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Full Length Article

Soil Microbial Community Diversity and Composition across a Range of *Eucalyptus grandis* Plantations of Different Ages

Wang Chunzi, Zhang Danju, Yu Junli, Tang Zhiqun and Zhang Jian*
Institute of Ecological Forestry, Sichuan Provincial Key Laboratory of Ecological Forestry Engineering, Sichuan Agricultural University, Wenjiang 611130, China
*For correspondence: sicauzhangjian@163.com

Abstract

Afforestation on farmland has been an important variation of land use in recent decades, and the government of China is engaged in the development of plantation projects at full blast. As a fast-growing exotic species, Eucalyptus is considered to reduce the biodiversity of an area. However, few detailed studies have been conducted on the changes of the soil microbial diversity and composition after Eucalyptus afforestation. Using the technique of Illumina Miseq sequencing, we studied the effects of a range of Eucalyptus plantations of different ages (2, 4, 6, 8, 10 years) on soil properties and bacterial diversity and composition. The results showed that as compared with the control abandoned farmland (AF), soil bacterial OTUs, ACE, chao1 and observed-species and Shannon index decreased. The principal co-ordinates analysis (PCoA) showed that the bacterial community composition at 4 years was similar to AF, and is significantly varied for other ages of plantation. On the phyla level, the relative abundance of Proteobacteria increased, while the trend of Gemmatimonadetes, Chloroflexi and Crenarchaeota was the opposite. Actinobacteria increased (2–4 years) and then decreased (6–10 years). The abundance of AD3 showed an increasing trend (2–6 years) and then declined (8–10 years). On the genus level, the abundance of the majority bacterial genus decreased. Especially after 6 years, the total kinds of genus turned to simplify further. The redundancy analysis (RDA) indicated that the main factors which related to the changes of the soil bacterial community were Eucalyptus growing age, soil moisture content (SMC), soil pH and nitrogen nutrients. Collectively, the results suggested that the variation of Eucalyptus growth stage caused changes in microenvironment, regulated the composition of soil bacterial community and lead soil bacteria trend to be simplified and steady on taxonomy.

Keywords: Soil bacterial community; Eucalyptus plantation age; Illumina miseq sequencing; Afforestation

Introduction

Soil microorganisms, as an important component of the forest ecosystem, play an important role in the decomposition of organic matter, nutrient cycling and the transit of energy (Coleman *et al*., 2008; Konopka, 2009). Maintenance of the diversity of composition of soil microbes is beneficial for the improvement of stability of an ecosystem and its buffer capacity against the deterioration of soil ecology (Kennedy and Smith, 1995). In natural conditions, the soil bacterial communities composition and diversity have been reported to be influenced by a wide range of biotic and abiotic factors (Staley and Reysenbach, 2002), such as ground vegetation (Carney and Matson, 2006), land use (Buckley and Schmidt, 2003; Jangid *et al*., 2008, 2011; Tripathi *et al*., 2012), soil properties (Fierer and Jackson, 2006; Rajaniemi and Allison, 2009; Bach *et al*., 2010), allelochemicals (Guo *et al*., 2011) and so on. The response of soil microbial communities can be used as a biological indicator of the environmental factors, and soil microorganisms can also be used to monitor environmental changes.

Land use change is regarded as a very important driver of environmental change. In recent decades, afforestation of agricultural land has been recognized as one of the major changes in land use in developing countries (*e.g*., Brazil, India and China). Afforestation of agricultural lands brings about a series of eco-environmental changes, which affect the soil biota in plantations. Buckley and Schmidt (2003) revealed that soil microbial community composition in 40 years successional forest soil was significantly different from that of farmland. While Liu *et al*. (2016) reported that soil bacterial community composition in forest succession had gradually changed to the native forest within 20 years. A few studies have reported higher levels of bacterial diversity in forest soils than farmland soils (Nogueira *et al*., 2006; Lagerlöf *et al*., 2014). However, most of the studies on the effects of afforestation on arable lands concerned plantations of a particular age prior to the rotation period, contributing little to comprehensively understand the changes in plantations forest soil through time.

Previously, techniques commonly used to determine soil microbial communities included dilution plate

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technique (Sun *et al*., 2013), Biolog plate (Salomo *et al*., 2009; Stefanowicz *et al*., 2012), PLFA (Chen *et al*., 2013a, b), denaturing gradient gel electrophoresis (DGGE) (Zhao *et al*., 2015), terminal-restriction fragment length polymorphism (T-RFLP) and 454 pyrosequencing analysis (Anderson *et al*., 2011). Recently, high-throughput sequencing has become more common and increasingly useful as a tool to examine the diversity and composition of soil microbes (Zheng *et al*., 2016, 2017; Sheng and Zhu, 2018). In order to help to improve our current understanding of the changes of soil biota in Eucalyptus plantations, we aimed to determine the changes in soil bacterial diversity and composition in *Eucalyptus grandis* plantations converted from agricultural land. In addition, we evaluated the relation of microbial taxonomy and soil properties.

Materials and Methods

Study Sites and Soil Sampling

The study was conducted in the Danling region located in the southwestern Sichuan Province (E 102º57′-103º04′E, N 29º55′N–29º59′E, 547–568 m.a.s.l.), belonging to subtropical zone, which mean annual temperature was 17.5°C and annual precipitation of 1397 mm (Zhang *et al*., 2014). The soil was classified as ferralsol (Gong, 2001), derived from pleistocene alluvium and has a yellow color, loam texture and granular structure. The 2–10 years plantations of *Eucalyptus grandis* were planted on more than 10 ha in size, which was developed from formerly agricultural lands. A "space-for-time substitution" approach was used to evaluate changes in microbiological and soil physico-chemical properties. Stands of *Eucalyptus grandis* aged 2, 4, 6, 8, 10 years and abandoned farmland (AF) were investigated. Three sites were selected in the same plantation age for sampling and investigation in this study. At each site, one 20 m × 20 m section was established in July 2016. Removing the litter layer, mixed soil samples at depths of 0–15 cm were collected in July 2016 from each Eucalyptus stand. The mixed soil samples were stored in sealed sterile plastic bags which were all stored in ice box and return to the lab as soon as possible. Each sample was parted in three portion, one part soil (50 g) was for DNA extraction frozen at -70°C, the other part soil was for soil chemical analysis after air-dried at room temperature and passed through a 0.2 mm sieve, the last part soil was stored at 4°C for soil analysis.

Soil Physico-chemical Analysis

Soil moisture content (SMC) was oven-dried at 105°C overnight to constant weight. The soil bulk density (SBD) was determined as the methods of Institute of Soil Science, Chinese Academy of Sciences (ISSCAS, 1978). Soil pH was conducted by using a Delta pH meter (Mettler-Toledo Instruments, Columbus, OH, USA), with 1:2.5 (w/w) soil/water suspension. Soil organic carbon (SOC) and total nitrogen (TN) contents were respectively measured *via* the methods of Nelson and Sommers (1982), Lu (1999). Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were determined in 1:10 (w/w) soil/water suspension (pH <3), before using vario TOC cube/vario TOC select (Elementar Analysensysteme GmbH, Hanau, Germany). The samples were filtered using filter membrane with a 0.45-μm pore size (Millipore™). Ammonium (NH_4^+) was measured by indigo blue colorimetric method. Nitrate (NO_3^-) was determined by phenol disulfonic acid colorimetry (Guan *et al*., 1986). Soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were measured by the methods described as Vance *et al*. (1987). Soil microbial respiration (SMR) was determined by dehydrogenase content converted into microbial respiration (Broberg, 1985).

DNA Extraction and Illumina Miseq Sequencing

0.25 g of soil samples was measured to extract the DNA by using the Mo Bio Powersoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA). The fineness of DNA was valued by GelDoc 2000 (Bio-Rad, Hercules, USA).

The amplification of the bacterial 16S rRNA gene V4 region was conducted with the primers 515f and 806r (Bates *et al*., 2011). There was a 6-bp error-correcting barcode in reverse primer which was unique to each sample. The amplification of DNA was measured by the methods of Magoč and Salzberg (2011). The 50 μL amplification mixture contained Premix Ex Taq 20 μL (Takara Biotechnology, Dalian, China), primers 515f and 806r 0.4 μL respectively (10 μM), five-fold diluted template DNA 4 μL (5 ng) and sterilized deionized water 25.2 μL. The PCR protocol were following conditions: initial denaturation at 95°C for 3 min, 25 cycles at 95°C for 30 s, annealing at 55°C for 30 s, chain extension at 72°C for 30 s, a final extension step at 72°C for 10 min. The PCR products were purified using a Wizard SV Gel and PCR Clean-up system (Promega, San Luis Obispo, CA, USA), and then sequenced by using the MiSeq platform (Illumina, San Diego, CA, USA) at Novogene, Beijing, China.

Through MiSeq paired-end sequencing, fast length adjustment of short reads (FLASH), with Q30 of clean full-length reads ranging from 95.0–95.8%, was used to merge raw sequences generated (Magoč and Salzberg, 2011). Operational taxonomic units (OTUs) at 97% similarity on the Bio-Linux platform (Field *et al*., 2006) was picked by UPARSE with a chimera filtering approach (Edgar, 2013). The QIIME pipeline was used to processe the representative sequences (Caporaso *et al*., 2010). Based on the latest Greengenes database (McDonald *et al*., 2012), PyNAST (Caporaso *et al*., 2009) alignment and ribosomal database project (RDP) assignment (Wang *et al*., 2007) was carried out. Before the downstream analyses, according to the minimum sequence numbers across all samples, resampling was conducted (Caporaso *et al*., 2010).

Classification information of different taxonomic levels can be provided by the community composition, which we analyzed on the phyla and genera level. Based on 97% OTU similarity across the soils, which were sampled from a range of ages of *Eucalyptus grandis*, principal co-ordinates analysis (PCoA) of the pairwise Bray-Curtis dissimilarity matrices was used to the visualization of the changes in soil bacterial community composition (Caporaso *et al*., 2010). The bacterial abundance and diversity indicated the values of different species. Based on the OTU level, we analyzed the abundance (OTUs, ACE, chao1 and observed-species), and Shannon and Simpson index.

Statistical Analysis

SPSS statistical software (version 20.0, IBM, USA) was conducted to the analyses of statistics. Mean values of three replicates with plus or minus one standard deviation were the final results listed in this study. Among different samples and the control AF, a one-way analysis of variance (ANOVA) with Duncan test was used to distinguish the significant differences ($P < 0.05$). OriginPro 9.0 was used to construct the bar graph of phyla.

Based on the obtained OTUs, the method of QIIME was used to create the rarefaction curves of each sample and community alpha diversity indices. To compare the principal co-ordinates analysis (PCoA) results at the OTU level with the community ecology package, Vegan 2.0 (Dixon, 2003), in R (V2.15.3, UniFrac was used as the method for the beta diversity analysis. For the evaluation of the environmental variables and Illumina Miseq sequencing data on the level of phyla and genera, redundancy analysis (RDA) with the Monte Carlo test of the significance of the environmental variables was conducted by using CANOCO for Windows 4.5 for Windows (Wageningen UR, Netherlands).

Results

Soil Physico-chemical Properties

The crown density, mean DBH and mean height of *Eucalyptus grandis* plantation in different ages were significantly increased. The SMC decreased along the increasing plantation age, and it is larger than that in AF (except SMC at 10 years). No significant difference was observed in SBD and SOC along different age plantations. The pH at different sites were acidic, with the pH reaching a minimum at 8 years. Compared with AF, soil TN declined after afforestation, although it increased with the age of Eucalyptus (Table 1). Except C/N ratio at 6 years, the ratio of C/N were larger than that in AF, and it significantly increased to top at 4 years. DOC and DON reached a minimum at 4 years and then increased with increasing plantation age. Moreover, the distributions of NH_4^+ at different ages were both higher than that in AF and their change trend decreased from 2 years to 4 years and

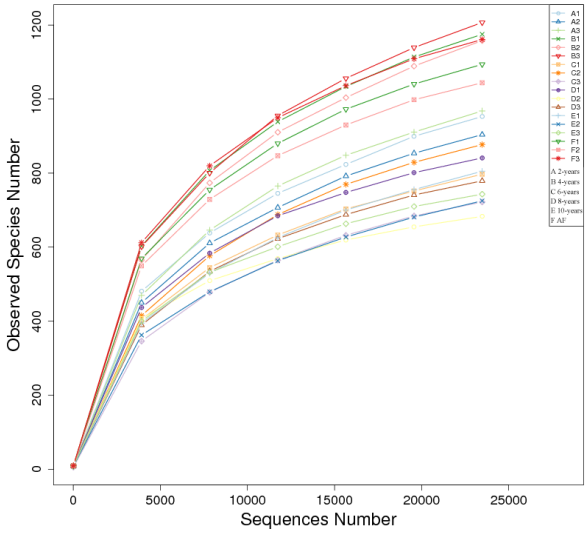

Fig. 1: Rarefaction on bacterial species-abundance data for individual samples (A includes A1, A2, A3-2years; B includes B1, B2, B3-4years; C includes C1, C2, C3-6years; D includes D1, D2, D3-8years; E includes E1, E2, E3-10years; AF includes F1, F2, F3-abandoned farmland)

increased from 6 years to 10 years. Both MBC and MBN of the differently aged plantations were higher than that of AF. In addition, MBC in the 6 years plantation soil was the highest, while MBN decreased with age. SMR significantly decreased from 2 to 6 years, and then increased (Table 1).

Bacterium Sequence Data and Alpha Diversity

High-throughput sequencing obtained 989889 16S rDNA sequences among all the tested soil samples. Each sample on average yielded 54994 sequences. Based on 97% OTU clustering, 1571 OTUs were left after the identification of sequence and the deletion of singletons and rarefaction at 23476 sequences each sample. The result of the rarefaction analysis revealed that the number of logged OTUs usually leveled off at 23476 stochastically selected bacterial sequences, indicating that the amount of data for the sequence was reasonable (Fig. 1).

The bacterial alpha diversity indices were significantly affected by Eucalyptus plantations, except the Simpson index. Though higher indices values were observed in the 4 years stands than in the other plantations, no significant difference was observed between 4 years and AF soils. The OTUs, ACE, chao1, observed-species and Shannon indices tended to decreased (except the 4 years) with increasing Eucalyptus age (Table 2).

Bacterial Community Structure and Composition

Shifts of bacterial communities were confirmed by PCoA. Obvious, soil bacterial community composition changes could be found in different age Eucalyptus plantations and AF. The bacterial community of 4 years and AF were

Table 1: Characteristics of *E. grandis* plantations with different stand age and abandoned farmland (mean values with standard error, N=3; SMC-soil moisture content, SBD- soil bulk density, SMR-soil microbial respiration)

site	Altitude (m)	Slope aspect	Crown density (ratio)	Mean DBH (cm)	Mean Height (m)	SMC (%)	SBD ($g \cdot cm^3$)	pH	SOC ($g \cdot kg^{-1}$)	TN ($g \cdot kg^{-1}$)	C: N	DOC ($mg \cdot kg^{-1}$)	DON ($mg \cdot kg^{-1}$)	NH_4^+ ($mg \cdot kg^{-1}$)	NO_3^- ($mg \cdot kg^{-1}$)	MBC ($mg \cdot kg^{-1}$)	MBN ($mg \cdot kg^{-1}$)	SMR ($nmol \cdot g^{-1} \cdot h^{-1}$)
2years	560.1	NE34	0.46 ± 0.04b	28.27 ± 5.18d	7.33 ± 0.76d	0.29 ± 0.01a	1.33 ± 0.03a	3.45 ± 0.11ab	10.45± 0.78a	1.60 ± 0.20b	6.56 ± 0.33b	323.54± 23.14ab	9.37 ± 4.38bc	5.39 ± 0.58b	2.45 ± 0.34c	85.59 ± 22.93b	2.19 ± 1.25a	0.14 ± 0.00b
4 years	560.7	NE31	0.52 ± 0.03b	48.97 ± 6.60c	14.50 ± 0.50c	0.29 ± 0.02a	1.33 ± 0.05a	3.30 ± 0.04bc	11.49± 2.97a	1.02 ± 0.03c	11.18± 2.58a	236.69± 42.77c	7.53 ± 2.93c	4.52 ± 0.44bc	4.30 ± 0.41c	76.87 ± 20.52b	0.43 ± 0.05b	0.10 ± 0.00c
6 years	564.3	NE33	0.53 ± 0.06b	64.93 ± 9.00b	17.67 ± 0.76b	0.29 ± 0.00a	1.31 ± 0.16a	3.34 ± 0.17bc	11.09± 1.49a	2.15 ± 0.14a	5.20 ± 1.03b	368.55± 43.33a	25.29 ± 8.32a	5.37 ± 0.52b	9.61 ± 2.12b	123.32± 22.10a	0.95 ± 0.34b	0.08 ± 0.01d
8 years	560.8	NE30	0.68 ± 0.09a	71.53 ± 6.21b	21.33 ± 0.29a	0.24 ± 0.00b	1.18 ± 0.02a	3.23 ± 0.05c	12.87± 1.47a	2.22 ± 0.11a	5.79 ± 0.45b	379.07± 38.21a	20.87 ± 6.36ab	6.04 ± 0.15b	14.72 ± 1.20a	53.40 ± 28.59b	0.40 ± 0.28b	0.18 ± 0.01a
10 years	567.8	NE29	0.76 ± 0.03a	83.30 ± 2.86a	21.50 ± 0.50a	0.23 ± 0.02b	1.28 ± 0.11a	3.53 ± 0.07a	12.10± 2.41a	2.04 ± 0.06a	5.96 ± 1.35b	346.75± 42.23a	25.77 ± 5.95a	22.72 ± 2.76a	10.96 ± 2.69b	88.70 ± 6.69ab	1.13 ± 0.52b	0.19 ± 0.01a
AF*	547.4	-	-	-	-	0.24 ± 0.01b	1.34 ± 0.05a	3.57 ± 0.09a	11.94± 0.88a	2.26 ± 0.11a	5.30 ± 0.43b	242.16± 72.41bc	11.65 ± 8.10bc	2.93 ± 0.28c	4.31 ± 2.07c	11.66 ± 3.31c	0.28 ± 0.16b	0.07 ± 0.01d

Different characters in a single column indicate significant difference between the treatments at $P < 0.05$

Table 2: Bacterial alpha diversity with 23476 sequences per community (mean values with standard error, N=3)

Sample name	OTUs	ACE	chao1	Observed-species	Shannon	Simpson
2 years	1248 ± 45b	1254 ± 60bc	1200 ± 71bc	942 ± 33b	6.38 ± 0.63ab	0.92 ± 0.05a
4 years	1547 ± 23a	1556 ± 54a	1522 ± 48a	1180 ± 25a	7.55 ± 0.31a	0.98 ± 0.02a
6 years	1040 ± 70bc	1024 ± 109cd	984 ± 120cd	799 ± 78bc	5.49 ± 1.10b	0.87 ± 0.13a
8 years	965 ± 124c	943 ± 115d	925 ± 110d	768 ± 80c	6.80 ± 0.51ab	0.97 ± 0.01a
10 years	950 ± 127c	1006 ± 106d	973 ± 115cd	758 ± 42c	6.05 ± 0.18ab	0.92 ± 0.03a
AF	1270 ± 157ab	1328 ± 61ab	1282 ± 74ab	1100 ± 59a	7.31 ± 0.08a	0.98 ± 0.00a

Different characters in a single column indicate significant difference between the treatments at $P < 0.05$

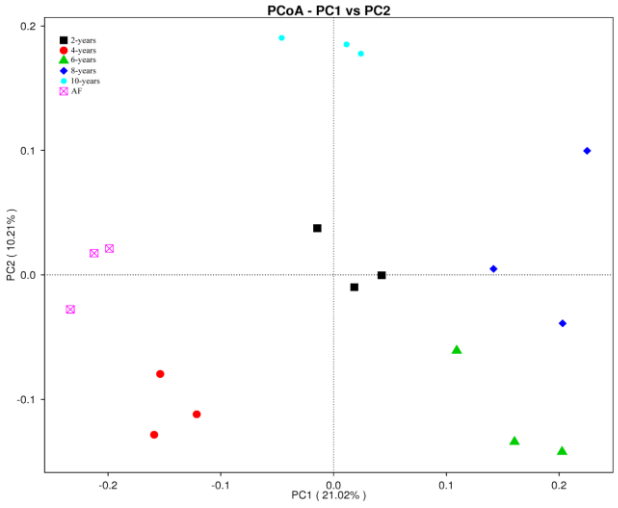

Fig. 2: Principal coordinate analysis (PCoA) of the relative abundance of bacterial genera (N=3)

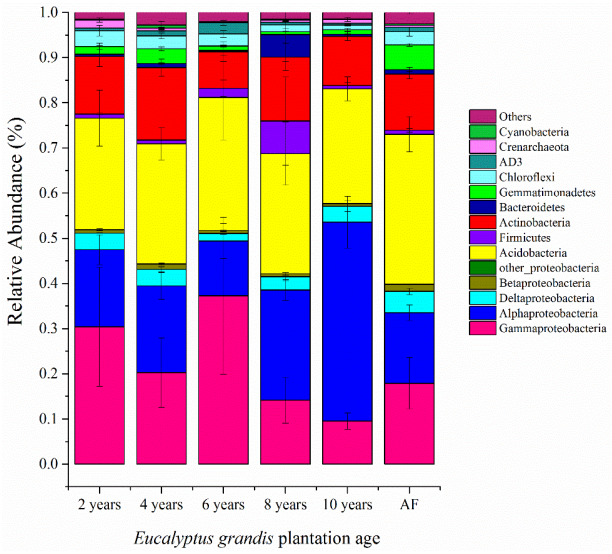

Fig. 3: The relative abundance of the dominant bacterial phyla for the soils of *E. grandis* across the range of ages (2, 4, 6, 8, 10 years; N=3; Proteobacteria contains Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Gammaproteobacteria and other_proteobacteria)

separated distinctly from the other years along the first axis. The second axis visually separated the bacterial community of 10 years from 4 years and 6 years (Fig. 2). The PCoA showed that the bacterial community composition at 4 years was was similar to AF, and the bacterial community composition at 2 years was also similar to the control AF. Bacterial community composition at different ages was dispersed in the four different quadrants.

38 phyla were classified by using the mothur program (Kim *et al.*, 2011) from all the samples. Proteobacteria, Acidobacteria, Actinobacteria, Firmicutes, Bacteroidetes, Gemmatimonadetes, Chloroflexi, AD3, Crenarchaeota and Cyanobacteria were the 10 dominant phyla, accounting for more than 97% of the reads (Fig. 3). Among them, Acidobacteria, Firmicutes, Bacteroidetes and Cyanobacteria had no significant differences with plantation age. In comparison with AF (39.82%), Proteobacteria (Alphaproteobacteria, Betaproteobacteria,

Deltaproteobacteria, Gammaproteobacteria and other_proteobacteria) in the 10 years plantation (57.66%) showed significant difference to the 8 years plantation (42.12%). Proteobacteria in other age plantations (2, 4, 6, 8 years) soil (42.12–51.89%) had no significant difference compared with AF (39.82%) (42.12–51.89%). Belonging to the Proteobacteria, Alphaproteobacteria had less proporation at 6 years (12.12%) than that in AF (15.63%), and it significantly increased from 2 years (17.03%) to 10 years (44.03%). Except Betaproteobacteria at 4 years (1.17%) significantly larger than that at other age plantation, there was no significant difference between the different Eucalyptus plantations. The abundance of Betaproteobacteria in Eucalyptus stands was significantly less than that in AF (1.54%). Gammaproteobacteria significantly increased to top at 6 years (37.25%) and it had no significant difference between 2 years (30.39%), 4 years (20.25%) and 6 years. From 8 years (14.16%) to 10 years (9.50%), the proporation of Gammaproteobacteria decreased. In addition, Gammaproteobacteria had no significant difference between that at 8 years 10 years and AF (17.88%). Deltaproteobacteria also belongs to Proteobacteria and it significantly decreased to the bottom at 6 years (1.71%), then recovered after that. The proporation of Deltaproteobacteria had no significant difference at 2 years (3.70%), 4 years (3.65%) and 10 years (3.54%). While in comparison with AF (4.73%), Deltaproteobacteria in Eucalyptus soil was less. Actinobacteria increased from 2 years (12.77%) to 4 years (16.03%), and then decreased, in particular, Actinobacteria at 6 years (8.1%) and at 10 years (10.9%) were significantly lower than that in AF (12.42%). Gemmatimonadetes in AF (5.50%) was significantly higher than that in Eucalyptus stands. Chloroflexi at 2 years (3.49%) was higher than that in the other Eucalyptus stands and AF (2.91%). With increasing age of Eucalyptus, Chloroflexi decreased significantly (0.98–2.85%). AD3 increased significantly from 2 years (0.55%) to 6 years (2.44%) and then decreased at 8 years (2.44%) and at 10 years (0.41%). AD3 at 4 years (1.12%) and 6 years were both higher than that in AF (1.03%). Except at 6 years (0.20%), Crenarchaeota declined from 2 years (1.81%) to 6 years (0.20%) and then recovered at 8 years (0.45%) and 10 years (0.87%).

285 genera were obtained among all the samples and the most abundant 35 genera were selected to test for significant differences listed in Table 3. Among them, the relative abundance of *Sinomonas*, *Streptomyces*, *Rhodoplanes*, *Conexibacter*, *Sutterella*, *Candidatus_Koribacter*, *Bradyrhizobium*, *Rhodanobacter*, *Pseudomonas*, *Streptacidiphilus*, *Shewanella*, *Serratia*, *Kaistobacter* and *Candidatus_Solibacter* had a significant difference in different processing samples. Moreover, the abundance of *Streptomyces*, *Rhodoplanes*, *Conexibacter*, *Bragyrhizobium* and *Serratia* generally increased, while the abundance of *Candidatus_Koribacter*, *Pseudomonas*, *Streptacidiphilus*, *Shewanella*, *Kaistobacter* and *Candidatus_Solibacter* decreased. *Sinomonas* increased from 2 years to 4 years, and then decreased. *Streptomyces* was highest at 2 years, then no significant difference was found from 4 years to 10 years. *Rhodoplanes* at 6 years was lower than that at other ages. The abundance of *Conexibacter* was highest at 8 years. Compared with AF, *Sutterella* reached a maximum at 8 years and then dropped significantly to the same level as in AF. *Candidatus_Koribacter* increased from 2 years to 4 years and decreased from 4 years to 10 years. Compared with AF, NO_3^- was significantly larger at 6 year to 10 years. *Bradyrhizobium* was relatively high at 4 years and 8 years. *Rhodanobacter* significantly decreased from 2 years to 6 years, and then recovered. The abundance of *Streptacidiphilus* was fluctuant. The minimum value of *Pseudomonas* was found at 6 years. *Shewanella* and *Kaistobacter* increased from 2 years to 4 years, and then decreased with increasing age. *Shewanella* and *Kaistobacter* abundance at different plantation ages was lower than in AF. *Serratia* reached a maximum at 6 years. The abundance of *Candidatus_Solibacter* was less at 8 years and 10 years.

Linking Taxonomic Distribution to Soil Properties

On the phyla level, the RDA analysis showed that the first and second axis were able to explain 89.0% of the total bacterial variation (Fig. 4a). The first axis (RDA1) explained 76.0% of the total variation of bacterial phyla. The bacterial communities in the 2 years and 6 years Eucalyptus stand soils were distinct from those in the 8 years and 10 years plantation soils, which were mainly distinguished by the RDA1, positively related to MBC, MBN, SMC and the ratio of C/N. The second axis (RDA2) explained 13.0% of the total variation of bacterial phyla. The bacterial communities in the 4 years planation and AF soils were different from those in the 6 years, 8 years and 10 years soils, which were mainly distinguished by the RDA2, had the negative relations with pH, the ratio of C/N and SMC. Based on the test of Monte Carlo permutation, the soil NH_4^+ revealed the variation in the biggest amounts of significant statistics (48.69%, $P < 0.01$). In addition, SMC, MBC, SMR and mean height followed by this order revealed 8.73% ($P < 0.01$), 8.41% ($p < 0.01$), 8.19% ($P < 0.01$) and 6.77% ($P < 0.01$) of the variation in soil bacterial composition. By their close grouping and the vectors, the result showed that bacterial phyla in the 2 years soils were dominated by Gammaproteobacteria, Crenarchaeota and AD3. The bacterial phyla in the 4 years soils were predominant by Chloroflexi, Gemmatimonadetes, Betaproteobacteria and Actinobacteria. Moreover, Gammaproteobacteria and Crenarchaeota dominated in the 6 years soil. The main dominant phyla in 8 years soils were Crenarchaeota, Deltaproteobacteria and Actinobacteria, while Alphaproteobacteria was dominant in the 10 years soils. The bacterial phyla in AF soils were mostly Gemmatimonadetes and Betaproteobacteria.

Table 3: The relative abundance of the dominant bacterial genera for the soils of *Eucalyptus grandis* across the range of ages (2, 4, 6, 8, 10 years) (mean values with standard error, N=3)

Taxon	Treatment					
	2 years (%)	4 years (%)	6 years (%)	8 years (%)	10 years (%)	AF (%)
Sinomonas	0.0007 ± 0.0002bc	0.0039 ± 0.0010a	0.0004 ± 0.0000bc	0.0012 ± 0.0004b	0.0001 ± 0.0000c	0.0012 ± 0.0003b
Staphylococcus	0.0003 ± 0.0002a	0.0005 ± 0.0003a	0.0103 ± 0.0175a	0.0002 ± 0.0000a	0.0002 ± 0.0002a	0.0018 ± 0.0027a
Oscillospira	0.0010 ± 0.0003a	0.0006 ± 0.0000a	0.0004 ± 0.0002a	0.0142 ± 0.0227a	0.0005 ± 0.0003a	0.0002 ± 0.0002a
Lactobacillus	0.0006 ± 0.0003a	0.0004 ± 0.0002a	0.0007 ± 0.0005a	0.0006 ± 0.0004a	0.0007 ± 0.0011a	0.0012 ± 0.0014a
Sphingobacterium	0.0000 ± 0.0000a	0.0002 ± 0.0000a	0.0000 ± 0.0000a	0.0039 ± 0.0067a	0.0000 ± 0.0000a	0.0023 ± 0.0039a
Streptomyces	0.0054 ± 0.0044a	0.0009 ± 0.0000b	0.0004 ± 0.0001b	0.0006 ± 0.0005b	0.0007 ± 0.0007b	0.0000 ± 0.0000b
Rhodoplanes	0.0059 ± 0.0010cd	0.0089 ± 0.0017ab	0.0041 ± 0.0021d	0.0080 ± 0.0012abc	0.0099 ± 0.0011a	0.0070 ± 0.0009bc
Akkermansia	0.0000 ± 0.0000a	0.0000 ± 0.0000a	0.0002 ± 0.0001a	0.0000 ± 0.0001a	0.0000 ± 0.0000a	0.0007 ± 0.0010a
Conexibacter	0.0028 ± 0.0002cd	0.0039 ± 0.0008c	0.0013 ± 0.0005e	0.0068 ± 0.0008a	0.0051 ± 0.0009b	0.0020 ± 0.0002de
Parabacteroides	0.0002 ± 0.0001a	0.0000 ± 0.0000a	0.0001 ± 0.0002a	0.0011 ± 0.0016a	0.0000 ± 0.0000a	0.0000 ± 0.0000a
Bacteroides	0.0000 ± 0.0000a	0.0000 ± 0.0000a	0.0005 ± 0.0004a	0.0031 ± 0.0033a	0.0003 ± 0.0004a	0.0002 ± 0.0001a
Corynebacterium	0.0005 ± 0.0001a	0.0005 ± 0.0002a	0.0012 ± 0.0016a	0.0004 ± 0.0001a	0.0009 ± 0.0005a	0.0012 ± 0.0012a
Sutterella	0.0001 ± 0.0001ab	0.0001 ± 0.0001ab	0.0002 ± 0.0003ab	0.0011 ± 0.0013a	0.0000 ± 0.0000b	0.0000 ± 0.0000ab
Candidatus_Koribacter	0.0020 ± 0.0003c	0.0041 ± 0.0003b	0.0007 ± 0.0003d	0.0004 ± 0.0000d	0.0022 ± 0.0003c	0.0084 ± 0.0010a
Bradyrhizobium	0.0032 ± 0.0008ab	0.0044 ± 0.0013a	0.0020 ± 0.0011b	0.0048 ± 0.0007a	0.0036 ± 0.0002ab	0.0025 ± 0.0005b
Novosphingobium	0.0002 ± 0.0000a	0.0006 ± 0.0004a	0.0002 ± 0.0001a	0.0000 ± 0.0000a	0.0003 ± 0.0000a	0.0011 ± 0.0012a
Mycoplasma	0.0000 ± 0.0000a	0.0019 ± 0.0023a	0.0000 ± 0.0000a	0.0000 ± 0.0000a	0.0000 ± 0.0000a	0.0015 ± 0.0021a
Rhodanobacter	0.0017 ± 0.0007a	0.0012 ± 0.0002ab	0.0002 ± 0.0000c	0.0012 ± 0.0003ab	0.0008 ± 0.0001bc	0.0006 ± 0.0001bc
Psychrobacter	0.0000 ± 0.0000a	0.0031 ± 0.0053a	0.0000 ± 0.0000a	0.0000 ± 0.0000a	0.0000 ± 0.0000a	0.0022 ± 0.0038a
Pseudomonas	0.0066 ± 0.0012ab	0.0087 ± 0.0007ab	0.0047 ± 0.0029b	0.0084 ± 0.0045ab	0.0075 ± 0.0009ab	0.0110 ± 0.0032a
Acinetobacter	0.0002 ± 0.0000a	0.0005 ± 0.0004a	0.0002 ± 0.0001a	0.0002 ± 0.0000a	0.0002 ± 0.0000a	0.0013 ± 0.0015a
Streptacidiphilus	0.0007 ± 0.0001cd	0.0018 ± 0.0004b	0.0004 ± 0.0002d	0.0008 ± 0.0002c	0.0006 ± 0.0000cd	0.0044 ± 0.0000a
Synechococcus	0.0004 ± 0.0003a	0.0044 ± 0.0068a	0.0001 ± 0.0001a	0.0004 ± 0.0003a	0.0000 ± 0.0000a	0.0005 ± 0.0006a
Roseburia	0.0000 ± 0.0000a	0.0000 ± 0.0000a	0.0002 ± 0.0002a	0.0011 ± 0.0011a	0.0000 ± 0.0000a	0.0001 ± 0.0000a
Jeotgalicoccus	0.0000 ± 0.0000a	0.0001 ± 0.0002a	0.0019 ± 0.0032a	0.0000 ± 0.0000a	0.0000 ± 0.0000a	0.0002 ± 0.0003a
Ruminococcus	0.0001 ± 0.0001a	0.0000 ± 0.0000a	0.0002 ± 0.0003a	0.0017 ± 0.0026a	0.0000 ± 0.0000a	0.0000 ± 0.0000a
Mucispirillum	0.0000 ± 0.0000a	0.0000 ± 0.0000a	0.0000 ± 0.0000a	0.0015 ± 0.0024a	0.0000 ± 0.0000a	0.0000 ± 0.0000a
Streptococcus	0.0004 ± 0.0004a	0.0003 ± 0.0002a	0.0005 ± 0.0004a	0.0007 ± 0.0006a	0.0016 ± 0.0028a	0.0005 ± 0.0002a
Anaerotruncus	0.0000 ± 0.0000a	0.0000 ± 0.0000a	0.0000 ± 0.0000a	0.0017 ± 0.0028a	0.0000 ± 0.0000a	0.0000 ± 0.0000a
Shewanella	0.0069 ± 0.0013bc	0.0092 ± 0.0017b	0.0067 ± 0.0018bc	0.0050 ± 0.0003c	0.0066 ± 0.0010bc	0.0173 ± 0.0015a
Serratia	0.0132 ± 0.0025b	0.0156 ± 0.0030b	0.0230 ± 0.0015a	0.0038 ± 0.0016c	0.0027 ± 0.0006c	0.0038 ± 0.0010c
Kaistobacter	0.0012 ± 0.0002b	0.0040 ± 0.0002a	0.0011 ± 0.0008b	0.0006 ± 0.0003b	0.0006 ± 0.0000b	0.0034 ± 0.0010a
Coprococcus	0.0002 ± 0.0003a	0.0003 ± 0.0000a	0.0002 ± 0.0000a	0.0014 ± 0.0019a	0.0003 ± 0.0004a	0.0000 ± 0.0000a
Prevotella	0.0002 ± 0.0000a	0.0001 ± 0.0000a	0.0003 ± 0.0002a	0.0031 ± 0.0040a	0.0005 ± 0.0005a	0.0000 ± 0.0001a
Candidatus_Solibacter	0.0131 ± 0.0018a	0.0163 ± 0.0032a	0.0139 ± 0.0043a	0.0027 ± 0.0007b	0.0041 ± 0.0004b	0.0129 ± 0.0016a

Different characters in a single column indicate significant difference between the treatments at $P < 0.05$

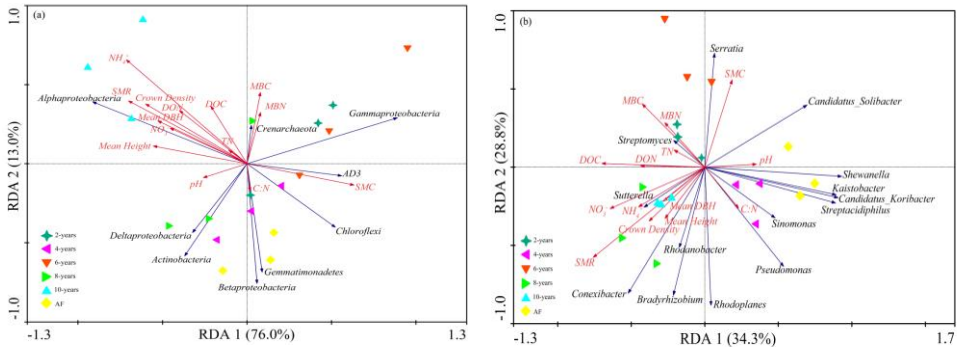

Fig. 4: The RDA of the abundant phyla (proteobacterial classes) in the bacterial phyla community (**a**) and genus community (**b**) and the environmental variables of the individual Eucalyptus plantation and AF soil samples (N=3)

On the genus level, the RDA analysis showed that the first and second axis were able to explain 63.1% of the total bacterial variation (Fig. 4b). The first axis (RDA1) explained 34.3% of the total variation of bacterial genera. Mainly separated by the RDA1, the bacterial communities in the 4 years and AF soils were distinguished from those in the other plantation soils, which had positive relations with SMC, pH and the ratio of C/N. The second axis (RDA2) explained 28.8% of the total variation of bacterial genera. Primarily distinguished by the RDA2, the bacterial communities in the 2 years and 6 years soils were distinct from those in the other years and AF soils, which had

positive relations with SMC, pH, MBC, MBN, TN, DOC and DON. Based on the test of Monte Carlo permutation, SMR revealed the variation in the biggest amounts of significant statistics (30.65%, $P < 0.01$). Moreover, NO_3^-, DOC and SMC explained 20.37% ($P < 0.01$), 18.11% ($p < 0.01$) and 17.18% ($P < 0.01$) of the changes of soil bacterial genera composition, respectively. In addition, *Streptomyces* dominated in 2 years soils. The dominant bacterial genera in the 4 years soils were *Sinomonas*, *Pseudomonas*, *Streptacidiphilus*, *Candidatus Korbacter* and *Kaistobacter*. The bacterial genus in the 6 years soils was mainly *Serratia*. *Sutterella*, *Conexibacter*, *Rhodanobacter* and *Bradyrhizobium* dominated the bacterial community composition in the 8 years soils, while *Sutterella* and *Conexibacter* dominated in the 10 years soils. The control AF was dominated by *Shewanella*, *Kaistobacter*, *Candidatus_Koribacter* and *Streptacidiphilus.*

Discussion

In this study, the range of different Eucalyptus plantation age sites provided a "space-for-time substitution" way to replace a long-term study on bacterial succession and soil physico-chemical properties (Pickett, 1989; Chauvat *et al*., 2003). It provided a possibility to identify the significant effects of different age planations on soil bacterial communities in Eucalyptus plantations cultivated from abandoned farmland. For this, we used MiSeq paired-end sequencing. These results supported our hypothesis that soil bacterial diversity and community were impacted to different degrees after Eucalyptus afforestation. Furthermore, along the growing age of Eucalyptus plantations, soil bacterial diversity and community significantly changed to relatively steady state.

Plenty of previous studies have suggested that soil microbial community diversity and composition have close relationship with environmental factors (Fierer and Jackson, 2006; Lauber *et al*., 2008; Rousk *et al*., 2010). In this study, soil bacterial diversity indices and community variations were the direct indicators reflected the ecology impacts due to different age of *Eucalyptus grandis*. The results suggested clear changes in soil bacterial abundance and diversity as indicated by the Illumina sequencing. In this study, soil bacterial diversity indices (OTUs, ACE, chao1, Observed-species and Shannon index) was increased to top at 4 years, and then decreased along the increasing plantation age (Table 2). This result was consistent with previous studies which indicated that Eucalyptus significantly affected microbial community composition, especially leaded to smaller community sizes and repressed functional activity (Sicardi *et al*., 2004; Berthrong *et al*., 2009; Chen *et al*., 2013a). Although this result was different from Cao *et al*. (2010), who suggested that the afforestation of Eucalyptus had no negative effects on the structure diversity of the soil microbial community. The soil nutrients showed lower content at 4 years (Table 1), while SMC was the important environment factor which limited the bacterial diversity at 8 years and 10 years in this study. A few studies have revealed the similar results that microclimate to deteriorate, such as decline in SMC inducing the decrease decomposition of organic matter (Kara *et al*., 2008; Zheng *et al*., 2017). Soil types and associated soil properties (such as SMC, pH) varied in different studies, which might contribute to the conflicting correlations of total bacterial species with SOC, TN and elemental contents (Cao *et al*., 2010). There also had the same result in our study (Table 1). In addition, previous studies have suggested that bacterial distribution inside the soil matrix was affected by SMC (Lombard *et al*., 2011; Preem *et al*., 2012). These results were consistent with our study (Fig. 4a). Furthermore, by influencing the availability of different elements in the soil, SMC can affect microbial community structure (Piña and Cervantes, 1996). This might be related to the decrease of soil microbial diversity indices (OTUs, ACE, chao1, Observed-species and Shannon index) after 6 years (Table 2) in this study. In addition, Fu *et al*. (2015) suggested that along the growing age of kiwifruit, relative utilization value of carbon sources showed that soil microbial communities tended less conducive to using polyphenols and polyamines. As is known to all, persistent organic matters, such as phenolic acids and volatile oils released from the Eucalyptus leaves, barks and roots were largely accumulated with planting age. And these chemicals might have deleterious effects on biodiversity (Florentine and Fox, 2003; Zhang and Fu, 2010), which could explain the decline of soil bacterial diversity indices (OTUs, ACE, chao1, Observed-species and Shannon index) after 6 years Eucalyptus afforestation in our study (Table 2).

As an important part of the biogeochemical cycle of terrestrial ecosystems, soil microbes actively participate in the ecosystem processes, such as organic matter decomposition, energy storage and transfer and almost all soil processes are related to soil microbes (Coleman *et al*., 2008; Konopka, 2009). Understanding soil microbial community variations could help to intuitively evaluate how biogeochemical cycles respond to land using transition (Chen *et al*., 2013b). In this study, the proportions of bacterial phyla found in Eucalyptus stands and AF soil samples were different (Fig. 3). The major phyla found, which showed significant differences, were Proteobacteria, Actinobacteria, Gemmatimonadetes, Chloroflexi, AD3 and Crenarchaeota. The most abundant clone sequences were affiliated with the phyla Proteobacteria and Acidobacteria (Fig. 3). This result was similar to that of previous studies which suggested that Proteobacteria and Acidobacteria were the primary phyla in bacteria of soils from different locations (He *et al*., 2008; Fierer *et al*., 2009; Preem *et al*., 2012). A previous study revealed that Proteobacteria can indicate nutrient status due to differences in lifestyles (Hartman *et al*., 2008). In this study, Alphaproteobacteria was dominant in the 10 years soils (Fig. 4a) which proporation significantly reached the top at 10 years (Fig.

3). Alphaproteobacteria positively correlated to soil nutrients such as NH_4+, DON, NO_3- and TN which reached the biggest content at 10 years (Table 1) in this study. This result suggested that Alphaproteobacteria was one of the main bacteria which participate in nitrogen cycling (Baker *et al*., 2013). In this study, the relative abundance of Alphaproteobacteria increased, while Betaproteobacteria decreased with increasing age of the plantations (Fig. 3) which suggested that Alphaproteobacteria and Betaproteobacteria occupied the similar niche space, and the similarity in reaction mode to biological, chemical or physical factors (Nielsen *et al*., 2014). As the main Proteobacteria in this study, Gammaproteobacteria decreased after 6 years (Fig. 3), which negatively correlated to pH (Fig. 4a). While soil pH increased after 6 years (Table 1) in this study, which suggested that Gammaproteobacteria contained plenty of bacteria which was acidophil (Bouchez *et al*., 2006; Danilova and Dedysh, 2014). Although pH is not the only dominator to determine the bacterial community in this study, previous studies reveal that soil pH is one of the important predictor of community structure at the continental scale (Sessitsch *et al*., 2001; Rousk *et al*., 2010; Bardhan *et al*., 2012) and soil pH on bacterial community composition can be evident in taxonomy (Lauber *et al*., 2009). In this study, the soil was most acidic at 8 years Eucalyptus plantation. While Deltaproteobacteria showed positive correlations to pH (Fig. 4a), and it reached to the bottom at 6–8 years (Table 1) which suggested that the abundance of Deltaproteobacteria was obviously limited by pH at 8 years. This result was consistent with Lauber *et al*. (2009) who have reported that Deltaproteobacteria was significantly restricted when soil pH<4. In addition, Actinobacteria showed the similar trend with Deltaproteobacteria (Table 3), and it was also positive correlated with pH (Fig. 4a). Lauber *et al*. (2009) suggested that the abundance of Actinobacteria was usually limited to different degrees when soil pH<6, which was consistent with our study result. Furthermore, Actinobacteria had negative relations to the soil nutrients (Fig. 4a), which suggested that Actinobacteria was dominant in which nutrient elements are relatively scarce environment (Sul *et al*., 2013; Calleja-Cervantes *et al*., 2015; Liu *et al*., 2017). In this study, the abundance of Chloroflexi had the similar trend with Actinobacteria (Table 3). We speculated that Eucalyptus might have phyto-chemical inhibition on the biodiversity in plantations (Zhang and Fu, 2010) and the compounds were difficult-to-decompose and accumulated with the growing age of Eucalyptus plantations. While in this study, the abundance of Chloroflexi and Actinobacteria were larger at the earlier afforestation, and they were benefit to remove the inhibition by these compounds (Sheng and Zhu, 2018). In comparison with the Eucalyptus plantations, the abundance of Gemmatimonadetes was the larger at 4 years and in AF (Fig. 3). Gemmatimonadetes was negatively correlated with the soil nutrients (Fig. 4a), which suggested that Gemmatimonadetes was more adaptable in less nutrients environment (Newsham *et al*., 2010). Considered as sulfurdependent extremophiles (Takai *et al*., 2004), Crenarchaeota was positively correlated with MBC and MBN in this study (Fig. 4a), which suggested that Crenarchaeota mainly participated in the turnover microbial carbon and nitrogen (Lam *et al*., 2007; Brochier-Armanet *et al*., 2008).

The functions of most bacteria which we obtained in this study were still unclear. On the level of genus, *Kaistobacter* was classified as a photosynthetic microbe which played an essential role in soil ecosystems (Waigi *et al*., 2015; Bastida *et al*., 2016). The abundance of *Kaistobacter* was larger at 4 years and AF. Rodriguez-Campos *et al*. (2014) reported that *Kaistobacter* can be used to expedite the removal of organic contaminants from soils, which might have participated in the decomposition of difficult-to-decompose compounds produced by Eucalyptus and decreased or removed the negative effects on microbial abundances in this study. More ingestion of photosynthetic products and more decline of inhibition on difficult-to-decompose compounds might be the reason why higher abundance and diversity of bacteria occurred in these soil samples (Table 2). Wide spreading in terrestrial environments, *Kaistobacter* and *Candidatus_Koribacter* were considered to be beneficial microorganisms. In this study, *Candidatus_Koribacter* was negatively related to NO_3- (Fig. 4b), which suggested that *Candidatus_Koribacter* played an important role in the conversion of N, and this microbe had been reported to contribute on the reduction nitrates and nitrites (Ward *et al*., 2009). *Streptomyces*, classified as Actinomycetes, which was considered as k-strategists (Lynch and Bragg, 1985; Benson and Silvester, 1993; Acosta-Martínez *et al*., 2008; Kiers *et al*., 2011). K-strategists usually have the responsibility on stability maintenance, which is their essential highlighting in ecosystems (Lebaron *et al*., 2001). *Streptomyces* had positive correlations with MBC and MBN (Fig. 4b), and it had largest proportion at 2 years (Table 3), which suggested that *Streptomyces* had excellent adaptability in earlier land using conversion. Previous studies also suggested that antibiotics were produced by *Streptomyces* as a by-product supplied them better competitiveness over other bacterium (Kämpfer, 2006; Acosta-Martínez *et al*., 2008; Shah *et al*., 2017).

Based on these results, we speculated that soil microbial communities in plantations are affected at different levels by multiple factors including forest type, vegetation age, plant growth stage, climate, soil nutrient conditions and management practices (Li *et al*., 2006; Burton *et al*., 2010; Bell *et al*., 2010; Chaparro *et al*., 2014; Hortal *et al*., 2015). The changes of relative bacterial abundance were influenced by the competition for resources in the same ecological niche. Meanwhile, chemical traits of Eucalyptus (*i.e*., phenolic acids and volatile oils released from the leaves, bark and roots) might have phyto-chemical inhibition on the undergrowth species (Zhang and Fu, 2010). While after afforestation of Eucalypyus, difficult-to-decompose

compounds such as phenols and terpenes might accumulate with the growing age of plants, which was consistent with the previous studies (Fu *et al*., 2015; Zheng *et al*., 2017).

Conclusion

In summary, the results showed that land using conversion to *Eucalyptus grandis* plantations significantly influenced the soil bacterial community diversity and composition. The soil bacterial community diversity and composition at 4 years were similar to that in AF. The variations of Eucalyptus growing age, soil pH, SMC and the nitrogen nutrients were the main factors affected the changes of bacterial community. With the increasing age of Eucalyptus plantations, the composition of soil bacterial community turned to simplified. The afforestation of *Eucalyptus grandis* had a screening effect on soil bacterial community composition. In addition, along the increasing age of Eucalyptus plantations, we suppose that the accumulation of allelochemicals produced by Eucalyptus might also bring about the decline of bacterial abundance and diversity. Furthermore, the improvement of soil physico-chemical properties might be related to fungi. In the future, our research with more environmental factors and larger age scales of Eucalyptus afforestation is advisable. In addition, the development and management of Eucalyptus could be considered in the management models of mixed forests with different tree species or eucalyptus-crops agroforestry with cash crops.

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