RESEARCH ARTICLE

Parental material and cultivation determine soil bacterial community structure and fertility

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One Sentence Summary: Thirty years of cultivation increased soil fertility and microbial richness across different soil types.

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ABSTRACT

Microbes are the key components of the soil environment, playing important roles during soil development. Soil parent material provides the foundation elements that comprise the basic nutritional environment for the development of microbial community. After 30 years artificial maturation of cultivation, the soil developments of three different parental materials were evaluated and bacterial community compositions were investigated using the high-throughput sequencing approach. Thirty years of cultivation increased the soil fertility and soil microbial biomass, richness and diversity, greatly changed the soil bacterial communities, the proportion of phylum \textit{Actinobacteria} decreased significantly, while the relative abundances of the phyla \textit{Acidobacteria}, \textit{Chloroflexi}, \textit{Gemmatimonadetes}, \textit{Armatimonadetes} and \textit{Nitrospira} were significantly increased. Soil bacterial communities of parental materials were separated with the cultivated ones, and comparisons of different soil types, granite soil and quaternary red clay soil were similar and different with purple sandy shale soil in both parental materials and cultivated treatments. Bacterial community variations in the three soil types were affected by different factors, and their alteration patterns in the soil development also varied with soil type. Soil properties (except total potassium) had a significant effect on the soil bacterial communities in all three soil types and a close relationship with abundant bacterial phyla. The amounts of nitrogen-fixing bacteria as well as the abundances of the \textit{nifH} gene in all cultivated soils were higher than those in the parental materials; \textit{Burkholderia} and \textit{Rhizobacte} were enriched significantly with long-term cultivation. The results suggested that crop system would not deplete the nutrients of soil parental materials in early stage of soil maturation, instead it increased soil fertility and changed bacterial community, specially enriched the nitrogen-fixing bacteria to accumulate nitrogen during soil development.

Key words: soil parental material; bacterial community; soil maturity; nitrogen accumulation
INTRODUCTION

The global decline of arable land resources has become a concern as the global population grows, which leads to an increasing demand for food. By 2050, the global population is projected to be 50% larger than it is presently, and global grain demand is projected to double (Alexandratos 1999; Cassman 1999; Cohen and Fedoroff 1999; Tilman et al., 2002; Green et al., 2005). In China, the arable land shortage is exacerbated because China feeds 22% of the world’s population with only 7% of the world’s arable land (National Bureau of Statistics of China 2009; Piao et al., 2010). Hence, the development and utilization of potential farmlands for crop production has become an alternative strategy for supplying grain. Red soil (Ultisols and Oxisols in US soil taxonomy) areas are widespread in southern and parts of central China, covering 11.8% of the national land area, and they are considered potential areas for developing agricultural production (Zhong and Cui 2007).

In the soil ecosystem, soil microorganisms are important for organic matter decomposition, nutrient cycling and plant nutrient availability (Paul and Clark 1989). Previous studies have shown that soil properties are correlated with the corresponding soil bacterial community (Steenwerth et al., 2002; Singh et al., 2006). With the application of culture-independent techniques such as phospholipid fatty acid analysis and high-throughput sequencing for soil microbial community analysis (Handelsman et al., 1998; Hill et al., 2002; Torsvik and Øvreås 2002; Tringe et al., 2005; Roesch et al., 2007), the crucial roles that microbes play in soil biogeochemical cycling and soil formation have been thoroughly explored (Rillig and Mummey 2006; Van Der Heijden, Bardgett and van Stralen 2008).

The variability in microbial communities during the development of soil parental materials has implications for the final community structure. Soil parental material provides the foundation elements that comprise the basic nutritional environment for microbial community development (Anderson 1988; Ulrich and Becker 2006). Many reports have stated that soil phosphorus and potassium are precipitated in soil mineral weathering (Arnold 1960; Stewart and Tiessen 1987; Holmquist et al., 2003; Olander and Vitousek 2005). Soil fertility is low during early stage of soil development (Yu et al., 2010), with nitrogen (N) in short supply (Vitousek and Farrington 1997), and most sedimentary rocks have extremely small amounts of N (Stevenson 1962). As a consequence, the accumulation of N during soil development comes from atmospheric N deposition and biological N fixation (Yavitt 2000). Therefore, nitrogen-fixing bacteria, an important part of bacterial community in soil parental materials, play a key role in soil development.

In addition, land management practices have significant and long-lasting effects on the accumulation of soil nutrient elements and bacterial community development (Doran 1989; Post and Mann 1990; Hedlund 2002; Bossio et al., 2005; Acosta-Martinez et al., 2008; Lauber et al., 2008). It has been reported that agricultural practices can rapidly influence soil properties and fertility compared with natural process (Knops and Tilman 2000; Li et al., 2014a). Without fertilization, cultivation may decrease the soil fertility through nutrient depletion; however, for the development of parental materials, cultivation alone may contribute to the soil fertility through the improvement of biological activities. So far, there are few studies to explore the effects of cultivation on the soil fertilities and bacterial communities during the development of different parental materials, which have important implication for the potential farmland development.

In this study, after 30 years of cultivation, the soil developments of three soil parental materials (quaternary red clay soil, granite soil and purple sandy shale) were evaluated, and bacterial abundance, taxonomic diversity and composition were investigated using the high-throughput sequencing technique. N-cycling-related genes, N-fixing bacteria and their variations in soil development were also explored. This study demonstrated that crop cultivation changed soil parental material properties, increased soil fertility and boosted soil microbial community, especially enriched soil nitrogen-fixing bacteria.

MATERIALS AND METHODS

Research site description

Agricultural soils in the Hunan province of southern China were originally developed from three major parental materials: quaternary red clay soil (hereby defined as Q), granite soil (hereby defined as G) and purple sandy shale (hereby defined as P). To accelerate the artificial maturation process for the rapid fertilization of raw lands, the Chinese Academy of Agricultural Sciences set up a long-term experimental station in 1982 in Qiyang (111°52′32″E, 45°26′42″N, 150–170 m a.s.l.), Hunan province in southern China (Gao et al., 2007), where the annual average temperature is 17.8 °C, and the mean annual rainfall is 1255 mm, 70%–80% of which occurs from April to October. To assess the effect of cultivation on soil maturation, two cultivation methods were selected for each parental material: removing all the aboveground biomass (hereby defined as T) and returning all the biomass to soil (hereby defined as R), no fertilizers were applied for all the treatments and the controls during the 30 years experiment. Three soil parental materials were collected on the bare hillsides near the experiment station, formed by the weathering of parental rocks and without the vegetation cover (Table S1’1, Supporting Information). The experimental plots were consisted of cement pools that were 4 m long × 2 m wide × 1 m deep, with open bottoms, the cement walls were about 10 cm above the soil surface to avoid the cross contamination of soils from different plots. All the plots were cultivated in the same way, using a Poaceae, Leguminosae, Cruciferae and Tuber crop rotation pattern (more details showed in Table S1’2, Supporting Information).

Soil sampling, soil properties and soil microbial biomass

Soil samples were collected in May 2012. When the experiment was initiated in 1982, there was only one cement pool for each treatment, which was divided into three parallel parts of similar size as biological replicate, and four subsamples of each replicate were collected from the top 20 cm of soil and immediately mixed into one composite sample. The three original parental materials were also collected in May 2012, at the same sites when the parental materials were collected in 1982. All of the collected samples were sieved (2 mm) and divided equally into two parts. Half of each sample was frozen at −80 °C until DNA extraction, and the other half part was used for the determination of soil characteristics and soil microbial biomass.

Soil pH was measured with a glass electrode (soil/water = 1:5). Total soil N was measured using the Kjeldahl method (Bremner and Mulvaney 1982). Other chemical properties were determined by routine methods (Bao 2010). Soil microbial biomass C (Cmic) was determined by the chloroform fumigation extraction method (Vance, Brookes and Jenkinson 1987). After 24
h fumigation, 12.5 g soil were extracted using 0.5 M K₂SO₄ with a 1:5 ratio for 60 min on a rotary shaker. The amount of organic C in the extract was measured by a Liqui TOC II total organic carbon analyzer (Elementar, Shanghai, China). Cmic was calculated from the following equation: Cmic = Eᵣ × kEc, where Eᵣ is the difference between the amount of C extracted from the fumigated and non-fumigated soils and kEc is 2.64 (Vance et al., 1987; Zhong and Cai 2007).

DNA extraction, PCR and sequencing

Soil DNA was extracted from 0.5 g of fresh soil using a PowerSoil DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer’s instructions, while the DNA of the three parental materials was extracted using a freeze-thawing method due to the low microbial biomass (Miller et al., 1999). The DNA extractions were quantified with a NanoDrop ND-2000 spectrophotometer (NanoDrop, Wilmington, DE, USA) and stored at −20 °C.

The primer set 27F: 5′-AGAGTTTGATCCTGGCTCAG-3′ and 533R: 5′-TACCCCGCGCTGCTGGCAC-3′ plus a Roche-454 sequencing adapter, a linker sequence and a unique, error-correcting barcode sequence (Ns) (Lane 1991; Weisburg et al., 1991), which target the V1–V3 hypervariable region of the 16S rRNA gene, were used for PCR. Amplification reactions were performed in a 20 μl volume with 2 μM of each primer, 0.25 μM dNTPs (Takara), 4 μl of 5 × FastPfu Buffer (TransGen, TransGen Biotech Co., Ltd, Beijing, China), one unit of FastPfu DNA polymerase (2.5 U μl⁻¹, TransGen) and 20 ng of soil DNA template. Amplification was initiated at 95 °C for 2 min, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s and a final elongation at 72 °C for 5 min. The PCR products were visualized on 2% agarose gels, pooled together (to minimize PCR bias), purified with a PCR Purification Kit (Axygen Bio, Union city, CA, USA), and quantified with PicoGreen® 130 (Molecular Probes, Eugene, OR, USA).

High-throughput sequencing was performed with the 454 GS-FLX Titanium Sequencer system at Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China) after an emulsion PCR to make single strands on beads, as required for the 454 pyrosequencing.

Sequence analysis

Raw sequence data generated from the 454 pyrosequencing were processed and analyzed using the bioinformatics platform Mothur (Schloss et al., 2009) as previously described; sequences with a minimum flow length of 450 flows were denoised using the Mothur-based reimplementation of the PyroNoise algorithm with the default parameters (Quince et al., 2011; Zhao et al., 2014a, b). Briefly, sequences were discarded if they contained any ambiguous base call, had more than two mismatches to the forward primer, one mismatch to the barcode sequence, or a homopolymer longer than 8 bp or shorter than 200 bp. After removing the barcode and primer sequences, the retained sequences were aligned against the Silva 106 reference database (Pruesse et al., 2007). After screening, filtering, pre-clustering, and chimera removing, the retained sequences were used to build a distance matrix with a distance threshold of 0.2. Using the average neighbor algorithm with a cut-off of 97% similarity, the sequences were clustered to operational taxonomic units (OTUs). The representative sequence of each OTU was picked and classified using the platform Ribosomal Database Project (RDP) with a confidence threshold of 80% (Wang et al., 2007).

Real-time PCR

Quantitative real-time PCR was carried out on ABI 7500 system (ABI, USA) using SYBR® Green as the fluorescent dye. The nifH gene was amplified with primer set PolF/PolR (Poly, Monrozier and Bally 2001), and 16S rRNA gene with primer set PRBA338F/PRUN518r (Sheik et al., 2011). PCR was performed in a 20 μl volume with 2 μl template DNA. Thermal conditions were set as follows: 30 s at 95 °C for initial denaturation, followed by 40 cycles of 95 °C for 5 s, 60 °C for 30 s, and 72 °C for 34 s. All PCR products were visualized using agarose gel electrophoresis and ethidium bromide staining.

A standard curve was obtained using gradient diluted plasmid DNA containing a fragment of the nifH gene or 16S rRNA gene (Poly et al., 2001), with an R² values of 0.9988 and 0.9981, respectively. The specificity of the amplification was verified using melting-curve analysis and agarose gels electrophoresis. Copy numbers of the nifH gene and 16S rRNA gene from each sample were calculated from the Ct values corresponding with the standard curve.

Statistical analyses

The relative abundance data of the OTUs were subjected to a ratio transformation. All statistics were performed using the program R (version 2.15.3) (R Development Core Team 2012). An OTU-based analysis was performed to calculate the richness, coverage and diversity in these samples with a cut-off of 3% dissimilarity. Rarefaction curves were generated using the program Mothur (Schloss et al., 2009). A hierarchical cluster analysis was performed using the hclust() function (Oksanen 2010), and a permutational multivariate analysis of variance (PERMANOVAs) was conducted using the Adonis () function by Bray–Curtis distances, Mantel tests, detrended correspondence analysis (DCA) and canonical correspondence analysis (CCA) in the vegan package of R (Oksanen et al., 2013). These analyses were conducted to compare bacterial community structures across all samples based on the retained OTU (removing singleton OTUs, hereby referred to as ‘retained OTUs’) composition and to visualize the relationship between bacterial communities and environmental factors (Hill and Gauch 1980; Oksanen et al., 2007). Moreover, the principal coordinates analysis (PCoA) with unweighted UniFrac was used to calculated taxonomic community similarity. Ternary plots were generated by using an attached hack function to call the command in the ggplot2 package (Mulcahy 2014). Heat map and variation partitioning analysis (VPA) (Oksanen et al., 2013) were performed in R (version 2.15.3.). (R Development Core Team 2012) with the gplots (Bolker et al., 2012) and vegan packages (Oksanen et al., 2013) to compare the nitrogen-fixing bacteria and the relative contributions of different factors to the bacterial community.

RESULTS

Soil chemical properties and microbial biomass

After 30 years of cultivation, the parental materials developed into agricultural soils to some extent. The soil chemical properties and soil microbial biomass carbon changed significantly. Soil OM (organic matter), TN (total nitrogen), AN (available N) and TP (total phosphorus) increased significantly (P < 0.05)
Table 1. The differences of total nitrogen and available nitrogen in all soil samples.

<table>
<thead>
<tr>
<th></th>
<th>Total nitrogen (g kg⁻¹)</th>
<th>Available nitrogen (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPM</td>
<td>0.120 ± 0.000(c)e</td>
<td>2.800 ± 0.026(c)h</td>
</tr>
<tr>
<td>GT</td>
<td>0.461 ± 0.020(b)c</td>
<td>38.104 ± 0.000(b)e</td>
</tr>
<tr>
<td>GR</td>
<td>0.503 ± 0.010(a)c</td>
<td>44.582 ± 1.905(a)c</td>
</tr>
<tr>
<td>PPM</td>
<td>0.380 ± 0.001(c)d</td>
<td>5.490 ± 0.010(c)g</td>
</tr>
<tr>
<td>PT</td>
<td>0.719 ± 0.003(b)b</td>
<td>27.435 ± 1.524(df)</td>
</tr>
<tr>
<td>PR</td>
<td>0.942 ± 0.036(a)a</td>
<td>41.915 ± 0.762(a)d</td>
</tr>
<tr>
<td>QPM</td>
<td>0.500 ± 0.020(b)c</td>
<td>3.300 ± 1.905(c)h</td>
</tr>
<tr>
<td>QT</td>
<td>0.707 ± 0.125(a)b</td>
<td>58.300 ± 1.143(b)b</td>
</tr>
<tr>
<td>QR</td>
<td>0.747 ± 0.026(a)b</td>
<td>64.396 ± 0.017(a)a</td>
</tr>
</tbody>
</table>

ANOVA: significant (P < 0.05).
The ANOVA results in brackets shows the difference within same soil type and the results without brackets mean the difference in all soil samples. GPM: granite parental material; GT: long-term treatment of granite soil, removing all the aboveground biomass; GR: long-term treatment of granite soil, returning all plant biomass to the soil; PPM: purple sandy shale parental material; PT: long-term purple sandy shale soil, removing all the aboveground biomass; PR: long-term treatment of purple sandy shale, returning all plant biomass to the soil; QPM: quaternary red clay parental material; QT: long-term treatment of quaternary red clay, returning all the aboveground biomass; QR: long-term treatment of quaternary red clay, returning all plant biomass to the soil.

Bacterial community composition
For all soil samples, 217 791 quality sequences were obtained, with 4931–14 697 sequences per sample (mean 8066), and 84.2% of those sequences were classified at the phylum level. There were 10 major bacterial taxa present within these 9 soil samples. The dominant phyla across all soils were Proteobacteria, Actinobacteria and Acidobacteria, representing approximately 24.0%, 20.0% and 17.6% of the bacterial sequences, respectively (Fig. 1). Phyla that were less abundant but still detectable in most of the soils included Firmicutes (7.8%), Bacteroidetes (4.2%), Chloroflexi (3.6%), Gemmatimonadetes (1.9%), Planctomycetes (1.6%), Armatimonadetes (1.2%), TM7 (0.8%) and Nitrospira (0.6%) (Table SI4, Supporting Information). A list of an additional nine rare phyla, which represented <0.5% of the sequences, is provided in (Table SI4, Supporting Information).

The relative abundances of the most dominant bacterial phyla significantly (P < 0.05) changed over the long-term soil maturation process except for Proteobacteria. The phylum Actinobacteria was dominant in the parental materials, and the relative abundance decreased significantly (P < 0.05) in the T and R treatments, while phyla Acidobacteria, Chloroflexi, Gemmatimonadetes, Armatimonadetes and Nitrospira were significantly (P < 0.05) increased. The variation of Firmicutes showed soil type-dependent trends; for the G soil, the relative abundance in the parental material (18.5%) was significantly (P < 0.05) higher than that in the GT (1.3%) and the GR (1.2%). Firmicutes was also more abundant in the Q parental material (14.7%) than in QT (0.2%) and QR (0.7%) treatments. However, in P soil, after long-term cultivation, the phylum Firmicutes was not changed (P > 0.05).

Bacterial diversity and richness
To calculate and compare the bacterial diversity and richness of the soil samples, a subset of 4931 sequences per sample were
Bacterial community structure

For bacterial community analysis, all singleton OTUs were removed from the original sequences because the single reads are likely to be non-informative and detected randomly (Yu et al., 2012). The 4403 retained OTUs were used for further analysis. The community composition of all samples was identified by hierarchical cluster analysis based on Bray–Curtis dissimilarities (Fig. 2a). The T and R cultivation treatments in each soil type were clustered together and were separated by soil type. Among all three soil types, the parental materials of G and Q were closely clustered, and the same was found for their maturing soils (GT, GR, QT, and QR). In addition, PERMANOVAs was performed to assess the effect of the two cultivation treatments and difference in soil parental material types, which showed that the parental materials had significant \( (P = 0.001) \) dissimilarities with T and R treatments; however, the dissimilarities between T and R treatments were not significant \( (P > 0.05) \). Likewise, P soils were significantly \( (P = 0.001) \) different with the G and Q soils, and the G and Q soils had no significant dissimilarity \( (P > 0.05) \).

DCA using retained OTUs was conducted to assess the main factors driving the formation of the soil bacterial community, and the analysis indicated that bacterial community variations in the three different soils were affected by different factors. The first two principal components explained 32.2% of the total bacterial community variations of the individual samples (Fig. 2b); among them, the first axis was most important, explaining 19.9% of the total variation, and the second axis explained 12.3%. The PCoA showed the similar trends (Fig. 2c). The bacterial community in P soils was separated from G and Q soils, and had its own phylogenetic direction. The results of the DCA and PCoA showed that different soil types had different variation directions. The parental materials and cultivation treatments of the G and Q soils were separated on the same axis, while in the P soils, they were distinguished on the other axis (Fig. 2b).

A ternary plot was used to show the OTU enrichment patterns among the parental material and the two cultivation treatments for each soil type (Fig. 3). In G soil, most OTUs existed in the GT and GR samples, and a small part of the OTUs were distributed among all three samples. Shared OTUs between the parental material and GT were more abundant than those between the parental material and GR, which indicates that the soil bacterial community structure of the GT was altered more than that of the GT during the cultivation process (Fig. 3a). In the P soil, OTUs were significantly enriched in the center of the triangle, and rare OTUs only existed in PT or PR, suggesting that the bacterial community development patterns of PT and PR were similar (Fig. 3b). The majority of OTUs in the Q soil were shared by QT and QR, indicating that the two cultivation methods had similar effects on soil bacterial community structure (Fig. 3c). The OTUs belonged to the species Arthrobacter, Aerococcus, Alcaligenes, Facklamia and Sporosarcina only existed in the G and Q parental materials and not in the cultivated soils. The OTUs that belonged to the Gp1 and Gemmatimonas species only existed in Q and G soils and were more abundant in the T and R treatments than in the parental materials, which suggested that both parental material and cultivation affected their abundances.
Figure 3. Ternary plots for three different soil types. The location of every OTU in each triangle denotes the OTU’s relative percentage in three samples. Location close to a vertex means that the OTU mainly appears in this soil sample. Ternary plots were prepared using the $100 \times x$ (OTU frequency/sum frequency across three treatments in same triangle)) and using the log $(100x + 1)$ values in colors.

GPM: granite parental material; GT: long-term treatment of granite soil, removing all the aboveground biomass; GR: long-term treatment of granite soil, returning all plant biomass to the soil; PPM: purple sandy shale parental material; PT: long-term purple sandy shale soil, removing all the aboveground biomass; PR: long-term treatment of purple sandy shale, returning all plant biomass to the soil; QPM: quaternary red clay parental material; QT: long-term treatment of quaternary red clay, removing all the aboveground biomass; QR: long-term treatment of quaternary red clay, returning all plant biomass to the soil.

Correlation between bacterial community and soil characteristics

The Mantel test showed significant ($r = 0.65$, $P < 0.001$) correlations between bacterial community and soil properties. The first two axes of the CCA explained 31.52 and 27.83% of the total variations (Fig. 4a). In the G and Q soils, parental materials were separated on the first axis with T and R treatments, while in the P soil, they were separated along the second axis. The effect of soil properties on the bacterial community is indicated by the length and direction of the vectors. The GT and GR bacterial communities were associated with higher contents of soil AK, AN and OM; soil TP and pH had close relationships with the P soil. All soil properties (except TK) had a significant ($P < 0.01$) effect on the T and R soil bacterial communities in all three soil types. The additional CCA relationships between relative abundance of phyla and the bacterial community in Fig. 4b indicated that all of the abundant phyla, except for Proteobacteria, had a significant ($P < 0.01$) correlation with the bacterial community. The phyla Actinobacteria, Bacteroidetes and Firmicutes were strongly correlated with the bacterial communities of the G and Q parental materials; Planctomycetes, Chloroflexi, Gemmatimonadetes, Armatimonadetes and Acidobacteria were more related with cultivated soils (QT, QR, GT and GR).

Pearson correlation coefficient was used to evaluate the correlations between soil characteristics and relative abundance of phyla, and the results showed that soil physicochemical properties had a close relationship with abundant bacterial phyla (Table SI 6, Supporting Information). The phylum Proteobacteria, one of the most abundant phyla, was positively and significantly

Figure 4. The CCA of bacterial community soil properties and the abundance of bacterial phyla. The influences of environmental parameters (a) and the relative abundance of phyla (b) on the structure of bacterial communities in three typical red soils were revealed by the CCA. GPM: granite parental material; GT: long-term treatment of granite soil, removing all the aboveground biomass; GR: long-term treatment of granite soil, returning all plant biomass to the soil; PPM: purple sandy shale parental material; PT: long-term purple sandy shale soil, removing all the aboveground biomass; PR: long-term treatment of purple sandy shale, returning all plant biomass to the soil; QPM: quaternary red clay parental material; QT: long-term treatment of quaternary red clay, removing all the aboveground biomass; QR: long-term treatment of quaternary red clay, returning all plant biomass to the soil.
correlated with soil TK (P < 0.05) and had a negative correlation with soil TN (P < 0.01). The phylum Acidobacteria had a significant positive relationship with soil OM, AN, AK (P < 0.01) and TN (P < 0.05), and a negative relationship with pH (P < 0.05). Another relatively abundant phylum, Actinobacteria, showed a significant negative relationship with soil OM, AN and AK. The phylum Firmicutes was significantly changed after long-term cultivation and had significant negative relationships with soil OM, AN and AK.

**Nitrogen-fixing bacteria**

Over 30 years of cultivation, soil total and available N contents increased significantly (P < 0.05). The Mantel test showed a significant (r = 0.59, P < 0.001) correlation between the soil bacterial community and soil N (TN and AN). In addition, the abundances of the nifH gene in the three soil parental materials were very low, but they were enriched by the cultivation treatments (Fig. 5). The nifH/rRNA gene ratios increased significantly in cultivation treatments, the T treatments had higher proportions than R treatments.

The 40 most abundant OTUs related to soil biological N-fixation are displayed in Fig. 6. The amounts of N-fixing bacteria in all cultivated soils were higher than those in the parental materials. In the G soils, Burkholderia, Rhizobacte and Bradyrhizobium increased significantly with long-term cultivation, in the Q soils, Burkholderia and Rhizobacte were enriched greatly, while in P soils, Microvirga, Rhizobacter, Azoarum and Paenibacillus were increased.

VPA was used to determine the relative contributions of soil N (TN + AN), soil type and cultivation on the soil bacterial community ( Fig. SI 3, Supporting Information). A subset of soil N (soil total N and soil available N) was selected and had a high Pearson correlation with bacterial communities (r = 0.5928, P = 0.006). The cultivation factor indicated that the influence of long-term cultivation on the soil bacterial community varied. The variation in the soil bacterial community structure was partitioned among soil N, soil type and cultivation treatment. The analysis could explain 43.15% of the observed variation in the soil bacterial community structure, leaving 56.85% unexplained. Soil type and soil N accounted for 5.10% (P = 0.025) and 18.38% (P = 0.005), respectively, and cultivation could explain 4.53% (P = 0.11) of the total variation. The interactions between soil type, soil N and cultivation accounted for 1.70% (P = 0.005), 4.80% (P = 0.005) and 13.29% (P = 0.005) of the variation, respectively.

**DISCUSSION**

In this study, soil fertility was significantly improved after 30 years of cultivation. Measurements of soil OM, total N, available N, total phosphorus and available potassium revealed that all of these nutrients especially the soil N were significantly accumulated, indicating that cultivation affected soil nutrients accumulation during soil parental material development. The soil total N and available N results suggested that returning the plant biomass into soil help the N-accumulation process. Long-term cultivation without fertilizer management has a positive impact on soil fertility accumulation, which could potentially provide an effective method for improving the fertility of unmatured soil. Results from this study also suggested that long-term cultivation greatly increased the soil microbial biomass in parental material and have a positive impact on soil microorganisms.

The soil bacterial community showed distinct variations during the maturation process of the parental materials. Buckley and Schmidt (2001) reported that the microbial community structures in long-term cultivated fields were significantly different from those in fields that had never been cultivated. In this study, abundant phyla became more diverse in cultivated soils, and the richness and diversity levels of soil bacterial assemblages were increased. The soil bacterial community composition results showed that the phyla Firmicutes and Actinobacteria were more abundant in...
soil parental materials because these phyla include some spore-forming species, which can survive in poor soils (Lazzarini et al., 2000; Stach et al., 2003; Onyenwoke et al., 2004). Previous studies have shown that the phylum Acidobacteria might be numerically dominant as well as metabolically active in soils and highly involved in the biogeochemical cycles of rhizosphere soil (Lee, Ka and Cho 2008). In our results, the relative abundance of bacteria belong to Acidobacteria increased significantly after long-term cultivation but was rare in the soil parental materials. This finding that the phylum Acidobacteria was dominated in high fertility soils, suggested that this phylum might be an indicator for soil maturation process. The correlation analysis of the soil physicochemical properties and the abundance of bacterial phyla suggest that the relative abundance of bacterial phyla had significant relationship with soil characteristics, which also indicate that the variation in soil characteristics caused by long-term cultivation had a significant effect on bacterial community development at the phylum level.

Across all the soil types, the greatest variation occurred between parental materials and long-term cultivated soils, while the T and R treatments only showed slight difference on soil bacterial α-diversity (Fig. 1 and Table SI 3, Supporting Information) and community structure at the OTU level (Fig. 2). Some investigations of different arable soils have demonstrated that the soil type is a primary determinant of the bacterial community (Girvan et al., 2003; Bossio et al., 2005; Ulrich and Becker 2006). In this study, bacterial communities of different soils were first separated by parental materials and then by cultivation. Our hierarchical clustering, DCA and PCOA results showed that soil bacterial community structures had different variation directions with different soil parental materials, which indicates that the soil bacterial community structure was also determined by their counterparts in the original soil parental materials. In conclusion, our results showed that cultivation changed the bacterial community, and this alteration was also affected by soil type.

Cultivation influenced not only the soil bacterial community but also the soil N accumulation. Previous studies reported that the plants could enhance parental soil weathering and nutrient release (Anderson 1988). In this study, soil N accumulation was related with the variation in the soil N-fixing bacterial composition. Long-term cultivation enriched the soil nifH gene and the N-fixing bacteria. The species Burkholderia and Bradyrhizobium have been reported to have roles in N-fixation (Garau et al., 2009), and they both changed actively in the rhizosphere soils with root growth (Li et al., 2014b). In our research, the genera Burkholderia was the most significantly increased, which could be a benefit in soil N accumulation. Members of the genera Azoarcus, Paenibacillus, Rhizobacter and Bradyrhizobium are reported to be involved in N-fixation (Sneath et al., 1986; Reinhold-Hurek et al., 1993; Steenhoudt and Vanderleyden 2000; Von der Weid et al., 2002), and they all were enriched in cultivated soils, suggesting that the N accumulation in the soil parental materials was influenced by the development of N-fixing bacteria. The accumulation of N also impacts the overall soil bacterial community, which gives bacteria an advantage in overcoming nutrient limitation (Vitousek and Farrington 1997). The VPA analysis indicates that soil N is the most important factor for the alteration of bacterial community structure, which is conducive to overcoming N limitations and the development of the whole soil bacterial community.
CONCLUSIONS

Our study demonstrated that 30 years of cultivation greatly increased soil parental material fertility and changed the structure of the soil bacterial community, making it more rich and diverse. A relationship between bacterial community development and N accumulation existed for all three soil parental materials. The results of this long-term experiment implicated that cultivation alone had considerable contribution to the artificial maturation of soil parental material, stimulated soil development rather than reduced soil fertility, which have important implication for the potential arable land development.

SUPPLEMENTARY DATA

Supplementary data is available at FEMS Journal online.

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