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Genetic diversity and community structure of soil bacteria in Chinese fir plantations

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Abstract: To explore the diversity of soil bacteria and changes in the bacterial community structure of Chinese fir plantations of different generations and developmental stages, the genetic diversity of soil bacteria was studied using the 454 sequencing technology. The results showed that the bacterial genetic diversity and community structure of Chinese fir plantation plots under monoculture planting and rotation planting practices were as follows: the Shannon diversity indices of first-generation young plantation of Chinese fir plantations (FYC), second-generation young plantation (SYC), and third-generation young plantation (TYC) initially decreased and then increased to 8.45, 8.1, and 8.43, respectively. Due to different management and tending measures, the phyla showing considerable differences in relative abundance were Cyanobacteria, Nitrospirae, Fibrobacteres, Thermotogae, and Planctomycetes. The bacterial genetic diversity and community structure of Chinese fir plantations at different developmental stages were as follows: the bacterial diversity and the number of operational taxonomic units (OTUs) decreased with increasing forest age; with the increasing forest age of Chinese fir, the bacteria with considerable differences in the relative abundance were Burkholderiales, Xanthomonadales, Ktedonobacteria, Nitrosomonadales, Anaerolineae, and Holophagae. The predominant bacteria of the Chinese fir plantations were Acidothermus, Bradyrhizobium, Lactococcus, Planctomyces, Sorangium, and Bryobacter.

Keywords: *Cunninghamia lanceolata*; soil bacteria diversity; 454 pyrosequencing

Chinese fir is a major afforestation species in South China, and its plantation area accounts for 21.35% of the total plantation area in China. The use of monoculture planting practices in Chinese fir for multiple generations has resulted in a decrease in community structure, single-tree species, site unsuitability to trees, land productivity, and other related issues. Furthermore, the decline in land productivity causes a series of changes in soil microbial diversity, soil physicochemical properties, and undergrowth vegetation.

Bacteria are an important part of the soil ecosystem (Gans *et al.* 2005); they decompose organic matter to provide nutrients for plants (Timonen *et al.* 1996; Guaa & Lindström 2009), ensure plant health (Doran *et al.* 1996; Smith & Goodman 1999; Yin *et al.* 2010), improve soil structure (Van Der Heijden *et al.* 2008; Kaschuk *et al.* 2011), and enhance soil fertility (Yao *et al.* 2000; O'Donnell *et al.* 2001). Soil bacterial diversity is the basis for the sustainable use of soils (Kaschuk *et al.* 2011), and a higher diversity favours stress tolerance (Zhou *et*

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al. 2002). However, traditional cultivation techniques can cultivate only 1% of the soil microorganisms and some bacteria cannot grow in nutritious media, and thus have limitations (Van'kova *et al.* 2013). The development of a large-scale high-throughput sequencing system based on pyrosequencing provides technical support to comprehensively assess soil bacterial diversity and community structure.

High-throughput sequencing is capable of sequencing hundreds of thousands to millions of DNA molecules at a time. The Genome Sequencer FLX (GS FLX; Roche,Switzerland) sequencing system has a read length of 400 bp, and the sequencing information it generates is more accurate and widely used in the study of genetic diversity and community of soil microorganisms, including biogeographical distribution (Fierer & Jackson 2006), the application of different management measures (Nacke *et al.* 2011), exposure to extreme environments (Hollister *et al.* 2010), and their correlation with various environmental factors (Yu *et al.* 2012).

In the present study, the structure and diversity of soil bacterial communities in Chinese fir plantations of different generations and forest ages and their relationship with environmental factors and soil productivity were evaluated using the GS FLX sequencing system.

MATERIAL AND METHODS

Study area overview. The study area is located in Huangfengqiao National Forest Farm, You County, Hunan Province, P.R. China. The geographical coordinates are 113°09'E–113°51'E, 26°46'N–27°26'N. This area has an elevation of 115–1270 m a.s.l., annual average temperature of 17.8°C, annual precipitation of 1410.8 mm, frost-free period of 292 days, and belongs to the subtropical monsoon humid climate zone. The Huangfengqiao forest farm exhibits zonal distribution across the east and west and generally depicts a medium-low mountain landscape, with a slope with a range of 25–35°. The parent rock is slate shale, followed by limestone, and the soil is predominantly upland red soil that developed from slate shale.

Sample plot settings. The first-, second-, and third-generation Chinese fir plantations with generally the same site conditions, stand structure, and tending and management measures as well as the first-generation Chinese fir plantations of different forest ages were selected as sample plots (Table 1). The species, number of plants, and coverage of the undergrowth vegetation in each plot were recorded. Determination of species diversity in communities was based on important values. There are 113 species of vascular plants in the Chinese fir plantation, which belong to 94 genera and 60 families. The dominant shrubs included *Maesa japonica*, *Pleioblastus amarus*, *Iitsea cubeba*, *Eurya muricata*, *Uncaria rhynchophylla*, *Clerodendrum cyrtophyllum* and the herbs included *Dryopteris subchampionii*, *Woodwardia japonica*, *Rubus rosifolius*, *Lophatherum gracile*, *Lamium barbatum*, *Clerodendrum cyrtophyllum*, *Miscanthus floridulus*.

Sample collection. Soil samples were collected in April 2016. In this study, soil cores (8.3 cm in diameter) were sampled with a soil sampler (SAT-002, Soao, P.R. China) at each corner and in the centre of each plot within a given area of 20 ×30 m, and soil samples were collected at a depth of 5 cm after the humus layer was removed. The soil sample was sieved to remove roots and stones (> 4 mm), mixed evenly, and stored in dry ice. The samples were then transported to the laboratory and stored in a freezer at –80°C. The remaining soil samples were placed in bags, transported to the laboratory, air-dried, and then used in the analysis of physicochemical properties.

Sample analysis. Analysis of soil physicochemical properties: Soil organic matter, total nitrogen (N),

Table 1. Characteristics of sample area

Plot	Altitude (m a.s.l.)	Slope (°)
FYC1	276	6
FYC2	276	6
FYC3	276	6
FMC1	347	9
FMC2	338	8
FMC3	326	7
FRC1	373	8
FRC2	284	7
FRC3	284	7
SYC1	276	6
SYC2	289	7
SYC3	276	6
TYC1	276	6
TYC2	312	7
TYC3	312	7

FYC – first-generation young plantation; FMC – first-generation middle-aged plantation; FRC – first-generation mature plantation; SYC – second-generation young plantation; TYC – third-generation young plantation available N, available phosphorus (P), and available potassium (K) were determined using the potassium dichromate-external heating method, Kjeldahl method (automatic analyzer KDY-9830, Hengrui, P.R. China), alkaline hydrolysis diffusion method, hydrogen chloride-ammonium fluoride method, and flame photometry method, respectively. Soil pH was measured using a pH meter (Mettler Toledo, Switzerland), and soil moisture content was determined by the drying method (105°C, 8 h).

Analysis of soil enzyme activity. Soil urease activity was determined by the method of Tabatabai and Bremner (1972), expressed as NH_4-N mg/kg/h. Catalase activity was determined using the permanganometric method (Samuel *et al.* 2015); Sucrase activity was measured by Hoffmann–Seegerer method (Zhou 1988). Cellulase activity was measured by the method described by Kanazawa and Mitashita (1986).

Soil bacterial DNA extraction and molecular identification. Total soil DNA was extracted using an E.Z.N.A. Soil DNA Extraction Kit (Omega, USA), and the 16S rDNA fragment of the extracted total soil DNA was amplified with the forward primer 27F(5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer 533R(5'-TTACCG CGGCTGCTGGCAC-3'). TransStart FastPfu DNA polymerase (TransGen, P.R. China) was used in amplification reactions, and each 20-μl reaction system contained 4 μl of 5× FastPfu Buffer, 2 μl of 2.5 mM of each of the deoxynucleotide triphosphates (dNTPs), 0.4 μl of the forward primer (5 μM), 0.4 μl of the reverse primer (5 μM), 0.4 μl of FastPfu polymerase, and 10 ng of template DNA, and ddH_2O was added to a final volume of 20 μl. Reaction mixtures were first incubated at 95°C for 2 min. Then 30 cycles were performed as follows: 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C. Reaction mixtures were then incubated at 72°C for additional 5 min. Each sample consisted of three replicates. The polymerase chain reaction (PCR) products of each sample were pooled and recovered using an Axy Prep DNA Gel Recovery Kit (Axygen, USA). Tris-HCl was used in elution, and the sample was detected using 2% agarose gel electrophoresis. Quantification was performed with a QuantiFluor™-ST blue fluorescence quantification system (Promega, USA), followed by emulsion PCR. The emulsion PCR-enriched products were pre-treated according to the sequencing method described in the XLR70 Kit (Roche, Switzerland), followed by high-throughput sequencing on a Roche GS FLX sequencing system (completed by Shanghai Majorbio Bio-Pharm Technology Co. Ltd.).

Data processing. Operational taxonomic unit (OTU) clustering: OTUs, similar to those employed for certain taxa (lines, genera, species, grouping, etc.), were used in this study for phylogenetic or population genetics assessment (Caron *et al.* 2009). The resulting sequences were screened for chimeras using UCHIME and excluded from further analysis (Edgar *et al.* 2011). The remaining sequences were then analysed by Mothur (Schloss *et al.* 2009) and compared with the Bacterial SILVA database (Ver. 106; http://www.arb-silva.de/documentation/background/release-106/). A 97% sequence similarity threshold was used in OTU clustering.

Diversity index analysis. Diversity index analysis was performed using Mothur. Chao 1 estimator and abundance-based coverage estimator (ACE) (Chao 1984; Chao & Lee 1992) were used to calculate community abundance indices; Shannon index (Hill 1973) and Simpson index (Simpson 1949) were employed to calculate community diversity indices; Pielou index (Liu *et al.* 1998) was used as community evenness index; and coverage (Esty 1986) was used to indicate sequencing depth.

Statistical analysis. Rarefaction and taxonomy analyses were performed using Mothur, and R language was utilized in graph plotting. SPSS (Ver. 19.0, 2010) was used for ANOVA and Spearman correlation analysis of the experimental data, and redundancy analysis (RDA) was conducted using CANOCO (Ver. 4.5, 2009).

RESULTS AND DISCUSSION

Soil physicochemical properties and enzymatic activity. The physicochemical properties and enzymatic activity of different plots are shown in Table 2. Significant differences in various indicators of soil physicochemical properties were observed between plots. Because the main soil type in the sample plots was acidic red soil, its pH levels were low. The second-generation young Chinese fir plantation showed the lowest pH (4.29). The first-generation young Chinese fir plantation exhibited the highest organic matter content, the highest N content, the highest urease activity, the highest sucrase activity and cellulase activity, and their average values were 88.02 g/kg, 3.56 g/kg, 6.67 mg/g, 7.63 mg/g, and 69.96 μg/g, respectively. The first-generation mature Chinese fir plantation had the highest available N content, with an average of 116.38 mg/kg. The first-generation middle-aged Chinese fir plantation presented

Table 2. Physicochemical characteristics and enzymatic activity of the analysed soil samples

Plot	pH	Organic matter	Total N	Available N	Available K	Available P	Moisture content	Urease	Catalase	Sucrase	Cellulase (µg/g)
		(g/kg)			(mg/kg)				(mg /g)		
FYC1	5.39 ± 0.05^{fg}	87.95 ± 9.34^{d}	3.93 ± 0.35^{k}	20.16 ± 3.13^{a}	82.14 ± 3.6^{1de}f	1.7 ± 0.10^{efgh}	25.78 ± 2.36^{ab}	6.13 ± 0.32^{c}	1.92 ± 0.23^{a}	7.74 ± 0.60^{b}	69.93 ± 4.90^{c}
FYC2	5.35 ± 0.02^{fg}	87.68 ± 16.92^{d}	3.20 ± 0.23^{h}	24.92 ± 0.76^{a}	85.28 ± 0.98^{ef}	2.03 ± 0.43^{h}	26.89 ± 0.98^{ab}	7.08 ± 0.23^{c}	1.84 ± 0.37^{a}	7.73 ± 0.25^{b}	68.98 ± 4.87^{c}
FYC3	5.72 ± 0.01^{g}	88.43 ± 8.88^{d}	3.55 ± 0.32^{j}	23.49 ± 1.86^{a}	87.45 ± 1.79^{fg}	1.57 ± 0.3^{6def}g	26.18 ± 0.61^{ab}	6.70 ± 0.35^{c}	1.92 ± 0.27^{a}	7.41 ± 0.32^{b}	70.98 ± 5.91^{c}
FMC1	4.44 ± 0.03^{abc}	28.5 ± 10.48^{a}	1.85 ± 0.10^{cd}	69.36 ± 1.11^{b}	97.66 ± 1.97^{hj}	1.09 ± 0.27^{bc}	23.33 ± 1.02^{a}	2.81 ± 0.37^{a}	1.91 ± 0.33^{a}	4.67 ± 0.08^{a}	34.32 ± 3.51^{a}
FMC2	5.04 ± 0.02^{ef}	38.96 ± 1.89^{a}	2 ± 0.17^{cde}	64.54 ± 0.98^{b}	92.37 ± 3.92^{gh}	1.04 ± 0.20^{bc}	25.23 ± 2.57^{ab}	2.94 ± 0.80^{a}	1.97 ± 0.34^{a}	3.82 ± 0.34^{a}	33.79 ± 3.56^{a}
FMC3	4.47 ± 0.46^{abcd}	38.83 ± 0.27^{a}	1.77 ± 0.17^{bc}	67.83 ± 1.79^{b}	99.85 ± 3.62^{j}	1.21 ± 0.22^{cd}	25.73 ± 2.06^{ab}	3.21 ± 0.11^{a}	2.02 ± 0.45^{a}	4.53 ± 0.38^{a}	34.99 ± 6.92^{a}
FRC1	4.9 ± 0.30^{cdef}	62.78 ± 8.46^{c}	2.15 ± 0.27^{de}	113.56 ± 7.89^{fg}	57.46 ± 2.31^{ab}	1.45 ± 0.31^{cdef}	27.51 ± 2.1^{ab}	5.40 ± 0.33^{b}	2.10 ± 0.21^{a}	4.23 ± 0.25^{a}	66.35 ± 4.30^{b}
FRC2	4.43 ± 0.82^{abc}	62.5 ± 5.88^{c}	2.52 ± 0.24^{fg}	120.12 ± 9.17^{g}	59.72 ± 5.10^{ab}	0.72 ± 0.20a^{b}	28.36 ± 1.49^{b}	5.39 ± 0.31^{b}	1.75 ± 0.12^{a}	5.51 ± 0.17^{a}	65.90 ± 2.32^{b}
FRC3	4.34 ± 0.28^{ab}	61.61 ± 4.24^{c}	1.22 ± 0.04^{a}	115.47 ± 6.49^{fg}	55.43 ± 2.74^{a}	1.28 ± 0.34^{cde}	27.28 ± 1.82^{ab}	5.22 ± 0.18^{b}	1.75 ± 0.31^{a}	5.56 ± 0.21^{a}	67.89 ± 4.58^{b}
SYC1	4.26 ± 0.04^{ab}	30.86 ± 3.64^{a}	1.34 ± 0.03^{a}	103.65 ± 8.61^{de}	78.27 ± 3.30^{de}	1.81 ± 0.09^{fgh}	23.46 ± 2.53^{a}	1.48 ± 0.30^{a}	0.87 ± 0.11^{a}	4.74 ± 0.36^{a}	26.07 ± 0.44^{a}
SYC2	4.36 ± 0.26^{a}	33.32 ± 3.14^{a}	2.03 ± 0.21^{cde}	107.34 ± 3.44^{ef}	79.59 ± 6.60^{de}	1.77 ± 0.19^{fgh}	25.37 ± 2.28^{ab}	1.73 ± 0.29^{a}	0.89 ± 0.25^{a}	5.53 ± 0.17^{a}	27.12 ± 4.23^{a}
SYC3	4.25 ± 0.01^{a}	27.45 ± 3.84^{a}	1.48 ± 0.03^{ab}	110.26 ± 8.22^{ef}	75.47 ± 2.38^{d}	1.97 ± 0.16^{gh}	26.28 ± 2.73^{ab}	1.63 ± 0.27^{a}	0.98 ± 0.30^{a}	4.01 ± 0.18^{a}	25.89 ± 4.34^{a}
TYC1	4.82 ± 0.02^{bcde}	40.78 ± 2.44^{ab}	2.63 ± 0.08^{g}	95.96 ± 2.37^{cd}	59.23 ± 6.40^{ab}	0.46 ± 0.11^{a}	29.41 ± 4.56^{b}	2.76 ± 0.11^{b}	1.68 ± 0.29^{b}	7.80 ± 0.31^{b}	47.20 ± 1.63^{b}
TYC2	4.88 ± 0.01^{cdef}	52.1 ± 5.09^{bc}	2.01 ± 0.12^{cde}	92.45 ± 2.75^{c}	63.16 ± 6.57^{bc}	0.56 ± 0.12^{a}	28.63 ± 0.67^{b}	2.83 ± 0.11^{b}	1.66 ± 0.11^{b}	7.07 ± 0.24^{b}	46.58 ± 2.86^{b}
TYC3	4.96 ± 0.02^{def}	52.83 ± 5.13^{bc}	2.27 ± 0.11^{ef}	88.67 ± 2.44^{c}	67.39 ± 2.19^{c}	0.68 ± 0.11^{ab}	27.16 ± 2.9	2.80 ± 0.28^{b}	1.71 ± 0.12^{b}	8.02 ± 0.35^{b}	47.79 ± 2.60^{b}

Data in this table are mean ± SD ($n = 3$); values within the same column followed by different letters are significantly different at $P < 0.05$ (ANOVA); FYC – first-generation young plantation; FMC – first-generation middle-aged plantation; FRC – first-generation mature plantation; SYC – second-generation young plantation; TYC – third-generation young plantation the highest available K content, with an average of 96.62 mg/kg. The second-generation young Chinese fir plantation showed the highest available P content, with an average value of 1.85 mg/kg. In this study, the organic content, total N, available N, urease, catalase, sucrase, cellulase activities of the third-generation Chinese fir plantations were higher than in the second-generation, which is contrary to previous studies (Wu *et al.* 2011). This was so because the third-generation plots were planted with *Pinus massoniana* before Chinese fir and *Pinus* has a strong ability to modify soil surface properties (Jeddi *et al.* 2009)

Diversity of undergrowth vegetation. The diversity index and the evenness index of undergrowth vegetation communities in 15 plots are shown in Table 3. Significant differences in the undergrowth vegetation diversity were observed between treatments, and the Shannon diversity index of the first-generation young Chinese fir plantation reached 1.74. The young Chinese fir plantation exhibited low canopy density, more species of undergrowth vegetation, and high biological diversity. The main species of undergrowth vegetation of the Chinese fir plantation in Huangfengqiao Forest Farm included *Maesa japonica*, *Eurya muricata*, *Macleaya cordata*, *Uncaria rhynchophylla*, *Dryopteris subchampionii* and *Woodwardia japonica*.

Table 3. The vegetation of the analysed soil samples

Plot	Shannon	Simpson	Pielou
FYC1	1.73 ± 0.05^{g}	0.58 ± 0.05^{ab}	0.88 ± 0.06^{cd}
FYC2	1.7 ± 0.0^{2f}g	0.54 ± 0.03^{a}	0.87 ± 0.0^{8c}d
FYC3	1.65 ± 0.0^{8e}f	0.57 ± 0.0^{4}a	0.91 ± 0.02^{c}
FMC1	1.21 ± 0.08^{a}	0.8 ± 0.03^{fgh}	0.68 ± 0.04^{ab}
FMC2	1.32 ± 0.02^{b}	0.77 ± 0.05^{fg}	0.65 ± 0.04^{a}
FMC3	1.27 ± 0.01^{ab}	0.77 ± 0.02^{fg}	0.68 ± 0.01^{ab}
FRC1	1.5 ± 0.04^{c}	0.85 ± 0.03^{gh}	0.7 ± 0.04^{ab}
FRC2	1.47 ± 0.02^{c}	0.81 ± 0.04^{gh}	0.7 ± 0.04^{ab}
FRC3	1.53 ± 0.03^{cd}	0.78 ± 0.03^{fg}	0.7 ± 0.04^{ab}
SYC1	1.59 ± 0.03^{de}	0.71 ± 0.03^{de}	0.72 ± 0.0^{1a}b
SYC2	1.5 ± 0.03^{c}	0.67 ± 0.01^{cd}	0.69 ± 0.04^{ab}
SYC3	1.48 ± 0.01^{c}	0.74 ± 0.02^{ef}	0.73 ± 0.04^{b}
TYC1	1.64 ± 0.04^{ef}	0.66 ± 0.03^{cd}	0.83 ± 0.02^{c}
TYC2	1.59 ± 0.03^{de}	0.63 ± 0.02^{bc}	0.86 ± 0.02^{cd}
TYC3	1.62 ± 0.02^{e}	0.69 ± 0.03^{de}	0.83 ± 0.04^{c}

Data in this table are mean ± SD (n = 3); values within the same column followed by different letters are significantly different at $P < 0.05$ (ANOVA); FYC – first-generation young plantation; FMC – first-generation middle-aged plantation; FRC – first-generation mature plantation; SYC – second-generation young plantation; TYC – third-generation young plantation

Soil bacterial diversity statistical analysis. Using the 454 sequencing method, the total of 185 256 sequences were generated after optimization, with an average length of 461 bp. Sequences with fragment lengths > 400 bp accounted for 76.62% of the total number of sequences.

The dilution curve showed that the number of OTUs in each sample increased with sequencing data. When the sequencing depth was 10 000, the dilution curve of each sample remained unsaturated, indicating that new species were continuously being detected in the sample with increasing sequencing data (Figure 1). Rarefaction is a technique to assess species richness from the results of sampling (Newton 2007). If a rarefaction curve becomes parallel to the x-axis, we can trust the observed level of richness. But in our study, the rarefaction curve was steep. Maybe it was so because we did not have a sufficient sequencing depth or the taxon was extremely rare or common (Bush *et al.* 2004). The analysis of Shannon diversity index curve depicted saturation, indicating that the amount of sequencing data obtained in this study basically reflected the bacterial diversity and composition of each soil sample (Figure 2, Table 4).

Analysis of bacterial diversity in Chinese fir plantations. The analysis of soil bacterial community diversity in Chinese fir plantations is shown in Table 3. The third-generation young Chinese fir

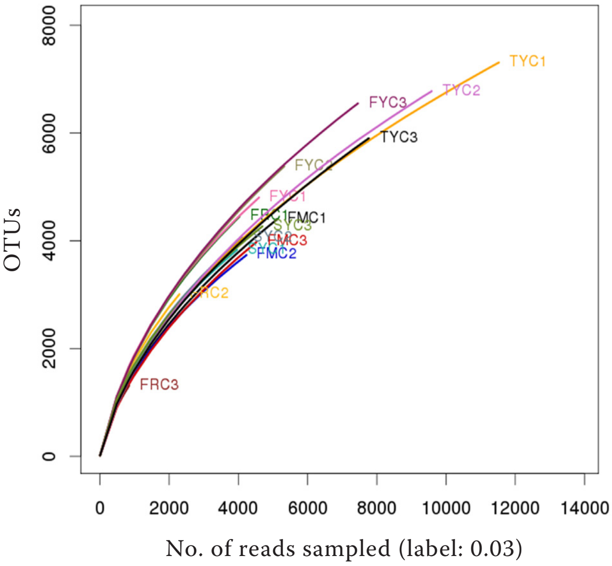
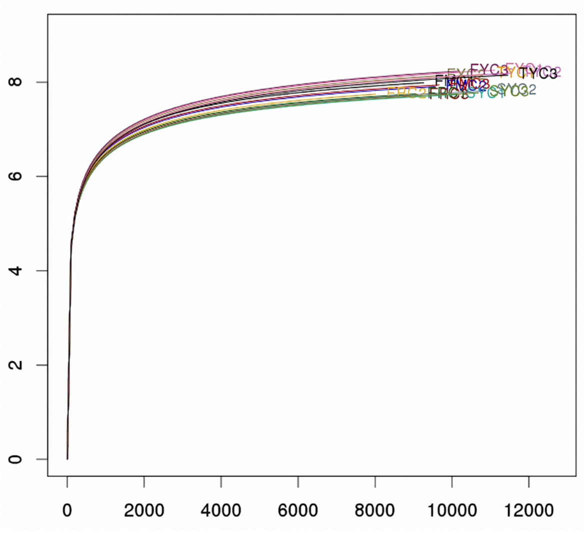


Figure 1. Rarefaction curves of 16S rRNA

OTU – operational taxonomic unit; for abbreviations explanation see Table 1



No. of reads sampled (label: 0.03)

Figure 2. Shannon-Wiener curves of samples
For abbreviations explanation see Table 1

plantations had the highest number of OTUs, with an average value of 7138, and the first-generation mature Chinese fir plantations showed the lowest number of OTUs (5075). The first-generation young Chinese fir plantations (FYC) with Chao index of 26 247 exhibited the highest number of bacterial species, whereas that of the other plantations in descending order was as follows: third-generation young Chinese fir plantations (TYC) > first-generation middle-aged Chinese fir plantations (FMC) > second-generation young Chinese fir plantations (SYC) > first-generation mature Chinese fir plantations (FRC). In terms of Shannon index and Simpson index, FYC exhibited the highest soil bacterial diversity, followed by TYC, and SYC had the lowest soil bacterial diversity, indicating that soil bacterial diversity was restored after the rotation planting of *P. massoniana*. At different developmental stages of the first-generation Chinese fir plantation, Shannon indices of the bacteria gradually decreased with increasing stand age, whereas Simpson indices gradually increased with decreasing diversity. With the growth of Chinese fir, the amount of litter increased, resulting in an increase in the levels of autotoxic phenolic compounds from Chinese fir, thereby reducing the abundance, distribution, and diversity of bacteria in the soil (Blum 1998; Liang *et al.* 2009).

Analysis of bacterial community characteristics in Chinese fir plantations. The predominant species in the soil of Chinese fir plantation included Proteobacteria, Acidobacteria, Chloroflexi and Actinobacteria, with relative contents of >5% (Figure 3). The relative abundances of Nitrospirae and Fibrobacteres in the first-generation Chinese fir plantation, the Chinese fir plantation after the rotation planting of *P. massoniana*, and the Chinese fir plantation under monoculture planting practice were high, low and high, respectively, and those of Thermotogae and Armatimonadetes in the above three planta-

Table 4. Analysis of the soil bacterial community diversity (3% genetic distance)

Plot	Reads	OTU	Ace	Chao	Shannon	Simpson
FYC1	11073	7152	54652 (52761, 56621)	27419 (25727, 29267)	8.49 (8.46, 8.51)	0.0004 (0.0004, 0.0005)
FYC2	9691	6383	48337 (46541, 50215)	25307 (23622, 27157)	8.4 (8.38, 8.42)	0.0005(0.0004, 0.0006)
FYC3	10113	6650	50635 (48834, 52513)	26015 (24327, 27864)	8.46 (8.43, 8.48)	0.0004 (0.0003, 0.0004)
FMC1	9262	5800	46312 (44672, 48021)	21722 (20250, 23345)	8.28 (8.26, 8.31)	0.0005 (0.0004, 0.0005)
FMC2	9585	5751	38780 (37331, 40296)	20527 (19167, 22025)	8.19 (8.17, 8.22)	0.0008 (0.0007, 0.0009)
FMC3	9608	5813	39391 (37896, 40955)	21271 (19846, 22842)	8.22 (8.19, 8.25)	0.0007 (0.0006, 0.0008)
FRC1	8989	5175	35968 (34645, 37350)	19210 (17822, 20750)	8.06 (8.03, 8.09)	0.0008 (0.0007, 0.0009)
FRC2	8005	4876	37112 (35627, 38670)	18278 (16931, 19775)	8.04 (8.01, 8.07)	0.0008 (0.0007, 0.0009)
FRC3	9035	5175	37679 (36291, 39130)	19714 (18270, 21318)	8.01 (7.98, 8.04)	0.0012 (0.001, 0.0013)
SYC1	9947	5670	36897 (35536, 38320)	19824 (18513, 21269)	8.06 (8.03, 8.09)	0.0013 (0.0012, 0.0015)
SYC2	10806	6262	44017 (42478, 45621)	21753 (20396, 23240)	8.15 (8.12, 8.18)	0.0014 (0.0013, 0.0016)
SYC3	10651	5944	37773 (36424, 39181)	20725 (19381, 22203)	8.09 (8.06, 8.12)	0.0015 (0.0013, 0.0017)
TYC1	10856	6935	45096 (43527 ,46733)	23817 (22418, 25343)	8.42 (8.39, 8.45)	0.0014 (0.0011, 0.0016)
TYC2	11541	7319	49150 (47502, 50865)	25940 (24423, 27592)	8.46 (8.43, 8.48)	0.0013 (0.0011, 0.0015)
TYC3	11367	7160	50566 (48885, 52315)	25890 (24338, 27583)	8.42 (8.39, 8.44)	0.0016 (0.0014, 0.0019)

FYC – first-generation young plantation; FMC – first-generation middle-aged plantation; FRC – first-generation mature plantation; SYC – second-generation young plantation; TYC – third-generation young plantation; OTU – operational taxonomic unit

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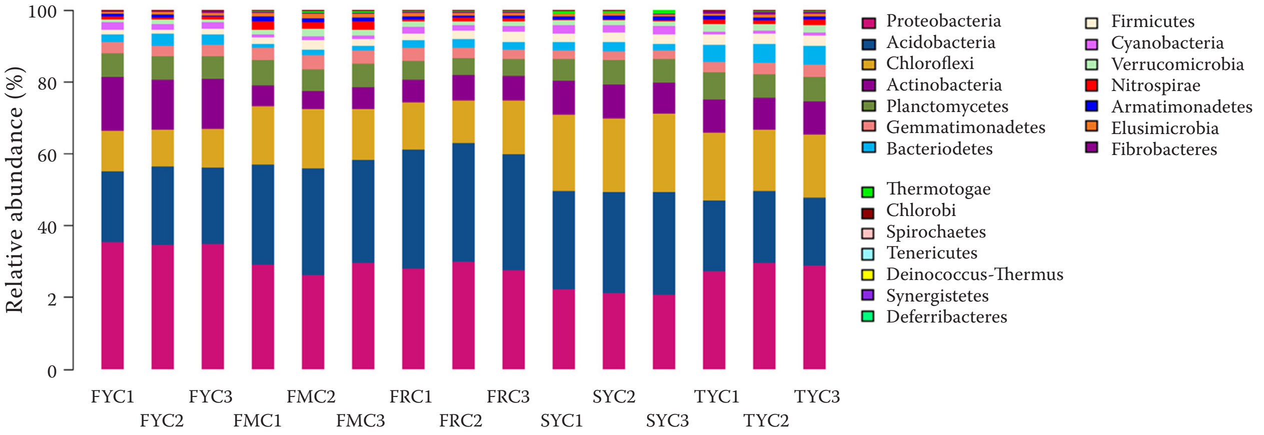

Figure 3. Bacterial composition barplot of the different plots

For abbreviations explanation see Table 1

tions were low, high and low, respectively. The relative abundance of Planctomycetes decreased (FYC 31.10%, SYC 23.96%, and TYC 21.62%). The relative abundance of Cyanobacteria in the plantation under rotation-planting practice was significantly lower than those of the first-generation plantation and second-generation plantation under monoculture planting practice. Therefore, Cyanobacteria, Nitrospirae, Fibrobacteres, and Thermotogae were more sensitive to the rotation planting of *P. massoniana*, whereas Planctomycetes were more sensitive to monoculture planting practices. Ammonia or ammonium salt could be transformed into nitrate by Nitrospirae in the soil. Nitrate can provide nitrogen for plants, improve alkaline soil and increase the utilization of phosphate fertilizer. In our study the increasing Nitrospirae showed that the soil environment was improved in the TYC. The ability of cellulolytic bacteria to degrade cellulose has been shown (Wilson 2008). Because of the large number of *Miscanthus floridulus* in the TYC plot, which provided abundant cellulose, the Fibrobacteres were higher than in SYC.

The relative abundances of Bacteroidetes (FYC 14.27%, FMC 6.34%, and FRC 11.76%) and Cyanobacteria (FYC 9.51%, FMC 4.55%, and FRC 8.79%) initially decreased and then increased, and that of Nitrospirae initially increased and then decreased (FYC 3.85%, FMC 9.87%, and FRC 3.91%). With increasing forest age, the relative abundances of Planctomycetes and Firmicutes decreased and increased, respectively. Both soil management history and compost amendment had significant effects on the Planctomycetes diversity, and variations in soil organic matter, Ca^{2+} content, and pH were associated with variations in the Planctomycetes community composition (Buckley *et al.* 2006). It is difficult to restore the abundance of Planctomycetes in the soil, so the relative abundances of *Planctomycetes* decline.

Effect of environmental factors on the soil bacteria of Chinese fir plantations. Based on bacterial community characteristics, we chose six bacterial communities (Burkholderiales, Acidothermaceae, Acidobacteria, Nitrosomonadales, Xanthomonadales and Rhizobiales) with significant changes in relative abundance or very important to plants for assessing the impact of the environment on bacteria (Moulin *et al.* 2001, Jiang *et al.* 2008 and Ward *et al.* 2009). Six soil bacterial communities in the Chinese fir plantation, three bacterial diversity variables and

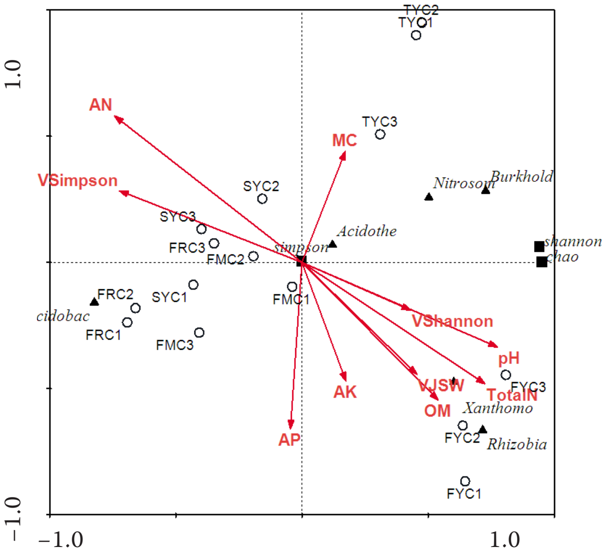

Figure 4. The ordination diagram of redundancy analysis with predominant soil bacterial communities

For abbreviations explanation see Table 1

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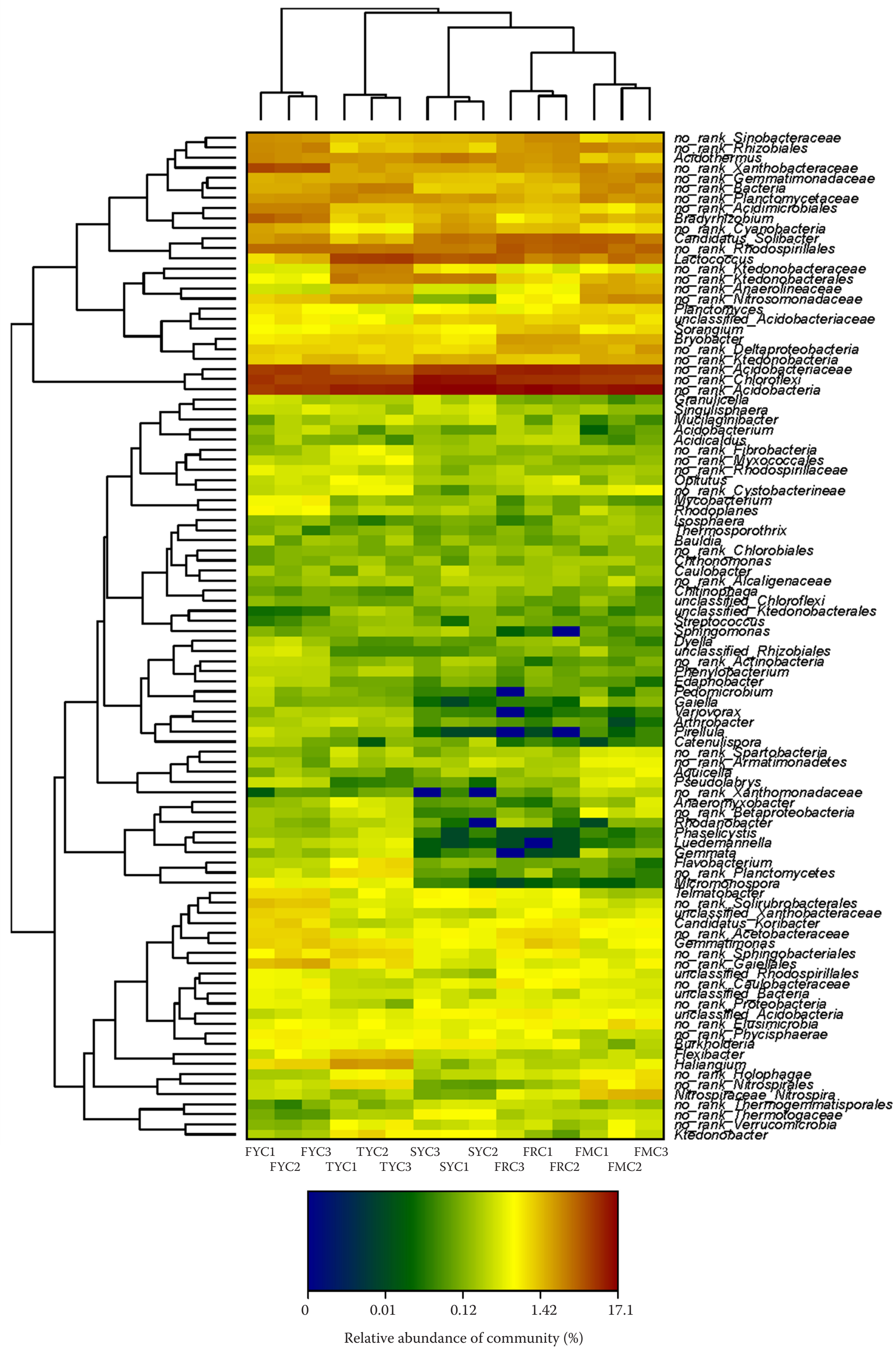

Figure 5. Heatmap of 15 soil samples based on abundance at the genus level; columns represent different samples, and rows indicate operational taxonomic unit (OTU)

14 environmental factors were selected for RDA analysis. The first axis could explain 90.0% of all the information and the second axis could explain 0.2%. The soil bacterial diversity and the spatial heterogeneity of the main communities were affected by soil physicochemical properties and undergrowth vegetation. The bacterial Simpson index was almost at the origin, indicating that it was influenced by various environmental factors. The bacterial Shannon index was significantly affected by pH, the Shannon index of undergrowth vegetation, and total N. Burkholderiales, Nitrosomonadales, and Acidothermaceae were considerably affected by soil moisture content, pH, and undergrowth vegetation. Rhizobiales and Xanthomonadales were more sensitive to environmental factors and significantly influenced by the Simpson index of undergrowth vegetation and available N (Figure 4).

Heatmap analysis of soil bacterial communities of Chinese fir plantations. Fifteen soil samples were clustered based on OTU abundance similarity at the genus level, and their clustering results were displayed using a heat map. FMC and FRC showed the most similar soil bacterial community structures, whereas FYC showed a relatively low level of similarity with the other bacterial communities. The relative abundances of *Micromonospora* and *Flavobacterium* in FYC and TYC were about 1%, whereas the corresponding relative abundances in SYC, FRC, and FMC were only about 0.1%. The relative abundances of *Pirellula*, *Phaselicystis*, *Luedemannella*, and *Gemmata* in FRC were lower than those in the other plots. The relative abundances of *Flexibacter* and *Haliangium* in TYC were higher than those in the other plots. The relative abundance of *Nitrospira* in FMC was higher than that in the other plots. In terms of bacterial abundance at the genus level, *Acidothermus*, *Bradyrhizobium*, *Lactococcus*, *Planctomyces*, *Sorangium*, and *Bryobacter* showed the highest counts (Figure 5).

References

Blum U. (1998): Effects of microbial utilization of phenolic acids and their phenolic acid breakdown products on allelopathic interactions. Journal of Chemical Ecology, 24: 685–708.

Buckley D.H., Huangyutitham V., Nelson T.A., Rumberger A., Thies J.E. (2006): Diversity of Planctomycetes in soil in relation to soil history and environmental heterogeneity. Applied and Environmental Microbiology, 72: 4522–4531.

Bush A.M., Markey M.J., Marshall C.R. (2004): Removing bias from diversity curves: the effects of spatially organized biodiversity on sampling-standardization. Paleobiology, 30: 666–686.

Caron D.A., Countway P.D., Savai P., Gast R.J., Schnetzer A., Moorthi S.D., Dennett M.R., Moran D.M., Jones A.C. (2009): Defining DNA-based operational taxonomic units for microbial-eukaryote ecology. Applied and Environmental Microbiology, 75: 5797–5808.

Chao A. (1984):Nonparametric estimation of the number of classes in a population. Scandinavian Journal of Statistics, 11: 265–270.

Chao A., Lee S. (1992): Estimating the number of classes via sample coverage. Publications of the American Statistical Association, 87: 210–217.

Doran J.W., Sarrantonio M., Liebig M.A. (1996): Soil health and sustainability. Advances in Agronomy, 56: 51–54.

Edgar R.C., Haas B.J., Clemente J.C., Quince C., Knight R. (2011): UCHIME improves sensitivity and speed of chimera detection. Bioinformatics, 27: 2194–2200.

Esty W.W. (1986): The efficiency of good's nonparametric coverage estimator. Annals of Statistics, 14: 1257–1260.

Fiere N., Jackson R.B. (2006): The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America, 103: 626.

Gans J., Wolinsky M., Dunbar J. (2005): Computational improvements reveal great bacterial diversity and high metal toxicity in soil. Science, 309: 1387–1390.

Guaa Y., Lindström K. (2009): Soil microbial biomass, crop yields, and bacterial community structure as affected by long-term fertilizer treatments under wheat-rice cropping. European Journal of Soil Biology, 45: 239–246.

Hill M.O. (1973): Diversity and evenness: a unifying notation and its consequences. Ecology, 54: 427–432.

Hollister E.B., Engledow A.S., Hammett A.J.M., Provin T.L., Wilkinson H.H., Gentry T.J. (2010): Shifts in microbial community structure along an ecological gradient of hypersaline soils and sediments. The ISME Journal, 4: 829.

Jeddi K., Cortina J., Chaieb M. (2009): *Acacia salicina*, *Pinus halepensis* and *Eucalyptus occidentalis* improve soil surface conditions in arid southern Tunisia. Journal of Arid Environments, 73: 1005–1013.

Jiang C.Y., Sheng X.F., Qian M., Wang Q.Y. (2008) Isolation and characterization of a heavy metal-resistant Burkholderia sp. from heavy metal-contaminated paddy field soil and its potential in promoting plant growth and heavy metal accumulation in metal-polluted soil. Chemosphere, 72: 157–164.

Kaschuk G., Alberton O., Hungria M. (2011): Quantifying effects of different agricultural land uses on soil microbial biomass and activity in Brazilian biomes: inferences to improve soil quality. Plant and Soil, 338: 467–481.

Kanazawa S., Miyashita K. (1986): A method for the determination of cellulase activity in forest soil. Soil Science and Plant Nutrition, 32: 71–79.

Liang C., Zhen W., Zhang C., Yin B. (2009): Determination of phenolic acids in decomposing products of maize straw and their allelopathy on pathogens of wheat soil-borne disease. Chinese Agricultural Science Bulletin, 25: 210–213.

Liu C., Ma K., Lu Y., Hang Y. (1998): Measurement of biotic community diversity. VI. The statistical aspects of diversity measures. Chinese Biodiversity, 6: 229–239.

Moulin L., Munive A., Dreyfus B., Boivinmasson C. (2001): Nodulation of legumes by members of the beta-subclass of Proteobacteria. Nature, 411: 948–950.

Nacke H., Thürmer A., Wollherr A., Will C., Hodac L., Herold N., Schöning I., Schrumpf M., Daniel R. (2011): Pyrosequencing-based assessment of bacterial community structure along different management types in German forest and grassland soils. PLoS ONE, 6: e17000.

Newton A. (2007): Forest Ecology and Conservation. Techniques in Ecology & Conservation Series. Oxford, Oxford University Press: 128–131.

O’Donnell A.G., Seasman M., Macrae A., Waite I., Davies J.T. (2001): Plants and fertilisers as drivers of change in microbial community structure and function in soils. Plant and Soil, 232: 135–145.

Samuel A.D., Blidar C.F., Domuța C., Șandor M., Vușcan A., Borza I., Brejea R. (2015): Influence of Heavy Metal Contamination on Soil Enzyme Activities. Social Science Electronic Publishing: 111–120.

Schloss P.D., Westcott S.L., Ryabin T., Hall J.R., Hartmann M., Hollister E.B., Lesniewski R.A., Oakley B.B., Parks D.H., Robinson C.J. (2009): Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology, 75: 7537.

Simpson E.H. (1949): The measurement of diversity. Nature, 163: 688.

Smith K.P., Goodman R.M. (1999): Host variation for interactions with benificial plant- associated microbes. Annual Review of Phytopathology, 37: 473–491.

Tabatabai M.A., Bremner J.M. (1972): Assay of urease activity in soils. Soil Biology and Biochemistry, 4: 479–487.

Timonen S., Finlay R.D., Olsson S., Söderström B. (1996): Dynamics of phosphorus translocation in intact ectomycorrhizal systems: non-destructive monitoring using a β-scanner. FEMS Microbiology Ecology, 19: 171–180.

Van Der Heijden M.G.A., Bardgett R.D., Van Straalen N.M. (2008): The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters, 11: 296–310.

Van’kova A.A., Ivanov P.I., Emtsev V.T. (2013): Filtrating forms of soil bacteria. Eurasian Soil Science, 46: 303–309.

Ward N.L., Challacombe J.F., Janssen P.H., Henrissat B., Coutinho P.M., Wu M., Xie G., Haft D.H., Sait M., Badger J., Barabote R.D., Bradley B., Brettin T.S., Brinkac L.M., Bruce D., Creasy T., Daugherty S.C., Davidsen T.M., DeBoy R.T., Detter J.C., Dodson R.J., Durkin A.S., Ganapathy A., Gwinn-Giglio M., Han C.S., Khouri H., Kiss H., Kothari S.P., Madupu R., Nelson K.E., Nelson W.C., Paulsen I., Penn K., Ren Q., Rosovitz M.J., Selengut J.D., Shrivastava S., Sullivan S.A., Tapia R., Thompson L.S., Watkins K.L., Yang Q., Yu C., Zafar N., Zhou L., Kuske C.R. (2009): Three genomes from the phylum Acidobacteria provide insight into the lifestyles of these microorganisms in soils. Applied and Environmental Microbiology, 75: 2046–2056.

Wilson D.B. (2008): Three microbial strategies for plant cell wall degradation. Annals of the New York Academy of Sciences, 1125: 289–297.

Wu Y.L., Wang B., Zhao C., Dai W., Li P. (2011): Comprehensive evaluation of soil fertility in different developing stages of Chinese Fir Plantations. Journal of Northwest A&F University, 39: 69–75.

Yao H., He Z., Wilson M.J., Campbell C.D. (2000): Microbial biomass and community structure in a sequence of soils with increasing fertility and changing land use. Microbial Ecology, 40: 223.

Yin C., Kennethl J., Dallase P., Karena G., Scoth H., Timothyc P. (2010): Members of soil bacterial communities sensitive to tillage and crop rotation. Soil Biology Biochemistry, 42: 2111–2118.

Yu Y., Wang H., Liu J., Wang Q., Shen T., Guo W., Wang R. (2012): Shifts in microbial community function and structure along the successional gradient of coastal wetlands in Yellow River estuary. European Journal of Soil Biology, 49: 12–21.

Zhou J., Xia B., Treves D.S., Wu L.Y., Marsh T.L., O’Neill R.V., Palumbo A.V. , Tiedje J.M. (2002): Spatial and resource factors influencing high microbial diversity in soil. Applied and Environmental Microbiology, 68: 326–334.

Zhou L.K. (1988): Soil Enzymology. Beijing, Science Press: 21–23.

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