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ORIGINAL ARTICLE

Impact of continuous *Salvia miltiorrhiza* cropping on rhizosphere actinomycetes and fungi communities

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Abstract *Salvia miltiorrhiza* Bunge (SMB) is an important herb that has been used in traditional Chinese medicine for centuries. Continuous SMB cropping can result in significant losses of yield and quality of the plant. The purpose of this study was to examine the variations in community structure of soil actinomycetes and fungi under continuous cropping of SMB in Sichuan, China. Four soil samples were analyzed by polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE). The microbial diversity indices (Shannon-Wiener index, richness, and evenness) of the soil actinomycetes decreased along with continuous SMB cropping and rebound at the 3rd year, while that of soil fungi declined. Cluster analysis showed that the similarity between uncultivated soils and cropping soils declined with subsequent cropping periods. Homology search of sequences recovered from the DGGE bands showed that the actinomycete community in the studied soils was dominated by *Actinobacteria*, while dominant species of the fungal community varied remarkably, including *Pleospora*, *Psathyrella*, *Pseudozyma*, *Rhizoctonia*, *Trichophaea* and unclassified groups. Overall, these findings demonstrate that continuous SMB cropping has a significant impact on soil actinomycete and fungal communities.

Keywords *Salvia miltiorrhiza* Bunge · Actinomycetes · Fungi · PCR-DGGE · 16S rRNA · 18S rRNA

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Introduction

Salvia miltiorrhiza Bunge (SMB), called 'Danshen' in Chinese, is one of the most widely used herbal medicines. The roots of SMB are used in traditional Chinese medicine for the treatment of numerous ailments, such as cardiovascular and cerebrovascular diseases (Lin and Hsieh 2010; Ho and Hong 2011), hypertension (Ng et al. 2011), ischemic stroke (Chung et al. 2012), breast cancer (Yang et al. 2010) and hepatitis (Lin et al. 2012). In the past decades, SMB cultivation areas in China have increased substantially due to rising demand for the crop as a source of active compounds for the pharmaceutical industry in addition to its cultivation for traditional Chinese medicines. Traditionally, farmers have used crop rotation as a method of managing the productivity of the soil and reducing the effects of crop pests and diseases, but increased demand for SMB and rising economic profits have urged farmers to specialize in SMB and cultivate the crop annually, leading to deterioration of yield and quality (Zhang et al. 2004). Similar findings have been reported in other crops grown annually, such as maize, soybean and wheat (Crookston et al. 1991; Kelley et al. 2003; Kirkegaard et al. 2008), all causing severe damage to agriculture and economics. In particular, it was reported that SMB cultivation can tolerate a maximum of only 3-years' continuous cropping, with yield and quality declining significantly annually and almost nothing being harvested at the 3rd year (Zhang et al. 2004; Lin 2010).

A number of factors have been considered as obstacles to continuous cropping, including abiotic factors such as soil quality degradation and field management practices, as well as biotic factors such as autotoxicity of the crop, plant pathogens, and shifts of the rhizosphere microbial community structure, which can deleteriously affect the crop (Gil et al.

2009). Attempts have been made to overcome such continuous cropping obstacles in agriculture, using inputs of fertilizers and pesticides to compensate for the lack of rotation (Bullock 1992); their effects, however, are temporary. Additionally, it was reported that the changes in the rhizosphere microbial community of crops made a major contribution to continuous cropping obstacles (González-Chávez et al. 2010; Chen et al. 2011; Bernard et al. 2012; Larkin et al. 2012). Yet, the precise nature of these changes and their effects are largely unknown, which hinders efforts to improve the soil microstructure.

To the date, there has been no investigation into the soil rhizosphere actinomycetes and fungi communities of SMB grown in fields with continuous cropping and different periods of fallow. This is of significant concern given the importance of this crop to agriculture and the pharmaceutical industry, as well as to the ecological functions of the microbial communities in soils (Qin et al. 2009; Wall et al. 2013). To be specific, continuous SMB cropping might result in the simplification of fungal diversity and/or prosperity of plant fungal pathogens (Chen et al. 2012), while the diversity and abundance of fungi have a substantial effect on plant growth and final crop quality (Bever et al. 2001; Liu et al. 2010). As for the actinomycetes, it is known that they can suppress soil-borne plant pathogens by the compounds they release, functioning as ecological balancer (Wang et al. 1999; Lee and Hwang 2002; Van Hop et al. 2011). Therefore, investigation of the change in actinomycete and fungal communities structure will benefit the understanding of their potential roles as continuous SMB cropping obstacles.

More recently, molecular fingerprinting using polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE) has been employed as an effective tool to characterize microbial community composition in soils and aquatic environments, over both space and time (Ben-David et al. 2011; García-Amado et al. 2011; Nimnoi et al. 2011; Singh 2012; Wu et al. 2013). PCR-DGGE was applied extensively to detect individual species and overall profiling of community structure. Primarily, this technique was carried out by amplifying specific genes of interest such as ribosomal RNA genes (González-Arenzana et al. 2013). It is a quicker and less labor-intensive approach than sequencing of clone libraries for comparing community composition in different samples. Besides, PCR-DGGE—a culture-independent technique—possesses the ability to characterize unculturable microorganisms and is more suitable for investigating soil microbial diversity (Hirsch et al. 2010).

In this study, PCR-DGGE was employed to investigate the profiles of rhizosphere actinomycete and fungal communities of SMB grown in fields with continuous cropping. Our objectives were to: (1) profile actinomycetes and fungi community in each soil samples; (2) investigate the potential structure changes of the actinomycetes and fungi communities by continuous SMB cropping.

Materials and methods

Sample collection

The field trials were established at a Good Manufacturing Practice (GMP) planting base of SMB in Shiquan Town, Zhongjiang County, Sichuan Province, China (31°00'N, 104°36′E; altitude, 750 m) between 2011 and 2013. The entire trial area was managed based on the typical approach of local best practice. Significant yield reduction was observed in the field trial, similar to the findings reported by Lin (2010).

The field trial was in its 3rd year when soil samples were collected during harvest period in December 2013. Soils were collected from fields subjected to 0- (uncultivated), 1-, 2-, and 3-year continuous SMB cropping. Each plot of the four trials had an approximate area of 20×10 square meters. Five soil samples were collected from the rhizosphere of SMB of each plot according to the double "S" method (Lin 2010). Soils adhering to the roots were also included in the composite rhizosphere sample of each plot by vigorously shaking. The soil samples were transported to the laboratory immediately after collection and stored at 4 °C prior to experimental analyses, which were conducted within 7 days. In the laboratory, soil samples were sieved and thoroughly mixed. Subsamples taken using quartering sampling (Lin 2010) were used for subsequent molecular analyses.

Soil DNA extraction

To extract bulk DNA of the microbial community from soil samples, 5 g of each sample was used separately for genomic DNA isolation. Total genomic DNA was extracted and purified using a PowerSoil DNA Isolation Kit (MO-BIO, Carlsbad, CA) according to the manufacturer's instructions. The quality and quantity of genomic DNA were examined by agarose gel electrophoresis and Nanodrop 1000 (Nanodrop Technologies, Wilmington, DE), respectively.

Polymerase chain reaction

The variable region of ribosomal RNA genes were amplified from the total DNA of each sample using specific sets of primers. The actinomycetes-specific forward primer Act243f (5′-GGATGAGCCCGCGGCCTA-3′) and reverse primer Act513r (5′-GC clamp-CGGCCGCGGCTGCTGGCACG TA-3′) were used for PCR amplification of 16S rRNA gene (Heuer et al. 1997). The primer pair NS1 (5′-GTAGTCATAT GCTTGTCTC-3′) and the fungi-specific primer GCFung (5′-GC clamp-ATTCCCCGTTACCCGTTG-3′) were used to

amplify the 5′ end of 18S rRNA gene (May et al. 2001). The GC clamp adding to primer Act513r and GCFung was 5′-CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCG-3′.

DNA amplifications were performed using a TProfessional thermocycler (Biometra, Goettingen, Germany) with 25-μL reaction mixtures containing 1–1.5 ng DNA, 0.1 mmol/L each dNTP, 0.3 μmol/L each primer, 21.5 μL Platinum PCR Supermix High Fidelity (Invitrogen, Beijing, China) and sterile double-distilled water. PCR cycling conditions consisted of denaturation at 94 °C for 4 min, 30 cycles of 94 °C for 30 s, 54 °C (for 16S rRNA) or 50 °C (for 18S rRNA) for 30 s, and 68 °C for 30 s, and final extension at 72 °C for 10 min.

Amplified PCR products were separated by 1 % agarose gel electrophoresis. DNA fragments of the correct size were excised from the gel and purified using a TIANgel Midi purification Kit (TIANGEN, Beijing, China) according to the manufacturer's protocol. PCR products were verified by 1 % agarose gel electrophoresis.

DGGE analysis

The D-code universal mutation detection system (Bio-Rad, Hercules, CA) was used to carry out DGGE fingerprints. An 8 μL aliquot of each of the purified PCR product was loaded onto the 16×16 cm DGGE gel. The products were resolved on 8 % (w/v) polyacrylamide gels with a 35–60 % denaturing gradient of urea plus formamide. Electrophoresis was performed in 1×Tris-acetate-EDTA (TAE) buffer at 60 °C and a constant voltage of 60 V for 12 h. Gels were stained for 30 min in 1×TAE buffer containing SYBR Green I (Sigma, St. Louis, MO) and visualized with UV transillumination (Gel Doc, Bio-Rad). The position and intensity of each band were determined using Quantity One v4.62 software (Bio-Rad).

Cluster analysis of DGGE banding patterns was performed automatically by Quantity One v4.62 software (Bio-Rad). Similarities among soil samples were displayed graphically as a dendrogram. The clustering algorithms used to calculate the dendrogram was an unweighted pair group method with arithmetic mean (UPGMA) based on the Dice coefficient. The diversity index of the soil actinomycetes and fungi communities was evaluated using the Shannon-Wiener index (H), richness (S), and evenness (E_H) based on the following equation (Saikaly et al. 2005):

$$H = -\sum_{i=1}^{S} Pi \ln Pi$$

$$E_H = H/H_{\max} = H/\ln S$$

where p_i is the ratio of the intensity of a single band to the total intensity of all bands within the same lane, and S is the total number of bands in each lane.

Sequencing and phylogenetic affiliation

Within the profile, one of the common bands and unique bands were selected for sequencing. The selected DGGE bands were excised from the gel and incubated overnight in 50 μL sterile water at 4 °C to dissolve. A 1 μL aliquot of the elution was sampled and sequenced by Beogene Biotech Corporation (Guangzhou, China). Nucleotide sequences generated in this study were all deposited in GenBank under accession numbers KJ561858 to KJ561886. The closest phylogenetic relatives were selected by subjecting the nucleotide sequences generated in this study to similarity searches using BLASTn (http://www.ncbi.nlm.nih.gov/blast).

All sequences recovered from DGGE bands and their closest reference sequences were included in phylogenetic analysis. Maximum-likelihood (ML) phylogenetic analyses were carried out using PhyML v3.0 (Guindon et al. 2010) and the substitution model GTR+G+I, chosen on the basis of the Akaike information criterion implemented in jModelTest2 (Darriba et al. 2012). Settings used in PhyML were as follows: BioNJ starting tree, four substitution rate categories, estimated proportion of invariable sites, and SPR and NNI tree improvement algorithms; 1,000 nonparametric bootstrap replications were used to assess support. Trees were drawn using Mega5 software (Tamura et al. 2011).

Results

Microbial diversity indices

Approximately 270 bp fragment from16S rRNA gene and 350 bp from the 18S rRNA gene were successfully amplified from bulk DNA isolated from rhizosphere soil with 0-, 1-, 2-, and 3-year continuous SMB cropping at four sites in Sichuan, China. The PCR products generated were separated by DGGE, respectively (Fig. 1). The DGGE patterns among samples exhibited differences regarding to the number, intensity and migration rates of bands, suggesting shifts in the actinomycete and fungal community structure in the bulk soils (Fig. 1).

With extended continuous SMB cropping periods, microbial diversity indices (H, S, and E_H) of soil actinomycetes showed various changes (Table 1). The three indices were highest for soil actinomycetes in uncultivated soil, and significant decreases occurred within the 1st year of continuous SMB cropping. However, the rate of decrease was lower in the 2nd year of continuous SMB cropping and then rose again after 2-years continuous cropping. The three diversity indices of soil actinomycetes under 3-year continuous cropping recovered slightly, but were significantly lower than those of uncultivated soils, except that the E_H value was marginally lower than that of uncultivated soil. The Shannon's diversities of all studied soils were significantly different from each other.

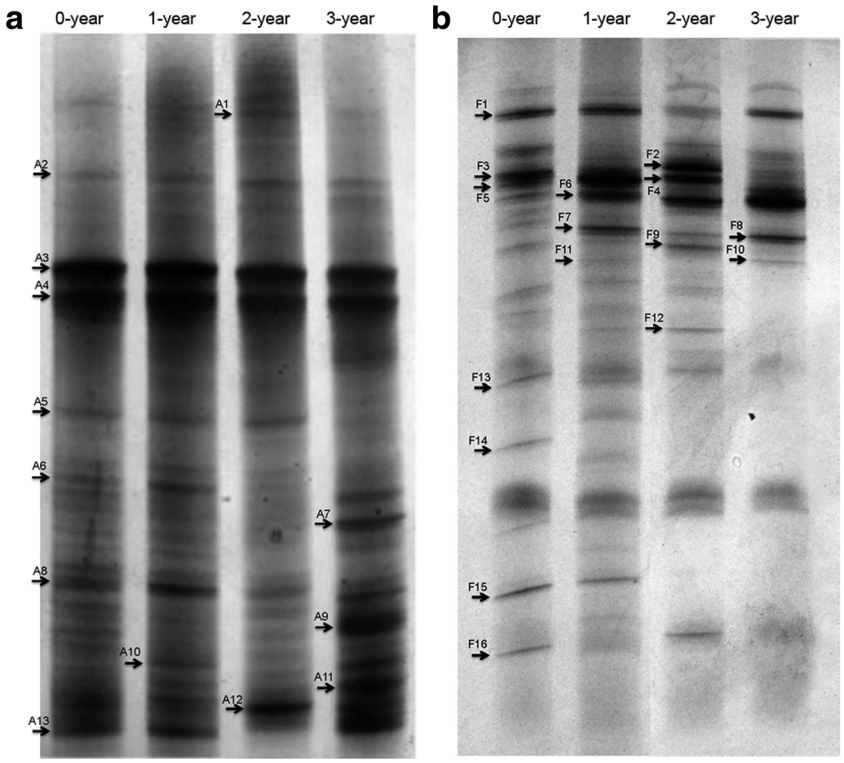

Fig. 1 Denaturing gradient gel electrophoresis (DGGE) profiles of the PCR products from **a** actinomycetes and **b** fungi in soils with 0-, 1-, 2-, and 3-year continuous *Salvia miltiorrhiza* Bunge (SMB) cropping. *Labels* indicate bands excised successfully for sequencing

The three indices of soil fungi also exhibited various changes with extended continuous SMB cropping periods (Table 1). The highest values of three indices were shown in uncultivated soil. Significant decreases occurred within the 1st year of continuous SMB cropping, while continuous decrease occurred at 2- and 3-year continuous cropping with a lower decreasing rate. The three diversity indices of soil fungi were all significantly lower than those of uncultivated soils after cultivation of SMB.

Cluster analysis and similarity

The cluster analysis showed that the DGGE patterns of soil actinomycetes were placed into four separate groups (Fig. 2a). The actinomycete community structure of the soil subjected to 1-year SMB cropping shared a 65 % similarity with that of soil subjected to 0-year SMB cropping, then the similarities dropped down with the extension of cropping periods. The sample of 3-year continuous cropping exhibited evident difference with other samples. Similarly, the DGGE patterns of soil fungi were also distributed into four separate groups (Fig. 2b), among which the sample of 3-year continuous cropping was the most divergent. In summary, there were large differences among the profiles of the actinomycete and fungi communities in soil samples.

Actinomycete community analysis and phylogenetic affiliation

DGGE analyses of 16S rRNA gene sequences revealed shifts in the actinomycete community structure in soils associated with continuous SMB cropping compared with uncultivated

Table 1 Diversity of soil actinomycetes and fungi obtained using denaturing gradient gel electrophoresis (DGGE) band pattern data associated with different succession cropping histories. Values were presented by means of three replicates± standard deviation. Values in a column are significantly different ($P<0.05$) if followed by different letters

Microbial community	Cropping history (year)	Shannon-Wiener index (H)	Richness (S)	Evenness (E_H)
Actinomycetes	0	2.51±0.09 a	14.00±1.00 a	0.960±0.000 a
	1	2.31±0.00 b	11.00±0.00 b	0.933±0.006 c
	2	2.16±0.04 c	9.67±0.58 c	0.943±0.007 b
	3	2.36±0.08 d	10.67±0.58 bc	0.953±0.006 a
Fungi	0	2.74±0.09 a	16.67±0.58 a	0.990±0.000 a
	1	2.40±0.06 b	11.67±0.58 b	0.977±0.006 b
	2	2.22±0.01 c	10.00±0.00 c	0.970±0.000 c
	3	2.06±0.00 d	9.00±0.00 d	0.980±0.000 d

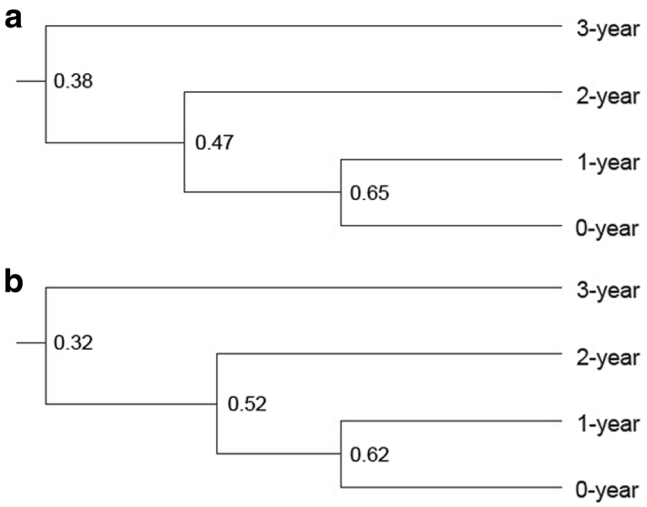

Fig. 2 Cluster analysis of DGGE profiles of **a** actinomycetes and **b** fungi in soils with 0-, 1-, 2-, and 3-year continuous SMB cropping

soils (Fig. 1a). The numbered bands in the DGGE gels were successfully sequenced. Bands A3-A4, dominant in all the studied soils, exhibited similar intensities across all the treatments, regardless of SMB cultivation, indicating that all the soils exhibited a similar predominant actinomycetes community. However, the soils with SMB cropping, especially the soil with 2-year continuous cropping, exhibited less diverse banding patterns compared with that of uncultivated soil. Among these banding patterns, A7 and A9 were absent in soil with 1-year continuous cropping, while A6- A7, A9-A10 and A13 in 2-year continuous cropping, and A5 in 3-year continuous cropping, suggesting lower actinomycete diversities, which were reflected in the lower Shannon's diversity index *H* (Table 1). Interestingly, bands A6-A7, A9-A10 and A13 that disappeared in the DGGE patterns were recovered afterwards, indicating the adaptation of those actinomycetes species to the environment of continuous cropping. The intensities of bands A7 and A9-A13 were clearly increased under 3-year continuous cropping, while the intensities of bands A6 and A8 were increased under 1-year continuous cropping and then decreased under 2- or 3-year continuous cropping. All these results indicated that SMB cropping had a significant impact on the actinomycetes community structure.

The phylogenetic tree reconstructed from partial 16S rRNA gene sequences is shown in Fig. 3. The 13 DNA sequences recovered from the DGGE gel shared 94–100 % sequence homology with the reference sequences retrieved from NCBI. The sequencing results showed that the actinomycete community in the studied soils was dominated by actinobacteria (bands A3-A4). The other bands were affiliated to other uncultured bacteria and actinobacteria, under *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Gemmatimonadetes*, *Proteobacteria* and *Verrucomicrobia*. Although band A6 and A12 were extracted from two different bands, both belonged to the same phylotype, which is common in similar studies using DGGE technology (Singh 2012, 2013; Wu et al. 2013).

Fungi community analysis and phylogenetical affiliation

DGGE analyses of 18S rRNA gene sequences revealed more changes in the fungal community than in the actinomycete community (Fig. 1b). The 16 bands in the DGGE gels were successfully sequenced. Only bands F1 and F6 were common to all the studied soils. Each soil was dominated by different bands, indicating that the distribution of the fungal community was influenced strongly by SMB cropping. Band F1 and F3-F5 were dominated in uncultivated soils, whereas F7, F2 and F8 newly emerged as one of the dominant bands in soils with 1-, 2- and 3-year continuous cropping, respectively. Bands F2, F7-F8, and F10-F12 were unique to the soils with SMB cultivation. Soils with 3-year continuous cropping seemed to have a much stronger impact on the fungal community structure, resulting in the least diverse banding patterns, with lower intensities or a lack of bands F13-F16, compared to uncultivated soil.

The phylogenetic tree reconstructed from partial 18S rRNA gene sequences is shown in Fig. 4. The 16 DNA sequences recovered from the DGGE gel shared 94–100 % sequence homology with the reference sequences retrieved from NCBI. The fungi community in the uncultivated soils was dominated by *Pleospora*, *Trichophaea* and unclassified groups (bands F1, F3-F5), while that of soils with 1-year cropping by *Psathyrella*, *Pseudozyma*, *Trichophaea* and unclassified groups (bands F1, F3, F5-F7), that of soils with 2-year continuous cropping by *Pleospora*, *Rhizoctonia* and unclassified groups (bands F2 and F4-F5), and that of soils with 3-year continuous cropping by *Psathyrella* and unclassified groups (bands F1, F5-F6 and F8). Compared to the uncultivated soils, the unique bands (F2, F7-F8, and F10-F12) of soils with SMB cultivation are affiliated to *Paecilomyces*, *Psathyrella*, *Pseudozyma*, *Rhizoctonia*, uncultured *Ascomycete* and unclassified groups. The unique band (F14) of uncultivated soils was distributed in unclassified groups.

Discussion

It is well known that continuous cropping of only one plant species has a strong influence on plant productivity and microbial community structure (Zhang et al. 2007; Urashima et al. 2012; Hilton et al. 2013). Results from our field trial support such reports that SMB cropping frequencies has an impact on yield (data not shown). However, the shifts in microbial population caused by continuously SMB cropping have not yet been investigated. In this study, we profiled the structure of actinomycetes and fungi community associated with 0-, 1-, 2-, and 3-year continuous SMB cropping and

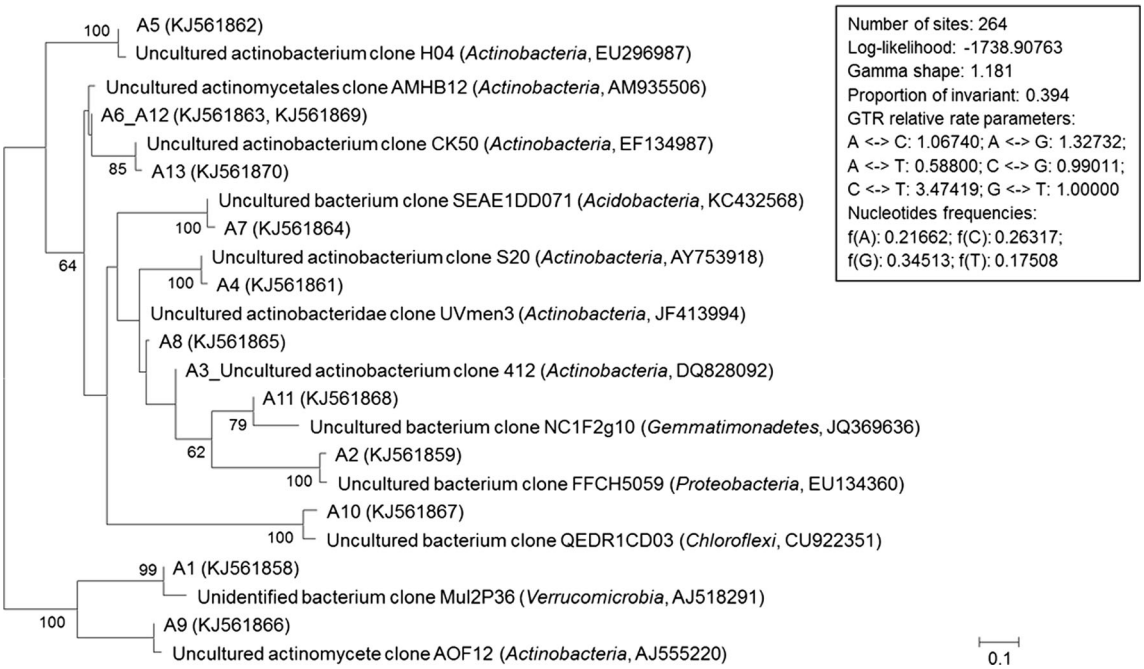

Fig. 3 Maximum-likelihood tree of 16S partial sequences for DGGE bands and references strains. Phylogenetical affiliation and accession numbers of reference strains are indicated in *parentheses*. Bootstrap values>50 % are shown at the nodes. *Scale bar* Estimated substitutions per site

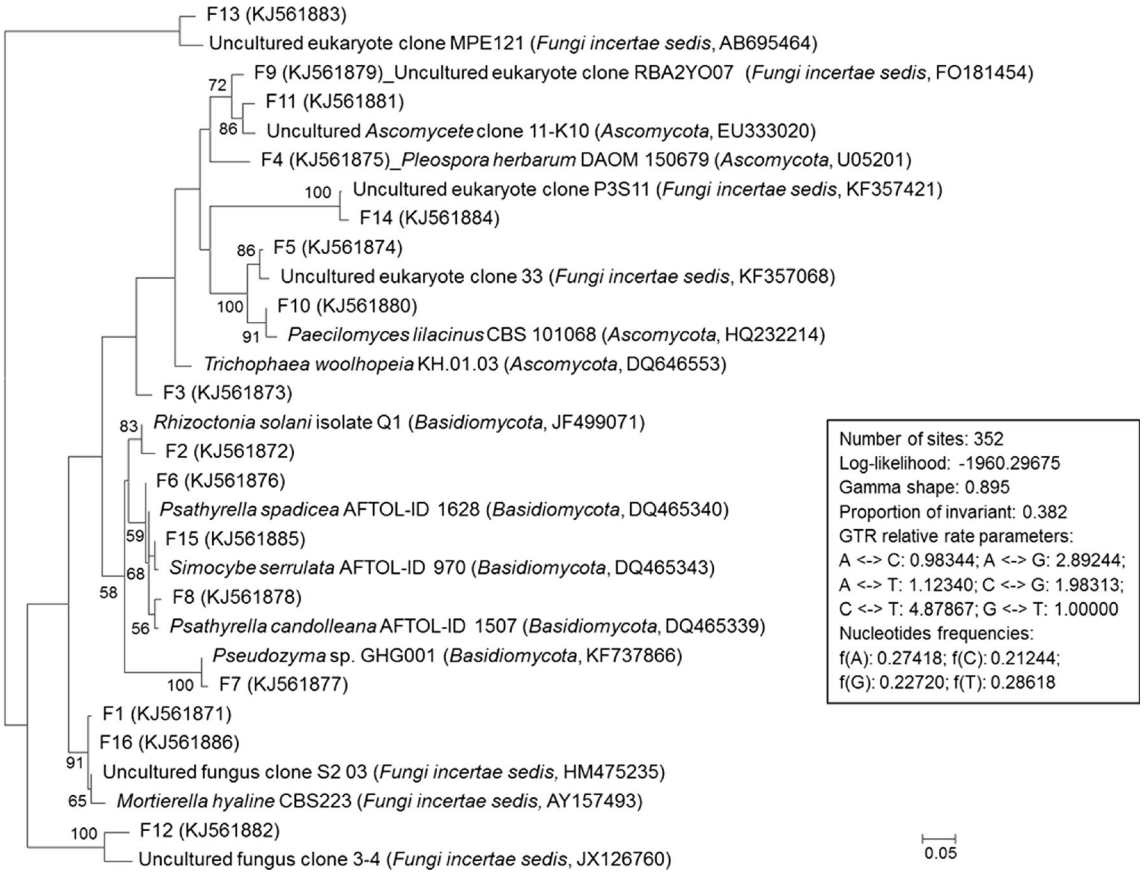

Fig. 4 Maximum-likelihood tree of 18S partial sequences for DGGE bands and references strains. Phylogenetical affiliation and accession numbers of reference strains are indicated in *parentheses*. Bootstrap values>50 % are shown at the nodes. *Scale bar* Estimated substitutions per site

outlined the community shifts, using PCR-DGGE and sequencing technologies.

This study showed that continuous SMB cropping caused a shift in rhizosphere actinomycetes 16S-DGGE profiles at the DNA level (Fig. 1a). In this process, the community structure of soil actinomycetes underwent the most rapid changes during the 1st year of SMB cultivation, mainly showing reduced diversity. The actinomycetes diversity continued decreasing during the 2nd year of SMB cultivation, reaching the least complex banding pattern. With the extension of continuous cropping, three actinomycetes diversity indices started to rebound after 3-year continuous cropping (Table 1), which is consistent with the reappearance of some species with even increased band intensities. One possible explanation for this finding is that those species were affected by root-released compounds of the newly introduced SMB and reduced to a small population size, but they gradually stabilized in the field after 2-year continuous cropping. Such variation is in agreement with shifts of actinomycetes communities associated with continuous cotton cropping (Zhang et al. 2013). In addition, cluster analysis indicated that there was a great difference among 16S rRNA gene pools of the studied soils (Fig. 2a). All these results suggested that SMB cultivation had a strong influence on the community structure of soil actinomycetes.

Seven 16S-DGGE band types observed in the present study (A1-A4, A8 and A11-A12), particularly the dominant bands, were shared by all soils studied, indicating that these actinomycetes species were stable. Sequencing of these bands revealed that *Actinobacteria*, *Gemmatimonadetes*, *Proteobacteria* and *Verrucomicrobia* were the predominant groups (Fig. 3), and these genera are commonly found in SMB fields, as reported in the previous study (Lin 2010). Those bands (A1 and A5-A13) with enhanced or decreased intensities were affiliated to *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Gemmatimonadetes*, *Proteobacteria* and *Verrucomicrobia*. However, at the species level, the closest relatives of those bands were all uncultured bacteria, which hinders our understanding of their function in this ecosystem. Further investigations are required to identify their ecological importance in SMB fields.

Molecular detection of actinomycetes colonizing soil often necessitates actinomycetes-specific primers, since the group is often present in low numbers (Muyzer and Smalla 1998). In addition, the relatively high G+C content of actinomycete DNA might reduce the competitiveness of their DNA targets in PCR (Rychlik and Rhoads 1989; Reysenbach et al. 1992). In the present study, the primers used have previously proved specific for the majority of actinomycetes (Heuer et al. 1997). However, the primers can also match the 16S rDNA of a few non-actinomycetes, as revealed by the detection of non-actinomycete strains in our study (Fig. 3). The high conservation of bacterial 16S rRNA genes is responsible for the non-specific PCR amplification. Besides, the specificity of primers relies on tested strains and known sequences from databases, which may not adequately reflect the occurrence of 16S rDNA sequences in nature. Therefore, future research could be done to improve the specificity of actinomycetes primers, based on a larger database and/or new technologies.

Contrary to the variation in actinomycetes diversity observed under continuous SMB cropping, the fungal community structure exhibited a different DGGE pattern (Fig. 1b), with a decreasing diversity index with the extension of continuous SMB cropping compared with the uncultivated soils (Table 1). The smaller number of fungal DGGE bands and decreased diversity provided more evidence of decreased fungal abundance under continuous cropping. Cluster analysis of fungal 18S rRNA gene DGGE profiles revealed clear separation of soils with continuous cropping compared with uncultivated soils (Fig. 1b). Additionally, the significantly negative correlations detected between continuous cropping cycles and the *H* fungal diversity index further supported the inhibitory effect of continuous SMB cropping on the fungal community.

It was reported that *Ascomycota* and *Basidiomycota* are important fungal groups in most soils (Carlile et al. 2001; Hussain et al. 2011). In the present study, *Ascomycota* and *Basidiomycota* were found to be predominant in all studied soils (Fig. 4), which suggested a ubiquity of these species and an important role in agroecosystems. Several bands (F2, F7-F8, and F10-F12) were found to be unique to soils with SMB cultivation. The appearance of these species could be responsible for the continuous cropping obstacle of SMB. For example, band F2 was dominated in the soils with 2-year continuous cropping and exhibited 99 % similarity with *Rhizoctonia solani* isolate Q1 (Huang et al. 2011), which is a plant pathogenic fungus with a wide host range and worldwide distribution (Parmeter 1970). In addition, the unidentified groups may play important role in continuous cropping obstacles. Further investigations are required to identify their functions associated with SMB cropping.

In conclusion, this study demonstrates that the continuous cropping of *Salvia miltiorrhiza* affects rhizosphere actinomycetes and fungal communities in both abundance and structure diversity. The relatively lower diversity of actinomycete and fungal communities in continuous cropping cycle suggest a causal link to the poor SMB growth performance in that cropping cycle, indicating that changes of actinomycetes and fungal communities may contribute to the soil sickness associated with SMB cultivation. Further monitoring using new fingerprinting methods (i.e., pyrosequencing) might be necessary to evaluate the dynamics in the soil microbial population.

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