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Effects of larch (*Larix gmelinii*) phenolic acids on manchurian ash (*Fraxinus mandshurica*) soil microbial community structure

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ABSTRACT

We investigated the effects of four *Larix gmelini* (larch) phenolic acids (2, 4-2 hydroxy benzoic acid, 7-hydroxyl coumarin, ferulic acid, abietic acid) on soil phospholipid fatty acid (PLFA) composition in *Fraxinus mandshurica* (Manchurian ash) soil. Sixteen combinations of 4- phenolic acids of larch rhizosphere and bulk soil were added to one year old Manchurian ash seedling pot cultured soil during Spring, Summer and Autumn 2011. We found that some of the larch rhizosphere and bulk phenolic acids combinations out of 16 groups acted as allelochemicals which stimulated and inhibited the Manchurian ash seedlings and microbial biomass of the soil. Larch rhizosphere phenolic acids combination significantly increased the seedling biomass and microbial biomass than control.. Specific phenolic acids were responsible to affect the abundance of specific microbe in the soil. Thus some phenolic acids in larch rhizosphere soil have stimulatory potential, which improved the productivity of Manchurian ash in inter-planting with larch. It is thus concluded from the experiment that the establishment and management of Manchurian ash can be improved by inter-planting with larch in northeast of China.

Key words: Abietic acid, allelochemicals, ferulic acid, 2,4-2 hydroxy benzoic acid, 7-hydroxyl coumarin, larch, Manchurian ash, phenolic acids, PLFA, rhizosphere

INTRODUCTION

Numerous of studies have shown that monocultures causes the decline in productivity and causes replantation problems (1,24,33,44). The most developed techniques to minimize and overcome the problems in managed tree plantations is mixed-species plantations instead of monocultures (11,19,25,44). Successful mixed-species plantations, based on carefully designed species mixtures, reveal many potential advantages in long-term practices (4,14,21,26,34).

Manchurian ash (*Fraxinus mandshurica*) and larch (*Larix gmelinii*) are two important timber species in the northeast China. The establishment and management of Manchurian ash plantations can be improved by inter-planting with larch. In certain experiments, the average collar diameter and height of Manchurian ash increased 0.46 cm and 14.26 cm, respectively in a 5-years-old mixed-species plantation than pure Manchurian ash plantation (17). The breast diameter, height and individual tree volume

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improved by 14%, 30% and 36%, respectively, in a 20-years-old mixed-species plantation in comparison to a Manchurian ash plantation (8,9). Therefore, larch has been advocated for inter-planting in Manchurian ash plantations; this has become a successful mixed-species plantation strategy in northeast China, but the potential mechanisms involved in the intra- and inter-specific interactions among the trees with environmental factors are largely unknown.

Soil microorganisms represent essential components of the biotic system where they are key players in nutrients turnover (15). However, the use of traditional counting and naming techniques have been excluded because only 6.5% of the microbes present in a soil sample can be characterized by selective cultures (3). Thereby, a culture-independent approach to study the composition of soil microbial communities has been offered by the analysis of the phospholipid fatty acid (PLFA) compounds of the microbial membranes. Subsets of the microbial community differ in their PLFA patterns, which makes it possible to plot signatures of the microbial communities in their habitats (7,16).

The phenolic acids have been studied as allelochemical for many years, which influences the growth and development of surrounding plants and soil microorganisms. However, the promoting or inhibitory effects on plant are controlled by the phenolic acids concentration (5,10,32,43,45,49). It has been suggested that there is likely to be a positive interference of larch with Manchurian ash in a mixed-species plantation. Few studies have shown that larch litter could stimulate the Manchurian ash seedlings growth and these compounds are some phenolic acids (46). In fact, larch soil contains a variety of compounds, such as 2, 4-2 hydroxy benzoic acid, ferulic acid, 7-hydroxyl coumarin, abietic acid, lariciresinol and secroisolariciresinol (48). In this study, we selected 4-kinds of commercial and typical compounds and added the exact concentrations to Manchurian ash soil which were measured in spring, summer and autumn in larch rhizosphere and bulk soil of mixed plantations. The study aimed to assess the effects of phenolic acids on the Manchurian ash seedling biomass and microbial biomass and to know which phenolic acid (or mixture) would be responsible for stimulation of microbial biomass.

MATERIALS AND METHODS

This study was conducted at the National Maoershan Experimental Station of Forest Ecosystem, Heilongjiang Province, China (45°16′ N, 128°34′E), in Changbai Mountains. This type of forest represents the typical forest types and landscapes in northeastern China at altitude of 300 m above sea level and mean slope of 10-15°. The parent material is granite bedrock and the soil is mostly Hap-Boric Luvisol. The climate is continental monsoon climate.

The Manchurian ash, larch and mixed-species plantations were established with their 1-year-old seedlings in the experimental station in spring 1987. Intra-row and inter-row spaces were 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. The Manchurian ash - larch mixed plantation increased the microbial biomasss and species abundance in the soil (Table. 1). Each plantation location was in a similar region, with an average gradient of 7°.

Table 1. Soil microbial profiles of pure Manchurian ash, pure larch and Manchurian ash - larch mixed plantations

Plantation	Bacteria	Gram-positive	Gram-negative	Actino-mycetes	Fungi	AM fungi	Protozoa	cy:Pre	Total sat./ Total mono
Ash	22.66 b	5.69 a	7.39 ab	2.92 b	2.02 b	1.17 b	0.17 b	0.19 b	1.17 a
Larch	19.37 b	4.88 a	5.73 b	2.47 b	1.84 b	0.92 b	0.09 b	0.17 b	1.62 a
Mixed	24.55 a	5.48 a	7.02 a	3.35 a	2.42 a	2.42 a	0.83 a	0.26 a	0.87 b

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

Table 2. Different combination concentrations of larch phenolic acids for Manchurian ash soil pot culture

Rhizosphere Order	Bulk order	Treatment combinations	Spring		Summer		Fall	
			Rhizosphere (g/L)	Bulk (g/L)	Rhizosphere (g/L)	Bulk (g/L)	Rhizosphere (g/L)	Bulk (g/L)
A1	B1	2, 4-2 hydroxy benzoic acid	1.88	1.12	2.34	2.03	3.28	2.42
A2	B2	7-hydroxyl coumarin	1.85	0.71	1.17	0.88	3.98	1.56
A3	B3	Ferulic acid	0.76	0.68	1.28	2.37	1.84	1.07
A4	B4	Abietic acid	1.76	1.17	1.98	2.52	2.40	2.58
A5	B5	2, 4-2 hydroxy benzoic acid + 7-hydroxyl coumarin	3.72	1.83	3.51	2.91	7.26	3.98
A6	B6	2, 4-2 hydroxy benzoic acid + ferulic acid	2.64	1.80	3.62	4.39	5.12	3.50
A7	B7	2, 4-2 hydroxy benzoic acid + abietic acid	3.64	2.29	4.32	4.54	5.67	5.01
A8	B8	7-hydroxyl coumarin + ferulic acid	2.61	1.39	2.46	3.24	5.83	2.63
A9	B9	7-hydroxyl coumarin + abietic acid	3.61	1.88	3.15	3.40	6.38	4.14
A10	B10	Ferulic acid + abietic acid	2.52	1.86	3.26	4.88	4.24	3.66
A11	B11	2, 4-2 hydroxy benzoic acid +7-hydroxyl coumarin + ferulic acid	4.49	2.51	4.80	5.27	9.11	5.05
A12	B12	2, 4-2 hydroxy benzoic acid +7-hydroxyl coumarin + abietic acid	5.49	3.00	5.49	5.42	9.66	6.56
A13	B13	2, 4-2 hydroxy benzoic acid + ferulic acid + abietic acid	4.40	2.97	5.60	6.91	7.52	6.08
A14	B14	7-hydroxyl coumarin+ ferulic acid+ abietic acid	4.37	2.57	4.43	5.76	8.22	5.22
A15	B15	2, 4-2 hydroxy benzoic acid +7-hydroxyl coumarin + ferulic acid + abietic acid	6.25	3.68	6.77	7.79	11.50	7.64
A16	B16	control	-	-	-	-	-	-

A: phenolic acid concentration of larch rhizosphere soils. B: phenolic acid concentration of larch bulk soils. We used permutation and combination method and divided the rhizosphere soil and bulk soil into 15 combinations, separately. A1 (B1) to A4 (B4) was single compound, A5 (B5) to A10 (B10) was combination of two phenolic acids group, A11 (B11) to A14 (B14) was combination of three phenolic acids group, A15 (B15) was combination of four phenolic acids group. On the basis of the phenolic acids may display synergic effects or antagonism actions in the soil, we used the single and combinations separately. The phenolic acid concentration of combinations were got by adding two, three and four phenolic acids, for example, the concentration of A11 (4.49) was the combination of three phenolic acids together [2, 4-2 hydroxy benzoic acid (1.88) + 7-hydroxyl coumarin (1.85) + ferulic acid (0.76)].

The experiment was designed with 16 testing blocks of different phenolic acids combinations including control group (Table. 2) and each block consisted of six pots.

Plant materials and chemicals

One year old seedlings of Manchurian ash obtained from Seedling Center (41°42′N, 126°35′E), Linjiang Forestry Bureau, Jilin Province, China were used. All solvents and chemicals used were analytical grade. Authentic 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 AccuStandard Inc. USA, and other chemicals were purchased from the local market. The required phenolic acid concentrations were prepared for the pot soil equal to the phenolic acids concentration of larch rhizosphere and bulk soil in larch rows in ash-larch mixed plantation.

Soil sampling and chemicals concentration

Soil were randomly collected at 0-20 cm depths for pot culture from pure Manchurian ash plantation in April 2011. The soil samples were sieved to remove plant residues, homogenized and transported to greenhouse for pot culture in Northeast Forestry University.

Six soil samples were randomly collected from pure Manchurian ash, larch and ash-larch rhizosphere and bulk soils to determine the phenolic acids concentration during Spring (April), Summer (June) and Autumn (August) 2011. Then these six samples were mixed to prepare a single composite soil sample and replicated three times. The rhizosphere soil was taken about 0 to 2 mm from the root surface and the bulk soil was taken about 0-20 cm depth in the central position between adjacent trees of each plantation. The samples were sieved (2-mm mesh) to remove plant residues, homogenized and taken back to the laboratory to determine the rhizosphere and bulk soil phenolic acids concentration in pure Manchurian ash, larch and ash-larch mixed plantations. 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 determination, the mobile phase was glacial acetic acid (3%) - methanol (75:25), flow rate was 1.0 ml/min, column temperature was 25° 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° and wave length: 241 nm (28,35,37,47). The quantifications of four kinds of phenolic acids are listed in Table 3.

Pot Culture

One year old Manchurian ash seedlings of uniform size (10 cm height & 3 mm stem diameter) was planted in each pots. Each pot was filled with 10 kg soil collected from pure Manchurian ash plantation in April 30, 2011. All pots were placed in greenhouse and were randomized. The phenolic acids concentration determined in the larch rows in ash-larch mixed plantation were only applied to seedling pot soil. One g/L stock solution of each phenolic acid was prepared using distilled water with slight warming and gentle stirring to dissolve the chemicals. Each stock solution was diluted to make the desire concentration solutions and 200 ml solutions were applied every two weeks. The plant pots

Table 3. Phenolic acids concentration in rhizosphere and bulk soil of Manchurian ash, larch and Manchurian ash - larch mixed Plantation in different seasons

Plantation	Season	Soil source	2, 4-2 hydroxy benzoic acid	7-hydroxyl coumarin	Ferulic acid	Abietic acid
	Spring	Rhizosphere	30.38±1.68g	39.22±0.74ab	21.48±1.08def	17.27±0.84ij
		Bulk	21.32±0.81h	12.45±1.17f	6.59±0.39	14.65±0.94ij
Ash	Summer	Rhizosphere	43.84±0.64cd	33.44±1.55cd	23.76±1.05de	31.13±2.16ef
		Bulk	29.96±2.90g	14.34±0.83f	20.53±0.83def	26.61±0.65fg
	Autumn	Rhizosphere	37.45±1.25ef	27.71±2.43d	31.31±1.41bc	29.98±1.79ef
		Bulk	31.05±0.89g	22.68±1.33e	21.32±1.19def	18.41±2.62hi
	Spring	Rhizosphere	34.64±0.51fg	27.44±0.62d	17.61±0.54ef	10.79±1.45j
		Bulk	21.41±1.14h	13.75±0.16f	7.18±0.73h	56.15±0.72a
	Summer	Rhizosphere	36.59±0.95f	30.25±0.74d	41.53±3.55a	32.38±0.65de
Larch		Bulk	29.04±1.80g	20.87±0.75e	20.45±0.87def	22.16±5.17gh
	Autumn	Rhizosphere	32.42±1.11g	35.45±0.55c	33.25±1.81b	38.45±1.09c
		Bulk	23.70±0.10h	15.27±0.66f	17.9±1.39ef	22.46±1.08gh
	Spring	Rhizosphere A	43.82±0.87cd	30.94±1.28d	27.75±1.33cd	32.63±1.03de
		Rhizosphere L	37.07±0.72e	36.75±1.09bc	15.78±0.88fg	35.82±1.02cd
		Bulk	22.34±0.51h	14.83±0.83f	13.33±0.73gh	23.88±0.87g
	Summer	Rhizosphere A	35.31±1.42fg	33.25±2.04cd	15.88±0.47fg	26.65±0.67fg
Mixed		Rhizosphere L	46.17±2.02bc	23.42±2.02e	40.94±7.92a	50.39±0.89b
		Bulk	40.59±1.10de	17.47±1.10f	26.12±1.25cd	40.05±1.45c
	Autumn	Rhizosphere A	54.53±0.67a	35.59±0.34	41.75±1.06a	34.12±1.34cd
		Rhizosphere L	56.73±0.94a	40.78±2.32a	36.91±1.01ab	47.23±1.54b
		Bulk	47.93±1.38b	21.67±0.67e	21.68±0.21def	50.49±1.57b

Rhizosphere A: Rhizosphere of Manchurian ash, Rhizosphere L: Rhizosphere of larch, Bulk: Bulk soil of Manchurian ash - larch mixed plantation.

were equally irrigated according to plants requirement. After 6-months, the Manchurian ash seedlings were harvested and dried at 60°C and total biomass was weighed.

Soil PLFAs analysis

Lipids from pot soil were extracted by a modified Bligh and Dyer method (13). 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 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 − (6,22,27,31,36,39).

Statistical analysis

In the study, significance of differences between treatments and the control were calculated using one-way analysis of variance (ANOVA) in the computer program SPSS 19.0. Duncan test was also performed for the experiments in SPSS 19.0 to compare the 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

Seedling biomass

Manchurian ash soil treated with different larch rhizosphere phenolic acids significantly affected the seedling biomass of Manchurian ash (Table. 4). The phenolic acid combination A6, A8, A9 and A10 significantly increased the seedling biomass of Manchurian ash than control, while, phenolic acids combination A7, A13, A14, A2 decreased the seedling biomass. The phenolic acid combination A6, A8, A9 and A10 stimulated the growth and trend to increase the seedling biomass 27.9%, 37.3%, 30.35% and 25.9% ($P<0.05$), respectively. While the larch bulk soil phenolics acid combinations inhibited the seedlings biomass (Table 5).

Soil PLFA

The larch rhizosphere phenolic acid significantly affected the total microbial biomass, bacteria, fungi, actinomycetes, G+ bacteria, G- bacteria and AM fungi of cultured pot soil (Table 4a-b). The phenolic acid combination A1, A9, A10, A14 stimulated the microbial biomass than control, while the rest of combinations were inhibitory to microbial biomass. The phenolic acid combination A1, A6, A9, A10, A 12, A14, A15 tend to stimulate the bacteria biomass than control, while the rest of combination showed

Table 4-a. Effect of larch soil rhizosphere phenolic acids on Manchurian ash seedling and soil microbial biomass

Treatment	Seedling biomass (g)	Total microbial biomass (μg/g)	Bacteria (μg/g)	Fungi (μg/g)	Actinomycetes (μg/g)	G+ Bacteria (μg/g)	G+ Bacteria (μg/g)
Control	20.1±1.9de	42.2±4.7def	25.7±3.8bc	11.7±2.5bc	0.5±0.2bcd	8.7±2.2bc	12.6±2.2de
A1	18.2±1.6cde	48.6±2.1f	31.0±4.1b	13.2±2.7b	0.7±0.3f	11.9±3.2bcd	13.2±2.6e
A2	16.7±3.7bc	26.4±1.9ab	17.2±3.2f	8.0±3.1d	0.4±0.1bc	5.9±2.5a	7.5±2.3ab
A3	18.7±9.2cde	33.9±2.5cd	21.1±4.7d	9.2±2.5cd	0.6±0.2def	6.7±2.7a	8.9±1.6b
A4	19.0±3.8de	21.7±3.5a	14.5±0.9g	6.3±1.9e	0.2±0.1a	4.8±2.1ab	6.4±1.5a
A5	19.4±4.5de	25.4±3.8ab	17.3±1.6f	6.4±1.2e	0.4±0.1bc	6.5±1.7a	6.7±2.2a
A6	25.7±3.0f	40.7±4.1de	26.7±2.8bc	11.4±1.9bc	0.3±0.1abc	10.8±3.2c	10.9±3.3cd
A7	9.8±5.6a	33.7±2.9c	20.3±3.8a	9.5±2.7cd	0.4±0.2bc	6.0±2.7a	9.6±3.5c
A8	27.6±4.1f	37.9±5.2d	25.6±2.0bc	10.5±2.1c	0.3±0.1bcde	10.3±1.5bc	11.1±2.4d
A9	26.2±4.5f	56.1±4.7g	35.9±4.6a	14.4±2.4b	0.4±0.2bc	16.2±4.1de	14.9±3.8e
A10	25.3±1.5f	61.0±5.5h	39.6±3.9a	17.2±1.5a	1.6±0.4f	16.9±1.6e	18.9±4.3f
A11	18.9±0.8cde	38.0±2.7de	24.9±5.1c	10.1±0.7c	0.4±0.1cde	10.8±2.8c	9.0±2.5bc
A12	18.7±4.8cde	36.4±4.6cd	26.4±4.7bc	8.5±1.4d	0.2±0.1cdef	13.0±3.4cd	8.7±2.9b
A13	16.2±4.1bc	37.8±4.4d	23.2±1.7c	10.8±3.2bc	0.3±0.1def	7.5±2.1b	11.1±3.1d
A14	11.9±5.9bc	44.3±6.2def	30.3±2.5b	9.8±2.3cd	0.4±0.1bc	14.7±4.3d	10.9±2.8cd
A15	20.9±4.3de	37.1±1.7cd	25.9±4.2bc	10.7±1.6bc	0.6±0.1def	11.5±1.5bcd	10.0±3.1cd

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

Table 4-b. Effect of larch soil rhizosphere phenolic acids on Manchurian ash seedling and soil microbial biomass

Treatment	AM fungi (μg/g)	Protozoa (μg/g)	Total sat.: Total mono	Cyclo: Precursor	Fungi: Bacteria	G+/G-
Control	3.0±0.7cd	2.7±0.7b	0.7±0.3a	0.3±0.1a	0.5±0.2a	0.7±0.2a
A1	2.9±1.1cd	1.4±0.7ab	0.9±0.2b	0.3±0.1a	0.4±0.1ab	0.9±0.2ab
A2	1.6±0.9a	1.3±0.3ab	0.7±0.1a	0.3±0.1a	0.5±0.2a	0.8±0.2a
A3	1.8±0.2ab	1.0±0.5ab	1.0±0.3b	0.4±0.1a	0.4±0.2ab	0.8±0.3a
A4	1.4±0.4a	0.3±0.1a	0.9±0.2b	0.4±0.2a	0.4±0.1ab	0.7±0.1a
A5	1.7±0.5ab	0.2±0.1a	1.0±0.3b	0.3±0.1a	0.4±0.1ab	1.0±0.2ab
A6	3.1±1.8cd	0.5±0.2a	0.8±0.2ab	0.3±0.1a	0.4±0.1ab	1.0±0.2ab
A7	2.4±1.3bc	0.8±0.2a	1.0±0.1b	0.3±0.1a	0.5±0.2a	0.6±0.1a
A8	2.8±1.3cd	1.2±0.4ab	0.7±0.2a	0.3±0.1a	0.4±0.2ab	0.9±0.3ab)
A9	4.3±1.5e	1.3±0.4ab	0.8±0.2ab	0.4±0.1b	0.4±0.1ab	1.1±0.3ab
A10	6.2±2.1f	1.4±0.5ab	0.5±0.1a	0.3±0.1a	0.4±0.2ab	0.9±0.2ab
A11	2.4±0.8bc	0.3±0.1ab	0.9±0.1b	0.3±0.1a	0.4±0.1ab	1.2±0.3b
A12	2.2±1.1b	0.3±0.1a	0.9±0.5b	0.4±0.1a	0.3±0.1b	1.5±0.5c
A13	3.1±1.2cd	1.0±0.4ab	0.8±0.3ab	0.5±0.2b	0.5±0.1a	0.7±0.2a
A14	3.0±1.5cd	0.5±0.2a	0.9±0.4b	0.5±0.1a	0.3±0.1b	1.3±0.4b
A15	2.1±1.1b	1.2±0.5ab	0.6±0.2a	0.2±0.1a	0.4±0.2ab	1.1±0.4ab

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

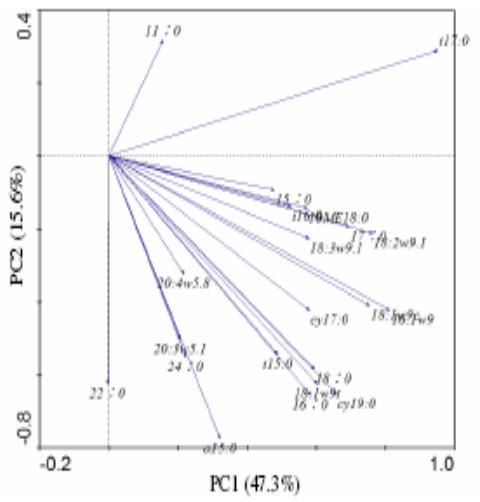

Figure 1. PLFA PCA on the soil treated by phenolic acids of larch rhizosphere soil

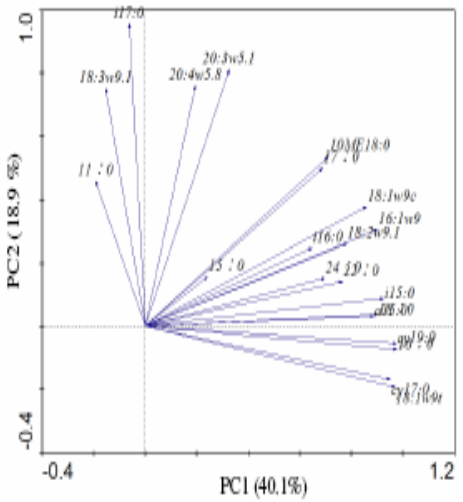

Figure 2. PLFA PCA on the soil treated by phenolic acids of larch bulk soil

inhibitory affect on the bacteria biomass. The phenolic acids combination A1, A9, A10 stimulated the fungi than control, while the rest of the combination were inhibitory to fungi. The combination A1, A3, A10 and A15 tend to stimulates actinomycetes as compared to control, while the rest of combination shows inhibitory affect. The G+ bacteria was stimulated by phenolic acid combination A1, A6, A8, A9, A10,A11, A12, A14, A15 than control, while the rest of combination inhibited the G+ bacteria. The G- bacteria was stimulated by phenolic acid combination A1, A9, A1, A10, while the rest of phenolic combination trend to inhibit G- bacteria. The AM fungi growth was stimulated by phenolic acids combination A6, A9, A10, A13 than control, while the rest of combination was inhibitory.

In case of larch bulk phenolic acids, Manchurian ash soil treated with different larch bulk phenolic acids increased the total microbial biomass, bacteria, fungi, actinomycetes, G+ bacteria and G- bacteria, respectively (Table. 5a-b).

Principal component analyses of soil PLFAs

The principal component analyses on the basis of the identified profiles of biomarker fatty acids of both soils were consistent. PC1 and PC2 of adding larch rhizosphere soil chemical(s) accounted for 47.3% and 15.6% of the total explained variation of PLFAs, respectively. All of the PLFAs were in the direction of the PC1 and the positive related PLFAs were i17:0, 10Me18:0, 18:2w9,1, 18:1w8, 18:0, 16:0 and cy19:0. PC1 and PC2 of adding larch bulk soil chemical(s) accounted for 40.1% and 18.9% of the total explained variation of PLFAs, respectively. The positive related PLFAs on the PC1 direction were 18:1w9c, 16:1w9, 18:2w9,1, i15:0, cy19:0, 16:0, cy17:0, 18:1w9t and the positive related PLFAs on the PC2 direction were 18:3w9,1, i17:0, 20:4w5,8, 20:3w5,1, 10Me18:0 and i17:0.

Table 5-a. Effect of larch bulk soil phenolic acids on Manchurian ash seedling and soil microbial biomass

Treatment	Seedling biomass (g)	Total microbial biomass (μg/g)	Bacteria (μg/g)	Fungi (μg/g)	Actinomycetes (μg/g)	G+ Bacteria (μg/g)	G+ Bacteria (μg/g)
B1	11.5±3.9ab	19.2±4.8ab	13.6±3.7a	5.7±2.7a	0.1±0.1a	4.8±2.1a	5.6±1.4a
B2	12.6±2.1ab	30.6±5.6de	19.4±4.2ab	9.3±4.4a	0.2±0.1a	6.3±2.7a	8.9±1.9a
B3	7.7±2.8ab	35.8±6.2de	23.0±5.7b	10.3±3.2ab	0.7±0.2bc	8.9±2.7b	11.0±2.3ab
B4	12.1±4.3a	55.3±4.7bc	34.1±6.3cd	15.0±4.8b	0.7±0.3bc	14.3±3.3d	16.2±3.5bc
B5	9.2±5.2a	26.7±5.3e	18.3±4.2ab	7.3±3.3a	0.2±0.1b	8.0±1.7ab	7.2±2.5a
B6	14.0±4.3b	38.5±4.8cd	25.6±5.9bc	12.2±5.2ab	0.4±0.2ab	8.9±1.9b	12.0±3.6b
B7	8.2±4.9a	28.3±3.7b	18.9±3.1ab	9.2±3.6a	0.1±0.1a	6.3±2.1a	8.8±2.4a
B8	10.4±3.9ab	34.1±5.6de	21.6±2.8ab	7.9±1.6a	0.4±0.2ab	8.7±2.5b	8.3±2.1ab
B9	11.6±5.5ab	48.9±5.5c	30.9±5.7c	14.5±2.4b	0.4±0.2ab	9.7±2.5bc	14.8±2.4bc
B10	8.4±3.2a	41.8±5.3cd	25.9±5.9bc	13.7±2.7ab	0.4±0.3ab	7.7±2.3ab	13.3±2.7b
B11	6.4±2.8a	26.6±4.8e	16.8±5.3ab	9.5±4.1a	0.2±0.1a	4.9±1.8a	8.7±2.9a
B12	8.1±5.8a	33.5±4.7de	20.9±3.2ab	9.5±3.9a	0.3±0.1ab	6.5±2.4ab	10.2±3.2ab
B13	10.8±5.5ab	36.3±4.6de	23.0±4.6b	10.5±4.5ab	0.4±0.2ab	7.1±2.1ab	10.8±2.6ab
B14	7.0±4.3a	62.7±6.5b	37.3±6.7d	20.4±3.6bc	0.6±0.4b	10.4±3.1bc	20.6±3.7c
B15	10.0±3.8ab	73.6±7.7a	44.5±5.9e	25.8±5.3c	0.8±0.1c	11.9±2.9c	24.2±3.9d
Control	15.0±3.4b	60.7±.9b	35.9±5.3cd	18.7±4.8bc	0.5±0.1ab	10.1±2.5bc	19.4±2.7c

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

Table 5-b. Effect of larch bulk soil phenolic acids on Manchurian ash seedling and soil microbial biomass

Treatment	AM fungi (μg/g)	Protozoa (μg/g)	Total sat.: Total mono	Cyclo: Precursor	Fungi: Bacteria	G+/G-
B1	1.1±0.5a	0.3±0.1a	0.8±0.1ab	0.5±0.2a	0.4±0.1b	0.9±0.3ab
B2	2.2±1.2ab	0.9±0.2a	0.8±0.2ab	0.3±0.1a	0.5±0.2ab	0.7±0.1a
B3	3.5±1.1ab	1.7±0.4b	0.5±0.2a	0.3±0.1a	0.4±0.2b	0.8±0.4ab
B4	4.9±1.5b	2.5±1.0c	0.6±0.2a	0.3±0.1b	0.4±0.2b	0.9±0.4ab
B5	1.7±1.1a	0.8±0.2a	0.7±0.2a	0.2±0.1b	0.4±0.1a	1.1±0.3b
B6	2.4±1.3ab	1.3±0.1b	0.6±0.1a	0.5±0.2a	0.5±0.1ab	0.7±0.2a
B7	2.2±1.2ab	0.1±0.1a	0.7±0.2a	0.5±0.2a	0.5±0.2ab	0.7±0.2a
B8	3.1±1.4ab	0.6±0.2a	0.9±0.3b	0.6±0.2a	0.4±0.2b	1.1±0.4b
B9	4.0±1.5ab	0.4±0.1a	0.8±0.2ab	0.6±0.2a	0.5±0.1ab	0.7±0.2a
B10	3.6±1.5ab	0.2±0.1a	0.6±0.2a	0.6±0.1a	0.5±0.1ab	0.6±0.1a
B11	1.8±1.1ab	0.3±0.1a	0.6±0.1a	0.6±0.1a	0.6±0.1a	0.6±0.1a
B12	2.9±1.2ab	0.3±0.1a	0.8±0.3ab	0.5±0.1a	0.5±0.1ab	0.6±0.3a
B13	2.9±1.9ab	0.3±0.1a	0.9±0.3b	0.4±0.2ab	0.5±0.2ab	0.7±0.3a
B14	5.2±1.4b	0.9±0.3ab	0.7±0.3a	0.6±0.2a	0.5±0.1ab	0.5±0.2a
B15	5.8±1.4b	0.9±0.2ab	0.6±0.2a	0.5±0.1a	0.6±0.2a	0.5±0.1a
Control	4.5±1.2b	2.0±0.4b	0.7±0.2a	0.5±0.1a	0.5±0.1ab	0.5±0.2a

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

DISCUSSION

Many kinds of phenolic acids can be detected from plant and often having allelopathic properties when released into the soil. The phenolic acids effect on the soil microorganisms was widely studied. Rhizosphere is the most complex part and its have impact on the surrounding due to different root exudates and their stimulatory effects on microbial activity have been explored (12,30,41,44,48). The larch rhizosphere phenolic acid application is more stimulative than bulk phenolic acids. These phenolic acids modify the microbial ecology of the soil as demonstrated (Table 4-5).

In the present study, the role of four phenolic acids (2, 4-2 hydroxy benzoic acid, ferulic acid, 7-hydroxyl coumarin and abietic acid) in different combination have unique effect on the growth and microbial biomass. The phenolic acids have unique mode of action and it has different response at different concentration. The Vanillic acid and PEDT application at different concentrations effectively changed the microbial biomass of the soil (38).

Many kinds of compounds in larch plantation soil have been reported (48), most of them, such as larixol and lariciresinol are not commercial. Four kinds of selected phenolic acids are commercially used for this experiment. Controlled experiments with allelochemicals require selection of concentrations for study, and selecting such concentrations is difficult. Actual uptake of compounds from the soil solution determines plant response (23,40). The concentrations of the phenolic acids used in this study were close to naturally occurring in the field because we imitated the exact concentrations of phenolic acids as in larch rhizosphere and bulk soil in different seasons. Phenolic acids are depleted rapidly from soil due to utilization of compounds by microbes under favorable environmental conditions, so the compounds were added to the system periodically to maintain desired levels according to seasonal change.

Differences in biochemical processes of the soil microbial may play a significant role in the degree to which allelopathic compounds inhibit/stimulate plant growth. A substantial amount of published information is available to explore the relationship between the concentrations of reactants and the intensity of the allelopathic effects (20). The material and energy exchange between the plant roots and soil occurs mainly in the rhizosphere and the chemical actions between plants and other soil organisms also occurs mainly in the rhizosphere (2,5,29). Plant species root exudates increase the growth of microbial organismd in the rhizosphere soil distinct from bulk soil (18,42).

In the study, although the chemicals concentrations in larch rhizosphere soil were higher than in bulk soil in mixed plantation, which effect the microbial biomass and seedling growth at a limited range of phenolic acid concentrations. We found most of phenolic acids combinations exudated from larch root rhizosphere improved Manchurian ash seedlings biomass and the total amount of microbal PLFAs, fungi, bacteria and actinomycetes. Thus larch rhizosphere have positive allepathic effects on Manchurian ash through root exudates as reported for other species (28). In the sharp contrast, almost of phenolic acids from bulk soil inhibited Manchurian ash seedlings biomass and microbes. Might be due to the material and energy exchange between plant roots and soil mainly occurs in the rhizosphere (2, 5).

Although we imitated chemicals exact concentrations in field conditions, but it did not imply that these chemicals would be fully exerting an allelopathic effect due to soil absorption etc (22). Therefore, further study on allelochemicals and their potential mechanisms in the Manchurian ash plantation inter-planting with larch are necessary.

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