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Full Length Research Paper

The effects of exotic and native poplars on rhizosphere soil microbe and enzyme activity

Fan-Juan Meng[1,2], Feng-Lan Huang[3,4] and Qiu-yu Wang[1,2]*

[1]Key Laboratory of Forest Tree Genetic Improvement and Biotechnology, Northeast Forestry University, Harbin 150040, P. R. China.
[2]College of Life Science, Northeast Forestry University, Harbin 150040, P. R. China.
[3]College of Life Science, Inner Mongolia University for Nationalities, Tongliao 028000, P. R. China.
[4]Inner Mongolia Industrial Engineering Research Center of University for Castor, Tongliao 028042, P. R. China.

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The relationship between tree species and soil microbial communities has attracted much attention in ecology. However, how different poplars species affect soil microbial community and soil enzymes activities are not well studied. Random amplified polymorphic DNA (RAPD) method was used to assess the effects of six plant species on soil microbial community. The results indicate that respirations were significantly higher in planted soil than that in control soil. The order of values of soil respiration rates was: eluosiyang > jiayang > xiaoqingyang > xinganyang > fenglanyang > xiaoyeyang > control. The different poplars species could change soil enzyme activities. The lowest phenol coxidase value was found for xiaoqingyang. The value of acid phosphomonoesterase was particularly high in the rhizosphere of eluosiyang, where it was 1.2 folds higher than that of control soil. In contrast to the other two enzymes, β-glucosidase activity did not differ significantly among parts of poplar species (p>0.05). From the dendrogram, cluster analysis unweighted pair-group method with arithmetic averages (UPGMA) resulted in a dendrogram with four main groups. Group I included jiayang, fenlangyang and xiaoyeyang. Eluosiyang and xinganyang were clustered into Group II. Group III included xiaoqingyang. Group IV contained control.

Key words: Exotic poplar, native poplar, rhizosphere soil, enzyme activities, random amplified polymorphic DNA (RAPD).

INTRODUCTION

Traditional forest industry is heavily dependent on the introduction of exotic tree species to improve genetic diversity or gain more benefits. However, introduced tree species may bring a series of ecological problems (Chapela et al., 2001; Kourtev et al., 2002; Marchante et al., 2008). Therefore, the potential effects of tree species on soil characters have been studied for a long time. Rhizosphere soil microbial communities are important to regulate plant growth and the decomposition of organic matter (Buchenauer, 1998; Smi et al., 2001; Wolfe and Klironomos, 2005; Christine et al., 2008; Xu et al., 2009). In addition, plant species can release a wide variety of compounds into the rhizosphere soil that create unique soil micro-environments (Pallant and Riha, 1990; Binkley and Valentine, 1991; Grayston et al., 1998; Porazinska et al., 2003; Priha et al., 1999; Grayston et al., 2001; Ushio et al., 2008, 2010). Many methods including dilution plate method, phospholipid fatty acids and molecular markers were used to analyze the characteristics of microbial communities in rhizosphere soil (Gao et al., 2010a; Wieland and Backhaus, 2001; Kourtev et al., 2003; Wu et al., 2009).

It is well known that poplar is one of the major planting trees in China. To date, poplar plantations have exceeded seven million hectares in China. However, poplar exhausts a lot of soil nutrients and easily causes a reduction in soil fertility, and now many regions of poplar

*Corresponding author. E-mail: mfj19751@163.com. Tel: +86 451 82191755. Fax: +86 451 86433905.

plantation in China have faced soil degradation, which will greatly affect the growth of poplar (Liu et al., 2007). These results may be ascribable to changes of rhizosphere soil microbial population and its enzyme activity. However, relationship between poplars species and soil properties remains largely unknown. In this paper, six poplars genotypes (three exotic poplars and three native poplars) grown in same soil types were used as materials, and the rhizosphere microbial communities diversity were examined by using random amplified polymorphic DNA (RAPD) technique. We also investigated the effects of different poplars species on enzyme activities. We aimed to analyze whether the exotic poplars would influence the local microbial community construction and the enzyme activities of the soil, and to know which poplar species are the most effective on rhizosphere soil properties.

MATERIALS AND METHODS

Soil characteristics and plant materials

Black soil used in this study was collected from the experimental fields at Northeast Forestry University (Harbin city, Heilongjiang Province in Northeast China, 45°41′ N, 126°37′ E) in February 2008. Black soil contained 2.14 g kg^{-1} of soluble soil N, 0.61 g kg^{-1} total soil P and 32.76 g kg^{-1} of total soil C, and had a pH of 7.2. The soil samples were dried for one month, sieved through a 2-mm sieve and stored at room temperature prior to use.

Three exotic poplar species included jiayang (*Poplar canadensis*), fenlanyang (*Poplar davidiana* Dode) and eluosiyang (*Poplar Russkii* Jabl). Three native poplar species included xiaoyeyang (*Poplar pseudo-simonii kitag*), xiaoqingyang (*Poplar pseudo* simonii) and xinganyang (*Poplar hsinganica C.Wang et Tung Plantations*).

Plant cultivation and soil samples

After storage period of one month, soils were transferred to pots (50 cm diameter and 50 cm depth). One pot consisted of 10 kg of soil. Then all trees (two-years old) were immediately sown into the black soil (one individual plant per pot) in March, 2008. All pots were placed in a greenhouse (night time temperature of 15 to 22°C, daytime temperature of 27 to 33°C, relative humidity of 70%). 30 plants were prepared for each genotype. No special treatment was carried out during the course of plant growth, except that plants were watered at seven days intervals.

Six treatments were assigned, in addition to control soil samples without plant, to rhizosphere soil of jiayang, fenglanyang, eluosiyang, xiaoyeyang, xiaoqingyang and xinganyang. After a period of eight months growth, rhizosphere soils adhering to the roots were sampled. The rhizosphere soils were selected from the 10 plants of genotype randomly by shaking off from the roots in the air to determine the effects of poplars genotype on rhizosphere soil microbial communities. After collection, rhizosphere samples were placed at -80°C prior to analysis.

Soil respiration rate and enzyme activity

Soil respiration rate was determined by the method described by Bringmark and Bringmark (1993). CO_2 evolved from samples was captured with alkali in closed vessels for 24 h. After precipitation of carbonate in $BaCl_2$, the remaining alkali concentration was determined by titration with HCl. Respiration rate was expressed as CO_2 evolution per gram dry weight and hour (mg CO_2/(g·h)).

Phenol oxidase activity was measured by the method of Floch et al. (2007). 0.1 g soil sample was incubated for 5 min at 30°C with 9 ml of Modified Universal Buffer (MUB, pH 2.0) and 200 µl of a 0.1 M 2,2′-azinobis-(-3-ethylbenzothiazoline-6-sulfononic acid) diammonium salt (ABTS) solution. The mixture was centrifuged at 11 300 *g* at 4°C for 2 min and the oxidation rate of ABTS to $ABTS^{+\cdot}$ released in the supernatant was measured at 420 nm (ε = 18 460 $M^{-1}cm^{-1}$).

β-Glucosidase activity was determined by the protocol of Eivazi and Tabatabai (1988). 0.5 g soil sample was supplemented with 2 ml of molasses-urea block (MUB) (pH 6.0) and 0.5 ml of 25 mM *p*-nitrophenyl-β-D-glucopyrannoside (PNG) solution. The mixture was incubated at 37°C for 1 h with continuous stirring and then treated with 0.5 ml of 0.5 M $CaCl_2$ and 2 ml of 0.1 M Tris buffer (pH 12). After centrifugation at 4000 *g* for 5 min, the supernatant was diluted with an adequate amount of 0.1 M Tris buffer (pH 10) and read at 400 nm.

Activity of phosphomonoesterase was assayed according to the method of Tabatabai and Bremmer (1969). One gram soil sample was incubated for 1 h at 37°C with 4 ml (MUB, pH 6.5) and 1 ml of 5 mM *p*-nitrophenyl phosphate (PNP). The reaction was stopped by adding 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH, and immediately centrifuged for 2 min at 12 000 *g*. The amount of *p*-nitrophenol released from PNP was measured in the supernatant at 412 nm.

Microbial community DNA extraction and polymerase chain reaction (PCR) amplification

Microbial community DNA was extracted from rhizosphere soil using CTAB method described by Jiao et al. (2004). DNA was amplified with 30 random primers. Names and sequences of the random primers used in this study are listed in Table 1. PCR reaction was performed in total volumes of 25 µl, containing 20 ng DNA template, 2.5 µl 10×PCR buffer (500 mM KCl, 15 mM $MgCl_2$, 100 mM Tris-HCl (pH9.0), 20 µM primer, 10 mM dNTP, 1.5 U of *Taq* DNA polymerase (TaKaRa Bio, Shanghai, China). PCR conditions contained a denaturation of 3 min at 94°C, 44 cycles of 1 min at 94°C, 1 min at 40°C and 1 min 30 s at 72°C, and a final extension of 7 min at 72°C in a thermal cycle (Applied Biosystems, Shanghai, China). PCR products were loaded into 1% (w/v) agarose gel containing ethidium bromide 1.0 µg·mL^{-1} and observed under UV light. The amplified products were recorded. To improve test reproducibility, every reaction was repeated twice.

RAPD data analysis

For RAPD analysis, all amplified fragments were transformed into a binary character matrix (“1” or “0” was used if a fragment was present or absent, respectively) using gel analyst software (Clara Vision, France). Then, the binary character matrices were compiled by the NTSYS 2.1 software package (Rohlf, 2001). The dendrogram was constructed using the unweighted pairs group method with arithmetic average (UPGMA) method (Van der Peer and Wachter, 1994).

Statistics

We performed post-hoc test (Tukey’ HSD for equal variance data) to test the difference among the tree species.

Table 1. Names and sequences of the random primers tested for this study.

Primer	Sequence 5 ' -3 '	Total band	Polymorphic band	Ratio of polymorphic band	Primer	Sequence 5 ' -3 '	Total band	Polymorphic band	Ratio of polymorphic band
S8	GTCCACACGG	38	31	0.82	S 1215	ACACTCTGCC	20	6	0.30
S32	TCGGCGTATA	29	8	0.28	S 1508	AAGAGCCCTC	16	9	0.56
S39	CAAACGTCGG	11	4	0.36	S 1367	CACGAGTCTC	29	15	0.52
S 236	ACACCCCACA	24	17	0.71	S 1387	CTACGCTCAC	37	9	0.24
S 261	CTCAGTGTCC	27	13	0.48	S 2125	CAAGCCGTGA	26	12	0.46
S 1024	CTATCCTGCC	17	10	0.59					

RESULTS

Soil respiration rate and soil enzyme activities

Soil respiration rate and soil enzyme activity were estimated in different poplar species (Figures 1 and 2). Respirations were significantly higher in planted soil than that in control soil. The behaviors of soil respiration rate showed significant difference among different poplars species. The order of soil respiration rate values was: eluosiyang > jiayang > xiaoqingyang > xinganyang > fenglanyang > xiaoyeyang > control.

In addition, the results show that the effects of different poplars species on soil enzyme activities were different (Figure 2). Phenol oxidase, phosphomonoesterase and β-glucosidase activities were significantly higher in planted treatment than in control soil. The lowest phenol oxidase value was found in xiaoqingyang, although it was still higher than in the control soil. The values of phenol coxidase were particularly high in the rhizospheres of jiayang and eluosiyang, where they were almost three-fold higher than that of the control soil.

Phosphomonoesterase was sampled under the different plant species. There were significant differences in acid phosphomonoesterase activity among the rhizosphere soil of all poplar species. The low acid phosphomonoesterase value was found in the rhizosphere of xiaoyeyang. The value of acid phosphomonoesterase was particularly high in the rhizosphere of eluosiyang, where it was 1.2-fold higher than that of control soil. In contrast to the other two enzymes, β-glucosidase activity did not differ significantly among parts of poplar species ($p>0.05$), but β-glucosidase activity was lowest for eluosiyang, where it was equal to that of control soil (Figure 2).

RAPD analysis

In this study, 30 primers were used to analyze the soil microbial community DNA samples, 11 produced well-defined and scorable bands (Table 1). A total of 274 bands were amplified, of which 134 were polymorphic (48.91%). The number of amplified bands ranged from 11 to 38. The number of polymorphic bands ranged from four to 31. Figure 3 shows parts of amplification profile by S1387 and S1508.

In order to obtain a relationship between all microbial community of rhizosphere soil, we constructed the dendrogram based on data from RAPD amplification (*Fig*ure 4). From the dendrogram, cluster analysis (UPGMA) resulted in a dendrogram with four main groups. Group I included jiayang, fenlangyang and xiaoyeyang. Eluosiyang and xinganyang were clustered into Group II. Group III included xiaoqingyang. Group IV was the control.

DISCUSSION

For a long time, scientists have discussed the influence of different tree species on biochemical processes of soil, because exotic plant species may alter soil characteristics (Chapela et al., 2001; Bhatnager and Bhatnager, 2005; Gyenge et al., 2008; Inderjit et al., 2010; Shi et al., 2011; Mubarak et al., 2012). In the past years, policies regarding ecology protection and increased timber production were imposed, many exotic tree species have been introduced to some countries, especially China (Wang et al., 1999; Tateno et al., 2007; Wan et al., 2009; Yang et al., 2009). However, there have been few studies on whether poplars establishment may lead to changes of soil characteristics (Hui et al., 2011), and the answer to this question could improve our understanding of the below-ground mechanism of exotic poplars trees introduction.

Soil respiration rate and enzyme activities have

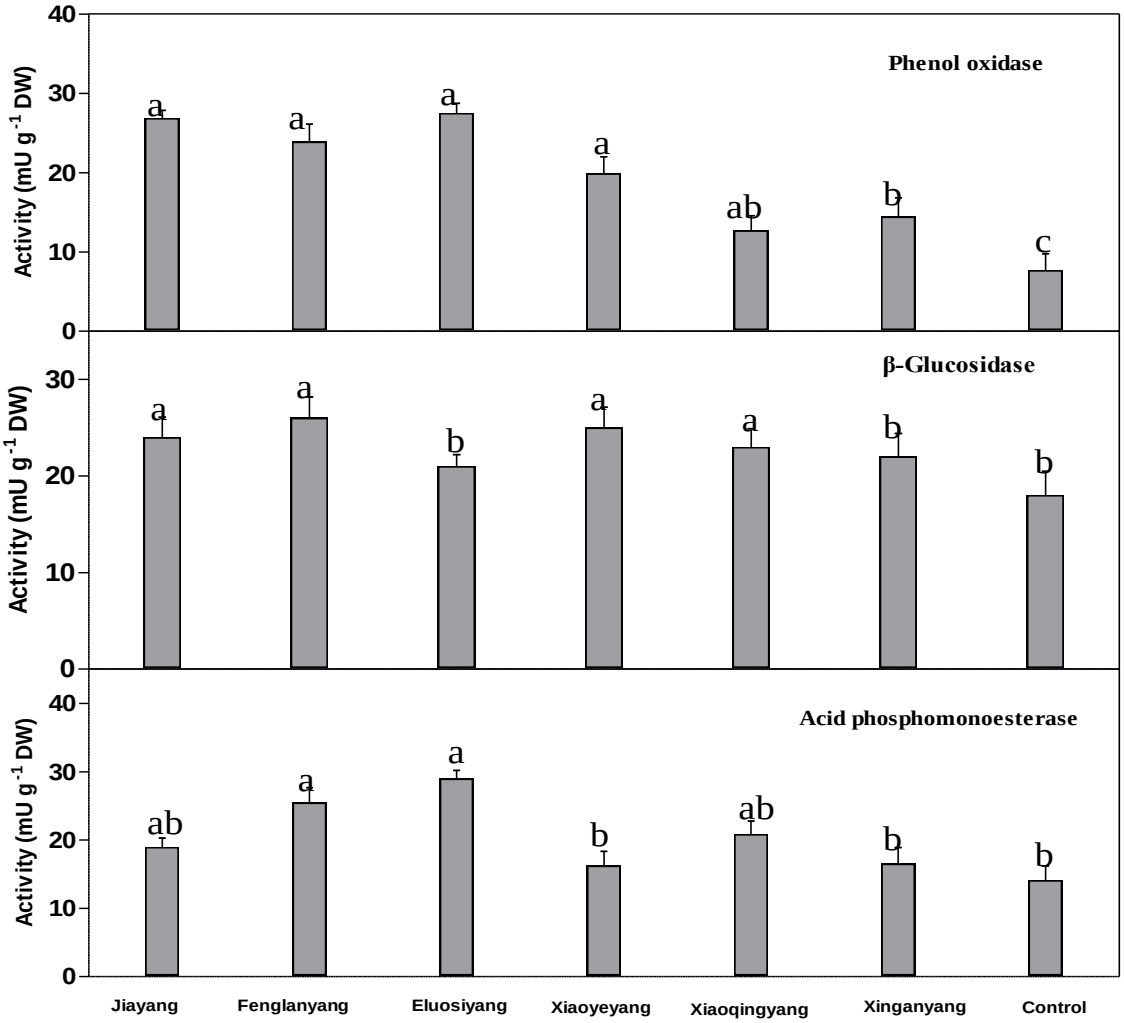

Figure 1. Soil respiration determined in the rhizosphere soil of different poplar species. Error bars represent the standard error of mean of three replicates (n = 3). Letters indicate significant differences by Tukey's HSD ($p < 0.05$).

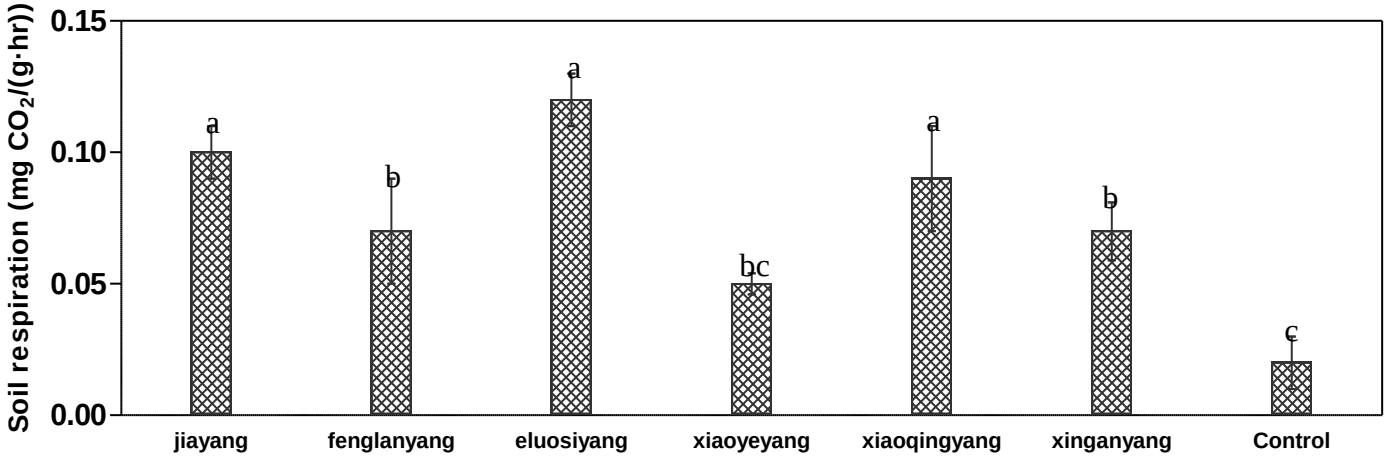

Figure 2. Effects of different poplars species on phenol oxidase, β-glucosidase and acid phosphoonoesterase activities of rhizosphere soils. Error bars represent the standard error of mean of three replicates (n = 3). Letters indicate significant differences by Tukey's HSD ($p < 0.05$).

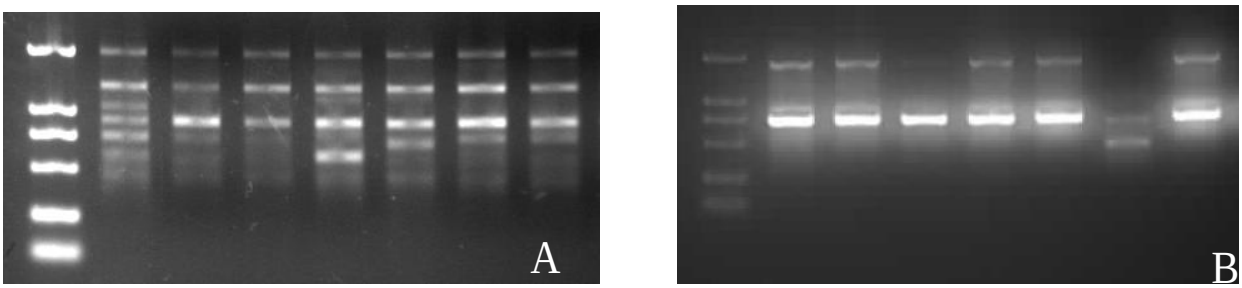

Figure 3. RAPD fingerprints of primer S1387 (A) and S1508 (B) from different poplars species. RAPD, Random amplified polymorphic DNA.

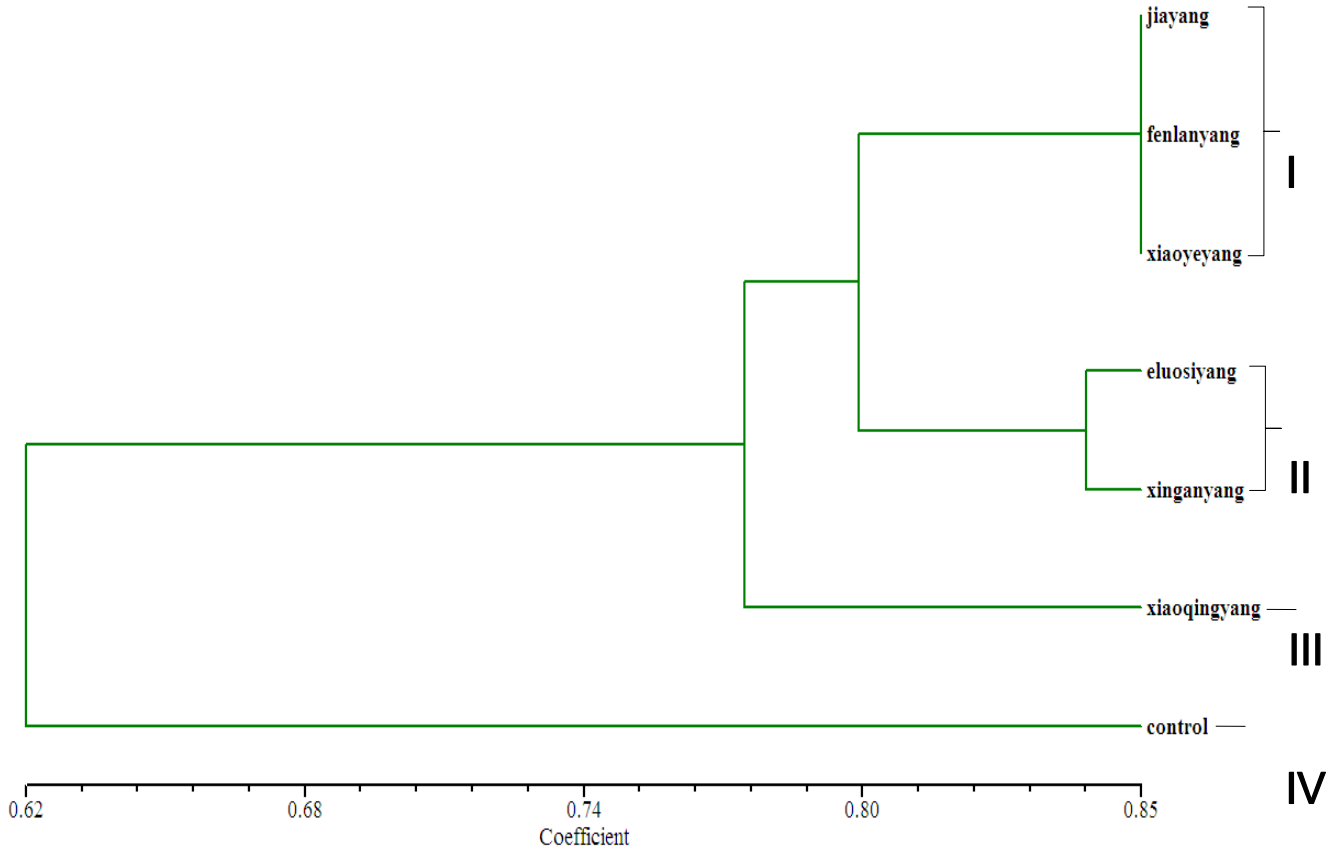

Figure 4. Dendrograms (UPGMA) based on data from RAPD amplifications. RAPD, Random amplified polymorphic DNA; UPGMA, unweighted pair-group method with arithmetic averages.

been related to soil physio-chemical characters, microbial community structure, vegetation and disturbance (Kowalchuk et al., 2002; Kourtev et al., 2003; Floch et al., 2007). These indexes were affected by different tree species, which have been reported by some researches. For example, as compared to unplanted soil, planted soils showed higher enzyme activity, which can be explained thus: as a result of the presence of additional surfaces for microbial colonization and organic compounds released by the plant roots (Dlorme et al., 2001). Kourtev (2002) also reported that enzyme activities in the four tree species changed significantly. Binkley and Giardina (1998) also reported that among different tree species (pine, birch, larch and alder), there are significant differences in basal respire-tion and enzyme activities. In this study, we found that the values of phenol coxidase changed significantly among different exotic and native poplars. In addition, β-glucosidase (C-

related enzymes) activity did not differ significantly among different poplar species. The mini-mum value of β-glucosidase activity was found in eluosiyang, probably due to the exudates of this species which may decrease the available substrates for β-glucosidase. This suggests that there may be qualities unique to each poplar species that increase the activity of specific enzymes. Phosphomonoesterase is involved in P cycling as it catalyzes the hydrolysis of organic P esters to inorganic P (Tan et al., 2001). Since the synthesis of phosphomonoesterase may be suppressed by the presence of inorganic P, high phosphomonoesterase activity may indicate insufficient P supply (Nannipieri et al., 1979). Eluosiyang have high P requirements, so the soil microbes in rhizosphere soil may face more intense competition for P than the other poplars. Therefore, there will be threat to induction of exotic poplars to native poplars.

RAPD is a relatively cost-effective and rapid technology of screening microbial communities and developing links between community structure and the soil characteristic (Thomas et al., 1996; McGregor et al., 2000; Lakhanpaul and Bhat, 2000; Yang et al., 2000; Dexter et al., 2010; Sharma et al., 2008). In the present study, the results of RAPD show that two exotic poplars (jiayang and fenlanyang) and one native poplar (xiaoyeyang) were clustered in one group. Moreover, eluosiyang and xingan-yang were also clustered in one group. These results suggest that exotic poplars may have similar effects on the soil microbial community with native poplar. However, RAPD is limited to some extent, since it is very likely that only dominant DNAs can be amplified and others are restrained or weakened (Xia et al., 1995). Consequently, it is necessary to integrate diverse approaches and per-spectives to understand more precisely the changes in the diversity of microbial communities.

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