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Effects of cultivation duration and mode on the microbial diversity of the *Amorphophallus konjac* rhizosphere

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Amorphophallus konjac **cannot be cultivated on the same field consecutively for extended periods, but can be cultivated on the same grove. The underlying mechanism for this is unknown and may involve the rhizosphere microbial community. Therefore, the different duration and mode for konjac were researched. Polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) showed that microbial communities varied by cultivation duration and mode. Seven bacterial phyla were detected in the soil and Proteobacteria was the most abundant of these phyla. The microbial metabolic diversity following three years of continuous cultivation (sample QXFH3) was the highest, and this sample may have contained pathogenic and antagonistic microorganisms. There was almost no disease in the soil sample from grove conditions (QXN0), which was readily distinguishable from soils from field conditions (QXF0). Amino acids, carboxylic acids, and miscellaneous carbohydrates were the main carbon sources utilized by microbes in these soils. The microbial diversity index and multivariate analyses revealed that bacterial diversity increased with cultivation duration.**

Key words: Discontinuous cultivation, metabolic function, microbial composition, *Amorphophallus konjac*.

INTRODUCTION

Amorphophallus konjac has been used for food, medicine and fodder, as well as in wine production (Gao, 2004). China is the main producer of konjac, ~1.13×10^5 hectares being cultivated (Wu *et al*., 2014). Due to the increasing demand for konjac glucomannan, konjac is now regarded by the Chinese government as an agronomically important crop having significant potential in both domestic and international markets. However, disease occurrence during continuous cultivation is 35 to 50% higher than in non-continuous cropping fields. This is the major factor threatening konjac production (Zhang et al., 2012). Multiple lines of evidence indicate that four major factors can result in discontinuous cultivation: deterioration of soil physicochemical characteristics, soilborne diseases, imbalance of the soil microbial community, and autotoxicity (Ding et al., 2014). When crop monoculture is practiced, the microbial community is continuously exposed to the roots of the same crop that

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selects and enriches certain groups of microorganisms including yield-debilitating populations (that is, soilborne pathogens) of that crop (Cook, 2006). Several studies have determined differences of soil microbial communities between rotation/mixed culture and monoculture of cotton (Acosta et al., 2010), maize (Ceja et al., 2010), wheat (and maize) (Govaerts et al., 2007), rice (Xuan et al., 2010), soybean (Li et al., 2010), oilseed rape (Hilton et al., 2013) and potato (Larkin, 2003). Despite some recent investigations into soil improvement and sterilization, the underlying mechanisms driving the relationship between microbial diversity and discontinuous cultivation are still poorly understood. Increasing evidence indicates that microorganisms in the rhizosphere play a vital role in nutrient cycling, organic matter decomposition, and the maintenance of soil fertility (Larkin, 2003). The soil microbial community is also an important bio-indicator of soil function (Zuppinger et al., 2014). Therefore, many studies of discontinuous cultivation have been focused on evaluating soil quality and microbial communities. Several previous studies have demonstrated that continuous farming leads to an imbalance in soil ecology and alterations of rhizosphere microbial diversity (Wu et al., 2009; Urashima et al., 2012). Although a number of microbial strains (<1% of total organisms) have been isolated from successively cultivated soil (Sang et al., 2008; Hoang et al., 2014), the study of most microbial community members is still difficult. Modern microbial ecology tools have enabled the study of microbial communities relating to plant growth and development, *in situ* localization of important forms, and alterations in abundance of soil microbes (Johri et al., 2003). In this study, we used molecular culture-independent methods based on 16S rDNA and 18S rDNA gene diversity (Lv et al., 2012; Ma et al., 2005), polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE), and random amplified polymorphic DNA to examine the microbial community and dynamics of the dominant microbial species in rhizosphere soil during growth (Li et al., 2012; Matsuyama et al., 2007).

Recently, a variety of culture-independent approaches, including random amplified polymorphic DNA, PCR-DGGE, and BIOLOG, have been used to investigate the diversity in bacterial structure and metabolic function of konjac soils (Bai et al., 2008). However, only a few trials have report the microbial community diversity of konjac soils with different cultivation duration using both PCR-DGGE and BIOLOG. The results of our study serve to elucidate the variations in soil microbial community and link those changes with continuous konjac farming; however, follow-up studies are required prior to modification of farming practices.

MATERIALS AND METHODS

Soil sampling and DNA extraction

The experiments were located in Quanxi village of Shiyan city in the Hubei province of China (B: 31°58.734′N, 109°40.213′E, and 1103 m elevation). This region has a typical subtropical monsoon climate with an average annual precipitation of 1000 mm and an average temperature of 14°C. The soils are loam. The grove was two years old kiwifruit orchards. The field was planted corn. The grove and the field contained 4 plots, respectively, and each plot was 5.0×10.0 m in size. Each year, starting from year 2013, konjac (*A. konjac* K. Koch ex N.E.Br.) was monoculture in field and interplant in grove. By 2014, all plots were used up. This experimental design provides opportunity to collect soil samples after konjac monoculture in field and interplant in two years old kiwifruit orchards from 0 to 3 years simultaneously.

Konjac was typically seeded in April 5 with a few days variation among years. Tuber pieces were buried on the top of raised paths (25 cm in height and 120 cm in bottom width) and plants were spaced 30 cm apart along the row. Two rows were planted on each raised path with 40 cm between the two rows. This resulted in a plant density of 225 plants per plot (equivalent to 45,000 plants ha^{-1}). Blended fertilizer (750 $kg \cdot ha^{-1}$) were applied before seeding, with the ratio $(NH_4)_2SO_4$:P_2O_5:K_2O being 15:15:15, additionally supplemented with blended fertilizer (75 $kg \cdot ha^{-1}$). All other field management activities were performed manually. Konjac was harvested in later October.

Soil samples were collected in July 20, 2015. In each plot, soil was collected at four diagonal points using a sterile plastic bag and combined into a single sample. Rhizosphere samples were collected by the root-shaking method (Kowalchuk et al., 2000). The bulk soil samples were obtained in depth 5 to 25 cm. The fresh soil samples were sieved through 2-mm meshes: The bulk soil samples were stored at 4°C for physical and chemical characterization, while the rhizosphere samples were stored at -70°C for DNA extraction (Ineceoglu et al., 2010). The physico-chemical properties of the soil are shown in Table 1 (Yu et al., 2014).

DGGE community fingerprints, DNA sequences, and phylogenetic analysis

PCR products were cleaned using the DNA Purification Kit [DP214, TIANGEN Biotech (Beijing) Co., Ltd., Beijing, China]. The variable V3 region of 16S rDNA was amplified using GC-338F and 518R primers, which were designed to be specific for most bacteria (Inceoglu et al., 2010).

The PCR products were analyzed with DGGE using a BioRad DCode Universal Mutation Detection System (Bio-Rad, Richmond,CA, USA). Samples were run on 8% (w/v) polyacrylamide gels in 1×Tris-acetate-EDTA solution. Optimal separation of the bacterial community was achieved with a 35 to 55% urea-formamide denaturing gradient [100% denaturant corresponds to 7 M urea and 40% (v/v) formamide]. Bacterial gels were run for 4 h at 150 V and 60°C. DGGE banding patterns were analyzed using QuantityOne-1-D (version 4.5; Bio-Rad Laboratories). Images were normalized using markers and the patterns were compared by clustering methods in CANOCO for Windows (version 4.5; Microcomputer Power, Ithaca, NY, USA). Similarity matrices, consisting of defined numbers within each gel, were generated using Pearson's correlation coefficient (*r*). Additionally, computer-assisted analyses of DGGE fingerprints, DNA sequences, and phylogenetics were employed according to previously described methods (Luo et al., 2010; Lyautey et al., 2005).

BIOLOG analysis

Functional diversity of the soil microbial community was characterized by community level physiological profiles using BIOLOG EcoPlates (BIOLOG, Hayward, CA, USA) (Schutter and Dick, 2001). All BIOLOG profiles were generated by a BIOLOG

Table 1. Soil samples used for analysis of diversity and main physicochemical characteristics.

Soil samples	Age (y)	Growth model	Health or ill	pH	Organic matter (g/kg)†	N	P	K
QXN0	0	Grove	-	6.20±0.01^{f}	2.56±0.02^{i}	106.63±0.95^{b}	5.19±0.51^{g}	81.00±3.06^{h}
QXNH1	1	Grove	Health	7.37±0.02^{a}	1.67±0.03^{k}	64.52±0.82^{h}	15.18±0.13^{d}	92.00±1.15^{g}
QXNI1	1	Grove	Ill	5.46±0.01^{i}	4.78±0.01^{a}	99.98±1.04^{c}	21.27±0.82^{c}	135.33±0.88^{d}
QXNH2	2	Grove	Health	5.44±0.02^{l}	3.38±0.03^{e}	56.35±0.81^{i}	16.50±0.13^{d}	59.67±0.88^{j}
QXNI2	2	Grove	Ill	5.47±0.01^{i}	2.84±0.02^{h}	76.30±1.13^{f}	22.01±2.36^{c}	117.67±0.88^{e}
QXNH3	3	Grove	Health	5.14±0.01^{c}	3.19±0.04^{f}	66.97±1.57^{gh}	17.75±0.48^{d}	53.33±0.67^{k}
QXNI3	3	Grove	Ill	4.96±0.04^{d}	3.37±0.02^{e}	85.17±0.42^{e}	10.63±0.53^{ef}	65.67±0.88^{i}
QXF0	0	Field	-	6.51±0.01^{e}	3.87±0.01^{b}	69.77±0.65^{g}	7.91±0.13^{f}	157.67±2.40^{c}
QXFH1	1	Field	Health	7.32±0.02^{b}	2.49±0.01^{j}	48.88±1.17^{j}	10.19±0.60^{ef}	79.67±1.20^{h}
QXFI1	1	Field	Ill	5.27±0.01^{j}	3.17±0.03^{f}	89.02±0.65^{d}	15.47±0.48^{d}	175.67±1.20^{b}
QXFH2	2	Field	Health	5.45±0.02^{i}	2.62±0.01^{i}	90.42±1.17^{d}	12.24±0.39^{e}	70.00±0.58^{i}
QXFI2	2	Field	Ill	6.00±0.01^{g}	2.98±0.02^{g}	165.20±0.73^{a}	23.77±0.34^{c}	109.67±3.53^{f}
QXFH3	3	Field	Health	5.74±0.02^{h}	3.78±0.02^{c}	67.78±2.30^{gh}	46.76±0.70^{b}	173.00±0.58^{b}
QXFI3	3	Field	Ill	5.22±0.01^{f}	3.66±0.01^{d}	107.68±0.62^{b}	91.26±1.98^{a}	194.33±2.67^{a}

†Letters indicate the Shortest Significant ranges (SSR) at P = 0.05 for different treatments. Different letters denote a significant difference at $p < 0.05$.

reader (ELx808BLG, BIO-TEK Instrument, Inc., Winooski, VT, USA) at 24 h intervals for 168 h (Li et al., 2012). The average well color development (AWCD), metabolic profile of microbial communities, and PCA were used to analyze the metabolic variance of rhizosphere soils. AWCD was calculated according to the procedure described by Garland et al. (1991). The total carbon substrate utilization ability of the microbial community was evaluated and the metabolic profiles of microbial communities were quantified via the Shannon (*H*) and evenness (*E*) indices. All community-level physiological profiles were calculated according to previously described methods (Li et al., 2012). The AWCD value at 120 h was used to calculate the Shannon index and IBM SPSS Statistics software (version 19.0; IBM, Armonk, NY, USA) was used for PCA analysis (Schutter and Dick, 2001).

RESULTS

Bacterial community structures in konjac rhizosphere soil as assessed by PCR-DGGE

Bacterial diversity of fourteen soil samples was evaluated using PCR-DGGE analysis of the amplified partial 16S rDNA genes (Figure 1). Overall, the bacterial community structures were relatively complex across different cultivation duration and modes. There were four bands (that is, band-1-1, band-1-4, band-3-4, and band-4-1) found in all samples (Figure 1A). All patterns derived from different planting configurations were generally similar, with an average similarity of 0.48 (using Unweighted Pair Group Method with Arithmetic Mean, a simple agglomerative [bottom up] hierarchical clustering method that is based on PCR-DGGE profiles). However, PCR-DGGE profiles from lower cultivation duration samples differed from higher cultivation duration samples, (average similarity rates of 0.56 to 0.74 and 0.65 to 0.76, respectively) (Figure 1B).

To evaluate the bacterial species, 17 bands common in the DGGE profiles were sequenced (Table 2). The similarity of all band sequences was ≥96% compared with those available in the GenBank database. Seven bacterial phyla (that is, Proteobacteria, Actinobacteria, Cellulomonadaceae, Acidobacteria, Firmicutes, Gemmatimonadetes, and uncultured bacteria) were detected; Proteobacteria was the most highly abundant. Alpha proteobacterium were most abundant and were found in all duration, modes, and healthly/disease sample. Firmicutes (that is, band-12-1 and band-12-2) were highly abundant in sample QXFI2; Gemmatimonadetes (that is, Band-14-1) were highly abundant in sample QXFI3; and Acinetobacter species (that is, Band-5-1) and uncultured proteobacterium (that is, Band-5-2) were highly abundant in sample QXNI2. Uncultured proteobacterium (Band-3-1) were not found in samples QXNI3 and QXNH3; this suggests that special groups, such as Gemmatimonadetes, Firmicutes, and uncultured proteobacterium were the main groups to change across samples.

Carbon substrate metabolic profiles of soil microbial communities

Functional diversity of the microbial community reflects the ecological function of the community. AWCD is one of the most important indices for determining the capacity for carbon utilization and is an important indicator microbial community activity (Zabinski and Gannon, 1997). The dynamics of AWCD were investigated with konjac soils cultivated for 24 h (Figure 2). In general, AWCD gradually

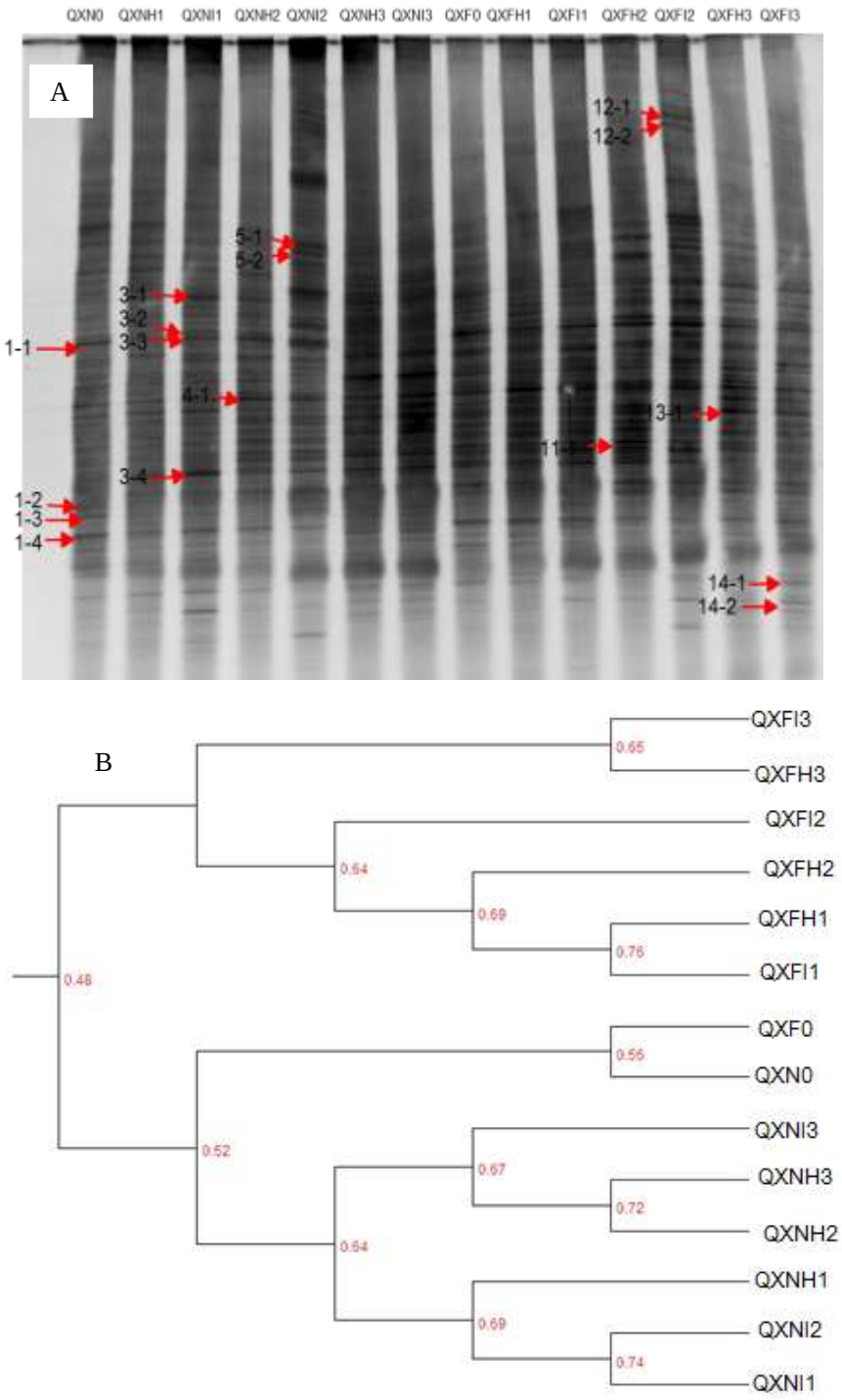

Figure 1. Denaturing gradient gel electrophoresis (DGGE) banding patterns of 16S rDNA fragments and the clustering of DGGE profiles in konjac soils of different cultivation duration and modes. Lanes corresponding to different soil samples are indicated by numbers at the top. The bands of DGGE profiles were detected, and some were excised, reamplified, and sequenced (Table 2). The arrow on the left indicates the direction of DGGE electrophoresis.

increased with the cultivation time. The carbon utilization was insignificant during the first 24 h. Soil microorganisms grew logarithmically from 24 to 120 h, and the AWCD of all soil samples increased to approximately 37. After 120

Table 2. Phylogenetic identification of selected denaturing gradient gel electrophoresis (DGGE) bands from the bacterial DGGE profiles in Figure 1[1)].

Band no.[2)]	Similar strain (NCBI accession No.)	Ident (%)	Classification[3)]
Band-1-1	Uncultured alpha proteobacterium (GQ383865.1)	99	Proteobacteria; alpha proteobacterium
Band-1-2	Uncultured bacterium (KX239244.1)	100	Bacteria
Band-1-3	Uncultured actinobacterium (HQ397176.1)	97	Actinobacteria; Acidimicrobium
Band-1-4	Uncultured actinobacterium (HM756016.1)	99	Actinobacteria; Acidimicrobium
Band-3-1	Uncultured proteobacterium (EU298748.1)	99	Proteobacteria; proteobacterium
Band-3-2	Oryzihumus leptocrescens (NR113000.1)	100	Cellulomonadaceae, Oryzihumus
Band-3-3	Uncultured alpha proteobacterium (GQ383865.1)	100	Proteobacteria; alpha proteobacterium
Band-3-4	Uncultured delta proteobacterium (EU299843.1)	99	Proteobacteria; deltaproteobacterium
Band-4-1	Uncultured Acidobacteria (EF663316.1)	98	Acidobacteriales; Acidobacteriaceae
Band-5-1	Acinetobacter spp. (KT825794.1)	100	Acinetobacter
Band-5-2	Uncultured proteobacterium (JQ910786.1)	99	Proteobacteria; proteobacterium
Band-11-1	Uncultured alpha proteobacterium (JF319258.1)	99	Proteobacteria; alpha proteobacterium
Band-12-1	Uncultured Firmicutes bacterium (JF269153.1)	99	Firmicutes; unknown species
Band-12-2	Uncultured Firmicutes bacterium (JF269153.1)	99	Firmicutes; unknown species
Band-13-1	Uncultured bacterium (KU930809.1)	100	Bacteria
Band-14-1	Uncultured Gemmatimonadetes bacterium (HG325756.1)	99	Gemmatimonadetes
Band-14-2	Uncultured alpha proteobacterium (KF183247.1)	99	Proteobacteria; alpha proteobacterium

[1)]Only the highest homology matches are presented. [2)]Bands are numbered according to Fig. 1. [3)]Classification represents phylum, order, and family of each strain.

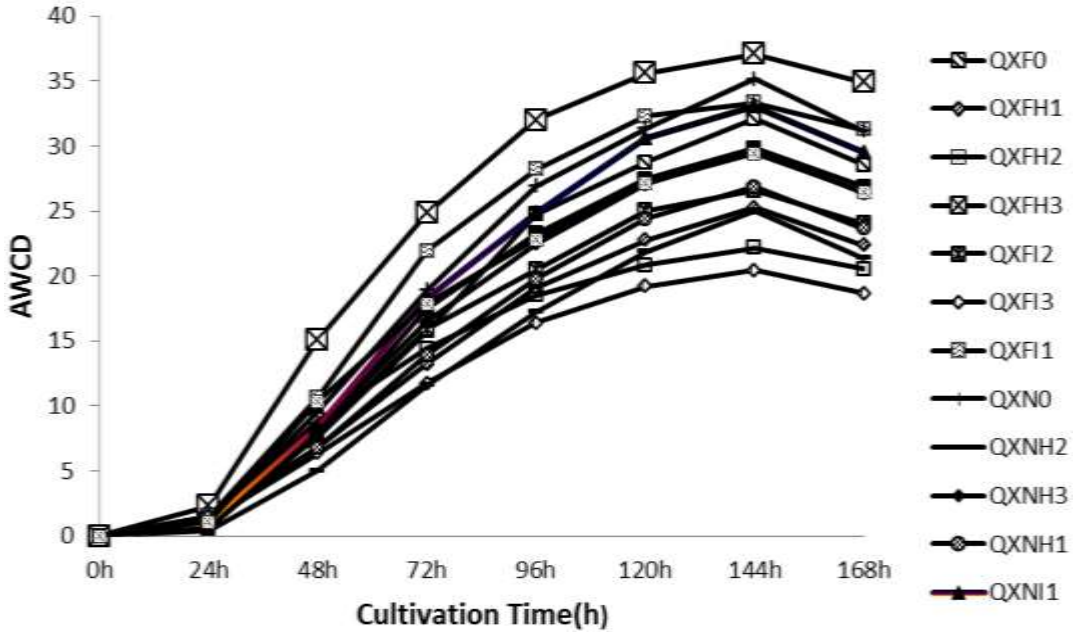

Figure 2. Average well color development (AWCD) development of soil microbial communities in konjac soil of cultivation duration and modes.

h, the rate of AWCD changed decreased because the soil microbes adapted to the environment of BIOLOG microplate. The rate of increase and final AWCD value depends on the abundance and activity of the microbial community (Garland and Mills, 1991). The AWCD values of konjac soils were between 19.18 and 35.6; these show that the utilization of a single carbon source by the soil microbial community significantly decreased in diseased

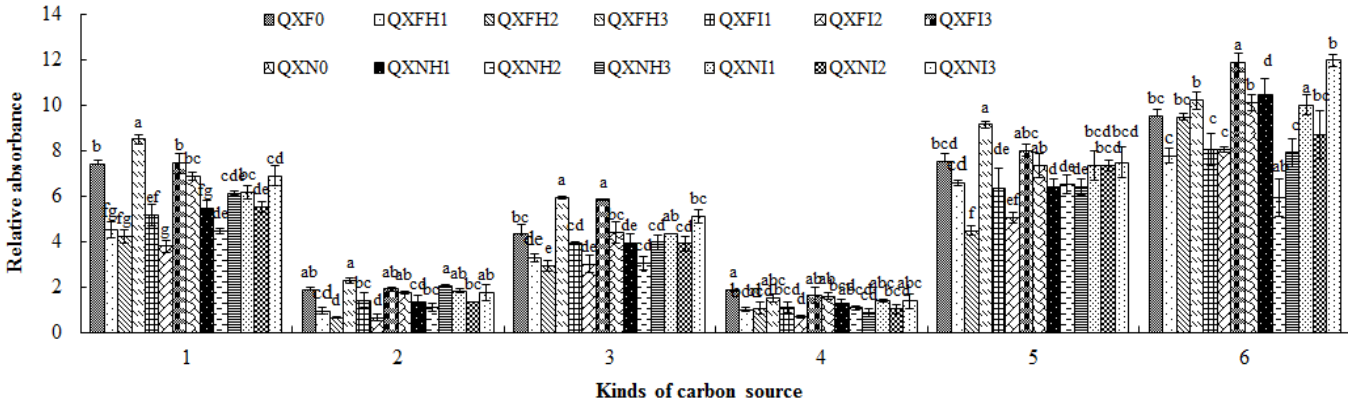

Figure 3. BIOLOG EcoPlate carbon utilization assay for soil microbial communities from different cultivation duration and modes: 1. amino acids, 2. amines/amides, 3. polymers, 4. phenolic acids, 5. carboxylic acids, 6. miscellaneous carbohydrates.

plants from field soils. From greatest to smallest, the AWCD in field samples was QXFH3>QXFI1>QXF0>QXFI2>QXFH1>QXFH2>QXFI3; in grove samples, the AWCD was QXN0>QXNI1>QXNI2>QXNI3>QXNH3>QXNH1>QXNH 2. The metabolic activity of the soil microbial communities changed with cultivation duration, and samples from healthy plants showed significantly higher metabolic activity than samples from diseased plants.

Specific substrate utilization of soil microbial communities

There are 31 types of different carbon sources in the BIOLOG EcoPlate (12 carbohydrates, six amino acids, four polymers, five carboxylic acids, two phenolic acids, and two amines/amides). Amino acids, polymers, carboxylic acids, and carbohydrates were the main carbon sources utilized by samples from different cultivation modes and health conditions (Figure 3). The relative absorbance of amino acids, polymers, and carboxylic acids was from the highest to lowest: QXFH3 >QXFH1>QXFH2, QXFI3>QXFI1>QXFI2, QXNH3>QXNH1>QXNH2, QXNI3>QXNI1>QXNI2; this indicates that the metabolic function of soil microbial communities first decreased and then increased with increases in cultivation duration. And the QXFH3>QXFI3>QXF0, QXNI3>QXN0>QXNH3, which indicated that the metabolic function of healthy soil microbial communities was higher than diseased communities in field conditions; however, there was no difference in grove conditions.

Diversity index of soil microbial communities based on PCR-DGGE and BIOLOG profiles

The overall species richness and catabolic diversity of microbial communities in konjac soil were evaluated by the number of major bands present in the PCR-DGGE and the AWCD value in 120 h BIOLOG profiles (Table 3). It was clear that the metabolic diversity of planted konjac was lower than unplanted; the diversity increased with continuous cropping. The microbial diversity index increased in diseased konjac. In the field, the microbial diversity index was (from highest to lowest): QXNH2 > QXNH1 > QXNH3; in groves the microbial diversity index was: QXFH3 > QXFH2 > QXFH1. Healthy konjac soils had a higher microbial diversity (3.27 < H < 3.18) than diseased konjac soils (3.34< H< 3.03) in grove conditions; this trend was also seen in field conditions (3.18 < H < 3.13 and 3.15 < H< 3.08 for healthy and diseased soils, respectively). Bacterial diversity differed between field (E=0.991-0.97) and grove soil samples (E=0.947-0.992); this indicates the presence of more homogeneous and stable ecosystems in the first year of planting. Increasing cultivation duration resulted in disequilibrium in microbial diversity; meanwhile, microbial diversity increased with field and grove soils, crop rotation following three years of konjac cultivation, and continuous cropping in grove soils.

Multivariate analysis of DGGE fingerprints and BIOLOG data

PCA analyses of relative band intensity (DGGE gel profiles) and carbon substrate utilization (via BIOLOG EcoPlates) were used to investigate correlations between cultivation duration, cultivation modes, and health of the konjac (Figure 4). Multivariate comparisons showed that the complex microbial communities differed with cultivation duration, cultivation mode, and health of the konjac (Figure 4). The first two components of the PCA plot of relative band intensity account for 50.52 and 27.49% of the variance. The QXN and QXF samples were separated on the PCA plot; this suggests that the bacterial

Table 3. Microbial diversity index of konjac soil calculated from DGGE fingerprinting and BIOLOG analysis.

Soil samples	Analysis by PCR-DGGE method		Analysis by BIOLOG method	
	Shannon index (H)	Substrate evenness (E)	Shannon index (H)	Substrate evenness (E)
QXN0	3.24±0.05^{abc}	0.991±0.003^{a}	3.29±0.004^{abc}	0.978±0.005^{a}
QXNH1	3.18±0.06^{bcd}	0.979±0.002^{abcd}	3.24±0.01^{cde}	0.973±0.002^{a}
QXNI1	3.15±0.02^{bcde}	0.969±0.006^{cd}	3.19±0.02^{ef}	0.990±0.012^{a}
QXNH2	3.18±0.04^{bcd}	0.967±0.006^{cd}	3.35±0.004^{a}	0.993±0.002^{a}
QXNI2	3.08±0.02^{def}	0.969±0.008^{cd}	3.23±0.02^{def}	0.983±0.006^{a}
QXNH3	3.13±0.05^{bcd}r^{f}	0.963±0.005^{d}	3.22±0.03^{def}	0.981±0.003^{a}
QXNI3	3.12±0.05^{cdef}	0.970±0.004^{cd}	3.33±0.01^{ab}	0.992±0.002^{a}
QXF0	3.00±0.04^{f}	0.947±0.008^{e}	3.30±0.02^{abc}	0.978±0.003^{a}
QXFH1	3.25±0.04^{abc}	0.972±0.008^{bcd}	3.18±0.02^{f}	0.975±0.003^{a}
QXFI1	3.03±0.07^{ef}	0.977±0.005^{abcd}	3.27±0.02^{bcd}	0.979±0.003^{a}
QXFH2	3.18±0.04^{bcd}	0.983±0.004^{abc}	3.20±0.04^{ef}	0.979±0.004^{a}
QXFI2	3.25±0.05^{abc}	0,989±0.004^{ab}	3.29±0.01^{bc}	0.983±0.006^{a}
QXFH3	3.27±0.04^{ab}	0.989±0.004^{ab}	3.30±0.01^{abc}	0.983±0.005^{a}
QXFI3	3.34±0.03^{a}	0.992±0.003^{a}	3.27±0.02^{bcd}	0.969±0.005^{a}

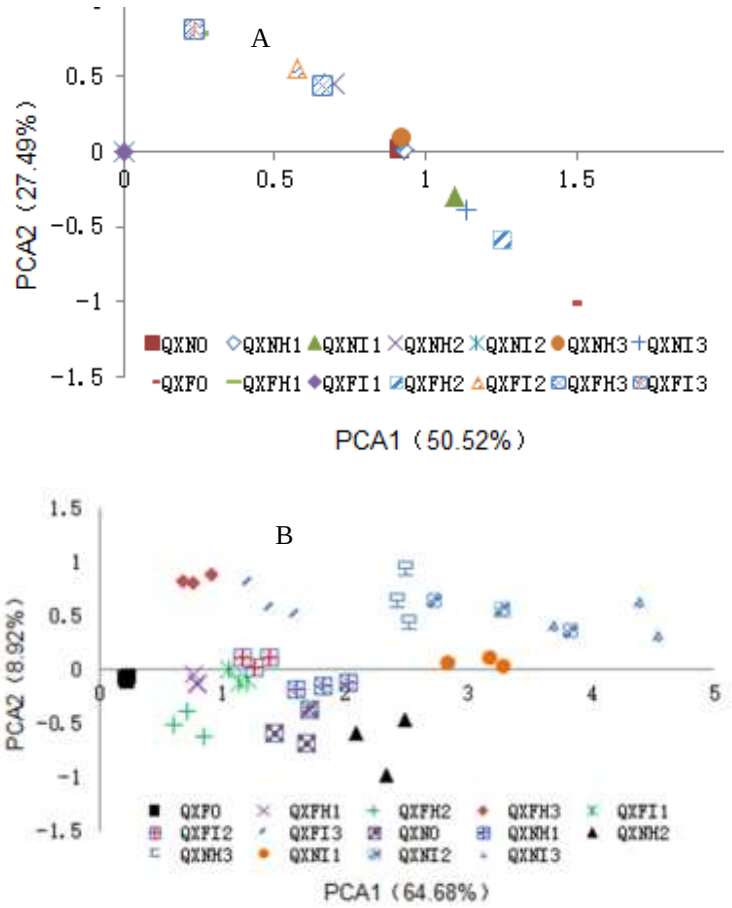

Figure 4. Principal component analysis (PCA) of the microbial composition of konjac soil samples from different cultivation duration and modes: (A) PCA of the bacterial composition of konjac soil samples by polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE), and; (B) PCA of the microbial composition of konjac soil samples by BIOLOG.

community structures of these soil samples were similar (Figure 4A). The first two components of the PCA plot of carbon utilization account for 64.68 and 8.92% of the variance. The PCA plot shows that the samples separated into three groups based on carbon utilization: Group 1 (QXFH3, QXFI3), Group 2 (QXNH3, QXNI1, QXNI2 and QXNI3), and Group 3 (QXF0, QXFH1, QXFH2, QXFI1, QXFI2, QXN0, QXNH1 and QXNH2) (Figure 4B). Collectively, the results of the PCA analyses indicate that functional metabolic diversity differed from metabolic bacterial community diversity. The primary drivers of differences in functional metabolic diversity between soil communities were konjac cultivation duration and mode.

DISCUSSION

The goal of this study was to investigate the effects of cultivation duration, cultivation mode, and plant health on the microbial genetic (via 16S rDNA gene profiles generated by PCR-DGGE) and functional diversity (via metabolic functional analyses generated with the BIOLOG EcoPlate) in konjac soils. The combination of these two methods was found to be useful for systematically understanding microbial communities in konjac soils. The combined approach is useful because soil functionality is thought to be dependent not only on the microbial species present, but also on the potential metabolic activities of the konjac soil microbiota. The BIOLOG profiles, which cannot separately represent the activity of bacterial and fungal communities, represent the total values of microbial communities. The main finding in this study was that the cultivation duration of konjac exerts the most profound influence on the genetic and functional diversity of konjac rhizosphere soils.

The bacterial community structure analyzed by PCR-DGGE was relatively complex, with significant diversity observed between different cultivation duration and modes. Seven bacterial phyla (that is, Proteobacteria, Actinobacteria, Cellulomonadaceae, Acidobacteria, Firmicutes, Gemmatimonadetes and uncultured bacteria) were detected. Proteobacteria dominated, suggesting that it is the major bacterial group in konjac soils. All members of Proteobacteria are gram negative (Berman, 2012); in the rhizosphere soil of plants, a selective effect favors Proteobacteria over Acidobacterium and gram-positive bacteria. This leads to the prevalence of the *Pseudomonas* group, which can cause disease or otherwise negatively impact plant development (Marilley and Aragno, 1999; Berggren et al., 2005). Therefore, we suspect that Proteobacteria play an important role during konjac growth. In this study, Actinobacteria represented an important component of the soil microbial population (Poltia et al., 2014) and is used as a biocontrolling agent for controlling soil- and seed-borne plant diseases (Priyadharsini and Dhanasekaran, 2015). Interestingly, some Actinobacteria species gradually appeared or disappeared with the konjac cultivation, suggesting that konjac soil significantly affects the Actinobacteria community.

To improve our understanding of the effect of cultivation duration and mode on microbial konjac soil communities, we applied the PCR-DGGE and BIOLOG methods to evaluate the metabolic activity and diversity indices. Wang et al. (2008, 2011) also used these two methods to investigate the effects of fertilization on bacterial community structure and function in black soils. The microbial metabolic activity in konjac soils was described by AWCD of substrates arranged on the BIOLOG EcoPlate. BIOLOG analysis indicated that the microbial activity of QXFH3 was the strongest; this could be due to the presence of pathogenic and antagonistic microorganisms following three years of continuous konjac cultivation. Konjac soft rot disease can cause losses of between 30 and 50% of total production; however, this can reach 80% or even complete destruction following three years of continuous cropping in field conditions (Xiu et al., 2006). However, there was almost no disease following continuous cropping years in grove conditions, and a grove soil sample (QXN0) was significantly stronger than the field soil sample (QXF0). Increase konjac cultivation duration in the same cultivation mode led to a decrease in microbial activity of diseased konjac soils (Figure 2). Amino acids, carboxylic acids, and miscellaneous carbohydrates were the main carbon sources utilized in konjac soils (Figure 3). Furthermore, the microbial diversity index and multivariate comparisons revealed that an increase in diseased konjac cultivation duration led to a larger increase in bacterial diversity than healthly konjac under field conditions. Furthermore, an increase in healthly konjac cultivation duration led to a larger increase in bacterial diversity than diseased konjac under grove conditions (Table 3 and Figure 4). Based on previous studies, cultivation duration was assumed to be an important factor that influenced microbial activity and diversity in the rhizosphere of plants (Yue et al., 2013; Ma et al., 2010). In addition, soil characteristics had a significant influence on soil microbial communities (Girvan et al., 2003), and soil pH was thought to be the primary driver of soil bacterial community composition (Landesman et al., 2014). However, in our study, soil pH decreased with increases in konjac cultivation (Table 1). Prior to our study, it was also thought that the rhizospheric microbial community and soil pH were influenced by the accumulation of root exudates (Wu et al., 2014). Therefore, root exudates could be a key factor that influences the microbial diversity of the konjac rhizosphere with increasing cultivation duration; however, the links between the microbial community composition and soil function were unclear (Anglet et al., 2014). Moreover, variations in the microbial community composition may not result in the alteration of soil function (Chapin et al., 1997). Thus, the underlying factors warranted further investigation.

The microbial structure of the rhizosphere was shown, particularly the outbreak of pathogenic bacteria, is driven by cultivation duration. Planting in groves effectively improved microbial diversity, and could potentially allow for continuous cultivation.

It was speculated that the key factors preventing continuous cultivation are changes in the microbial community structure and a microecological imbalance of the rhizosphere caused by the accumulation of root exudates. A more detailed examination of the correlation between a certain soilborne diseases and the relevant konjac root exudates is necessary; the elimination of konjac root exudates or addition of adsorption material would also be informative studies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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