acids, A11 (B11) to A14 (B14) is combination of three phenolic acids, A15 (B15) is combination of four phenolic acids. On the basis of the compounds displaying synergistic orantagonistic actions in the soil, we used the single and combinations separately.

Green house experiment: One year old Manchurian walnut seedlings of uniform size were planted in pots. Each plant was grown in a single pot and each pot was filled with 5 kg soil collected from pure Manchurian walnut plantation site during May 2011. All pots were placed in greenhouse and randomized. The required phenolic acid concentrations were applied to the pot soil equal to the natural phenolic acids concentration of larch rhizosphere and bulk soil of larch manchurian walnut mixed plantation. One g/L stock solution of each phenolic acid was prepared using distilled water with slight warming and gentle stirring to dissolve chemicals. Each stock solution was diluted to make the desired concentration solutions. The larch rhizosphere and bulk soil phenolic acid concentrations were added to pots in early June, mid-July and September. Phenolic acid solutions were applied periodically after two weeks to avoid the depletion of phenolic acids (Shafer & Blum, 1991). The control group was irrigated with distilled water only. All seedling pots were irrigated weekly. The Manchurian walnut seedlings were harvested and dried at 60^{o}C and total biomass was weighed at the end of October.

Table 1. Different combinations of phenolic acids concentrationsfor Manchurian walnut pot culture.

Rhizosphere	Bulk	Chemicals combination
A1	B1	2, 4-2 hydroxy benzoic acid
A2	B2	7-hydroxyl coumarin
A3	B3	ferulic acid
A4	B4	abietic acid
A5	B5	2, 4-2 hydroxy benzoic acid + 7-hydroxyl coumarin
A6	B6	2, 4-2 hydroxy benzoic acid + ferulic acid
A7	B7	2, 4-2 hydroxy benzoic acid +abietic acid
A8	B8	7-hydroxyl coumarin +ferulic acid
A9	B9	7-hydroxyl coumarin +abietic acid
A10	B10	ferulic acid + abietic acid
A11	B11	2, 4-2 hydroxy benzoic acid + 7-hydroxyl coumarin +ferulic acid
A12	B12	2, 4-2 hydroxy benzoic acid + 7-hydroxyl coumarin + abietic acid
A13	B13	2, 4-2 hydroxy benzoic acid + ferulic acid + abietic acid
A14	B14	7-hydroxyl coumarin + ferulic acid +abietic acid
A15	B15	2, 4-2 hydroxy benzoic acid +7-hydroxyl coumarin + ferulic acid + abietic acid
A16	B16	Control

Note: A: larch rhizosphere phenoilc acids combination, B: larch bulk phenoilc acids combination

Phospholipid fatty acid analysis: Lipids from soil in pots were extracted by a modified Bligh and Dyer method. The fatty acid methyl esters were separated by a Hewlett-Packard 5890 gas chromatograph fitted with a flame ionization detector and mass spectrometric (GC-MS) system (Frostegård *et al.*, 1993).Fatty acids methyl esters (FAMEs) were identified by chromatographic retention time comparison with a standard mixture composed of 37 different FAMEs ranging from C11 to C24 (Sigma corporation, USA). The sum of PLFAs i15:0, a15:0, 16:0, i17:0, 18:0, 17:0, 15:0, 11:0, 14:0, 16:0, 22:0, 24:0, 16:1ω9, cy17:0, cy19:0 were used as source of bacterial fatty acid. The sum of PLFAs 18:3ω6, 18:2ω6, 18:1ω9c, 18:1ω9t, 20:0 were used as source of fungal fatty acid, 10Me17:0 as source of actinomycete fatty acid. The sum of PLFAs i15:0, a15:0, 16:0, i17:0, 18:0 were marked for Gram-positive (G^+) bacteria, and 16:1ω9, cy17:0, cy19:0 were marked for Gram-negative (G^-) bacteria. Furthermore, the fatty acids 20:4ω6 and 20:3ω6 were used as indicators of soil microfauna (protozoa and nematodes). The proportion of fungi to bacteria (F/B) was calculated as ratio of the sum of fungi to the sum of bacteria, as the same to G^+/G^- (Buyer, 2010; Kong, 2004; Li *et al.*, 2009; Qin *et al.*, 2012).

Statistical analysis: Data were subjected to statistical analysis for significant of differences between the treatments and control by using one-way analysis of variance (ANOVA) in the computer program SPSS19.0. Duncan test was also performed for experiments to compare treatments with each other. Multivariate principal component analysis was performed in Canoco for Windows 4.5. Data were log-transformed to obtain a normal distribution of residuals. For all analyses, significance levels were set at $p<0.05$.

Results

Phenolic acid concentration in larch soil: Seasonal fluctuations of total root exudates detected in the larch rhizosphere and bulk soil are enlisted in Table 2. Total root exudates were higher ($p<0.05$) in autumn and summer than spring. Four phenolic acids concentration had no relation with the seasonal variation. In larch soil, eleven kind of phenolic acids were identified e.g ferulic acid, 2,4-2 hydroxy benzoic acid, abietic acid, shikimic acid, β-sitosterol, phloroglucinol, linoleic acid, oleanolic acid, stearic acid, cinnamic acid and 7-hydroxyl coumarin, but only four phenolic acids concentration of our interest are enlisted in Table 2.

Effect of larch rhizosphere and bulk phenolic acids on Manchurian walnut seedlings: The Manchurian soil treated with larch rhizosphere phenolic acids A1 and A3 slightly increased seedling biomass compared to the control. The rest of phenolic acids combinations exhibited different degree of reduction, particularly A12, reduced seedling biomass up to 244.63% (Table 3a). In contrary, the larch bulk phenolic acids combination (B1, B2, B4, B6, B7, B8, B10) exhibited higher degree of increase in the Manchurian walnut seedling biomass compared to control (Table 3b), the phenolic acids combination, especially B8, exhibited highest increase in the biomass (39.91 g), which increased 26.82% seedling biomass.

Effect of larch rhizosphere and bulk phenolic acids on soil PLFA: Manchurian soil treated with larch rhizosphere phenolic acids single combination A1 and A3 only exhibited a slight increase in the total amount of PLFAs and bacteria PLFAs than the control, but the rest of treatments tend to lower than control (Table 3a). All larch rhizosphere phenolic acids combinations decreased the abundance of Gram-negative bacteria, Gram-positive bacteria, fungi and protozoa compared to control. The phenolic acids combination A12 exhibited an elevated inhibitory trend to decrease the total number of bacteria actinomycetes, fungi and protozoa. Rhizosphere phenolic acids combination A1, A3, A4, A6, A7 and A12 decreased fungi- bacteria ratio than control. In addition, G^+:G^- ratio was significantly higher than control in all treatment except A5 and A6.

The total PLFA, bacteria, gram positive bacteria, gram negative, bacteria, actinomycetes, fungi and gram-positive bacteria: gram-negative bacteria ratio were significantly affected by larch bulk phenolic acids combination. Phenolic acids combination (B2, B3, B4, B5, B6, B7 and B8) increased the total PLFAs than control in Manchurian walnut soil. Phenolic acids combination (B10, B11, B12 and B14) exhibited inhibitory trend and minimized the abundance of bacteria than control. The phenolic acids combination (B11 and B12) exhibited inhibitory response for gram-positive bacteria than the rest of combinations. The gram negative bacteria were increased by phenolic acids combination (B2, B6, B7 and B8) in the Manchurian walnut soil. All the phenolic acids combinations had a stimulative effect on actinomycetes except B9, B10, B11, B12, B13 and B14. The phenolic acids combination (B6 and B8) exhibited stimulatory effect on the abundance of fungi. Protozoa exhibited inhibitory response for all phenolic acids combination treatments. Gram positive and gram negative ratio exhibited stimulatory response for all phenolic acids combination.

Table 2. The phenolic acid concentrationsin larch soil in different seasons (μg▪g^{-1} Freeze-dried soil).

Date	Sample	Total Exudates	2, 4-2 hydroxy benzoic acid	7-hydroxyl coumarin	Ferulic acid	Abietic acid
Spring	Rhizosphere	293.75 ± 39.52c	98.8 ± 9.29a	58.66 ± 46.0b	ND	59.79 ± 10.82a
	Bulk soil	306.45 ± 35.81c	71.63 ± 4.96b	29.06 ± 3.53d	20.15 ± 2.39b	48.57 ± 7.30b
Summer	Rhizosphere	470.43 ± 34.31a	61.91 ± 3.56bc	40.84 ± 0.99cd	38.59 ± 3.19a	30.49 ± 12.03c
	Bulk soil	376.95 ± 28.44b	55.96 ± 3.18c	44.15 ± 2.23c	39.18 ± 3.15a	32.12 ± 13.80c
Autumn	Rhizosphere	489.82 ± 47.26a	100.8 ± 2.34a	72.04 ± 2.56a	19.32 ± 0.46b	24.53 ± 8.62d
	Bulk soil	358.48 ± 38.18b	71.75 ± 1.23b	40.29 ± 1.09cd	19.2 ± 0.62b	27.79 ± 10.57cd

Data with different letters are significantly difference in the column at 5% level

Table 3a. Effect of larch rhizosphere phenolic acid concentrations on Manchurian walnut seedling and microbial biomass.

Treatment	Biomass	Total microbial	Bacteria	Gram-positive (G^+)	Gram-negative (G^-)	Actinomycetes	Fungi	Soil fauna	Fungal: Bacterial ratio	G^+: G^- ratio
A1	36.18±3.03a	38.87±4.83a	27.25±2.59a	6.88±0.84ab	7.26±1.05bc	2.03±0.35a	8.54±1.35abc	1.05±0.54bcde	0.31±0.02f	0.95±0.02ef
A2	28.61±5.43bcd	31.82±4.10bc	20.30±1.76cd	6.53±0.57abc	6.19±0.63cde	1.63±0.29abc	8.56±1.66abc	1.33±0.39bc	0.42±0.05bcd	1.06±0.02c
A3	34.79±4.16ab	38.64±2.93a	25.64±1.14a	6.93±0.44ab	5.29±0.37ef	1.53±0.33abc	10.08±1.11a	1.39±0.35bc	0.39±0.03cde	1.31±0.01a
A4	24.52±3.99cdef	28.68±3.55cde	19.21±1.68d	5.89±0.62bcd	7.09±0.45bc	1.45±0.17bcd	7.46±1.53bcd	0.56±0.17ef	0.39±0.05cde	0.83±0.03h
A5	24.53±4.01cdef	23.82±3.73efg	14.81±2.17fg	4.10±0.79fgh	5.28±0.83ef	1.35±0.11cdef	6.45±1.09cd	1.21±0.36bcd	0.43±0.01abcd	0.77±0.03i
A6	22.92±3.43def	27.66±5.34cdef	18.39±3.29de	4.76±1.14efg	6.57±1.26bcd	1.56±0.22abc	7.28±1.41bcd	0.44±0.11f	0.40±0.01cde	0.72±0.04j
A7	31.37±1.59abc	35.45±2.50ab	24.63±1.45ab	7.54±0.51a	7.43±0.61b	1.61±0.10abc	8.50±0.73abc	0.72±0.22def	0.35±0.01ef	1.02±0.01d
A8	20.80±1.52ef	22.41±2.57fg	14.23±1.69fg	4.01±0.52gh	4.36±0.48fg	0.92±0.28efg	6.66±0.68bcd	0.6±0.12ef	0.47±0.01ab	0.92±0.02fg
A9	25.34±3.93cde	21.19±2.45fg	13.19±1.46fg	4.53±0.39efg	4.66±0.42fg	0.89±0.19fg	6.17±0.65d	0.95±0.15cdef	0.47±0.01ab	0.97±0.01e
A10	19.82±2.02ef	24.77±2.31def	16.02±0.94ef	5.52±0.35cde	4.35±0.28fg	1.38±0.23cdef	6.65±0.90bcd	0.71±0.24def	0.41±0.03bcd	1.27±0.01b
A11	26.67±3.10cde	21.94±3.27fg	13.17±1.61fg	4.41±0.43fg	4.10±0.57g	1.01±0.33def	6.57±1.03cd	1.18±0.30bcd	0.50±0.02a	1.08±0.01c
A12	9.77±5.87g	14.65±2.44h	10.02±0.81h	3.04±0.32h	2.78±0.27h	0.33±0.08h	3.84±1.24e	0.46±0.11f	0.38±0.09de	1.09±0.01c
A13	26.14±3.00cde	30.77±2.84bcd	18.98±1.02de	6.94±0.46ab	5.49±0.37def	1.41±0.44cde	8.81±1.05ab	1.56±0.33ab	0.46±0.03ab	1.26±0.01b
A14	20.00±4.19ef	18.42±1.71gh	11.87±0.63gh	3.69±0.21gh	4.06±0.27g	0.49±0.13gh	5.34±0.62de	0.71±0.17def	0.45±0.03abc	0.91±0.01g
A15	17.95±4.56f	30.63±2.73bcd	18.94±1.11de	5.14±0.41def	5.39±0.47ef	1.65±0.26abc	8.46±1.12abc	1.59±0.24ab	0.45±0.03abc	0.95±0.01ef
Cont.	33.67±3.34ab	36.77±4.27ab	22.43±1.98bc	7.20±0.79a	8.83±0.75a	1.95±0.38ab	10.45±1.49a	1.95±0.42a	0.46±0.03ab	0.81±0.02h

Data with different letters are significantly difference in the column at 5% level

Table 3b. Effect of larch bulk phenolic acids concentrations on Manchurian walnut seedling and microbial biomass.

Treatment	Biomass	Total microbial	Bacteria	Gram-positive (G^+)	Gram-negative (G^-)	Actinomycetes	Fungi	Soil fauna	Fungal: Bacterial ratio	G^+: G^- ratio
B1	35.26±2.73abc	33.97±4.33bcdef	22.29±2.28cdef	7.02±0.95d	6.61±0.74bcd	1.85±0.48bc	8.56±1.33bcde	1.26±0.24cde	0.38±0.02bcd	1.06±0.03f
B2	31.67±1.70bcd	37.86±4.77abc	26.22±2.31b	8.68±0.84c	9.59±0.97a	2.05±0.56abc	9.07±1.71bcd	0.52±0.19f	0.34±0.04def	0.91±0.01h
B3	29.55±5.01bcde	34.74±3.73bcde	24.63±1.87bcd	10.10±1.05ab	5.29±0.44ef	1.82±0.41bc	7.62±1.23cde	0.66±0.22f	0.31±0.03f	1.91±0.04b
B4	32.63±5.67bcd	35.61±3.69bcd	24.61±1.58bcd	10.49±0.86a	5.81±0.31de	1.83±0.17bc	7.72±1.53cde	1.46±0.41bcd	0.31±0.04f	1.80±0.05c
B5	29.47±3.86bcde	35.05±3.60bcde	23.12±1.79bcde	9.01±0.84bc	7.51±0.52b	1.90±0.36bc	8.42±1.09bcde	1.60±0.36bc	0.36±0.02bcde	1.20±0.03d
B6	35.41±4.87abc	39.62±4.35ab	24.25±2.25bcd	8.57±0.96c	7.76±0.88b	2.04±0.27abc	11.49±1.41a	1.85±0.42b	0.47±0.01a	1.10±0.01e
B7	36.34±3.45ab	37.81±2.77abc	25.33±1.41bc	8.44±0.63c	7.73±0.57b	2.45±0.19ab	9.30±0.95bc	0.73±0.22ef	0.37±0.02bcde	1.09±0.01ef
B8	39.91±4.98a	43.76±3.66a	29.16±2.07a	10.18±0.88ab	10.28±0.95a	2.64±0.55a	10.04±0.66ab	1.92±0.38ab	0.34±0.01def	0.99±0.01g
B9	25.46±2.70de	31.13±1.93def	21.66±1.14defg	9.40±0.62abc	4.35±0.33fg	1.39±0.19cd	7.50±0.45cde	0.58±0.15f	0.35±0.01cdef	2.16±0.02a
B10	31.96±4.75bcd	27.73±2.45f	18.79±0.94g	5.86±0.31d	7.14±0.47bc	1.09±0.23de	6.84±1.04de	1.01±0.24def	0.36±0.04bcde	0.82±0.01i
B11	23.71±2.33e	17.56±1.90g	11.79±0.85h	3.65±0.24e	3.69±0.36g	0.67±0.11e	4.52±0.64f	0.58±0.30f	0.38±0.03bcd	0.99±0.03g
B12	27.76±3.66de	30.81±2.86def	20.46±1.36efg	6.93±0.58d	6.89±0.42bcd	1.44±0.28cd	8.15±1.11bcde	0.76±0.11ef	0.40±0.03b	1.01±0.02g
B13	27.72±2.15de	32.50±3.15cdef	21.68±1.24defg	8.43±0.52c	7.54±0.47b	1.51±0.25cd	8.51±1.33bcde	0.80±0.33ef	0.39±0.04bcd	1.12±0.01e
B14	30.55±1.69bcde	28.92±1.95ef	19.60±0.87fg	7.08±0.33d	6.25±0.36cde	1.59±0.29cd	6.46±0.62e	1.28±0.17cde	0.33±0.02ef	1.13±0.01e
B15	29.00±2.72cde	32.78±3.1cdef	20.94±1.56efg	6.96±0.49d	6.32±0.53cde	1.77±0.26c	8.30±1.12bcde	1.77±0.24bc	0.40±0.02b	1.10±0.02e
Cont.	31.47±4.35bcd	34.44±4.14bcde	20.75±1.95efg	6.05±0.78d	7.69±0.89b	1.60±0.41cd	9.69±1.47abc	2.40±0.52a	0.47±0.03a	0.79±0.01i

Data with different letters are significantly difference in the column at 5% level

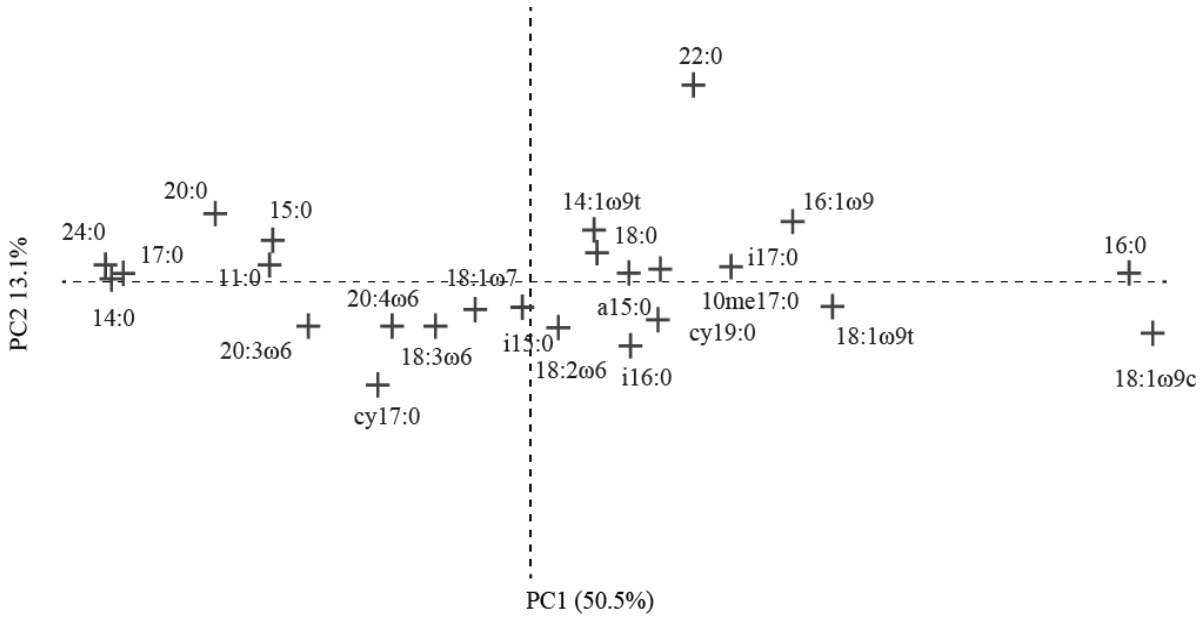

Fig. 1. Ordination plots of the principal component analyses based on phospholipid fatty acid profiles of the cluster of biomarker fatty acids to identify microbial groups responsible for distinction between different treatments of larch rhizosphere phenolic acid(s).

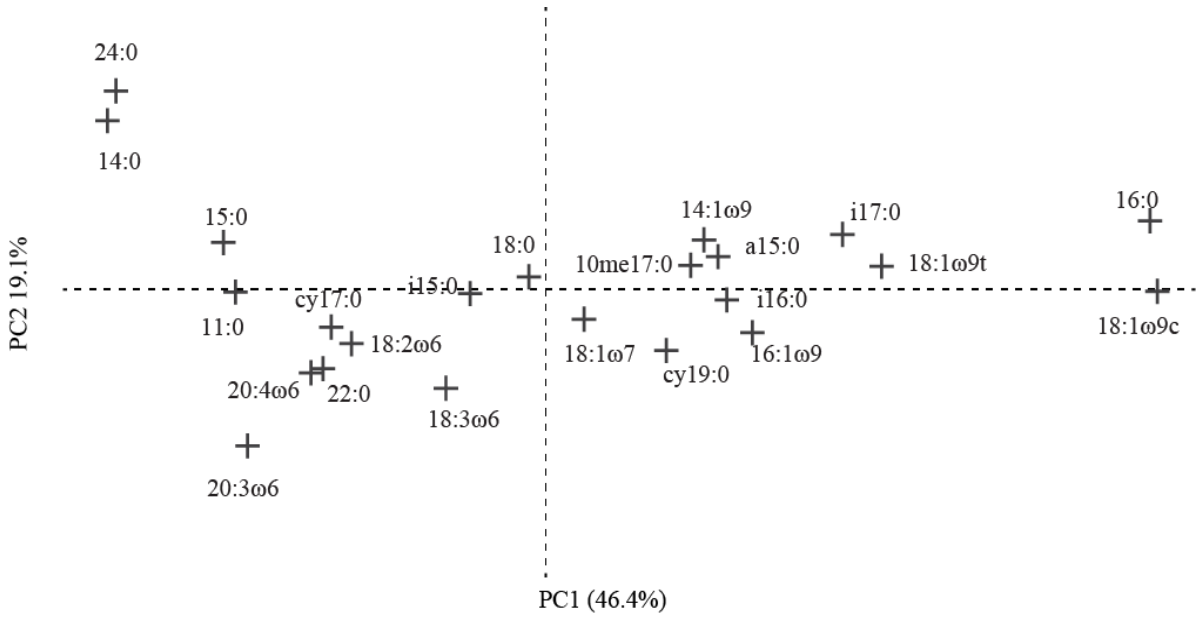

Fig. 2. Ordination plots of the principal component analyses based on phospholipid fatty acid profiles of the cluster of biomarker fatty acids to identify microbial groups responsible for distinction between different treatments of larch bulk phenolic acid(s).

Principal component analysis of soil PLFA'S: The principal component analyses on the basis of identified profiles of biomarker fatty acids of both larch rhizosphere and bulk soils were consistent (Figs. 1 and 2). PC1 and PC2 of larch rhizosphere soil phenolic acids accounted for 50.5 % and 13.1 % of total explained variation for PLFA. The major contribution to PC1 included bacteria 16: 0, 22: 1, gram-positive bacteria of a15: 0, i16: 0, i17: 0,18: 0, gram-negative bacteria 16: 1ω9, cy19: 0, actinomycetes 10Me17: 0, fungi 18: 1ω9c, 18: 1ω9t etc.,while 18: 1ω9c and 16: 0 was the biggest factor of variation. The fatty acids 11: 0, 14: 0, 15: 0, 17: 0, 20: 0 and 24: 0 which represent bacteria were impacted on PC2 significantly.

Discussions

In the present study, we used a wide range of larch phenolic acid concentrations to identify their role and effect on Manchurian walnut seedling and microbial biomass in the soil. Phenolic acids used in the experiment had similar concentration to those occurr naturally in soil (Yan, 2003).

Seasonal fluctuation and phenolic acids: The available source data about seasonal phenolic acids concentration in the soil is inconsistent. The phenolic acid concentrations in soil is influenced by biotic and abiotic factors, such as drought, temperature, nutrient deficiency, disease and insect damage (Cseke *et al.*, 2006; Vidal & Bauman, 1997). The age of plant species also effects chemical variation in soil (Fisher, 1987; Melkania, 1992; Khan *et al.*, 2014). Seasonal variations of phenol content were found higher in autumn than summer in soil (Muscolo & Sidari, 2006). The quantity of phenolic acids positively linked with the seasonal variation which had a strong impact on the ecosystem (Strobe, 2001). In contrast, phenolic acids concentration and composition in soil do not depend on the seasonal variation (Gallet & Pellissier, 1997). In our study, the total root exudates were higher in autumn but individual four phenolic acids concentration in soil had no relation with seasonal variations.

Phenolic acids relation with microbial community and plant growth: Plant root exudates have been studied for a long time as allelochemicals which play an allelopathic potential role in the soil (Fiamegos *et al.*, 2004; Zhang T-T *et al.*, 2010). Allelochemicals are generally believed to promote plant growth in low concentration but can inhibit, promote or had no effect on the plant growth in high concentration (Fries *et al.*, 1997). In our study, phenolic acids exhibited different degrees of concentration dependent inhibition or promotion in Manchurian seedling biomass and microbial profile.

Phenolic acids are the major source for microbes which activated in the rhizosphere and bulk soil (Blum *et al.*, 2000; Blum, 2004; Schmidt, 1999). Phenolic acids changes the microbial biomass, which influences the plant biomass (Zhou *et al.*, 2012a, 2012c). The phenolic substances stimulate plant growth (Bais *et al.*, 2003; Hattenschwiler & Vitousek, 2000). However, the plant growth response varies at different concentration (Muscolo *et al.*, 2006). Different allelochemicals act additively to effect the plant growth in field soil because simple chemical not enough to change the plant growth (Belz, 2007; Kruse *et al*., 2000; Seigler, 1996; Khattak *et al*., 2015). In our results, different phenoilc acid combinations induced negative and positive effect on the seedling biomass.

Larch root exudates were capable to significantly increase Manchurian walnut seedlings growth, which especially accelerates the degradation of juglone in the soil and increases the total microbial biomass and its activity in the soil (Yang *et al.*, 2010). Cotton root exudates affected the abundance of the bacteria, fungi and actinomycetes in soil, which had significant role in promoting the abundance of fungi at low concentrations but had no significant effect on actinomycetes and bacteria.

Phenolic acids at low concentration in soil playes important role in promoting plant growth and microbial biochemical process. It also changes the structure, composition and microbial activity in the soil (Inderjit, 2001; Blum *et al.*, 2000; De Nobili *et al.*, 2001; Qu & Wang, 2008; Zhou *et al.*, 2012b; Aziz & Shaukat, 2014). Our study finding are also consistent with previous studies that phenolic acids (bulk soil concentration) promote the biomass at low concentration and phenolic acids (rhizosphere soil concentration) inhibits the growth of microorganism at high concentration (Qu & Wang, 2008). Soil has complex and dynamic environmental ecology, which usually control soil microbial activities. When the root exudate chemicals are released in to soil, the microbes in the soil utilized the organic molecules to avoid the accumulation of such substances to toxic levels (Inderjit, 2005).

Bacteria and fungi play crucial role in soil processes, such as C and N circulation. The diversity and activity of bacteria and fungi reflect the richness of the soil and thus beneficial for plant growth (Milne & Haynes, 2004; Xie *et al.*, 2011). Soil contains more fungi (18: 1ω9c, 18: 1ω9t) can promote the growth of arbuscular mycorrhizal and improving the nutrient utilization in the soil (Jeffries *et al.*, 2003; Stover *et al.*, 2012; Rillig & Mummey, 2006; Aleklett & Wallander, 2012). Bulk soil phenolic acids application also increase the abundance of bacteria and fungi in the soil.

Actinomycete is capable of producing more biologically active metabolites and branched mycelium that can produce a variety of extracellular hydrolytic enzymes. It also plays an important role in mineralization and the degradation of a variety of insoluble organic matter in soil that needs a variety of nutrients for cellular metabolism (Winding *et al.*, 2004; Compant *et al.*, 2005). Phenolic acids also limited the mycelial growth of the soil pathogen (Ling, 1997). Phenolic acids (A1, B2, B4, B5, B6, B7 and B8) stimulated more actinomycetes in the soil which promoted the growth of seedlings.

In this study, larch root exudates varies with seasonal fluctuation, highest in autumn and summer than spring, but individual phenolic acid contents inconsistent with seasonal variation. Larch bulk phenolic acids concentration in different combination promotes the seedling biomass, microbial activity and microbial biomass. Although, larch phenolic acids concentrations were inferior in the bulk soil as compared to rhizosphere soil. It is concluded that Larch bulk soil phenolic acids have great influence and role in making a successful combination of larch-Manchurian walnut mixed plantation

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