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ORIGINAL ARTICLE

Effects of different concentrations of phosphorus on microbial communities in soybean rhizosphere grown in two types of soils

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Abstract Microbial communities in plant rhizosphere interact in a complicated manner among soil types, plant genotypes and soil environmental conditions. In this study, the effects of phosphorus (P) nutrition on microbial community structures in soybean rhizosphere were examined in a pot experiment. Soybean genotypes of Dongsheng 1 and Suinong 14 grew in two kinds of sand/soil (4:1, v/v) mixed cultural media with application of three P levels of P_0 (0 mg kg^{-1}), P_1 (15 mg kg^{-1}) and P_2 (40 mg kg^{-1}). The bacterial and fungal communities in soybean rhizosphere at the start of the pod stage (R_3) were examined based on the polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) banding patterns of partial 16S rDNA and 18S rDNA ITS regions and sequencing methods. Principal component analysis based on DGGE banding patterns showed that the soil type was the dominant factor in changing both bacterial and fungal communities in the soybean rhizosphere. However, separated principal component analysis based on individual soil showed that the microbial community structures were also influenced by soybean genotypes and soil P concentrations, and the influences of P were greater than those of genotypes. BLAST search of sequence data generated from excised DGGE bands indicated that bacteria belonging to Proteobacteria, Actinobacteria, Verrucomicrobia, Bacteriodetes and Gemmatimonadetes, and fungi belonging to Ascomycetes and Basidiomycetes commonly inhabited the soybean rhizosphere.

Keywords Bacterial community · Fungal community · DGGE · rDNA · Soybean genotype · Soil type

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Introduction

Soil microorganisms play an intrinsic role in nutrient cycling and crop productivity (Sun et al. 2004). The rhizosphere is the soil region that is influenced by plant roots and is characterized with a high microbial activity where soil microbes transform organic substrates, release mineral elements, and hence strongly influence plant growth (Atkinson and Watson 2000). A number of studies have been demonstrated that plants have a major influence in shaping rhizosphere microbial communities (Smalla et al. 2001), as it is commonly recognized that root exudates vary according to plant species. In other cases, the influence of soil types on the microbial community is greater than that of the specific plant species (Singh et al. 2007), as different soils show different particle size distribution, pH, aeration, and physico-chemical characteristics that can affect microbial communities either directly, e.g., by providing a specific habitat for selecting specific microbes, or indirectly, e.g., by affecting plant root exudation (Garbeva et al. 2004).

P is one of the most essential nutrient elements for plant growth and development. Despite its wide and abundant distribution in soils in both inorganic and

organic forms, the majority of them are immobilized in soils and become unavailable to plants (Holford 1997; Raghothama and Karthikeyan 2005). However, on the other hand, some plants or genotypes can secrete organic acids into the rhizosphere to mobilize P efficiently under P-deficiency condition (Jones 1998). Among them, white lupin (*Lupinus albus* L.), a kind of legume, secretes large amounts of citric and malic acids from cluster roots has been widely reported (Keerthisinghe et al. 1998; Neumann et al. 1999). Since the rhizosphere microbes respond quickly to the changes of root exudates (Singh and Mukerji 2006), it is speculated that different microbial communities in the rhizosphere are developed under different levels of soil P nutrition; however, the link between the two items is unclear.

Soybean is an important crop in northeast China, and its development and yield are very sensitive to soil P availability (Xu et al. 2003). Field experimental screening showed that different soybean genotypes differed in the ability to use P efficiently (Pan et al. 2008), and the most efficient genotypes secrete large amounts of organic acids into the rhizosphere (Sheokand et al. 2009). In the present study, we investigated the effects of P applications on the microbial communities in the rhizosphere of two soybean genotypes grown in two types of soils in a pot experiment. The bacterial and fungal communities were estimated by using PCR-DGGE for community evaluation and sequencing of DGGE bands for phylogenetic determination of dominant members.

Materials and methods

Soil samples

Samples of Black soil (Mollisol) and Dark Brown soil (Alfisol) were collected from farmland at Hailun Agro-Ecological Experimental Station, Chinese Academy of sciences (47°26′N, 126°38′E) and Lanling village, Jixi city (45°17′N, 130°42′E), respectively. Both sites were located in the Heilongjiang Province in northeast China. The Black soil had a pH of 7.01 and total soil N, P and K of 2.12 g kg^{-1}, 0.87 g kg^{-1} and 19.9 g kg^{-1}, respectively. The Dark Brown soil had a pH of 5.2 and total soil N, P and K of 0.87 g kg^{-1}, 0.31 g kg^{-1} and 22 g kg^{-1}, respectively. The available P concentrations for Black soil and Dark Brown soil were 16.9 mg kg^{-1} and 8.82 mg kg^{-1}, respectively.

Experimental design

The pot experiment was conducted in the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin (45°41.8′N, 126°38.1′E). Soil samples were filtered through a 4-mm sieve prior to use. In order to decrease the soil available P concentrations and to enlarge the effects of P treatments on microbial community structures in soybean rhizosphere, a sand/soil (4:1, v/v) mixed cultural medium was adopted in this study (Marschner et al. 2002). The cultural medium was fertilized with (mg kg^{-1}) $Ca(NO_3)_2$ 1,200, K_2SO_4 200, $MgSO_4$ 250, Fe (EDTA) 15, $ZnSO_4$ 10, and $CuSO_4$ 8 as basic fertilizers. After thorough mixing, 15 kg of mixed soil was transferred into a pot of 25.5 cm diameter and 29.0 cm depth, and the soil moisture content was adjusted to 70% of the field capacity. P treatments of P_0 (0 mg kg^{-1}), P_1 (15 mg kg^{-1}) and P_2 (40 mg kg^{-1}) calculated as pure P contents were added to the pots with KH_2PO_4 as phosphorus nutrition, respectively.

Two soybean genotypes of Dongsheng 1 and Suinong 14 were chosen for this study. Six seeds with uniform size were sown in each pot. After the seed germination, seedlings were thinned to three plants per pot. All pots were placed in a greenhouse with daytime temperature of 24–28°C and nighttime temperature of 16–20°C.

Rhizosphere soil samplings

At the soybean R_3 growth stage, the plant shoots were cut off and the roots were carefully separated from the soil by inverting the pots. Only the soil adhering to the roots was considered as rhizosphere soil, and the rhizosphere soil was collected by shaking it from the roots in the air (Nazih et al. 2001). A portion of the composite soil samples was immediately placed into autoclaved microcentrifuge tubes (2 ml) and stored at –80°C until use.

DNA extraction and purification

DNA was extracted from the soil samples (0.5 g wet weight) by bead-beating method of Zhou et al. (1996). DNA extracts were dissolved in 100 μl of TE buffer (10 mM Tris-HCL, 1 mM EDTA, pH 8.0) and purified with Sephadex G-200 (Cahyani et al. 2003).

PCR-DGGE

Partial bacterial 16S rDNA was PCR amplified with the primers GC-357f and 517r (Muyzer et al. 1993) according to the method described by Cahyani et al. (2003). A nested PCR amplification targeting fungal rDNA internal transcribed spacer (ITS) regions (Bastias et al. 2006) was conducted in this study. Fungal-specific primers ITS1-F and ITS4, and GC-ITS1-F and ITS2 (Gardes and Bruns 1993) were used for the first and second round of PCR amplifications, respectively, and PCR protocols were followed to the description by Bastias et al. (2006). DGGE for bacterial community was performed by using a 8%

(w/v) acrylamide gel with a 30-70% denaturant gradient [100% denaturant gradient was defined as 7 M urea and 40% (v/v) deionized formamide] and run in a 1×TAE (Tris-acetate-EDTA) buffer for 14 h under conditions of 60°C and 100 V; DGGE for fungal community was performed by using a 8% (w/v) acrylamide gel with a 20–60% denaturant gradient and run in 1 × TAE buffer for 16 h under conditions at 60°C and 75 V. After the electrophoresis, the gel was stained in 1:3,300 (v/v) GelRed (Biotium, USA) nucleic acid staining solution for 20 min. DGGE profiles were photographed by using a Bio-Rad transilluminator (Bio-Rad Laboratories, Segrate, Italy) under UV light.

Analysis of the DGGE profile

Banding patterns of the DGGE profiles were analyzed by the Quantity one software (version 4.5). The position and intensity of each band were determined automatically. The density value of each band was divided by the average band density of the lane in order to minimize the influence of loaded DNA amount among samples (Graham and Haynes 2005). Normalized data were used for principal component analysis as described previously (Matsuyama et al. 2007).

Isolation and sequencing of DGGE bands

Several common or special bands were carefully excised from the DGGE gel and DNA was eluted by incubation of a band in 30 μl of sterilized distilled Milli-Q water overnight at 4°C. One microlitre of eluted DNA was used as a template for PCR amplification with the same primers and program as described above. The resulting amplicons were electrophoresed again on a DGGE gel to verify their position to match the original bands. This operation was repeated three or four times until the band appeared to be a single band. After this, the PCR product was directly sequence with the same primer sets. However, when the sequencing procedure failed owing to the presence of several ambiguous peaks, the amplified DNA was cloned into pMD18-T plasmid vector system (TaKaRa, Dalian, China) according to the manufacture's instructions. The plasmid were transformed into competent cells of *Escherichia coli* DH5α. Plasmid with the correct insert, as determined by DGGE was selected, and insert was sequenced with the plasmid standard primers RV-M and M13-47.

Phylogenetic analysis

The closest relatives and phylogenetic affiliation of the obtained DNA sequences were determined by using the BLAST search program at the NCBI web site. All sequences obtained from this study were deposited in GenBank under accession numbers from HM008215 to HM008254.

Results

DGGE fingerprints of the bacterial community and principal component analysis

The DGGE profiles of bacterial communities in soybean rhizosphere are shown in Fig. 1. In total, 58 bands with different mobility were observed. The average number of bands across P_0, P_1 and P_2 treatments for genotype Dongsheng 1 and Suinong 14 were 35.5±2.7 and 41.3±2.2 in cultural medium of Black soil, and were 37.2±1.5 and 38.8±3.1 in Dark Brown soil, respectively. The number of bands in the rhizosphere of Suinong 14 was a few more than that in Dongsheng 1 under same P treatment, exception is both genotypes in Dark Brown soil under P_0 condition. In addition, more bands were observed in Suinong 14 in the P_2 treatment than that in P_0 and P_1 treatments under the same soil conditions, but this tendency was not detected in Dongsheng 1 (Fig. 1).

DGGE banding patterns showed that bacterial communities in soybean rhizosphere varied with soil types, soybean genotypes and P treatments. For example, bands P, Q, R and T were specific to Black soil, bands Z and M were restricted to Dark Brown soil, band U showed the highest intensity in the P_2 treatment compared with the P_0 and P_1 treatments, band C was detected only in the Suinong 14 rhizosphere, and greater abundances of bands X and M were observed in the genotype of Dongsheng 1 than those in Suinong 14 in the P_0 and P_1 treatments (Fig. 1).

Principal component analysis clearly separated bacterial communities in soybean rhizosphere into Black and Dark Brown soil groups, irrespective of genotypes and P treatments (Fig. 2). Within the Dark Brown soil group, the bacterial communities for all samples were very similar. Within the Black soil group, although the bacterial communities in the P_2 treatment differed from those in the P_0 and P_1 treatments, the differences of the bacterial communities among the three P treatments were difficult to determine, as the first principal component rate (48.2%) was remarkably higher than the second one (9.3%) (Fig. 2).

In order to better illustrate the effects of P treatments on bacterial communities in soybean rhizosphere, separated principal component analyses based on individual soil type were conducted and the results are shown in Fig. 3. The figure clearly shows that the bacterial communities were influenced both by genotypes and P treatments. The arrows

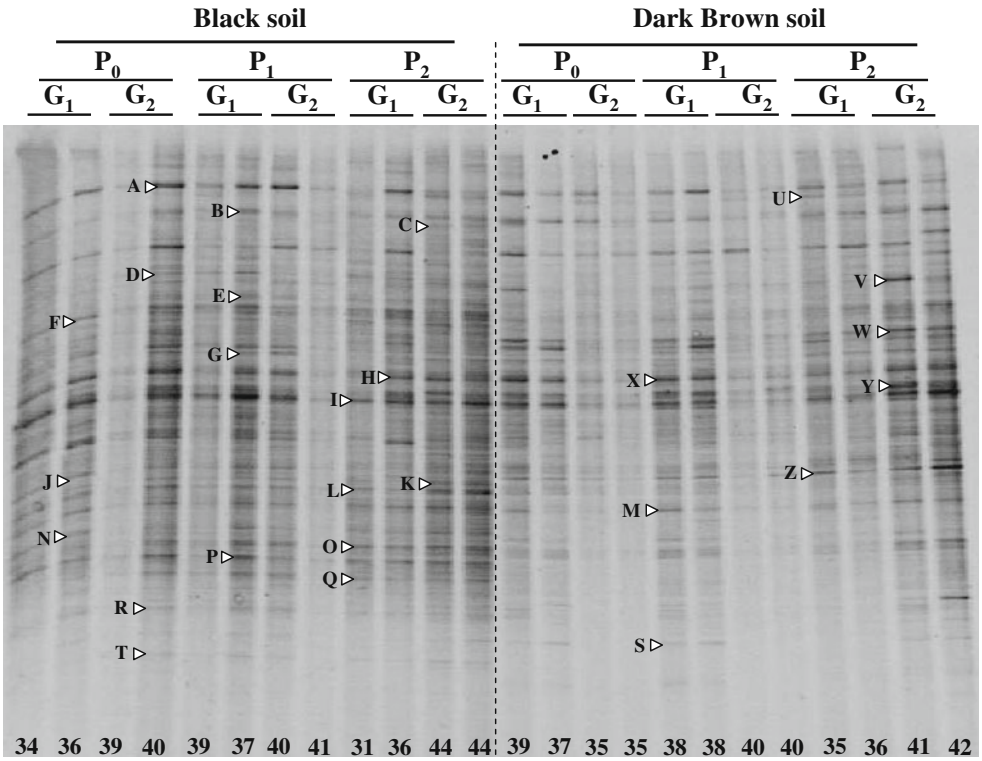

Fig. 1 Effect of different phosphorus concentrations on bacterial communities in soybean rhizospheres of two genotypes Dongsheng 1 and Suinong 14 grown in Black soil and Dark Brown soil estimated by DGGE profiles. G_1 and G_2 represent soybean genotypes of Dongsheng 1 and Suinong 14, respectively. *Numbers* below the profiles indicate the DGGE band abundances of the lane, and *arrows* indicate the bands excised from the gel for sequencing

in the figures indicate the shift of the bacterial community from treatments P_0 to P_2.

DGGE fingerprints of the fungal community and principal component analysis

Figure 4 shows the DGGE profiles of fungal communities in soybean rhizospheres. In total, 57 bands with different mobility were obtained in this study. The average number of bands across the P_0, P_1 and P_2 treatments for genotypes of Dongsheng 1 and Suinong 14 were 33.1±1.7 and 34.2±3.4 in the cultural medium of Black soil, and 37.0±1.3 and 36.7±0.5 in Dark Brown soil, respectively. The number of DGGE bands was very stable for all samples; the exception is that the number of bands for Suinong 14 was larger in the P_2 treatment than that in the P_0 and P_1 treatments grown in Black soil (Fig. 4).

DGGE banding patterns showed that fungal communities in soybean rhizosphere were very complicated and affected by soil types, genotypes and P treatments. For example, bands *f*G and *f*Q were specific to Black soil, bands *f*O, *f*R and *f*S were restricted to Dark Brown soil, band *f*A showed relative higher intensity in the P_0 and P_1 treatments than in the P_2 treatment in Black soil, but band *f*T displayed a reverse tendency showing the highest intensity in the P_2 treatment. Bands *f*P and *f*U were detected only in Suinong 14 in the P_2 treatment in Dark Brown soil and Black soil, respectively. And greater abundance of band *f*C was observed in Dongsheng 1 than in Suinong 14 in the P_0 treatment in Dark Brown soil (Fig. 4).

Similar to the bacterial communities (Fig. 2), the principal component analysis showed that the fungal communities in soybean rhizosphere were also significantly affected by soil types, and the communities were clearly separated into Black soil and Dark Brown soil groups (Fig. 5). In individual groups, the fungal communities of the two genotypes between treatments P_0 and P_1 were similar, and differed from those in the P_2 treatment. Separated principal component analyses based on individual soil type showed that the fungal community structure

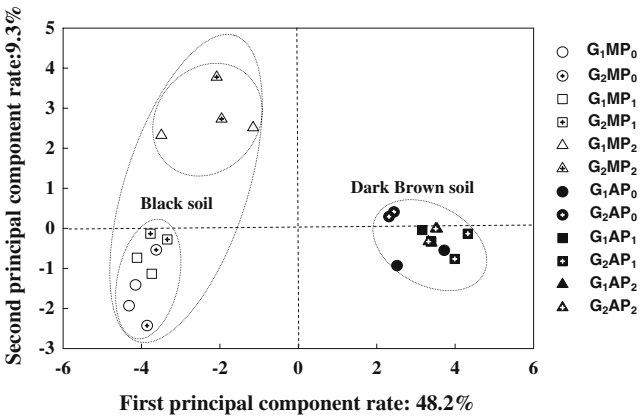

Fig. 2 Principal component analysis of DGGE banding pattern of bacterial communities in soybean rhizospheres of the two genotypes Dongsheng 1 (G_1) and Suinong 14 (G_2) grown in Black soil (*M*) and Dark Brown soil (*A*)

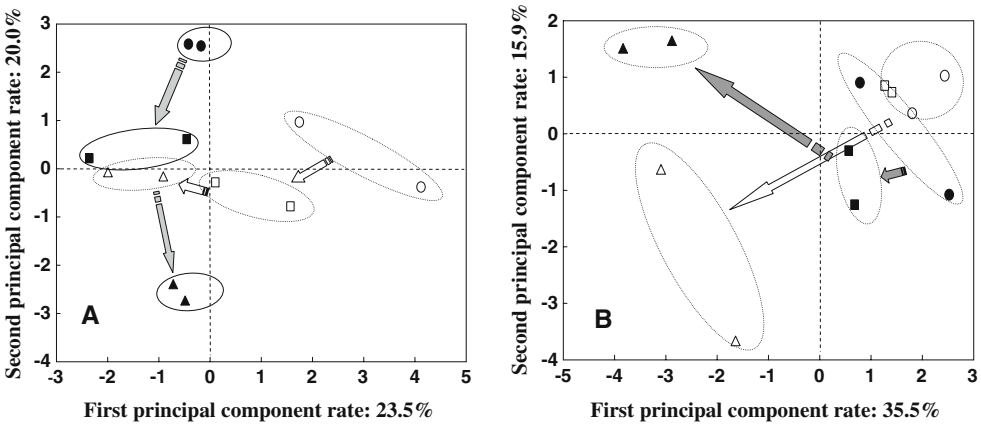

Fig. 3 Separated principal component analysis of DGGE banding patterns of bacterial communities in rhizospheres of soybean grown in Dark Brown soil (**A**) and in Black soil (**B**). *Circles*, *squares* and *triangles* indicate treatments of P_0, P_1 and P_2, respectively. *White* and *black symbols* indicate soybean genotypes of Dongsheng 1 and Suinong 14, respectively

was significantly affected by genotypes and P treatments (Fig. 6). All samples in the P_2 treatment were plotted on separated quadrants indicated that high P concentrations had a significant influence on the fungal community. The arrows in Fig. 6 indicate the succession of the fungal community along the P treatments from low to high levels.

Sequence analysis of DGGE bands

Several bacterial common, dense bands, such as A, B and H, and specific bands such as C, Y and K in Fig. 1 were excised. In total, 22 bands were sequenced and their tentative phylogenetic affiliations are shown in Table 1. Eight out of 22 sequenced DGGE bands were affiliated with Proteobacteria (α-, β- and γ-), 3 bands were affiliated with Actinobacteria and 5 bands with Gemmatimonadetes, 3 bands with Verrucomicrobia, and 1 band with Bacteroidetes.

Eighteen fungal common, dense and soil-specific bands in Fig. 4 were excised for sequencing. BLAST search indicated that all bands have the closest relatives with fungal clones or isolates (Table 2). Among them, 6 bands were affiliated with Ascomycota, 6 bands with Basidiomycota, 2 bands with Oomycetes, 1 band with Zygomycota and 3 bands with uncultured soil fungal clones.

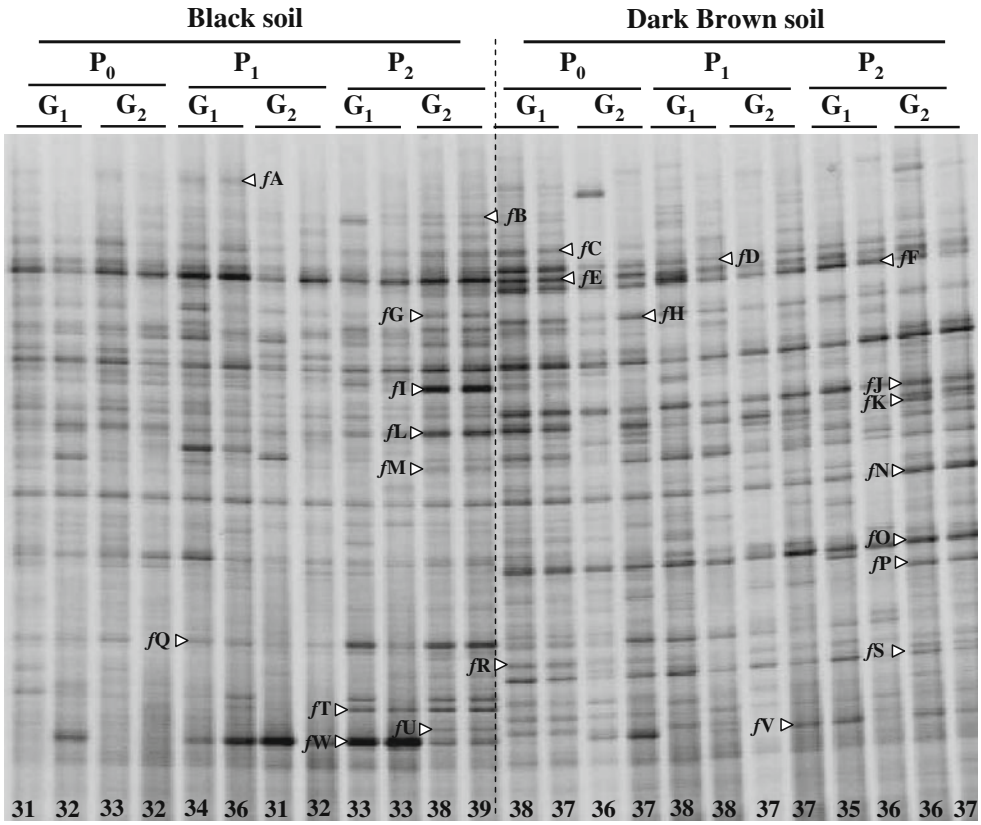

Fig. 4 Effect of different phosphorus concentrations on fungal communities in soybean rhizospheres of two genotypes Dongsheng 1 and Suinong 14 grown in the Black soil and the Dark Brown soil estimated by DGGE profiles. G_1 and G_2 represent the soybean genotypes of Dongsheng 1 and Suinong 14, respectively. *Numbers* below the profiles indicate the DGGE band abundances of the lane, and *arrows* indicate the bands excised from the gel for sequencing

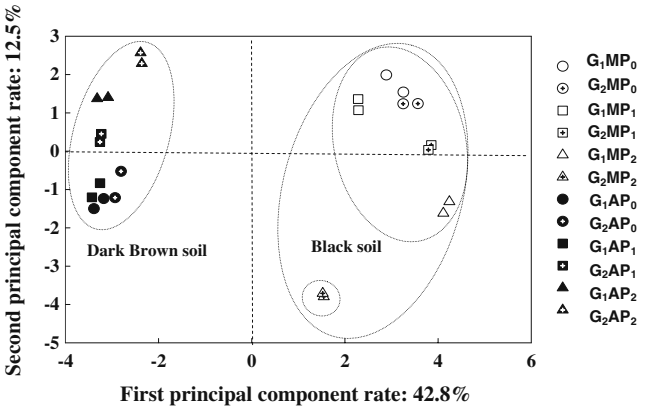

Fig. 5 Principal component analysis of DGGE banding patterns of fungal communities in soybean rhizospheres of two genotypes Dongsheng 1 (G_1) and Suinong 14 (G_2) grown in Black soil (M) and Dark Brown soil (A)

Discussion

Soil type as the major factor in determining microbial community

Our previous research has indicated that the soil type is the dominating factor in determining bacterial and fungal communities in soybean rhizosphere (Xu et al. 2009; Wang et al. 2009b). It is commonly recognized that the microbial community is relatively stable in a soil with more clay and more organic matter content (Marschner et al. 2004). Therefore, in the present study, in order to weaken the impact of soil types and to enlarge the effects of the P treatments on the microbial community in the soybean rhizosphere, the method of dilution soil with sand was employed (Marschner et al. 2002). The results clearly showed that both bacterial and fungal communities were separated into Black soil and Dark Brown soil groups (Figs. 2 and 5), indicating that the influence strength of the P treatments or of soybean genotypes on the microbial community in the soybean rhizosphere is weaker compared with soil types, even when 4:1 (sand:soil) cultural medium was used. These findings suggest that different microbial members in Black soil and Dark Brown soil are easily inhabiting the soybean rhizosphere and form different microbial communities.

Microbial communities influenced by genotype and phosphorus concentration

Although soil type as a dominating factor in determining microbial communities in the soybean rhizosphere was observed in this study, the results of separated principal component analyses based on Black soil or Dark Brown soil clearly indicated that the bacterial and fungal communities in the soybean rhizosphere were influenced both by the P treatments and the soybean genotypes (Figs. 3 and 6).

Different plants select different microbial communities in their rhizosphere due to the differences in the amount and composition of root exudates (Garcia et al. 2001; Soederberg et al. 2002). In the present study, significant differences in bacterial and fungal communities between two genotypes were developed by observation of DGGE banding patterns (Figs. 1 and 4), and separated principal component analyses (Figs. 3 and 6). This finding is inconsistent with our previous studies that bacterial and fungal communities in the soybean rhizosphere were very similar among three soybean genotypes (Xu et al. 2009; Wang et al. 2009b). We ascribed this disagreement to the use of diluted soil medium in this study, which might enlarge the effect of the soybean genotype.

Marschner et al. (2006) reported three *Poaceae* genotypes forming different microbial communities in the rhizosphere under P-limiting conditions, and the difference was more pronounced in the treatment P120 (120 mg P kg^{-1}) than in P0 (no added P). Similar results were also detected in this study, as the separated principal component

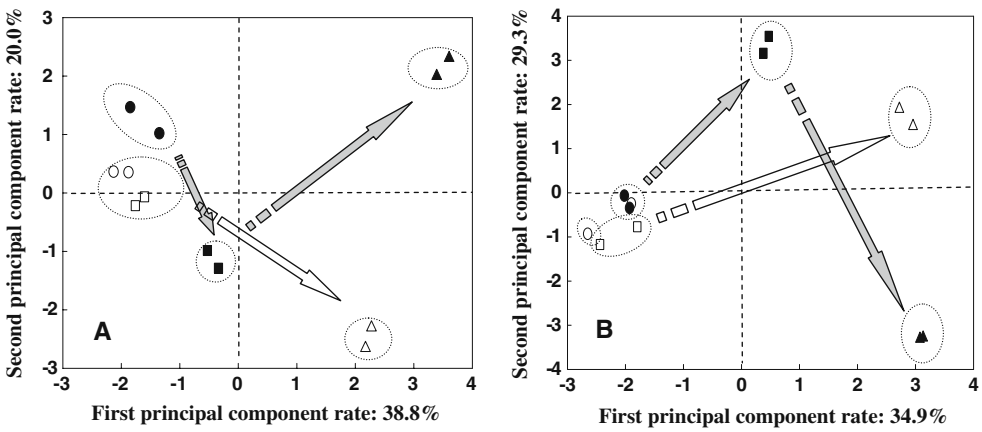

Fig. 6 Separated principal component analysis of DGGE banding patterns of fungal communities in rhizospheres of soybean grown in Dark Brown soil (**A**) and in Black soil (**B**). *Circles*, *squares* and *triangles* indicate treatments of P_0, P_1 and P_2, respectively. *White* and *black symbols* indicate soybean genotypes of Dongsheng 1 and Suinong 14, respectively

Table 1 Closest relatives of partial of 16S rDNA sequences derived from DGGE bands

DGGE band	Seq (bp)	Closest relatives			Similarity (%)	Alignment
		Microorganisms	Phylogenetic affiliations	Accession number		
A	160	Uncultured betaproteobacterium clone BIqi38	*Betaproteobacteria*	AJ318162	98	158/160
B	139	Uncultured Actinobacterium	*Actinobacteria*	EF074674	95	120/126
C	155	*Flavobacterium* sp. EMB117	*Bacteroidetes*	DQ372986	100	155/155
D	159	Uncultured *Verrucomicrobia* clone FI-1M_C06	*Verrucomicrobia*	EF220577	100	159/159
E	159	Uncultured *Verrucomicrobia* clone AKYG804	*Verrucomicrobia*	AY922067	93	145/155
F	159	Uncultured *Verrucomicrobia* clone lhac15	*Verrucomicrobia*	DQ648941	100	159/159
G	160	Uncultured betaproteobacterium clone M3sb8	*Betaproteobacteria*	AF424752	97	155/159
H	135	Uncultured proteobacterium	*Alphaproteobacteria*	FJ854722	100	135/135
I	135	Uncultured proteobacterium clone RBE1CI-63	*Alphaproteobacteria*	EF111072	100	135/135
J	147	Uncultured actinobacterium clone LW10	*Actinobacteria*	DQ973235	96	137/142
K	161	Uncultured proteobacterium	*Proteobacteria*	DQ828988	100	161/161
L	149	Uncultured *Gemmatimonadetes* clone F07_MO02	*Gemmatimonadetes*	EF220857	100	149/149
M	160	Uncultured betaproteobacterium clone LF089	*Betaproteobacteria*	EF417778	100	139/139
N	152	Uncultured *Gemmatimonadetes* clone G03_SGPO01	*Gemmatimonadetes*	EF221038	94	144/152
O	152	Uncultured *Gemmatimonadetes bacterium*	*Gemmatimonadetes*	EU297951	100	152/152
P	130	Uncultured actinobacterium clone A21YB07RM	*Actinobacteria*	FJ568800	97	97/100
Q	149	Uncultured proteobacterium	*Proteobacteria*	EF019642	96	144/149
R	152	Uncultured *Gemmatimonadetes* clone AUVE_11G10	*Gemmatimonadetes*	EF651462	100	152/152
S	139	Uncultured soil bacterium clone L4-B04	*Bacteria*	FJ930532	97	136/139
T	117	Uncultured *gammaproteobacterium* cloneSII-12	Gammaproteobacteria	AJ853877	100	117/117
U	140	Uncultured soil bacterium clone 8 F-519R-16	*Bacteria*	EU703597	100	140/140
V	161	Uncultured *Gemmatimonadete*s clone OS-C21	*Gemmatimonadetes*	EF612384	100	161/161

analyses clearly illustrated that the difference of both bacterial and fungal communities between the two genotypes was remarkably great in the P_2 treatment than that in the P_0 and P_1 treatments (Figs. 3 and 6), except for the bacterial communities of the two genotypes grown in Dark Brown soil (Fig. 3A). Although we did not investigate the amount and composition of root exudates in this study, a larger soybean biomass was observed in the P_2 treatment (data not shown), which inferred a greater abundance of root exudates released into the rhizosphere and resulting in a greater influence on the microbial community by the P_2 treatment. Direct evidence had already demonstrated that the soil microbial community can be affected by adding different amounts of citrate (Marschner et al. 2002) and artificial root exudates (Baudoin et al. 2003).

Comparison of the relative influence strength between the P treatments and genotypes on the microbial community composition suggested that the influence of the P treatment is greater than that of the soybean genotype, as the succession of the microbial community with P treatments was explained mainly by the first principal component rate (Figs. 3 and 6). Changed microbial communities in the soybean rhizosphere by P treatments inferred that the overall microbial functions are also changed, since a study by Marschner et al. (2006) indicated that microbial community composition in the rhizosphere was affected by P addition, and a positive correlation between microbial community composition and acid phosphatase activity was revealed. The contribution of changed microbial communities to P availability in the soybean rhizosphere needs to be studied further.

Table 2 Closest relatives of partial of 18S rDNA sequences derived from DGGE bands

DGGE band	Seq (bp)	Closest relatives			Similarity (%)	Alignment
		Microorganisms	Phylogenetic affiliations	Accession number		
*f*A	174	*Mortierella alpina*	*Zygomycota*	EF519903	100	174/174
*f*B	172	Uncultured soil clone 14-19	Fungi	DQ420853	100	172/172
*f*M	178	Uncultured soil clone 57F33	Fungi	FJ626936	100	178/178
*f*E	185	Uncultured clone Singleton	Fungi	FJ779614	86	169/147
*f*H	185	*Phaeosphaeria lindii*	*Ascomycota*	AF439489	98	183/185
*f*F	223	Uncultured *Ascomycota* clone A18YM12RM1	*Ascomycota*	FN294291	99	179/180
*f*K	147	*Trichocladium opacum*	*Ascomycota*	AM292049	100	147/147
*f*L	167	*Verticillium catenulatum*	*Ascomycota*	AY555966	100	167/167
*f*P	183	Epacrid root endophyte E2-1-6	*Ascomycota*	AF098291	90	166/184
fU	172	*Pseudeurotium bakeri*	*Ascomycota*	AY129287	100	165/172
*f*C	148	*Guehomyces pullulans*	*Basidiomycota*	AF444418	99	147/148
*f*D	151	Uncultured basidiomycete clone BF-OTU112	*Basidiomycota*	AM901869	86	132/152
*f*Q	143	Uncultured basidiomycete A18YM12RM1	*Basidiomycota*	FN295472	96	180/186
*f*I	183	Uncultured *Ceratobasidium*	*Basidiomycota*	EF154356	100	183/183
*f*N	138	*Cryptococcus fragicola*	*Basidiomycota*	AB035588	82	91/110
*f*T	167	*Phallus rugulosus*	*Basidiomycota*	AF324170	97	171/175
*f*O	159	*Pythium myriotylum*	*Oomycetes*	DQ102701	100	159/159
fV	170	*Pythium myriotylum*	*Oomycetes*	AM396958	100	170/170

Sequence analysis of the dominant DGGE bands

Twenty-two bacterial DGGE bands were sequenced, and their phylogenies represented members of seven distinct phyla: α-Proteobacteria, β-Proteobacteria, γ-Proteobacteria, Actinobacteria, Gemmatimonadetes, Verrucomicrobia, Bacteroidetes, and unclassified bacteria (Table 1). The most predominant bacterial group was Proteobacteria, which accounted for about 36% of sequenced DGGE bands in this study. The Proteobacteria are commonly found in agro-ecosystems (Smit et al. 2001; Sun et al. 2004; Wang et al. 2009a), which comprised 35% of the bacterial community in a wheat field (Smit et al. 2001) and 37% in a manure-treated agro-ecosystem (Sun et al. 2004), and 21% of sequenced DGGE bands in Black soils (Wang et al. 2009a). The Acidobacteria group comprised 14% of sequenced DGGE bands (Table 1). The Acidobacteria are a group of Gram-positive bacteria, which are diversified genetically and metabolically and widely spread in environments (Sun et al. 2004). However, despite Acidobacteria being widespread and abundant in soils, their role is still unknown (Aislabie et al. 2006). The phyla Verrucomicrobia and Gemmatimonadetes were also well represented in the agricultural agro-ecosystems (Brons and Elsas 2008). In this study, 3 and 5 bands were sequenced as Verrucomicrobia and Gemmatimonadetes, respectively (Table 1). Members of the Verrucomicrobia are known to be abundant in agricultural grassland soils (Kielak et al. 2010) and black soils (Wang et al. 2009a), whereas bacteria belonging to the Gemmatimonadetes group have been found in various soils and have the ability to accumulate polyphosphates (Zhang et al. 2003).

Different from bacterial 16S rDNA, an analysis of the diversity of the fungal community based on 18S rDNA sequences is more problematic due to the relative lack of variation within 18S rRNA genes between closely related fungal species (Anderson and Cairney 2004). Many primers have been designed for amplification of environmental fungal DNA, and the fungal ITS region was found to be important in taxonomy and has proved to be a very powerful tool in fungal ecology research (Bastias et al. 2007). The ITS1 primer has been used in conjunction with ITS4 specifically to amplify fungal templates from mixed community DNA samples, and increase specificity for amplification of Ascomycete DNA (Larena et al. 1999), while it was particularly true for most Ascomycota and Basidiomycota which were dominant in soil environments (Anderson et al. 2003; Bastias et al. 2006). In the present study, those primer sets were used for analysis of the soybean fungal community, 18 fungal DGGE bands were sequenced, and about 67% of sequenced bands belonged to the Ascomycete and Basidiomycete (Table 2). The fungi belonging to Ascomycete and Basidiomycete dominating in

soil environments were also observed in transect ecosystems from moorland to forest by Anderson et al. (2003), and in the soybean rhizosphere in our previous reports (Wang et al. 2009b). In this study, several bands which were changed with P treatments, soybean genotypes and soil types were sequenced (Table 2), but their roles are still unknown. In addition, three ITS sequences, *f*B, *f*M and *f*E, obtained in this study had high similarity with uncultured fungal ITS sequences in the databases, which inferred that some unknown fungal groups exist in the soybean rhizosphere (Table 2).

Conclusion

It is concluded that the bacterial and fungal community structure in the soybean rhizosphere is strongly affected by soil type, and also changed with soybean genotype and soil P concentration. Interestingly, the influence strength of P treatment on the microbial community is greater than that of the soybean genotype. Sequence analysis of the DGGE bands revealed that bacteria belonging to the Proteobacteria, Actinobacteria, Verrucomicrobia, Bacteriodetes, and Gemmatimonadetes commonly inhabit the soybean rhizosphere, while fungi belonging to the Ascomycetes and Basidiomycetes predominantly inhabited the soybean rhizosphere.

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