Impact of monoculture of poplar on the rhizosphere microbial communities over time

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ABSTRACT

Research on the adverse effects of continuous cropping was so far focused on field crops; plantations, despite their very significant economic and ecological value, have received less attention. The evolution of microbial community in the rhizosphere of plantations, in particular, has rarely been examined. Changes in the size and composition, or structure, of bacterial and fungal communities in the rhizosphere of poplar plantations were studied using real-time polymerase chain reaction (PCR) and PCR-denaturing gradient gel electrophoresis (DGGE) and compared with the composition of the microbial community in bare land next to the plantation sites through sequencing analysis and by constructing phylogenetic trees. The numbers of bacteria and fungi increased significantly with successive generations of poplar, although the increase in the two groups was not parallel: compared to the control (soil from bare land), the bacterial community increased greatly in the second generation and the most significant increase occurred in the third generation whereas the most significant increase in the fungal community occurred in the first generation, the increase in the third generation being insignificant. In terms of community composition, the first generation showed little difference in either communities compared to the control but the second generation showed remarkable changes in the bacterial community and the third, in the fungal community. Compared to the soil microflora of bare land, the numbers of *Gammaproteobacteria*, *Alphaproteobacteria*, and *Actinobacteria* increased by 10.9%, 7.4%, and 5.5%, respectively, whereas those of *Acidobacteria* decreased by 18.4%. These changes in the numbers and composition of microbial communities in the rhizosphere could be the one of the reasons for the decline in yield and quality associated with long-term monoculture.

Keywords: continuous cropping, bacterial community, fungi, PCR-DGGE, *Populus deltoides*

INTRODUCTION

Two major problems caused by the degradation of forest soils are poor regeneration of the forests and declining yields of plantations. So far, studies on the degradation of forest soils have focused mainly on environmental and human factors, including nutrients, light, water, the method of harvesting and tending to the plants, and characteristics of seed including seed vigour. However, little is known of the ecological functions of rhizosphere microorganisms in forest soils. Recent advances in molecular biology and chemical ecology keep adding to the evidence that shows that plant allelopathy and autotoxicity could be the key factors in the degradation of forest soils (LI et al., 2011; MALLIK et al., 2000; PAN et al., 2009) and that rhizosphere microorganisms are sensitive to plant allelopathy (LIN et al., 2010; QU et al., 2008; WANG et al., 2008; WARDLE et al., 1997; ZHOU et al., 2011).

Soil microorganisms are important components of soil and play the role of decomposers in the soil ecosystem. Besides decomposing organic compounds and providing nutrients to plant, they fulfil other ecological functions. Growth hormones and vitamins produced by soil microorganisms promote seed germination and subsequent growth and development of the plant. Different types of organic matter, the moisture content and pH of soil, and parent material of soil result in different kinds and numbers of soil microorganisms, of which bacteria are the largest group and are widely distributed in soil. Soil bacteria play an important role in recycling soil nutrients. Soil fungi can be serious phytopathogens but are also indispensable to the nitrogen and carbon cycles in soil. Especially in the early stages of decomposition of plant matter, fungi are more active than bacteria and Actinobacteria. Changes in soil bacterial and fungal communities can reflect changes in the soil environment to some extent. Monoculture, or continuous cropping with a single crop, has been shown to change the composition of microbial communities in soil including the rhizosphere microbial community (BRESOLIN et al., 2010; OLSSON et al., 2000, HE et al., 2015). In recent years, polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) have facilitated the study of the composition and function of soil microflora, and such studies have laid a solid foundation for in-depth analyses of the characteristics of microbial community, its status, and its role in biochemical processes.

The failure of coniferous forests in North America and Europe to regenerate themselves has been studied extensively and, so far, is believed to be due to allelopathy and autotoxicity (MALLIK, 2003). For example, *Pinus halepensis* is not only one of the most important forest tree species in the Mediterranean basin but also serves as a pioneer tree species to bring abandoned land under cultivation in the south of France. However, the species has often failed to regenerate itself in later years. The roots of *P. halepensis* and extracts of its needles inhibited the germination of pine seeds and the growth of its seedlings. Such inhibitory effects, or autotoxicity, are particularly important to forests raised on relatively sterile soils (FERNANDEZ et al., 2008). The root system and residual litter from monoculture accumulate large quantities of autotoxins (LI et al., 2011). With successive generations of forest trees, these autotoxins gradually affect the evolution of microbial populations in the rhizosphere and, ultimately, the growth of plants (LIN, et al., 2010).

Forest plantations in China are some of the largest in the world. Poplar plantations in China cover about 700 hm^2. Poplar being one of fast-growing timber species, its plantations are the largest forests in the plains in the middle latitudes, and their timber yields are the highest. In China, poplar has always been planted and raised scientifically because it grows fast, matures early, gives high output, and regenerates easily. However, monoculture of poplar has often proved problematic. One of the studies focused on lack of soil nutrients and poor management as possible causes (Kong and Lou, 2010). In recent years, chemical aspects of intra- and interspecific relationships have been studied in an attempt to elucidate the mechanisms involved in allelopathy and autotoxicity in forest plantations. So far, most of the studies on the problems of monoculture were focused on Chinese fir, poplar, eucalyptus, and a few other forest species. Several researchers studied autotoxicity in relation to the dynamics of available nutrients and soil microorganisms (BONANOMI et al., 2005; TAN et al., 2008a; YANG et al., 2012a). However, changes in the numbers and composition of microbial communities in the rhizosphere over time in monoculture of poplar have received less attention—the present study sought to redress this imbalance by studying the bacterial and fungal communities in the rhizosphere soil across several generations of poplar in a monoculture as a contribution to sustainable management of poplar plantations.

MATERIALS AND METHODS

Sampling overview

Huang-huai-hai plain is the main poplar belt of China, and one forest farm in this belt, namely the Da Sha-he forest farm (34 ° 79' N, 116 ° 57' E) in the north-western part of Xuzhou, in Jiangsu province, was chosen for the study. The site lies in the Huang Pan alluvial plain, where the terrain slopes gently, and is part of a warm temperate zone with semi-humid monsoon climate. The annual average temperature is 14 ℃, with annual sunshine duration of 2284–2495 h and relative sunshine of 52%–57%; the average frost-free period is 200 d; and annual average precipitation is 784 mm, 56% of which is received during the rainy season.

Three generations of poplar were selected for the study: the first (Gen I) comprised 10-year-old trees from a site on which poplar had been grown for 10 years but had never been subjected to clear-cutting; the second (Gen II) comprised 7-year-old trees from a site on which poplar had been grown for 30 years and had been subjected to clear-cutting once; the third (Gen III) also comprised 7-year-old trees but the site had been under poplar cultivation for 40 years and had been subjected to clear-cutting twice. For comparison, soil samples were also collected from bare land adjacent to the experimental plots; these samples formed the control samples. All the sampled forests were managed on similar lines. Trees from Gen I were *Populus deltoides* and those of Gen II and III were *P. euramericana* 'San Martino' (Table I). All had been originally imported from Italy.

TABLE I
Characteristics of the selected trees

Generation	Species sequence	Age (years)	Height (m)	Diameter at breast height (cm)	Spacing (m)	Soil density (g/cm^3)
I	*Robinia pseudoococia* followed by poplar	10	14.7	20.09±4.56	3.0 × 5.0	1.46 ± 0.07
II	*Amorpha fruiticosa* followed by poplar	7	13.0	18.47±3.47	3.5 × 7.0	1.49 ± 0.11
III	Poplar throughout	7	12.3	18.11±2.55	3.0 × 3.0	1.49 ± 0.07

Sampling method

Ten strains of poplar were selected at random from each generation and five samples of rhizosphere soil were collected from every strain within the quincunx. The soil attached to fine roots (diameter smaller than 0.5 cm) was collected from every soil sample by sonication. Soil from all the five samples was thoroughly mixed and that composite sample was used for analysis. Ten soil samples were collected at random from the bare land. All samples, duly labelled, were stored in a refrigerator.

Experimental method

Quantitative Real-time PCR analysis. Total genomic DNA was extracted from the soil samples and synthesized using specific primers. The target fragment was obtained by PCR amplification with the extracted DNA as a template and 16s rRNA-F/R ITS1/ITS4 as primers and cloned using TA cloning. The primer sequences are as follows:

Primer	sequence
16s rRNA-F	CCTACGGGAGGCAGCAG
16s rRNA-R	CCATGTGTAATCCCAGCAGC
ITS1	ATGTGGATCAGCAAGCAGGA
ITS4	AAGGGTGTAAAACGCAGCTCA

Plasmids were extracted and a series of standard products were obtained by the gradient method.

Absolute quantification of 16S rRNA and ITS rRNA was carried out for each soil sample by using of real-time polymerase chain reaction (PCR) on SYBR® Green I dye. The reagents, consumables, and instruments were provided by Zoonbio Biotechnology Company, China. Data were analyzed following analysis of variance (ANOVA) and mean comparison between treatments was performed based on the was based on the least significant difference (LSD) test with SPSS 19.0 software.

DNA extraction and PCR-DGGE. Total genomic DNA was extracted using proteinase K, sodium dodecylsulfate, and cetyltrimethylammonium bromide concomitant with chloroform extraction and isopropanol precipitation, as described by Zhou et al. (1996). Partial 16S rRNA gene fragments were PCR-amplified using the bacteria-specific primer F341 (5′ -CCTACGGGAGGCAGCAG-3′) with a 40 bp GC-clamp attached to its 5′ end and the universal primer R907 (5′ -CCGTCAATTCMTTTGAGTTT-3′) (Muyzer et al., 1993). The products of PCR from each sample were loaded onto 6% (w/v) polyacrylamide gels (acrylamide and bisacrylamide in a ratio of 37.5 to 1) cast in 1×TAE buffer (40 mmol/L Tris, 20 mmol/L acetic acid, and 1 mmol/L EDTA; pH 8.0), and made with denaturing gradients ranging from 40% to 65%; DGGE was performed with a DGGE-2001 system (CBS Scientific Co., San Diego, California). The gels were run at 60 ℃ initially at 20 V for 15 min and then at 100 V for 16 h. The gels were stained with SYBR Green I solution (1:10000, Amresco, Solon, Ohio) for 30 min, rinsed with Milli-Q water, and photographed with Omega Lum^{TM} gel imaging system (Ultra-Lum Inc).

Analysis of DGGE banding pattern. To assess the bacterial community in each soil sample, data from DGGE were used for generating a matrix of relation band intensities and a binary matrix according to the presence (1) or absence (0) of bands determined with a gel documentation system, GelComparII (Applied Maths, Austin, Texas).

Differences in the number of DGGE bands among the four soil samples (conrol, Gen I, Gen II, and Gen III) were tested by one-way analysis of variance, with the least significant difference calculated at $P < 0.05$. Clustering analysis of the DGGE banding pattern obtained from the four soil samples was performed using the unweighted pairwise grouping method with mathematical averages (UPGMA).

Construction of clone libraries. Two 16S rRNA gene clone libraries were constructed with sequences amplified from the control group and from Gen II separately. A fragment, about 1500 bp long, of a bacterial 16S rRNA gene was amplified in a thermocycler using the eubacterial forward primer 27F (5′ -AGAGTTTGATCMTGGCTCAG-3′) and the universal reverse primer 1492R (5′ -GGTTACCTTGTTACGACTT-3′) (Newton et al., 2006). Each 50 μL of the PCR mixtures contained DNA templates, 5 μL 10× PCR buffer, 4 μL of dNTPs (2.5 mM each, TaKaRa), 2 μL of each primer (10 μM), and 0.5 μL of Ex Taq TM enzyme (5 U/μL, TaKaRa Bio, Tokyo). For PCR cycling, the conditions were as follows: 95 ℃ for 5 min, 30 cycles at 94 ℃ for 30 s, 53 ℃ and 72 ℃ for 45 s, with an additional 10 min of final extension at 72 ℃.

The products of PCR were examined by gel electrophoresis on 1.0% agarose gel and then purified

immediately with the E.Z.N.A.®Cycle Pure Kit (Omega Bio-Tek, Norcross, Georgia, USA), and the 16S rRNA gene fragments were cloned into a pMD18-T vector (TaKaRa) according to the manufacturer's instruction. The ligated DNA was transformed into *E. coli* TOP10 supercompetent cells. Randomly chosen clones were re-amplified with PCR (as described above) by using the primers RV-M and M13-47 that targeted the vector sequences and ignored false-positive clones. In all, 69 representative clones from Gen II and 65 representative clones from the control group were selected for sequencing.

Sequencing and phylogenetic analysis. Representative clones from the two clone libraries were sequenced on an ABI PRISM 3730 automated DNA capillary sequencer (Applied Biosystems, Foster City, California) by Nanjing Genscript Biological Technology Co. Ltd, China. The sequencing primer was the primer 27F (5'-AGAGTTTGATCMTGGCTCAG-3'). All partial 16S rRNA sequences were edited manually using BioEdit (ver. 7.0.9), which generated high-quality reads. Sequences with 97% sequence similarity to one another were treated as a single phylotype. The 16S rRNA gene sequences obtained were checked for potential chimeric sequences using the CHECK_CHIMERA program (http://rdp8.cme.msu.edu/cgis/chimera.cgi) (Maidak et al., 2000). Sequences containing no chimera were compared to the 16S rRNA sequences of the closest organism from GenBank and Ribosomal Database Project databases to obtain a preliminary phylogenetic affiliation of the clones. The phylogenetic trees were constructed using Molecular Evolutionary Genetics Analysis (MEGA) ver. 4.0 (available at www.megasoftware.net) (Tamura et al., 2007). The robustness of the phylogenetic tree topology was confirmed by maximum parsimony analysis with 1000 bootstrap replications. Evolutionary distances were calculated using the maximum composite likelihood method and the neighbor-joining algorithm was used to generate the initial tree (Tamura et al., 2004).

Nucleotide sequence accession numbers. A total of 134 partial 16S rRNA gene sequences determined in the present study were submitted to the GenBank database (accession numbers KT998669 to KT998802).

RESULTS

Changes in the number of bacteria and fungi in rhizosphere soil from different generations of poplar plantation

Assuming that every bacterium contained one copy of a 16S rRNA gene and that every fungus contained one copy of an ITS rRNA gene, the numbers of bacteria and fungi in the different samples were estimated based on the standard curve.

Every gram of dry soil in the control samples contained 2.03×10^6–4.37×10^6 copies of the 16S rRNA gene (the mean was 3.60×10^6 copies) and 1.14×10^3–3.69×10^3 copies of the ITS rRNA gene (the mean was 2.52×10^3 copies) (Table II) whereas in the plantation soils, the numbers were much higher and increased with every successive generation, which also meant longer periods over which the sites had been cultivated (see Table III for Gen I, Table 4 for Gen II, and Table 5 for Gen III).

The pattern of the increase in bacteria was different from that in fungi (Fig. 1). The number of bacteria (Fig. 1a) in Gen I samples was not significantly different from that in the control samples ($P > 0.05$); by Gen II, however, the number of bacteria had increased markedly ($P < 0.01$); in Gen III, the increase was even more, the number being 6-fold of that in Gen II. The number of fungi (Fig. 1b) in Gen I, on the other hand, was markedly higher than in the control ($P < 0.001$) and remained higher in the next two generations also ($P < 0.01$). Between generations, the increase was significant from Gen I to Gen II ($P < 0.01$) but not from Gen II

to Gen III ($P > 0.05$)

Table II

Numbers of copies of 16S bacterial gene and of ITS rRNA fungal gene per gram of soil (dry weight) from the control group (bare land)

Sample	Total number of bacteria(Copies g^{-1} soil)	Total number of fungi(Copies g^{-1} soil)
1	4268511.93	3378.867
2	4056995.558	3692.96
3	4365177.042	2919.032
4	3335305.01	1604.699
5	3803036.07	2875.157
6	2665409.338	2151.982
7	2033852.645	1137.46
8	4106392.693	2832.476
9	3160112.805	2499.591
10	4174035.431	2091.178
average number	3596882.852	2518.34

Table III

Numbers of copies of 16S bacterial gene and of ITS rRNA fungal gene per gram of soil (dry weight) from Gen I plantations

Soil sample of Gen I	Total number of bacteria (Copies g^{-1} soil)	Total number of fungi (Copies g^{-1} soil)
1	4734031.926	3328.4561
2	5087814.734	9726.9433
3	2058634.498	7563.32976
4	2581900.863	5892.98137
5	4411847.277	7521.43121
6	4037400.79	5922.50659
7	3609857.034	5889.7226
8	5592195.158	4592.83219
9	5273486.903	8112.5562
10	4442982.246	9358.03423
average number	4183015.143	6790.87936

Table IV

Numbers of copies of 16S bacterial gene and of ITS rRNA fungal gene per gram of soil (dry weight) from Gen II plantations

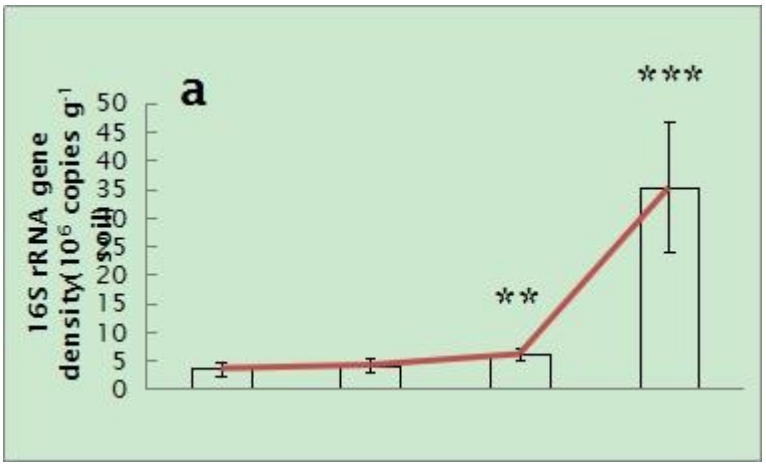
Soil sample of Gen I**I**	Total number of bacteria	Total number of fungi

	(Copies g^{-1} soil)	(Copies g^{-1} soil)
1	5104075.631	7723.503322
2	7178846.499	12154.68941
3	7414356.81	17245.17699
4	7043346.611	15470.76383
5	5521644.942	7773.469819
6	5928394.466	13038.2013
7	5261981.168	10271.70771
8	6258705.977	12700.70321
9	3666183.52	5355.613925
10	7531713.221	15781.80528
average number	6090924.885	11374.91272

Table V

Numbers of copies of 16S bacterial gene and of ITS rRNA fungal gene per gram of soil (dry weight) from Gen III plantations

Soil sample of GenⅢ	Total number of bacteria (Copies g^{-1} soil)	Total number of fungi(Copies g^{-1} soil)
1	43942976.92	15033.2909
2	10189539.493	6142.69151
3	20199485.29	9492.850696
4	46238228.091	18212.336898
5	40970084.847	10051.08755
6	41234415.492	14343.92712
7	45709389.0351	16253.80956
8	39002255.826	13065.10299
9	28200247.482	11444.1081
10	36231960.591	14575.32622
average number	35191858.373	11861.45316



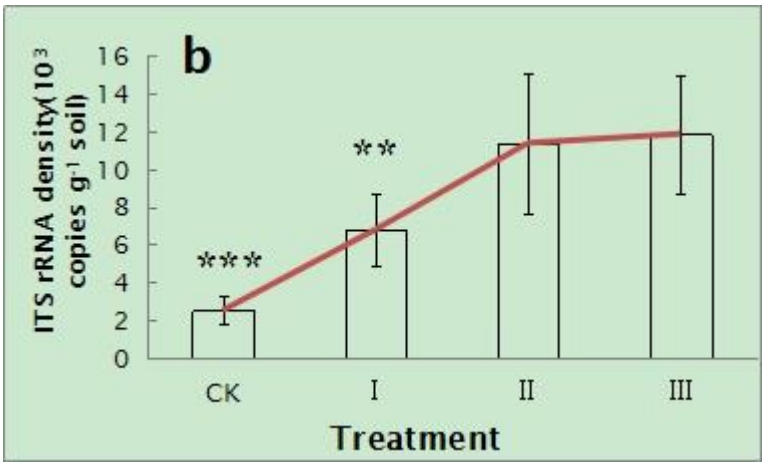


Fig.1 Populations of rhizosphere bacteria (a) and fungi (b) in poplar plantation in successive generations. ***, extremely significant difference ($P < 0.001$); **, highly significant difference ($P < 0.01$); *, significant difference ($P < 0.05$); no asterisk, no significant difference ($P > 0.05$).

Changes in composition of bacterial and fungal communities in rhizosphere soil from different generations of poplar

The degree of similarity in bacterial communities from the rhizosphere soil in different poplar generations ranged from 46% to 91% (Fig. 2) and that in fungal communities from 40% to 88% (Fig. 3), indicating that the composition of the two communities differed with the generation. The phylogenetic tree divided the population roughly into three clusters, indicating that the bacterial and fungal species in the rhizosphere were distinct from those in control sample. Based on the similarity matrix generated by DGGE electrophoresis, average values were computed for comparing the microbial communities in the rhizosphere of different generations with those in the soil from bare land.

In the case of bacteria, the degree of similarity between the communities in the rhizosphere of poplar Gen I and those in the soil from bare land was 91% whereas that between Gen I and Gen II was only 46%. The first figure indicates that the composition of the bacterial community had changed little up to Gen I whereas the second figure indicates marked changes. The degree of similarity between Gen II and Gen III was 52%, only slightly higher than that between Gen I and Gen II, indicating that the community composition had stabilized (Fig. 2 and Tab.6).

The similarity for fungal communities between Gen I of poplar rhizosphere soil and bare land soil sample was 86% (Fig. 3). The high similarity indicated that the fungal community was as diverse as that in the control sample. The similarity between fungal communities in Gen II of poplar rhizosphere soil and those in Gen I was 52% and that between Gen I and Gen III was 40%, indicating that the composition of the fungal community in Gen III changed more slowly (Fig. 3 and Tab.7).

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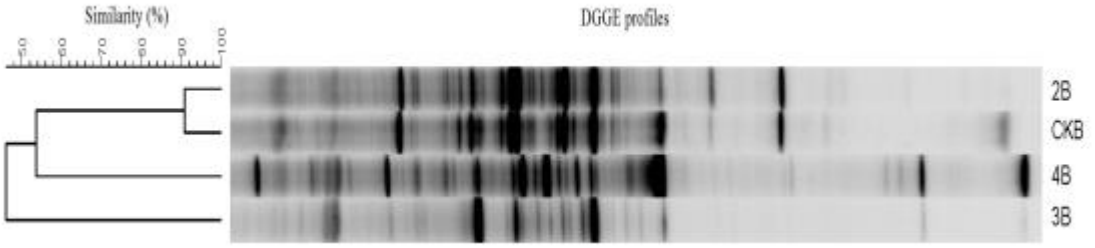


Fig. 2 Composition of bacterial communities as reflected in DGGE profiles and cluster analysis (CKB represents control group, and 2B, 3B, and 4B represent Gen I, Gen II, and Gen III, respectively).

Table VI

Analysis of similarity (R values) between bacterial communities in soil from the control group and those from three generations of poplar in terms of their DGGE profiles

Comparison	Peak area data	Presence-absence data
CKB vs. 2B	–0.105	–0.098
CKB vs. 3B	0.313*	0.302*
CKB vs. 4B	0.452**	0.539**

Note: *, $p < 0.05$; **, $p < 0.01$

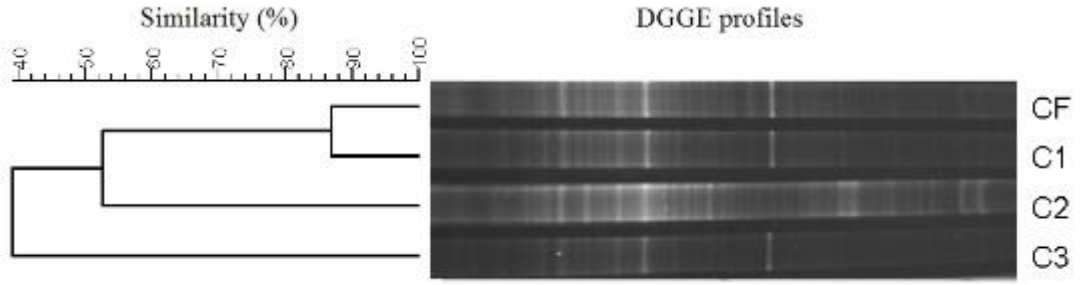


Fig.3 Composition of fungal communities as reflected in DGGE profiles and cluster analysis (CF represents control group, and C1, C2, and C3 represent Gen I, Gen II, and Gen III, respectively).

Tab.7

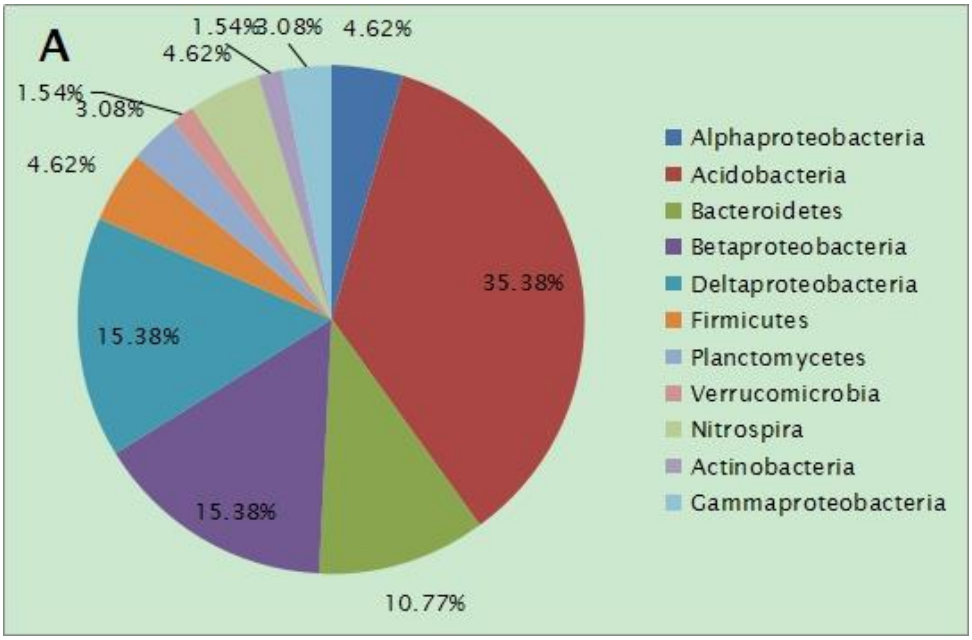
Analysis of similarity (R values) between fungal communities in soil from the control group and those from three generations of poplar in terms of their DGGE profiles

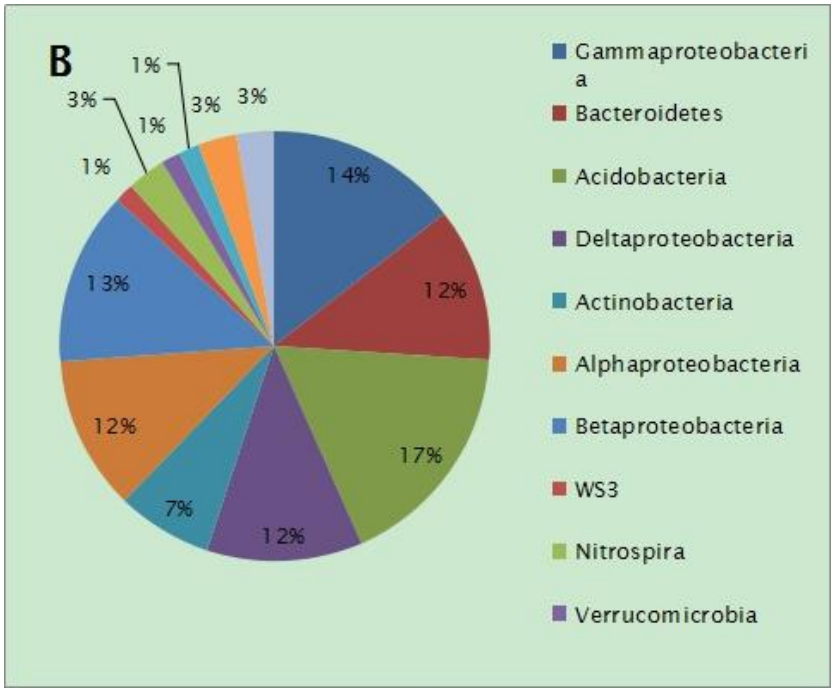
Comparison	Peak area data	Presence-absence data
CF vs. C1	–0.108	–0.098
CF vs. C2	0.314*	0.278*
CF vs. C3	0.456**	0.421**

Note: *, $p < 0.05$; **, $p < 0.01$

Composition of bacterial communities in rhizosphere of poplar plantations

The composition of the bacterial community in the rhizosphere of Gen II – in contrast to that of either Gen I or Gen III – was markedly different from that in the control group (soil from bare land) and was therefore selected for the comparison. The phylogenetic tree showed that dominant phyla in the control were Acidobacteria, Deltaproteobacteria, and Betaproteobacteria (Fig. 4A) whereas in Gen II Gammaproteobacteria had supplanted Deltaproteobacteria (Fig. 4B). In terms of numbers, compared to the control group, members of Gammaproteobacteria, Alphaproteobacteria, and Actinobacteria had increased by 10.92%, 7.38%, and 5.46%, respectively, whereas those of Acidobacteria had decreased by 18.38% (Fig. 4C).





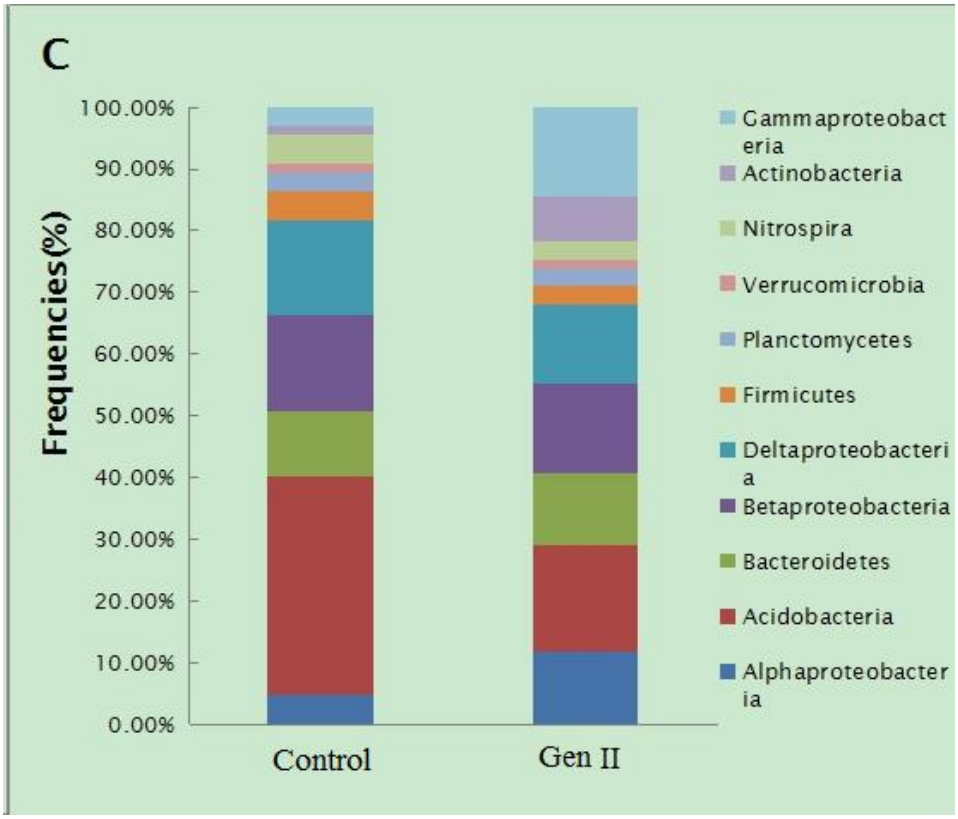


Fig.4 Composition, at phylum level, of bacterial communities in the rhizosphere of poplar Gen II and in the control group (soil from bare land). A, control; B, Gen II; C, control and Gen II.

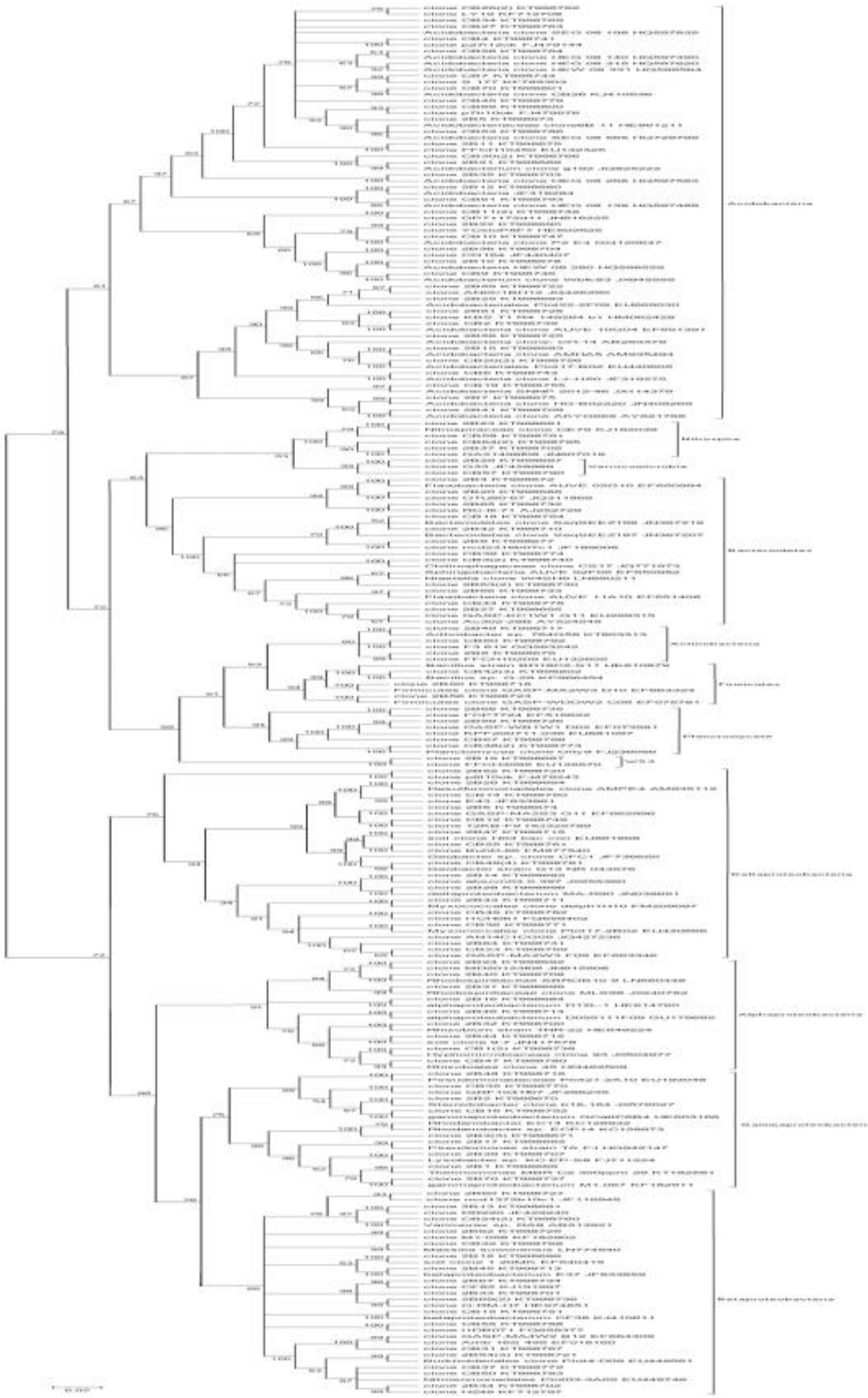


Fig.5 Phylogenetic tree of bacteria in the rhizosphere of poplar Gen II

DISCUSSION

Changes in the number of bacteria and fungi in rhizosphere soil from different generations of poplar plantation

The soil microbial community evolves with the plants growing on that soil and, in turn, affects them. For example, in natural ecosystems, *Streptomyces* sp. protects plants from the parasitic nematode *Pratylenchus penetrans* indirectly by modifying the composition of the microbial community in rhizosphere and promoting plant growth (CARAVACA et al., 2015). Bringing land under cultivation has major impacts on the micro-ecology of soil and especially of the rhizosphere. Soil microbial community is sensitive to monoculture. Such monoculture of *Rehmannia glutinosa*, for example, upset the balance among microbial species in the rhizosphere, and the numbers of bacteria and fungi decreased slightly after a year of monoculture (CHEN et al., 2007). In the case of cucumber (*Cucumis sativus*), the content of microbial biomass carbon and the abundance of active bacteria in the rhizosphere were significantly affected by the number of times the crop had been grown ($P < 0.05$): both the values increased when the crop was grown for the third time in succession but decreased thereafter up to the ninth crop (ZHOU et al., 2014). In the present study, the number of bacteria increased slowly at first and then sharply: in the first 10 years, little difference was seen between the plantation and the control (bare land); after 30 years of cropping (Gen II), the number of rhizosphere bacteria had increased although not by an order of magnitude—after 40 years (Gen III), however, it had increased sharply, by an order of magnitude. Changes in the numbers of fungi followed a different pattern: a sharp initial increase, which slowed down later; in other words, the number of fungi in Gen II was much greater than that in Gen I but not very different from that in Gen III.

In general, the numbers of both bacteria and fungi increased with time despite the monoculture, an observation consistent with an earlier report (WU et al., 2013a). Microorganisms in the rhizosphere compete with plants for nitrogen (N) (INDERJIT, 2005a; MEIER et al., 2008), and increased activity, numbers, and biomass of rhizosphere microflora could indicate aggregation and increase in soil N but its decreased supply to plants. In the present experiment, all the three generations received the same dose of N; however, because of the larger bacterial population – and hence greater competition – N uptake by plants was probably affected, reflected in lower yields as a result of continuous cropping. A striking increase in the populations of soil microflora increased the consumption of phenolic acids by the microbes substantially (YANG et al., 2012b). Another study showed that rhizosphere soils from monoculture of poplar contained ferulic acid, which is toxic to poplar (WANG et al., 2010). Therefore, the increased number of rhizosphere microorganisms could be related to the absorption of phenolic acids.

The differences in the patterns of increase shown by bacteria and fungi may indicate the difference in the response of the two groups to the artificial planting environment even in the short term (MAZZOLA et al., 2000). Intercropping could increase the numbers of ectomycorrhizal fungi (MORTIMER et al., 2015), and the proportion of fungi to bacteria could change as a result of the accumulation of plant autotoxins during continuous cropping (ZHOU et al., 2012). Bacterial communities proved more sensitive to continuous cropping (ZHOU et al., 2014), whereas fungi were affected more by soil pathogens and soil sickness; the population of *Fusarium crassistipitatum*, for example, increased significantly during continuous cropping (PEREZ-BRANDAN et al., 2014). Fungi and bacteria may also differ in their response to different forms of N: higher levels of nitrate-N, in particular, lowered the ratio of fungal biomass to bacterial biomass (BARDGETT et al., 1999). Forms of N pool in soil influence plant growth. For example, the pool of soluble N was dominated by inorganic N (especially nitrate N) in highly productive grasslands but by amino-acid N in less

productive grasslands (BARDGETT et al., 2003). Therefore, the significantly greater number of bacteria and the more or less stable number of fungi reflected the absorption of exogenous nitrate N.

Changes in composition of bacterial and fungal communities in rhizosphere soil from different generations of poplar

Changes in the composition of the microbial communities – as seen in the PCR-DGGE analysis in the present study – could be the result of long-term continuous cropping of poplar. Many studies have shown that continuous monoculture affects the composition of soil microbial communities. Benizri et al. (2005) isolated bacteria of different genera from the rhizosphere of healthy peach trees and that of replanted peach trees (to replace those that had died due to a disease), and found that the pathogenic bacteria had changed the composition of the bacterial community. Zhang et al. (2011) found that the diversity of rhizosphere fungal communities decreased with time because of continuous cropping of *Rehmannia*. Liu et al. (2014) studied microbial communities in a 7-year monoculture of potato, using the high-throughput technique of pyrosequencing, and found that both bacterial and fungal communities had been affected. LI et al. (2013) also reported that long-term rotation greatly impacted the composition of microbial community in the rhizosphere. The results of the present experiment indicate that the effects of poplar monoculture on the diversity and abundance of rhizosphere microorganisms peaked at 30 years, which is consistent with the time by which yields begin to decline in the region in which the research was carried out.

The relations between soil and plants are relatively stable under continuous cropping. Some root exudates affected the bacterial taxa selectively (SHI et al., 2011) and some exudates selectively inhibited or stimulated some bacterial and fungal species (LI et al., 2014). Some bacterial taxa specifically prefer some simple carbon sources to others (glucose, glycine, and citric acid) (EILERS et al., 2010). These factors may also lead to changes in the composition of soil bacterial communities. Simple sources of carbon and root exudates from 30-year-old poplars favoured some groups of bacteria, which gradually became dominant. The same phenomenon was observed in monocultures of *Rehmannia glutinosa* (WU et al., 2013b).

Not only root exudates but also allelochemicals accumulate in the rhizosphere as a result of long-term monoculture (RANAGALAGE et al., 2015; SMITH et al., 2015; WU et al., 2015; XIA et al., 2015). Allelochemicals are small but active molecules, which are often found in the rhizosphere of soils that have been subjected to monoculture (ZHOU et al., 2013), and interact closely with soil microorganisms (INDERJIT, 2005b). Root exudates of *Centaurea diffusa*, which contain 8-hydroxyquinoline, changed the composition of soil microbial communities after invading a site (VIVANCO et al., 2004). Allelochemicals extracted from *Mikania micrantha* H.B.K. also changed the composition of a soil microbial community (NI et al., 2006), and p-hydroxybenzoic acid affected the composition of bacterial and fungal communities in the rhizosphere in soils that had been under a monoculture of cucumber (ZHOU et al., 2012). Microbial community in the rhizosphere of poplar plantation is also affected by allelopathy (WANG et al., 2010). Different types of phenolic acid produce different effects on bacteria, fungi, and actinomycetes (TAN et al., 2008b). The present study revealed that in Gen II, by which time the site had been under poplar for 30 years, the composition of microbial community in the rhizosphere had changed significantly, possibly because of accumulated allelochemicals in the rhizosphere.

Composition of bacterial communities in rhizosphere of poplar plantations

The composition of bacterial community in the rhizosphere of Gen II plantation and in the control group (bare land) was analysed using sequencing technology. Members of Gammaproteobacteria and Actinobacteria were present in the highest numbers, which may reflect changes in total phosphorus and organic matter in the soil (GUO et al., 2015). Actinobacteria are widespread in soil and can inhibit plant pathogens and promote plant growth (PALANIYANDI et al., 2013). A striking feature of Acidobacteria, which have been studied relatively recently, is their slow growth. Acidobacteria can grow even in poor soils and have therefore been used as markers of poor soil PEIFFER et al. (2013). In the present experiment, the numbers of Actinobacteria increased in Gen II whereas those of Acidobacteria decreased, indicating that by Gen II, the soil had become richer than what it had been initially (as reflected in the control samples, from bare land.. On the contrary, the adverse effects of monoculture had set in by Gen II and Gen III; therefore, the cause of those effects was not lack of soil nutrients but allelopathy.

CONCLUSION

Both bacteria and fungi in the rhizosphere soil of poplar plantations increased with time, or with each succeeding generation of poplar, but the increase was not parallel: bacteria increased in Gen I and fungi in Gen II. The increase may be attributed to the accumulation of autotoxins secreted by poplar and of exogenous nitrate, both of which served as substrates for the microorganisms.

The increase in numbers was not uniform over time but most marked in Gen II, probably because of the levels of root exudates and autotoxins that stimulate or inhibit soil bacteria and fungi selectively.

The diversity of the rhizosphere microbial community was maximum in Gen II (30 years of poplar monoculture) but fairly small in Gen III (40 years of monoculture): this may signal the onset of the decline due to monoculture. Therefore, we suggest that monoculture of poplar be limited to 30 years. Intercropping or crop rotation could restore the lost diversity and improve the rhizosphere environment, thereby arresting the decline due to continuous monoculture.

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