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# Effects of microbial inoculants on phosphorus and potassium availability, bacterial community composition, and chili pepper growth in a calcareous soil: a greenhouse study

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## Abstract

**Purpose** Phosphorus (P) and potassium (K) are two important essential nutrient elements for plant growth and development but their availability is often limited in calcareous soils. The objective of this study was to determine the effects of applying microbial inoculants (MI, containing effective strains of *Bacillus megaterium* and *Bacillus mucilaginous*) on the availability of P and K, plant growth, and the bacterial community in calcareous soil.

**Materials and methods** A greenhouse experiment was conducted to explore the effects of the addition of MI (control: without MI addition; treatment: with MI addition at the rate of 60 L $ha^{-1}$) on the concentrations of P and K in soil and plant, soil bacterial community diversity and composition, and chili pepper (*Capsicum annuum* L.) growth.

**Results and discussion** The results showed that MI inoculation significantly increased the fruit yields by 28.5% ($p < 0.01$), available P and K in the rhizosphere soil by 32.1% and 28.1% ($p < 0.05$), and P and K accumulation in the whole plants by 40.9% and 40.2%, respectively ($p < 0.05$). Moreover, high-throughput sequencing revealed that *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Chloroflexi*, and *Gemmatimonadetes* were the dominant phyla of soil bacteria. MI application did not significantly impact the diversity and composition of soil bacterial communities, but increased relative abundances of bacterial genera *Flavobacterium* responsible for promoting root development across growing stages ($p < 0.05$), and changed the soil bacterial community structure associated closely with soil properties of available P, K, and pH in soil.

**Conclusions** The application of MI improved the bioavailability of P and K and plant growth due to its impact on the soil bacterial community structure.

**Keywords** Available P and K in soil · *Capsicum annuum* · Microbial inoculants · P and K accumulation in plant · Soil bacterial community composition · Soil bacterial community structure

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## 1 Introduction

Chili pepper (*Capsicum annuum* L.) is an important and popular vegetable worldwide (Wu et al. 2015; Wang et al. 2018). P and K are two major essential macronutrients and play an important role in plant development and reproduction (Nath et al. 2017; Pande et al. 2017). For the intensive vegetable production system in a greenhouse, a large amount of chemical fertilizers are applied in soil to achieve high yields, including chemical P and K fertilizers (Abou-el-seoud and Abdel-megeed 2012). However, more than 80% of fertilizer P applied to calcareous soils is normally immobilized and unavailable for plant uptake shortly after application via sorption, precipitation, or other pathways (Roberts and Johnston 2015). Meanwhile, only 2% of the total soil K exists as soluble $K^+$ in soil solution and most of the soil K is in the insoluble or non-exchangeable

mineral or structural forms (Shanware et al. 2014). The practice of farmer's traditional fertilization resulted in plenty of P and K nutrients fixed in the soil and roots could not take up enough nutrients from soil for the growth and development, which would be not beneficial for sustainable cultivation of vegetables in a greenhouse (Zhu et al. 2017). Therefore, the mobilization of soil insoluble P and K is very important to supply P and K to crops via the use of bio-fertilizers or microbial inoculants (MI) which have the ability to mobilize P and K from solid phases in calcareous soils (Ahmad et al. 2016). It was shown that the application of bio-fertilizers including P- and K-solubilizing bacteria could be a sustainable pathway to improve plant growth, plant competitiveness, and resistance to environmental stresses (Han and Lee 2006; Singh and Reddy 2011; Das and Pradhan 2016). Most previous studies focused on the impacts of bio-fertilizers with P- and K-solubilizing bacteria on plant growth and soil physicochemical characteristics (Han and Lee 2006; Abou-el-seoud and Abdel-megeed 2012; Das and Pradhan 2016). However, agricultural intensification and traditional farmer's practices have generally affected soil microbial composition and biodiversity (Zhu et al. 2016), which is not beneficial to keep the sustainable development of greenhouse production. The previous studies found that inoculation of rhizobia increased the phylotype richness of the bacterial communities and changed the beneficial soil functions of bacteria (Trabelsi et al. 2011). The inoculation of beneficial bacteria into soil with tomato planting changed the composition of microbial communities in the rhizosphere soil as well (Xue et al. 2013). Therefore, it should be paid more attention to investigate the effects of exogenous microbial inoculants including P- and K-solubilizing bacteria applied to calcareous soils on the diversity and composition of the soil bacterial community in greenhouse. Recently, the 16S rRNA pyrosequencing approach was used to explore the variation of the soil bacterial communities (Shen et al. 2014; Ling et al. 2014; Zhu et al. 2016). In general, Biolog, phospholipid fatty acid (PLFA) analysis, PCR-DGGE, and pyrosequencing are typically used to analyze microbial community composition and structure in soil. However, more detailed taxonomic information should be determined to identify the specific microbial groups that may respond to bio-inoculations. Illumina MiSeq sequencing has provided unprecedented insights into the diversity of soil microbial communities, and it is now commonly used to characterize bacterial, archaeal, and fungal communities in soil or related environments (Smets et al. 2016; Yi et al. 2018). Thus, the objectives of the study were to investigate (1) the effects of applying microbial inoculants (MI) containing P- and K-solubilizing bacteria on the availability of P and K and plant growth, (2) whether and how MI application influence the diversity and composition of the indigenous bacterial community in soil from greenhouse, and (3) the linkages between soil bacterial community structure and soil environmental factors related to MI application.

## 2 Materials and methods

### 2.1 Field experimental site

The field experiment was conducted in a typical greenhouse with chili pepper (Hengjiao No. 1) cultivation in a village (39° 113′ 10″ N, 116° 27′ 50″ E) of Yongqing County, Hebei Province, from March to August, 2016. The region belongs to the temperate continental monsoon climate. The mean annual temperature is 11.5 °C. The annual precipitation is 508.7 mm with most of the rainfall occurring from June to August. It is a cinnamon soil, a very common soil type on the North China Plains. The soil had been used to produce vegetables for 8 years.

### 2.2 Experimental treatments and maintenance

The chili pepper seeds were sown in floating polystyrene trays. After growth for nearly 2 months, seedlings were transplanted into the field. The field experiment was set up with the following treatments of control (CK): the traditional fertilization without the application of MI containing P- and K-solubilizing bacteria and treatment with microbial inoculation application (MI: the traditional fertilization with microbial inoculant addition at the rate of 60 L $ha^{-1}$ when chili pepper seedlings were transplanted and irrigated). There were three replicates for each treatment, giving a total of six plots. Each plot was approximately 39.2 $m^2$ (length × width = 14 m × 2.8 m) with 140 pepper seedlings. The treatments were allocated to the plots in a fully randomized block design in one greenhouse. The microbial inoculants contain the effective strains of *Bacillus megaterium* ($7.0 \times 10^8$ cfu $mL^{-1}$) and *Bacillus mucilaginous* ($2.0 \times 10^8$ cfu $mL^{-1}$) were supplied by Runwo Biotechnology Co., Ltd. in Hebei Province of China. The total nutrient application rates followed 383 kg N $ha^{-1}$, 174 kg $P_2O_5$ $ha^{-1}$, and 556 kg $K_2O$ $ha^{-1}$ according to the traditional fertilization (Li 2014).

In addition, the management of irrigation and pesticides spraying for all treatments followed conventional practices by local farmers.

### 2.3 Soil and plant sampling and analyses

Prior to the setup of the plots, soil samples were collected at 0–20-cm layer with randomly five points following a Z-type pattern in the whole greenhouse for analyses. Soil chemical properties and plant tissue nutrients were determined by routine methods (Abou-el-seoud and Abdel-megeed 2012). Soil alkaline phosphatase activity (ALP) was measured spectrophotometrically by monitoring the release of paranitrophenol from paranitrophenyl phosphate (Khadem and Raiesi 2019). The basic properties of the soil are shown in Table 1.

**Table 1** The characteristics of soil from the greenhouse of chili pepper in the study

| Soil properties | Values |
|---|---|
| Soil pH | 8.08 |
| Organic matter (g $kg^{-1}$) | 32.21 |
| Total N (g $kg^{-1}$) | 2.17 |
| Total P (g $kg^{-1}$) | 3.17 |
| Total K (g $kg^{-1}$) | 17.16 |
| Available N (mg $kg^{-1}$) | 141.37 |
| Available P (mg $kg^{-1}$) | 321.43 |
| Available K (mg $kg^{-1}$) | 433.59 |
| Soil alkaline phosphatase activities (mg $g^{-1}$) | 1.17 |

Soil samples (non-rhizosphere) at 0–20-cm layer were collected by soil auger with 2 cm diameter after chili pepper seedling transplanting and MI application for 20 (D20), 40 (D40), and 147 (D147, chili pepper fruit maturity) days. After chili pepper was harvested, the non-rhizosphere soil (D147NR) and rhizosphere (D147R) soil were sampled separately (Wu et al. 2014). Each rhizosphere sample per replicate was collected from roots of three randomly selected plants. The roots were shaken vigorously to separate soil not tightly adhering to the roots. Then, soil still tightly adhering to the roots was classified as rhizosphere soil (Wu et al. 2014). All soil samples were sieved through a 2-mm mesh and visible plant residues and stones were manually removed. One portion of each soil sample was placed into a 50-mL centrifuge tube and stored at − 80 °C for soil DNA extraction, and the remaining soil was air-dried at room temperature to measure the soil chemical and biological characteristics.

## 2.4 Soil DNA extraction and PCR amplification

Microbial DNA was extracted from frozen soil samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's protocols. The final DNA concentration and purification were determined by NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis. The extracted DNA was stored at − 20 °C until use.

The V3–V4 hyper variable regions of the bacteria 16S rRNA gene were amplified with primers 338F (5′-ACTC CTACGGGAGGCAGCAG-3′) and 806R (5′-GGAC TACHVGGGTWTCTAAT-3′) (Mori et al. 2013) by thermo cycler PCR system (GeneAmp 9700, ABI, USA). The PCR reactions were conducted using the following program: 3 min of denaturation at 95 °C, 27 cycles of 30 s at 95 °C, 30 s for annealing at 55 °C, and 45 s for elongation at 72 °C, and a final extension at 72 °C for 10 min. All PCR reactions for each sample were performed in triplicate 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA. The resulted PCR products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA) according to the manufacturer's protocol (Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China).

## 2.5 Illumina MiSeq sequencing

Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP151750). The raw fastq files were demultiplexed, quality-filtered by trimmomatic, and merged by FLASH with the following criteria: (i) the reads were truncated at any site receiving an average quality score < 20 over a 50-bp sliding window; (ii) primers were exactly matched allowing two nucleotide mismatching, and reads containing ambiguous bases were removed; (iii) sequences whose overlap longer than 10 bp were merged according to their overlap sequence. The operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/) and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by Ribosomal Database Project (RDP) Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (SSU123) 16S rRNA database using confidence threshold of 70%.

## 2.6 Statistical analysis

The diversity indices Chao1 estimator of richness, Good's coverage, and Shannon and Simpson diversity index were calculated in Mothur and used to compare soil bacterial alpha diversity. The significant differences in soil characteristics, plant biomass, P and K accumulation in plant tissues, bacterial alpha diversity, and the relative abundances of different taxonomic levels of bacteria between the treatment and control were determined by Student's *t* test using the SPSS software (Version 17.0). Non-metric multidimensional scaling (NMDS) ordination plots were used to identify the differences in bacterial community structure between the treatment and control. Redundancy analysis (RDA) was conducted to determine the linkages between soil bacterial community structure based on the abundant phyla and soil environmental factors (soil pH, available P, available K, and ALP). In addition, the SPSS software was also used to conduct the Spearman correlation analysis to identify

correlations between environmental factors and the relative abundances of abundant phyla and classes.

## 3 Results

### 3.1 Effects of MI application on chili pepper biomass

Compared with the control, the MI treatment significantly increased the yield of chili pepper fruit by 28.5% ($p < 0.01$) (Table 2). The biomass of chili pepper shoots and roots was also increased by 14.1% and 8.4% for the MI application treatment, although these increases were not statistically significant compared with the control. In addition, the ratio of fruit yield to biomass of whole plant increased from 0.51 in the control to 0.54 in the MI treatment. In general, the MI application promoted the growth and development of chili pepper plants (Table 2).

### 3.2 Effects of MI application on the bioavailability of P and K in soil and chili pepper tissues

After the MI addition for 20 days (D20), soil available K (AK) concentration was significantly increased by 25.2% compared with the control. Likewise, the levels of soil available P (AP) and AK at D40 were also significantly increased by 24.5% and 14.8%, respectively ($p < 0.05$). At chili pepper harvesting stage, MI addition significantly increased AP and AK contents in the rhizosphere soils (D147R), accounting for 32.1% and 28.1% compared with the control, but there was no difference between the MI treatment and control in the non-rhizosphere soils (D147NR) (Table S1, Electronic Supplementary Material - ESM). In short, exogenous microbial inoculants indeed enhanced the availability of P and K in soil from chili pepper greenhouse.

The amounts of P and K in the different tissues of chili pepper were affected by the MI addition (Table 3). Compared to the control, the application of MI significantly increased P and K amounts in the chili pepper fruits and the whole plants, accounting for 59.0% and 58.7% and 40.9% and 40.2%, respectively ($p < 0.01$) (Table 3). It indicated that application of MI increased the accumulation of P and K in the fruits of chili pepper.

### 3.3 Effects of MI application on soil bacterial community diversity

The results illustrated that MI application increased chili pepper yields and the availability of P and K. It was wondered whether exogenous microbial inoculant addition in soil affected soil bacterial community diversity. Therefore, the bacterial richness and diversity in the soils collected from the CK and MI treatment at the different growth stages were calculated based on 30,920 randomly selected sequences (the minimum number of bacterial sequence of this study) (Table 4). The results showed that the coverage values of all samples were more than 97%, indicating that the current sequencing depth was sufficient to capture the bacterial community diversity in this study. The numbers of observed OTUs were significantly lower in the MI treatment than those of the control except for non-rhizosphere soils at D147 (D147NR). The Shannon or Simpson diversity index was only significantly lower in the MI treatment than that in the control in the rhizosphere or non-rhizosphere soil at D147, respectively (Table 4). However, there was no difference in the Chao1 index between the MI treatment and control.

### 3.4 Effects of MI application on soil bacterial community composition

A total of 1,033,684 quality sequences were obtained across all soil samples and 30,920 to 44,769 sequences were obtained per sample (mean = 38,284). The dominant bacterial phyla (relative abundance > 5%) were *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Chloroflexi*, and *Gemmatimonadetes*, accounting for 82.97% of the total sequences and with relative abundances from 28.21 to 36.94%, 25.97 to 34.09%, 8.61 to 11.88%, 6.26 to 10.42%, and 5.08 to 7.19%, respectively, regardless of the treatments (Fig. 1, Table S2 - ESM). Although the phylum distribution varied in the control and MI treatment at different growth stages (Fig. 1), application of MI did not significantly influence the relative abundances of dominant bacterial phyla of *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, and *Gemmatimonadetes* through the growth stages except for *Chloroflexi* that MI application significantly decreased the relative abundances by 45.4% at D40 ($p < 0.05$) (Table S2 - ESM). Moreover, MI addition increased the relative

**Table 2** Biomass (dry weights) of chili pepper with the MI application (means ± SE, $n = 3$)

| Treatments | Fruits (kg ha$^{-1}$) | Shoots (kg ha$^{-1}$) | Roots (kg ha$^{-1}$) | Ratio of fruits yield to biomass of whole plant |
|---|---|---|---|---|
| CK | 4718 ± 71 | 4189 ± 400 | 443 ± 15 | 0.51 ± 0.02 |
| MI | 6063 ± 79** | 4778 ± 334 | 480 ± 19 | 0.54 ± 0.02 |

The "**" indicated significant differences between MI treatment and control ($p < 0.01$) (Student's $t$ test); fruit yield: the biomass (dry weights) of chili pepper fruits; whole plant biomass including total biomass (dry weights) of chili pepper fruits, shoots, and roots

**Table 3** The amounts of P and K accumulation (kg $ha^{-1}$) in different tissues (fruits, shoots, roots) of pepper plants (means ± SE, $n = 3$)

| Treatments | | Fruits | Shoots | Roots | Whole plant[a] |
|---|---|---|---|---|---|
| CK | P | 26.48 ± 0.75 | 19.76 ± 1.61 | 1.22 ± 0.06 | 47.45 ± 1.55 |
| MI | | 42.09 ± 4.27* | 23.34 ± 2.02 | 1.41 ± 0.18 | 66.85 ± 2.52** |
| CK | K | 246.79 ± 6.67 | 250.31 ± 17.93 | 10.76 ± 0.31 | 508 ± 23.07 |
| MI | | 391.61 ± 41.51* | 307.96 ± 19.88 | 11.97 ± 0.90 | 712 ± 22.19** |

The "*" and "**" indicate significant differences at $p < 0.05$ and $p < 0.01$ between the MI treatment and control (Student's $t$ test)

[a] Whole plant includes fruits, shoots, and roots

abundance of *Planctomycetes* by 91.4% in the rhizosphere soil at D147 ($p < 0.05$), but the relative abundances were very lower than the dominant bacterial phyla.

The bacterial classes of *Acidobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria*, and *Gemmatimonadetes* were abundant (relative abundance > 5%), with the relative abundances ranging from 25.97 to 36.72%, 8.03 to 12.15%, 7.63 to 9.41%, and 5.08 to 7.19% of the total bacterial communities, respectively (Fig. 2, Table S3 - ESM). The MI addition did not significantly influence their relative abundance at different growth stages except for *Alphaproteobacteria*. MI application significantly increased relative abundance of *Alphaproteobacteria* at D40 ($p < 0.05$), which was kept consistent with that variation of phylum *Proteobacteria* (Table S2). For other bacterial classes, MI inoculation reduced relative abundance of *Anaerolineae* at D20 ($p < 0.05$) and D40 ($p < 0.01$), whereas increased relative abundance of *Flavobacteriia* at D40 and D147R ($p < 0.05$).

Taxonomical classification at the genus level showed that more than 500 bacterial genera were detected in this study. There were 11 bacterial genera with relative abundances more than 1.0% at least in one treatment (Table 5), which were Subgroup6, Subgroup4, *Sphingomonas*, *Blastocatella*, *AKYG587*, *Woodsholea*, *Chryseolinea*, *Steroidobacter*, *Bryobacter*, *Lysobacter*, and *Flavobacterium* (Table 5). Although MI application significantly increased the relative abundance of *Flavobacterium* for more than twofold except for D147R ($p < 0.05$) and the relative abundance of *Lysobacter* at D40 by 54.8% ($p < 0.05$), and significantly decreased the relative abundance of the genus *Anaerolineaceae_norank* at D20 ($p < 0.05$) and D40 ($p < 0.01$), there were no significant differences in the dominant genus (relative abundances > 5%) between MI treatment and control across the whole growth period.

### 3.5 Effects of MI application on soil bacterial community structure

Non-metric multidimensional scaling (NMDS) analysis was used to reveal the difference of the soil bacterial community structure between control and MI treatment based on the abundance of OTUs (Fig. 3). The NMDS plot illustrated that the bacterial community structure of the three replicates from each treatment was usually located closely, while the bacterial community structure of MI treatment was distinctly different from that of CK along the NMDS2 axis. Moreover, the bacterial community structure at D20, D40, and D147 was well separated from each other along the NMDS1 axis (Fig. 3). It indicated that MI application changed the soil bacterial community structure across the growth stages.

Redundancy analysis (RDA) was used to examine the linkages between soil bacterial community structure based on the abundant phyla and soil environmental factors related to MI application (Fig. 4). The first two RDA components could explain 33.65% of the total variation of the community structure. The soil AK ($R^2 = 0.64$, $p = 0.001$) and pH ($R^2 = 0.33$, $p = 0.017$) were relatively near the RDA1 which explained 31.38% of the community variations, and AP ($R^2 = 0.47$,

**Table 4** The richness and diversity indices in soils with and without MI application at 97% sequence similarity based on 16S rRNA sequencing (means ± SE, $n = 3$)

| Sampling date | Treatments | OTUs | Chao1 | Shannon | Simpson | Coverage |
|---|---|---|---|---|---|---|
| D20 | CK | 1760 ± 20 | 2123 ± 12 | 6.31 ± 0.06 | 0.9948 ± 0.0005 | 97.64 ± 0.03 |
| | MI | 1685 ± 18* | 2104 ± 21 | 6.23 ± 0.07 | 0.9941 ± 0.0007 | 97.59 ± 0.04 |
| D40 | CK | 1713 ± 25 | 2117 ± 34 | 6.19 ± 0.04 | 0.9941 ± 0.0003 | 97.53 ± 0.05 |
| | MI | 1631 ± 12* | 2043 ± 27 | 6.08 ± 0.03 | 0.9930 ± 0.0005 | 97.52 ± 0.04 |
| D147NR | CK | 1763 ± 8 | 2138 ± 3 | 6.27 ± 0.01 | 0.9945 ± 0.0001 | 97.53 ± 0.03 |
| | MI | 1654 ± 53 | 2044 ± 60 | 6.13 ± 0.06 | 0.9937 ± 0.0003* | 97.61 ± 0.07 |
| D147R | CK | 1658 ± 6 | 2071 ± 1 | 6.17 ± 0.01 | 0.9941 ± 0.0002 | 97.63 ± 0.02 |
| | MI | 1566 ± 23* | 1942 ± 40 | 6.11 ± 0.02* | 0.9943 ± 0.0001 | 97.75 ± 0.05 |

The "*" indicated significant differences between MI treatment and the control ($p < 0.05$) (Student's $t$ test)

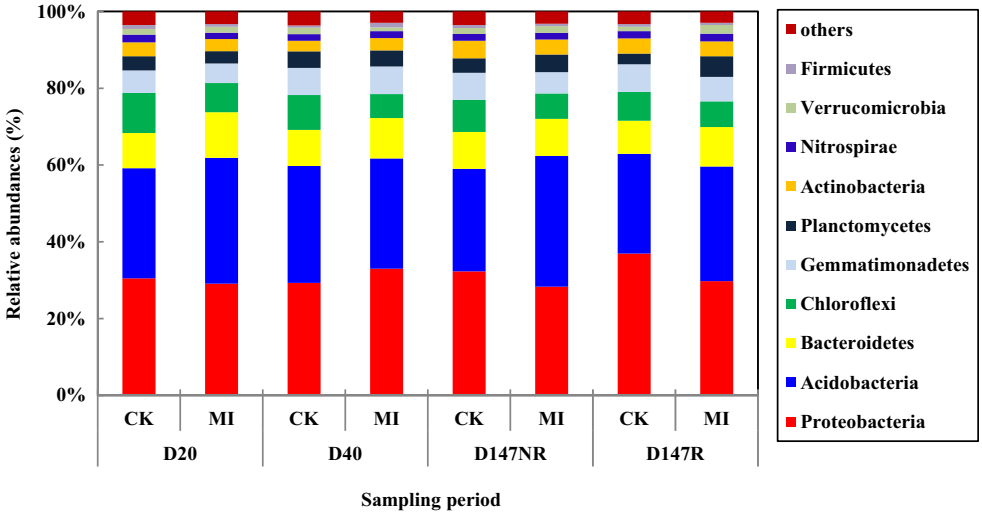


**Fig. 1** Relative abundance of the dominant bacteria phyla in the soil samples in the control (CK) and MI treatment at different chili pepper growth stages

$p = 0.004$) and ALP ($R^2 = 0.29$, $p = 0.032$) also had a significant effect on shifting the bacterial communities along the RDA2 which accounted for 2.27% of the community variations, this indicating their importance in shifting the bacterial community structure with MI application. There were different correlations between soil properties and different soil bacterial phyla, so the Spearman rank-order correlation was also used to further evaluate relationships between abundant phyla, classes, and soil environmental factors (Table 6). It was found that the relative abundances of *Proteobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria*, and *Firmicutes* were positively correlated with soil pH, while the relative abundances of *Acidobacteria* and *Verrucomicrobia* were negatively correlated with soil pH. Moreover, the relative abundance of *Chloroflexi* was negatively correlated with soil AP and AK, while the phylum of *Planctomycetes* appeared in the opposite condition. The relative abundance of *Actinobacteria* was negatively correlated with soil AK and ALP, while the relative abundance of *Bacteroidetes* and soil ALP was positively correlated. This indicated that the alteration of the soil properties by the MI application would change the soil bacterial community structure.

## 4 Discussion

Results from this study clearly demonstrated that MI inoculation was an effective approach in promoting chili pepper growth. The increased plant growth following the MI addition may have arisen from a combination of mechanisms. Firstly, the application of MI significantly increased the concentrations of soil available P and K (Table S1 - ESM). The increased available P and K also confirmed the functions of MI inoculation, which is the solubilization of slowly available P and K sources (Supanjani et al. 2006). The enhanced availability of P and K increased uptakes of both nutrients by the chili pepper

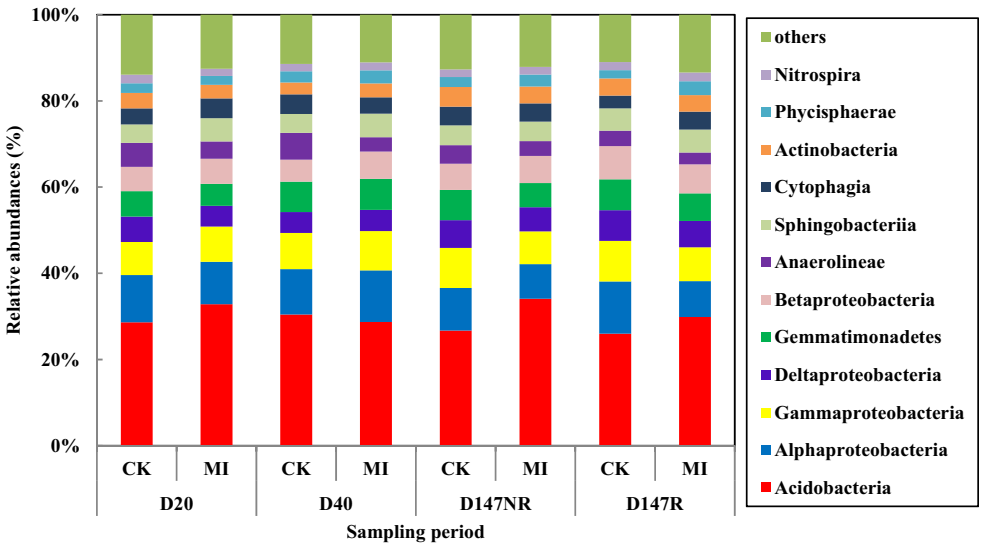


**Fig. 2** Relative abundance of the dominant bacteria classes in the soil samples in the control (CK) and MI treatment at different chili pepper growth stages

**Table 5** Relative abundances (%) of the dominant bacteria genus in the soil samples (means ± SE, $n = 3$)

| Phylum | Class | Genus | D20 | | D40 | | D147NR | | D147R | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | CK | MI | CK | MI | CK | MI | CK | MI |
| *Acidobacteria* | *Acidobacteria* | Subgroup6 | 13.78 ± 1.39 | 17.71 ± 2.54 | 15.70 ± 2.50 | 13.77 ± 3.07 | 13.85 ± 3.49 | 18.93 ± 1.72 | 13.48 ± 4.04 | 15.88 ± 1.81 |
| *Acidobacteria* | *Acidobacteria* | Subgroup4 | 6.63 ± 0.31 | 7.35 ± 0.65 | 6.17 ± 1.03 | 7.14 ± 1.52 | 4.96 ± 1.05 | 6.50 ± 0.51 | 4.22 ± 1.14 | 5.32 ± 0.15 |
| *Chloroflexi* | *Anaerolineae* | *Anaerolineaceae_norank* | 5.54 ± 0.37 | 4.00 ± 0.09* | 6.23 ± 0.35 | 3.31 ± 0.45** | 4.26 ± 0.67 | 3.43 ± 0.5 | 3.54 ± 0.58 | 2.74 ± 0.25 |
| *Proteobacteria* | *Alphaproteobacteria* | *Sphingomonas* | 2.78 ± 0.22 | 2.69 ± 0.30 | 3.46 ± 0.46 | 5.13 ± 0.75 | 2.60 ± 0.25 | 1.98 ± 0.18 | 3.71 ± 0.84 | 2.26 ± 0.50 |
| *Acidobacteria* | *Acidobacteria* | *Blastocatella* | 1.99 ± 0.22 | 2.73 ± 0.23 | 2.59 ± 0.51 | 2.62 ± 0.67 | 1.91 ± 0.48 | 2.50 ± 0.36 | 1.58 ± 0.44 | 2.24 ± 0.32 |
| *Planctomycetes* | *Phycisphaerae* | *AKYG587* | 1.73 ± 0.23 | 1.66 ± 0.11 | 2.08 ± 0.08 | 2.47 ± 0.15 | 1.81 ± 0.08 | 2.30 ± 0.24 | 1.51 ± 0.18 | 2.53 ± 0.11** |
| *Proteobacteria* | *Alphaproteobacteria* | *Woodsholea* | 1.65 ± 0.46 | 0.78 ± 0.11 | 1.51 ± 0.19 | 1.01 ± 0.19 | 1.12 ± 0.16 | 1.03 ± 0.08 | 1.60 ± 0.18 | 1.09 ± 0.29 |
| *Bacteroidetes* | *Cytophagia* | *Chryseolinea* | 1.36 ± 0.17 | 1.16 ± 0.14 | 2.34 ± 0.77 | 1.17 ± 0.24 | 1.86 ± 0.36 | 1.50 ± 0.03 | 1.28 ± 0.22 | 1.44 ± 0.23 |
| *Proteobacteria* | *Gammaproteobacteria* | *Steroidobacter* | 1.73 ± 0.12 | 1.36 ± 0.09 | 1.70 ± 0.05 | 1.63 ± 0.27 | 2.79 ± 0.46 | 2.07 ± 0.21 | 2.58 ± 0.35 | 1.44 ± 0.13* |
| *Acidobacteria* | *Acidobacteria* | *Bryobacter* | 1.32 ± 0.06 | 0.94 ± 0.10* | 1.38 ± 0.10 | 1.16 ± 0.06 | 1.36 ± 0.26 | 1.11 ± 0.07 | 1.62 ± 0.24 | 1.08 ± 0.13 |
| *Proteobacteria* | *Gammaproteobacteria* | *Lysobacter* | 0.91 ± 0.15 | 1.07 ± 0.18 | 1.24 ± 0.07 | 1.92 ± 0.22* | 1.50 ± 0.29 | 0.96 ± 0.12 | 2.03 ± 0.28 | 1.67 ± 0.09 |
| *Bacteroidetes* | *Flavobacteriia* | *Flavobacterium* | 0.62 ± 0.17 | 1.36 ± 0.11* | 0.29 ± 0.06 | 1.12 ± 0.11** | 0.32 ± 0.16 | 0.78 ± 0.24 | 0.27 ± 0.13 | 0.68 ± 0.04* |

The "*" and "**" indicate significant differences at $p < 0.05$ and $p < 0.01$ between the MI treatment and control at the same sampling time (Student's $t$ test)

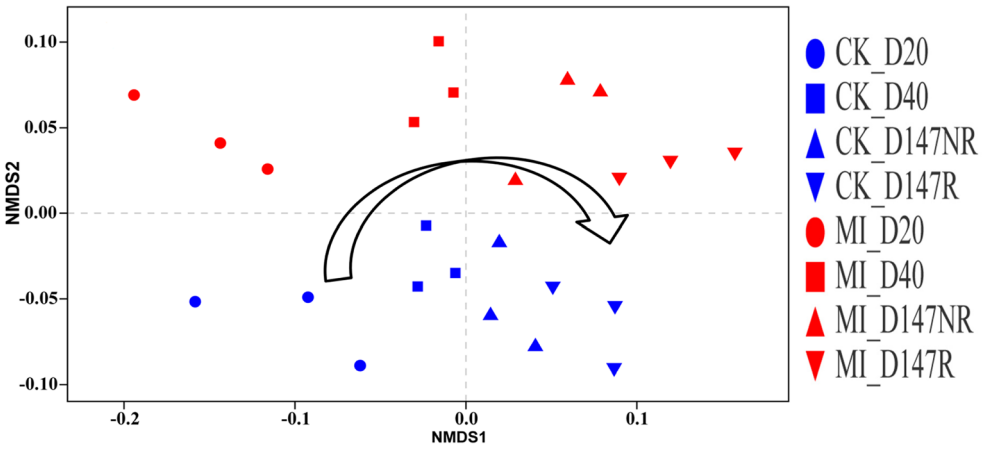


**Fig. 3** Non-metric multidimensional scaling (NMDS) plot of soil bacterial community structure in the control (CK) and MI treatment; the thick arrow in the plot indicated the change tendency of soil bacterial community structure from D20 to D40 with the growth stage. The soil samples were marked with four different symbols according to sampling date and two colors (blue and red) according to control and treatment, respectively

plants (Table 3). It was hypothesized that the MI inoculants, *Bacillus megaterium* and *Bacillus mucilaginous* may release organic compounds which are able to solubilize P- and K-containing soil components. These findings are also supported by some other studies which also showed P and K solubilization by inoculated microorganisms in soil (Basak and Biswas 2010; Abou-el-seoud and Abdel-megeed 2012). Subsequently, the enhancement of soil P and K would stimulate P and K uptake by roots, plant growth and pepper fruit yields, and plant tissues (Rafique et al. 2017; Etesami et al. 2017).

Secondly, the increased biomass production of chili pepper fruits may have been due to the release of plant growth-stimulating substances by the inoculated *Bacillus megaterium* and *Bacillus mucilaginous,* such as IAA, gibberellins, and abscisic acid, which can stimulate the fruit development and growth (Basak and Biswas 2010; Ibrahim et al. 2010). Singh and Reddy (2011) reported that inoculation of the phosphate-solubilizing fungus *Penicillium oxalicum* significantly increased the yields of wheat and maize but not in the biomass of shoots and roots compared to the control in alkaline soil. Inoculation of the *Rhizobium* also significantly increased the total biomass yield of lentil not in shoot dry weight under greenhouse conditions (Zafar et al. 2012). The MI inoculation may also promote the growth of autochthonous bacteria with specific growth-promoting functions. For example, the increased relative abundance of *Flavobacterium* in the MI treatment (Table 5) could produce auxin which can stimulate plant growth and drive developmental plasticity in the roots of plant (Verbon and Liberman 2016; Tsukanova et al. 2017).

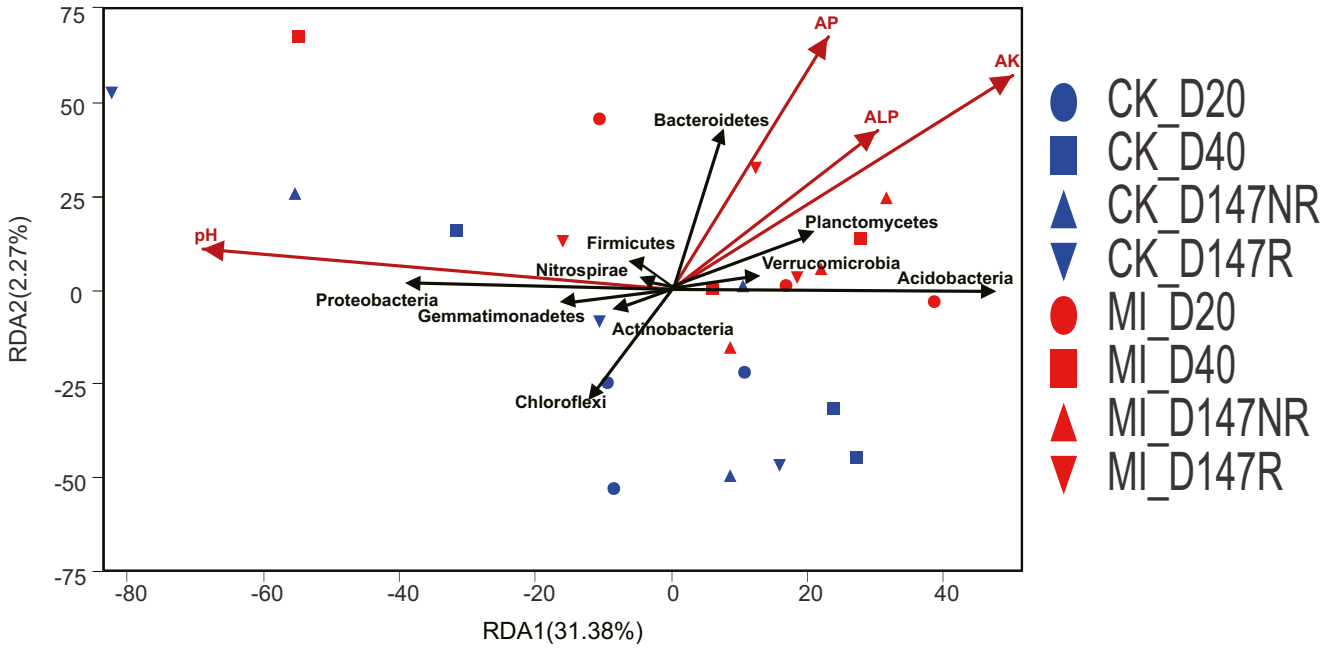


**Fig. 4** Redundancy analysis (RDA) of the abundant phyla and environmental variables for soil samples from control (CK) and MI treatment. The soil samples were marked with four different symbols according to sampling date and two colors (blue and red) according to control and treatment, respectively

**Table 6** Spearman's rank correlations ($r$) between abundant phyla (relative abundance > 1% in all samples combined) (classes) and soil properties

| Phylum (class) | pH | AP | AK | ALP |
|---|---|---|---|---|
| *Proteobacteria* | 0.535** | − 0.187 | − 0.175 | − 0.141 |
| *Alphaproteobacteria* | 0.573** | − 0.282 | − 0.369 | − 0.081 |
| *Gammaproteobacteria* | 0.412* | − 0.195 | 0.35 | − 0.291 |
| *Deltaproteobacteria* | 0.274 | − 0.197 | − 0.411* | − 0.381 |
| *Betaproteobacteria* | 0.064 | 0.133 | − 0.067 | − 0.2 |
| *Acidobacteria* | − 0.541** | 0.154 | 0.286 | 0.19 |
| *Bacteroidetes* | 0.027 | 0.338 | 0.237 | 0.432* |
| *Chloroflexi* | 0.161 | − 0.479** | − 0.578* | − 0.266 |
| *Gemmatimonadetes* | 0.282 | − 0.14 | − 0.24 | − 0.296 |
| *Actinobacteria* | 0.246 | − 0.113 | − 0.428* | − 0.453* |
| *Planctomycetes* | − 0.358 | 0.449* | 0.434* | 0.03 |
| *Verrucomicrobia* | − 0.405* | 0.29 | 0.128 | − 0.124 |
| *Firmicutes* | 0.466** | 0.145 | − 0.177 | 0.052 |
| *Nitrospirae* | 0.239 | − 0.192 | 0.249 | 0.117 |

*AP*, soil available phosphorus; *AK*, soil available potassium; *ALP*, soil alkaline phosphatase activity; "*" and "**" indicate significant correlations ($p < 0.05$ and $p < 0.01$)

Thirdly, the increased chili pepper biomass yield in the MI treatment may have been due to the stimulated growth of beneficial microbes, such as *Lysobacter*, known as an antagonistic bacteria, in MI treatment was higher during the initial stages of plant growth (Table 5), which can significantly inhibit the growth of various phytopathogenic bacteria and fungi (Ji et al. 2008; Postma et al. 2009).

Soil microbial diversity is an important soil attribute related to the sustainability of soil management practices (Garbeva et al. 2006; Chaer et al. 2009). The MI application in this study had a minor effect on the microbial diversity by decreasing the Shannon or Simpson diversity index in the rhizosphere or non-rhizosphere soil at the chili pepper mature stage (Table 4). Similarly, rhizospheric soil analyses of faba beans (*Vicia faba* L.) inoculated with *R. leguminosarum* bv. *viciae* CCBAU01253 showed a decrease in bacterial diversity that was negatively correlated with microbial biomass (Zhang et al. 2010). To promote plant growth, inoculants must either establish themselves in the soil or become associated with the host plant; however, the permanence of these inoculants in the soil has potential to cause disturbances on the native microbial populations (Ambrosini et al. 2016). On the one hand, the *Bacillus megaterium* and *Bacillus mucilaginous* in the MI can release plant growth-stimulating substances to help the development of plant. On the other hand, plant root exudates may also impact on the composition of microbial communities in the soil (Hartmann et al. 2009; Wu et al. 2016). This probably explains that the relative abundance of *Flavobacterium* and *Lysobacter* was significantly increased in MI treatment than in CK (Table 5).

It is well known that environmental factors can shape the microbial community structure (Rousk et al. 2010; Brockett et al. 2012; Li et al. 2015). This hypothesis was confirmed by the significant correlations between the bacterial communities and soil properties (Fig. 4). It was found that soil pH was significantly correlated with the abundant phyla *Acidobacteria* and *Proteobacteria*; this result also occurred in the study by Lauber et al. (2009) and Rousk et al. (2010). However, the relative abundances of *Acidobacteria* and *Proteobacteria* were not significantly affected in the calcareous soil after MI addition in this study, probably because the initial pH of calcareous soil was alkaline and not significantly changed by the MI application (Table S1 and Table S2 - ESM). In addition, the soil available P and K were significantly correlated with the abundant phyla (Table 5). For instance, the relative abundance of *Chloroflexi* was negatively correlated with soil available P and K, while the relative abundance of *Planctomycetes* was opposite (Table 6). This finding may be a good explanation for the relative abundance of genus *Anaerolineaceae_norank*, which belongs to the phylum *Chloroflexi*, significantly decreased at D40, while the relative abundance of genus *AKYG587*, which belongs to the phylum *Planctomycetes*, significantly increased at D147R (Table S2 and Table S3 - ESM, Table 5). Similarly, Zhao et al. (2014) reported that the relative abundance of *Chloroflexi* was negatively correlated with soil available K. Therefore, the changes of soil microbial populations were probably due to the various soil properties related to MI application—an indirect MI effect.

## 5 Conclusions

Results from this study clearly showed that MI inoculation is a viable approach to increasing plant yield. It is possible that the increased yield resulted from the increased available P and K in the soil and the release of plant growth-promoting compounds by the MI inoculants. The MI inoculation did not significantly alter the soil microbial diversity except for a minor effect at the chili pepper maturity stage. The clear correlations between the microbial communities and the soil properties indicate the importance of soil properties in shaping microbial communities in the environment and soil microbial population changes in the MI treatment may have been partly because of the MI effect on the soil P and K status and other soil properties.

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## References


Abou-el-seoud, Abdel-megeed A (2012) Impact of rock materials and biofertilizations on P and K availability for maize (Zea maize) under calcareous soil conditions. Saudi J Biol Sci 19:55–63

Ahmad M, Nadeem SM, Naveed M, Zahir ZA (2016) Potassium-solubilizing bacteria and their application in agriculture. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, New Delhi, pp 293–313

Ambrosini A, Souza RD, Passaglia LMP (2016) Ecological role of bacterial inoculants and their potential impact on soil microbial diversity. Plant Soil 400:1–15

Basak BB, Biswas DR (2010) Co-inoculation of potassium solubilizing and nitrogen fixing bacteria on solubilization of waste mica and their effect on growth promotion and nutrient acquisition by a forage crop. Biol Fertil Soils 46:641–648

Brockett BFT, Prescott CE, Grayston SJ (2012) Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. Soil Biol Biochem 44:9–20

Chaer G, Fernandes M, Myrold D, Bottomley P (2009) Comparative resistance and resilience of soil microbial communities and enzyme activities in adjacent native forest and agricultural soils. Microb Ecol 58:414–424

Das I, Pradhan M (2016) Potassium-solubilizing microorganisms and their role in enhancing soil fertility and health. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, New Delhi, pp 281–291

Etesami H, Emami S, Alikhani HA (2017) Potassium solubilizing bacteria (KSB): mechanisms, promotion of plant growth, and future prospects—a review. J Soil Sci Plant Nutr 17:897–911

Garbeva P, Postma J, Van Veen JA, Van Elsas JD (2006) Effect of above-ground plant species on soil microbial community structure and its impact on suppression of *Rhizoctonia solani* AG3. Environ Microbiol 8:233–246

Han HS, Lee KD (2006) Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of cayenne pepper and cucumber. Plant Soil Environ-UZPI (Czech Republic) 52:130–136

Hartmann A, Schmid M, Tuinen DV, Berg G (2009) Plant-driven selection of microbes. Plant Soil 321:235–257

Ibrahim HIM, Zaglol MMA, Hammad AMM (2010) Response of balady guava trees cultivated in sandy calcareous soil to biofertilization with phosphate dissolving bacteria and/or VAM fungi. J Am Sci 6: 399–404

Ji GH, Wei LF, He YQ, Wu YP, Bai XH (2008) Biological control of rice bacterial blight by *Lysobacter antibioticus* strain 13-1. Biol Control 45:288–296

Khadem A, Raiesi F (2019) Response of soil alkaline phosphatase to biochar amendments: changes in kinetic and thermodynamic characteristics. Geoderma 337:44–54

Lauber CL, Hamady M, Knight R, Fierer N (2009) Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl Environ Microbiol 75: 5111–5120

Li BW (2014) Safe and efficient vegetables fertilization. China Agriculture Press, Beijing (in Chinese)

Li Y, Chen L, Wen H (2015) Changes in the composition and diversity of bacterial communities 13 years after soil reclamation of abandoned mine land in eastern China. Ecol Res 30:357–366

Ling N, Deng K, Song Y, Wu Y, Zhao J, Raza W, Huang QW, Shen QR (2014) Variation of rhizosphere bacterial community in watermelon continuous mono-cropping soil by long-term application of a novel bioorganic fertilizer. Microbiol Res 169:570–578

Mori H, Maruyama F, Kato H, Toyoda A, Dozono A, Ohtsubo Y, Nagata Y, Fujiyama A, Tsude M, Kurokawa K (2013) Design and experimental application of a novel non-degenerate universal primer set that amplifies prokaryotic 16s rRNA genes with a low possibility to amplify eukaryotic rRNA genes. DNA Res 21:217–227

Nath D, Maurya BR, Meena VS (2017) Documentation of five potassium-and phosphorus-solubilizing bacteria for their K and P-solubilization ability from various minerals. Biocatal Agric Biotechnol 10:174–181

Pande A, Pandey P, Mehra S, Singh M, Kaushik S (2017) Phenotypic and genotypic characterization of phosphate solubilizing bacteria and their efficiency on the growth of maize. J Genet Eng Biotechnol 6: 1–13

Postma J, Stevens LH, Wiegers GL, Davelaar E, Nijhuis EH (2009) Biological control of *Pythium aphanidermatum* in cucumber with a combined application of *Lysobacter enzymogenes* strain 3.1 T8 and chitosan. Biol Control 48:301–309

Rafique M, Sultan T, Ortas I, Chaudhary HJ (2017) Enhancement of maize plant growth with inoculation of phosphate-solubilizing bacteria and biochar amendment in soil. Soil Sci Plant Nutr 63:460–469

Roberts TL, Johnston AE (2015) Phosphorus use efficiency and management in agriculture. Resour Conserv Recycl 105:275–281

Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, Knight R, Fierer N (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J 4:134–151

Shanware AS, Kalkar SA, Trivedi MM (2014) Potassium solublisers: occurrence, mechanism and their role as competent biofertilizers. IJCMAS 3:622–629

Shen Z, Wang D, Ruan Y, Xue C, Zhang J, Li R, Shen Q (2014) Deep 16S rRNA pyrosequencing reveals a bacterial community associated with banana *Fusarium* wilt disease suppression induced by bio-organic fertilizer application. PLoS One 9:e98420

Singh H, Reddy MS (2011) Effect of inoculation with phosphate solubilizing fungus on growth and nutrient uptake of wheat and maize plants fertilized with rock phosphate in alkaline soils. Eur J Soil Biol 47:30–34

Smets W, Leff JW, Bradford MA, McCulley RL, Lebeer S, Fierer N (2016) A method for simultaneous measurement of soil bacterial abundances and community composition via 16S rRNA gene sequencing. Soil Biol Biochem 96:145–151

Supanjani HH, Jung JS, Lee KD (2006) Rock phosphate-potassium and rock-solubilising bacteria as alternative, sustainable fertilizers. Agron Sustain Dev 26:233–240

Trabelsi D, Mengoni A, Ben AH, Mhamdi R (2011) Effect of on-field inoculation of *Phaseolus vulgaris* with rhizobia on soil bacterial communities. FEMS Microbiol Ecol 77:211–222

Tsukanova KA, Meyer JJM, Bibikova TN (2017) Effect of plant growth-promoting rhizobacteria on plant hormone homeostasis. South Afr J Bot 113:91–102

Verbon EH, Liberman LM (2016) Beneficial microbes affect endogenous mechanisms controlling root development. Trends Plant Sci 21: 218–229

Wang X, Zou C, Zhang Y, Shi X, Liu J, Fan S, Wu C (2018) Environmental impacts of cayenne pepper (*Capsicum annuum* L) production affected by nutrient management: a case study in southwest China. J Clean Prod 171:934–943

Wu K, Yuan SF, Wang LL, Shi JX, Zhao J, Shen B, Shen QR (2014) Effects of bio-organic fertilizer plus soil amendment on the control of tobacco bacterial wilt and composition of soil bacterial communities. Biol Fertil Soils 50:961–971

Wu Y, Zhao C, Farmer J, Sun J (2015) Effects of bio-organic fertilizer on cayenne pepper growth and *Fusarium* wilt biocontrol. Sci Hortic 193:114–120

Wu B, Wang X, Yang L, Yang H, Zeng H, Qiu YM, Wang CJ, Yu J, Li JP, Xu DH, He ZL, Chen SW (2016) Effects of *Bacillus amyloliquefaciens* ZM9 on bacterial wilt and rhizosphere microbial communities of tobacco. Appl Soil Ecol 103:1–12

Xue QY, Ding GC, Li SM, Yang Y, Lan CZ, Guo JH, Smalla K (2013) Rhizocompetence and antagonistic activity towards genetically diverse *Ralstonia solanacearum* strains—an improved strategy for selecting biocontrol agents. Appl Microbiol Biotechnol 97:1361–1371

Yi B, Zhang QC, Gu C, Li JY, Abbas T, Di HJ (2018) Effects of different fertilization regimes on nitrogen and phosphorus losses by surface runoff and bacterial community in a vegetable soil. J Soils Sediments 18:3186–3196

Zafar M, Abbasi MK, Khan MA, Khaliq A, Sultan T, Aslam M (2012) Effect of plant growth-promoting rhizobacteria on growth, nodulation and nutrient accumulation of lentil under controlled conditions. Pedosphere 22:848–859

Zhang N, Sun YM, Li L, Wang ET, Chen WX, Yuan HL (2010) Effects of intercropping and Rhizobium, inoculation on yield and rhizosphere bacterial community of faba bean (Vicia faba, L.). Biol Fertil Soils 46:625–639

Zhao J, Zhang R, Xue C, Xun W, Sun L, Xu Y, Shen QR (2014) Pyrosequencing reveals contrasting soil bacterial diversity and community structure of two main winter wheat cropping systems in China. Microb Ecol 67:443–453

Zhu YG, Su JQ, Cao ZH, Xue K, Quensen J, Guo GX, Yang YF, Zhou JZ, Chu HY, Tiedje JM (2016) A buried neolithic paddy soil reveals loss of microbial functional diversity after modern rice cultivation. Sci Bull 61:1–9

Zhu YG, Reid BJ, Meharg AA, Banwart SA, Fu BJ (2017) Optimizing peri-urban ecosystems (pure) to re-couple urban-rural symbiosis. Sci Total Environ 586:1085–1090