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Effect of Long-Term Different Fertilization on Bacterial Community Structures and Diversity in Citrus Orchard Soil of Volcanic Ash

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This study was conducted to assess bacterial species richness, diversity and community distribution according to different fertilization regimes for 16 years in citrus orchard soil of volcanic ash. Soil samples were collected and analyzed from Compost (cattle manure, 2,000 kg/10a), 1/2 NPK+compost (14-20-14+2,000 kg/10a), NPK+compost (28-40-28+2,000 kg/10a), NPK (28-40-28 kg/10a), 3 NPK (84-120-84 kg/10a), and Control (no fertilization) plot which have been managed in the same manners with compost and different amount of chemical fertilization. The range of pyrosequencing reads and OTUs were 4,687–7,330 and 1,790–3,695, respectively. Species richness estimates such as Ace, Chao1, and Shannon index were higher in 1/2 NPK+compost than other treatments, which were 15,202, 9,112, 7.7, respectively. Dominant bacterial groups at level of phylum were *Proteobacteria*, *Acidobacteria*, and *Actinobacteria*. Those were occupied at 70.9% in 1/2 NPK+compost. Dominant bacterial groups at level of genus were *Pseudolabrys*, *Bradyrhizobium*, and *Acidobacteria*. Those were distributed at 14.4% of a total of bacteria in Compost. Soil pH displayed significantly closely related to bacterial species richness estimates such as Ace, Chao1 ($p<0.05$) and Shannon index ($p<0.01$). However, it showed the negative correlation with exchangeable aluminum contents ($p<0.05$). In conclusion, diversity of bacterial community in citrus orchard soil was affected by fertilization management, soil pH changes and characteristics of volcanic ash.

Keywords: bacterial community structure, bacterial diversity, citrus orchard, volcanic ash soil

Introduction

Organic matter will be decomposed in soil and then can be used as nutrients for crops and microorganism growth. Microorganisms play an important role in nutrient cycles occurring within ecosystems. There were many reports about microorganisms fixing nitrogen and decomposing organic materials (Jung *et al.*, 2012). However, studies about how many and where these microorganisms are distributed are still in progress (Hill *et al.*, 2003). Pyrosequencing analysis is the method to interpret the community structure and diversity of bacteria and fungi by analyzing DNA sequence and distribution ratio of microorganisms (Devine *et al.*, 2004; Huse *et al.*, 2007; Liu *et al.*, 2007; Roesch *et al.*, 2007; Ahn *et al.*, 2012; Oh *et al.*, 2012; Jeong *et al.*, 2013). Agriculture brings major changes in the soil bacterial community structure and composition (Buckley and Schmidt, 2003). Amount of applied compost or chemical fertilizers and long-term application may affect the bacterial community and composition. Bacteria were distributed the most abundant in the soil (Gans *et al.*, 2005; Lauber *et al.*, 2009). Bacteria are the species richness in the soil (Devine *et al.*, 2004). According to Gans *et al.* (2005), 2,000–8,300,000 species of bacteria are distributed per 1 g of soil and diversity of bacterial community can be assessed with 16S rRNA sequence analysis of DNA extracted from soil and statistical inference. However, there are many difficulties to evaluate the diversity of bacteria. Youssef and Elshahed (2008) estimated species richness in a library of 13,001 near full-length 16S rRNA clones derived from soil, as well as in multiple subsets of the original library. Species richness estimates obtained increased with the increase in library size.

Several researchers have reported that the soil microbial community structure (Fierer *et al.*, 2007), the difference in diversity of microbial community between farmland and forest soil, seasonal changes (Buckley and Schmidt, 2003; Lipson and Schmidt, 2004; Upchurch *et al.*, 2008), ecological diversity assessment using bacterial communities (Hill *et al.*, 2003). It has been reported that soil management and land use affect soil bacterial diversity (Roesch *et al.*, 2007; Martinez *et al.*, 2008) and the soil bacterial diversity has high correlation with pH (Lauber *et al.*, 2009). Fierer *et al.* (2007) has reported that the correlation between soil chemical properties and relative abundance of six dominance genera such as *Acidobacteria*, *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *α-Proteobacteria*, and *β-Proteobacteria* is high and the biological and environmental factors affect survival and community structure of bacteria (Langenheder *et al.*, 2006). *Actinobacteria* is abundant involved in weathering process in volcanic rock (Cockell *et al.*, 2013). It were dominated *Chloroflexi*, *Proteobacteria*, and *Actinobacteria* due to long-term fertilization regimes in the rice field soils of non-volcanic ash (Ahn *et al.*, 2012). Soil type and land use becomes an

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important factor to determine the structure of microbial community in soil (de Ridder-Duine *et al.*, 2005; Nunan *et al.*, 2005; Mercier *et al.*, 2013; Singh *et al.*, 2013). Soil microbial density and enzyme activities were low due to organic complexes between organic matter and allophane with high phosphate retention characteristics (Allbrook, 1985; Song, 1990; Nanzyo, 2002; Shoji and Takahashi, 2002). Nitrogen (Deenik, 2006) and organic carbon (Takenaka and Hayano, 1999) mineralization rate was low in volcanic ash soil than non-volcanic ash soils. Low density of soil microorganisms was caused by organic complexes showed non soluble characteristics and much aluminium ion released from allophane in volcanic ash soil of Jeju region in Korea (Song, 1990; He *et al.*, 2012). Soil bacterial diversity was reduced by the highly toxic effect of metal (Gans *et al.*, 2005; He *et al.*, 2012). The composition and diversity of bacterial communities was closely affected by soil pH (Kemmitt, 2006; Nicol *et al.*, 2008; Rousk *et al.*, 2010; Li *et al.*, 2012). It was found that *Proteobacteria* and *Actinobacteria* were more sensitive to pH variation (Li *et al.*, 2012). Bacterial community structures were significantly affected by organic C content (Marschnera *et al.*, 2003; Chaudhry *et al.*, 2012; Cockell *et al.*, 2013; Mercier *et al.*, 2013; Coats *et al.*, 2014). Fertilization regimes and continuous crop cultivation affected soil organic carbon content and bacterial community structure (Marschner *et al.*, 2003), microbial community structure changed by nitrogen fertilization in grassland and forest soils (Clegg, 2006; Fredrik *et al.*, 2008). Organic and inorganic fertilization or soil management practices significantly affects community and diversity of soil microorganisms. Little ecological studies on bacterial community structure and species richness and diversity in citrus orchard soil of volcanic ash. This study was conducted in citrus orchard soil of volcanic ash to assess bacterial species richness, diversity, and community structure.

Materials and Methods

Experiment design and soil management

This study was performed at the Citrus Experiment Station (33°18′22″N, 126°36′33″E) located 190 m above sea level in Jeju Special Self-Governing Province. It has applied compost and different amount of chemical fertilization for 16 years with 20 years citrus tree (*Citrus unshiu* M.). Experimented soil showed particle density of 2.11 g/cm^3 and bulk density of 0.75 g/cm^3 as volcanic ash (Jung bang series) of silt loam soil. In March, June and November, nitrogen fertilizer applied at a rate of 50, 20, and 30%, potassium applied 30, 40, and 30%, respectively. The total amount of phosphate and cattle manure as compost applied in March. Agricultural chemicals sprayed 10–12 times a year to protect disease and pest of citrus. Soil samples were taken two replicate from six plots such as Compost (cattle manure, 2,000 kg/10a), 1/2 NPK+compost (14-20-14+2,000 kg/10a), NPK+compost (28-40-28+2,000 kg/10a), NPK (28-40-28 kg/10a), 3 NPK (84-120-84 kg/10a), and Control (no fertilization) in May 2010. Soil sample was passed through sieve of 2 mm and then stored in the state of wet soil at -80°C. After that DNA extraction from soil was performed. The remainder was used for soil chemical property analysis after air drying.

Soil chemical analysis

For chemical analysis, soil samples were air dried at room temperature in the shade and sieved through a 2-mm screen. Soil physicochemical properties were analyzed in accordance with standard analytical technique in Rural Development Administration of Korea (NIAST, 2000). Soil pH value was measured by a ratio of soil:distilled water in 1:5. The contents of organic materials were measured by Walkley-Black method. The content of total nitrogen was measured by Kjeldahl method. Available phosphate was measured by Bray. No-1 method. Cations such as exchangeable potassium, calcium and magnesium was measured by 1 N ammonium acetate (pH 7.0) infusion method. For exchangeable aluminum, 10 g of soil was added in 50 ml of 0.2 M ammonium oxalate/oxalic acid solution and incubated at 30°C for 1 h at 200 rpm. After filtration, it was analyzed by using ICP (GBC Integra XL, Australia).

Pyrosequencing analysis

Soil DNA extraction: Ultra clean soil DNA extraction kit (MoBio Inc., USA) was used for soil DNA extraction. 0.3 g of frozen soil was added into 2 ml bead solution tube and mixed well for 5 sec. After that, 250 µl of S1 solution, IRS (Inhibitor removal solution) solution and S2 solution were added and mixed well for 5 sec. After that, it was incubated at 4°C for 5 min. According to kit manual, it was centrifuged and S3, S4, and S5 solutions were added in order. After DNA was extracted. After 1% agarose gel was made, genomic DNA was loaded and electrophoresis was performed at 100 V for 2 h. PCR products were confirmed by staining gel with ethidium bromide.

Bacterial 16S rRNA gene PCR Amplification: For FLX amplicon sequencing, The 50 µl PCR reaction mixture contained 10× PCR buffer+$MgCl_2$ 5 µl, 10 mM dNTPs 1 µl, 20 pM Primers 2 µl, 5U *taq* DNA polymerase 0.25 µl, 100 ng template DNA 1 µl, ddH_2O 40.75 µl. The 49 µl of mixture was added into each PCR tube. After that, 1 µl of template DNA was added and mixed well for 30 sec with pipetting. After mixing, it was put into PCR (PTC-200, Applied Biosystems, USA). PCR amplification was performed using the following PCR protocol. After initial incubation at 94°C for 5 min, denaturation at 94°C (30 sec), annealing at 60°C (45 sec) and extension at 72°C (90 sec) were performed for 10 cycles. After that, denaturation at 94°C (30 sec), annealing at 55°C (45 sec) and extension at 72°C (90 sec) were performed for 20 cycles. PCR reactions were ended at 4°C. PCR amplification used fusion primers specially made for bacterial 16S rRNA genes were amplified using the primers. Tags-linker (AC)-27f Forward: CCTATCCCCTGTGTGCCTTGGCAGTCTCAGACGAGTTTGATCMTGGCTCAG, Tags-linker (AC)-518r Reverse: CCATCTCATCCCTGCGTGTCTCCGACTCAGATCAGCACACWTTACCGCGGCTGCTGG. After PCR amplification, DNA samples were run on the gel at 120 V for 30 min to check whether PCR was performed well or not.

Pyrosequencing data processing: The PCR products were gel-purified with the QIAquick gel extraction kit (QIAGEN,

Table 1. Chemical properties of soil used this experiment

Treatment	pH	O.M.	Av.P_2O_5	T-N	Exch. Cations			Exch. Al
					K	Ca	Mg	
	1:5	g/kg	Mg/kg	%		$cmol_+$/kg		Mg/kg
Compost	5.7±0.4	135.5±2.1	182.9±46.9	0.60±0.0	0.56±0.3	7.7±0.6	1.7±0.4	455.5±100.2
1/2 NPK+compost	6.0±0.1	136.6±0.8	238.8±16.2	0.62±0.0	0.50±0.2	9.0±2.4	2.5±0.4	409.6±113.5
NPK+compost	5.9±0.1	130.8±3.0	328.4±181.1	0.60±0.0	0.62±0.0	13.3±1.8	3.5±0.5	416.8±60.4
NPK	5.9±0.1	135.3±0.1	279.9±15.3	0.59±0.0	0.41±0.1	12.3±0.2	2.7±0.0	506.5±148.0
3 NPK	5.9±0.4	129.8±4.7	517.9±238.5	0.58±0.0	0.80±0.3	16.7±5.7	5.1±0.7	504.3±68.4
Control	5.5±0.1	134.1±0.8	83.9±35.6	0.54±0.0	0.29±0.0	4.1±0.5	1.0±0.1	497.4±77.5

Germany) and pyrosequencing was performed by using a 454 GS FLX Titanium Sequencing System (Roche, Germany), according to the manufacturer's instructions in Chunlab Inc. (Korea). Pyrosequencing analysis was performed according to methods presented by Chun *et al.* (2010). Chimeric sequences were removed using UCHIME (Edgar *et al.*, 2011) and DECIPHER (http://decipher.cee.wisc.edu) (Wright *et al.*, 2012). The rarefaction curve at 3% dissimilarity level, ACE and Chao1 estimates were analyzed using the obtained sequences. Qualified sequences from each sample were merged, and the pair-wise distances were calculatedusing the "pairwise.seqs" command in the Mothur software Package (version 1.23.1) (Schloss *et al.*, 2009), which aligns two sequences using the Needleman algorithm. Sequences were then clustered into operational taxonomic units (OTUs) at the cut-off of 97% similarity using the furthest-neighbor algorithm.

Statistical analysis

Correlation coefficients between soil chemical properties (pH and Al) and Ace, Chao1, Shannon index in the citrus orchard soil of volcanic ash were analyzed at (p<0.01) and (p<0.05) of statistical significance levels using SAS Enterprise guide 4.2 (SAS Inst., USA).

Results and Discussion

Soil chemical properties

The soil pH of Control was low slightly at 5.8 compared to Compost (6.0), NPK (6.3), 1/2 NPK+compost (6.2), NPK+compost (6.0), 3 NPK treatment (6.1) (Table 1). Soil organic matter content was similar between treatments. Chemical fertilizer application rate was higher in 3 NPK treatment than others. However, total nitrogen content of soil was similar at 0.6% between treatments. Available phosphate in control was lower at 70.9 mg/kg than in compost (127.2) and NPK (202.3). However, in 3 NPK (423.2 mg/kg) was two times higher than in NPK. Exchangeable calcium and aluminum content were higher at 11.7 $cmol_+$/kg and 480.7 mg/kg in 3 NPK than other treatments. Soil pH and available phosphate is low in control. However, those were higher including exchangeable aluminum content in 3 NPK. Application of nitrogen fertilizer affected soil pH (Barak *et al.*, 1997) and the composition of soil microbial and bacterial communities influenced by soil pH (Kemmitt, 2006; Rousk *et al.*, 2010; Li *et al.*, 2012). Nitrogen content was alike between treatments because of nitrogen mineralization rate was low in volcanic ash soil (Deenik, 2006). More genera were discovered in A1-non treated soil than in Al-treated soils (He *et al.*, 2012). As above results, soil physicochemical properties affected significantly soil bacterial community structure (Mercier *et al.*, 2013).

Species richness estimate and diversity index

Rarefaction curves can expect whether diversity of species is large or small based on slope of graph. OTUs are groups of nucleotide sequence based on similarity between nucleotide sequences and used as the concept of particular classification group. Rarefaction curves representing 97% sequence similarity were quite different between treatments (Fig. 1). The range of clones of pyrosequence analyzed in May was from 4,687 to 7,330 and OTUs showed 1,790–3,695 of distribution. Average length of pyrosequence ranged from 447–450, it was similar between treatments. Shannon index as bacterial diversity ranged from 6.9–7.7 and Coverage was high at 78.7% in Compost. OTU (97% similarity level) per sample analyzed in rarefaction curves was more diverse in 1/2 NPK+compost treatment than others. OTU was the lowest in Compost treatment. Shannon index was the highest at 7.7 in 1/2 NPK+compost treatment. It is thought that these results are caused by pH, exchangeable aluminum contents, and non-soluble organic matter complex combining allophane. Marschnera *et al.* (2003) were reported that bacterial community structures were significantly affected by organic C content through long-term fertilization

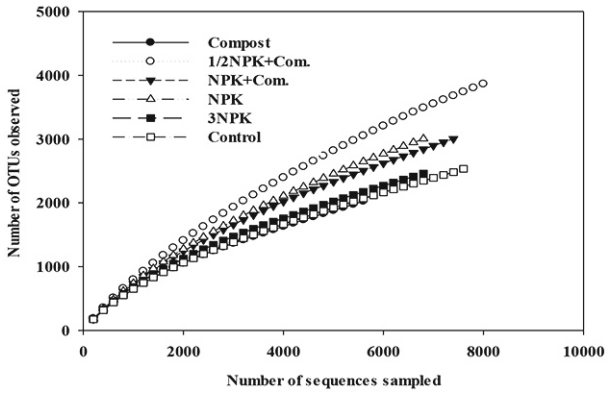

Fig. 1. Rarefaction curves at the 97% sequence similarity level analyzed from the Mothur program in May. Data for the curves represent an average of 1,000 simulations performed using the software Mothur.

Table 2. Bacterial richness estimators and diversity indices as affected by different fertilization management practices in citrus orchard soil of volcanic ash

Treatment	Total no. of reads	No. of OTUs	Average length	Ace	Chao1	Shannon index	Coverage (%)
Compost	4,687±1530.9	1,790±553.0	449.6±1.3	4,518±1835.6	3,290±1234.6	6.9±0.3	78.7±0.4
1/2 NPK+compost	7,330±922.8	3,695±590.4	449.9±0.9	15,202±5882.4	9,112±2537.8	7.7±0.1	65.9±3.2
NPK+compost	6,674±189.5	2,967±316.8	447.0±1.9	10,453±518.3	6,722±668.9	7.5±0.2	71.5±2.1
NPK	5,892±2118.5	2,624±895.9	449.4±2.2	9,174±3883.4	5,878±2252.8	7.3±0.3	71.7±0.3
3 NPK	5,451±2100.1	2,121±737.5	450.1±2.9	5,441±1620.0	3,852±1261.5	7.1±0.4	77.8±1.3
Control	5,423±3219.5	1,964±910.8	449.7±1.5	5,249±2122.0	3,637±1413.5	6.9±0.3	78.3±4.2

Estimates of Chao1 richness, Ace richness, Shannon index were all based on 3% differences in nucleic acid sequence alignments. Numbers were calculated from the Mothur program.

management practices. This result showed that applied compost dissolved in soil and changed slowly during long-time in volcanic ash soil. Soil microbial density was low due to organic complexes between organic matter, aluminum toxicity, and allophane with high phosphate fixation (Allbrook, 1985; Song, 1990; Nanzyo, 2002; Deenik, 2006; He *et al.*, 2012). Giller *et al.* (2009) reported that microbial diversity may become affected by long-term chronic toxicity (stress) which accumulates gradually in soil.

Ace, Chao1 and Shannon are used to represent species richness estimate and diversity index, respectively. Table 2 showed species richness estimate of bacteria and diversity index by using OTUs, Ace, Chao1, and Shannon. The number of pyrosequens analyzed was low in Compost compared with 1/2 NPK+compost. It is thought that application of nitrogen and phosphate fertilizer affects supply of nutrients required for microbial growth and soil fertility. It has been reported that continuous use of nitrogen fertilizer affects bacterial community structure and bacterial biomass content is decreased by 40% (Clegg, 2006) and change in pH significantly affects the function of microorganism (Kermmit *et al.*, 2006). In both 3 NPK and NPK+compost, microbial community simplifies by continuous fertilization management and diversity decreased. Ace and Chao1 species richness and Shannon diversity index were the highest in 1/2 NPK+compost treatment by exchangeable aluminum contents. Ace estimate in 1/2 NPK+compost treatment were at 15,202. It was about 3 times higher than in Compost treatment. Chao1 estimate in 1/2 NPK+compost treatment was 9,112, which was twice as high as in Compost treatment. Ace species richness and Shannon diversity index in 1/2 NPK+compost treatment were at 15,202 and 7.7, respectively. Ace species richness estimate showed significant difference between treatments. Estimated OTU numbers ranged from 1,790 (1/2 NPK+compost)-3,695 (Compost). It was very low compared to the 2,000–52,000 species reported by Roesch *et al.* (2007). That is thought to be caused by difference calculation method. Soil bacterial diversity and species richness were affected by chemical properties such as soil pH (Rousk *et al.*, 2010; Li *et al.*, 2012), exchangeable aluminium content and fertilization regimes. Agriculture gives a large impact to bacterial community structure and composition by various activities such as crops cultivation, soil management and land use (Buckley and Schmidt, 2003). Bulk soil chemistry had more effect on the bacterial community structure than the fungal community (Coats *et al.*, 2014). It is estimated that community structure and composition of fungi are different those of bacteria. It suggests that fertilizer management mixing chemical fertilizer and organic materials is required to increase the diversity of microorganisms in citrus orchard soil rather than single use of chemical fertilizer.

Changes of bacterial community by taxonomic groups

Comparison of relative abundance of major bacteria at level of phylum can be used to understand soil management, land use and the degree of mineralization of soil nutrients (Fierer *et al.*, 2007; Roesch *et al.*, 2007; Martinez *et al.*, 2008). Relative abundance of dominant bacteria at the level of phylum was shown in Table 3. The relative abundance of bacterial phyla was as follows: *Proteobacteria*>*Acidobacteria*>*Actinobacteria*>*Chloroflexi*>*Gemmatimonadetes*. Among major bacterial phylum, *Proteobacteria* showed the distribution range of 39.1–41.2%. *Acidobacteria* showed the range of 13.9–18.9% and in 3 NPK showed at 13.9%, which was the lowest. *Actinobacteria* showed the range of

Table 3. Relative abundance of dominant bacterial phylla as affected by 16 years different fertilization management in citrus orchard soil of volcanic ash

Phylum	Compost	1/2 NPK+compost	NPK+compost	3 NPK	NPK	Control
Proteobacteria	40.2±7.4	41.2±3.7	39.8±0.7	40.6±2.9	39.1±2.4	39.8±3.0
Acidobacteria	18.7±5.6	17.0±1.6	14.6±3.4	13.9±2.3	18.9±1.0	18.7±2.4
Actinobacteria	10.8±0.4	13.0±1.1	16.0±1.5	16.2±0.1	12.2±0.3	10.0±2.6
Chloroflexi	5.4±1.0	5.3±2.2	6.3±0.3	6.2±0.4	5.1±0.1	6.6±2.2
Gemmatimonadetes	4.0±0.1	3.8±0.5	4.0±2.0	5.0±0.3	3.9±0.3	3.4±0.2
Firmicutes	4.2±0.4	2.0±0.5	2.6±1.2	1.3±0.5	2.8±0.1	3.4±0.7
Nitrospirae	2.7±1.0	3.5±1.5	2.3±0.2	3.2±0.8	3.9±0.2	3.0±0.9
Bacteroidetes	2.9±0.1	4.2±0.8	2.5±0.1	3.7±0.9	3.3±1.0	2.3±0.8
Planctomycetes	1.0±0.3	1.3±0.5	1.4±0.3	1.0±0.4	1.5±0.1	1.3±0.5
Thermobaculum	1.0±0.1	1.0±0.1	1.6±0.8	1.6±0.2	1.1±0.1	0.9±0.3
Others	9.6±1.0	8.3±0.5	8.8±3.7	7.1±1.8	8.6±0.0	10.6±0.4

Table 4. Relative abundance of dominant bacterial genera by 16 years different fertilization management in citrus orchard soil of volcanic ash in May

Genus	Compost	1/2 NPK+compost	NPK+compost	3 NPK	NPK	Control
			%			
Acidobacteria[a]	5.6±1.3	3.1±0.3	4.2±2.5	2.9±1.8	3.8±1.2	5.6±1.8
Pseudolabrys	6.0±1.1	4.9±0.6	5.6±0.1	3.9±1.3	5.7±0.6	5.0±0.6
Koribacter[a]	2.3±0.4	2.1±1.1	1.4±0.6	0.6±0.1	2.1±1.1	3.5±2.2
Bradyrhizobium	2.8±1.1	2.7±0.3	1.9±0.1	1.4±0.5	2.1±0.8	2.5±0.4
Arthrobacter	0.8±0.6	1.7±0.2	1.1±0.2	1.6±0.4	0.9±0.3	2.4±2.0
Nitrosospira	2.3±0.6	1.0±0.1	1.4±0.7	1.0±0.4	1.8±0.4	2.2±0.9
Rhodospirillaceae[a]	2.1±0.8	1.1±0.3	1.5±0.7	0.8±0.1	1.0±0.1	2.0±0.3
Afipia	1.7±0.0	1.9±0.5	1.4±0.1	1.2±0.1	1.4±0.4	1.7±0.6
Solibacteres[a]	2.6±2.8	1.4±1.2	1.0±0.4	1.5±1.2	2.1±1.5	1.6±0.5
Ktedonobacterales[a]	0.6±0.6	0.7±0.6	0.7±0.1	0.6±0.1	0.6±0.1	1.5±1.3
Betaproteobacteriav[a]	1.1±0.4	1.2±0.4	0.8±0.3	1.5±0.6	1.7±0.4	1.4±0.4
Gemmatimonadetes[a]	1.5±1.1	1.0±0.8	1.0±0.4	1.5±1.8	1.4±1.1	1.4±0.4
Tremblaya[a]	1.3±0.1	0.6±0.0	0.5±0.2	0.5±0.2	0.6±0.0	1.4±0.6
Tepidimonas[a]	1.3±0.2	1.1±0.1	1.0±0.1	1.6±0.4	0.9±0.1	1.4±0.5
Rubrobacterales[a]	1.7±0.7	1.1±0.6	1.2±0.5	1.8±0.4	1.4±0.6	1.3±0.1
Sinobacteraceae[a]	1.5±0.2	0.6±0.0	1.2±0.8	0.7±0.1	0.9±0.0	1.2±0.1
Burkholderia	0.6±0.4	0.6±0.1	0.2±0.1	0.4±0.1	0.6±0.2	1.1±0.4
Pseudomonas	0.5±0.2	0.6±0.6	0.5±0.6	1.0±0.4	0.5±0.0	1.0±0.4
Others	64.1±0.2	72.9±0.6	73.7±4.8	71.0±1.0	76.1±2.6	62.4±4.3

[a] Not classified at the genus level

10.0–16.2% and 3 NPK treatment showed at 16.2% which was the highest. *Chloroflexi*, *Gemmatimonadetes*, *Firmicutes*, *Nitrospirae*, *Bacteroidetes*, *Planctomycetes*, and *Thermobaculum* showed the range of 5.1–6.6%, 3.4–5.0%, 1.3–4.2%, 2.3–3.9%, 2.3–4.2%, 1.0–1.5%, and 0.9–1.6%, respectively. Li *et al.* (2012) reported that Abundance of *Actinobacteria* was more sensitive to pH variation. The bacteria which cannot be classified to any of existing phylum ranged from 7.1% to 10.6% and 3 NPK treatment showed at 7.1%, which was the lowest. It was similar to the results reported by Gans *et al.* (2005) in which *Alphaproteobacteria*, *Betaproteobacteria*, and *Bacteroidetes* were the most abundant bacterial groups in farmland soil. It is thought to be caused by fertilization regime, seasonal factors, crop growth stage, volcanic ash characteristic and pH. Nanzyo (2002) reported that andisol of volcanic ash soil has more anaerobic bacteria and *Actinobacteria*. Buckley and Schmidt (2003) have reported that major bacterial group including *α-Proteobacteria*, *β-Proteobacteria*, and *Actinobacteria* was changed by temporal shift of soil ecosystem and relative richness of bacterial community was affected by practices management. The distribution of *Acidobacteria, Actinobacteria* is significantly varied depending on pH of soil (Lauber *et al.*, 2009; Li *et al.*, 2012). *Actinobacteria* was high in NPK+compost (16.0%) and 3 NPK (16.2%). It was due to high soil pH than in Control (10.0%) and Compost (10.9%) treatment. Based on above results, occupation ratio of *Proteobacteria*, *Acidobacteria*, and *Actinobacteria* phylum between treatments were similar from 68.5% (Control) to 71.1% (1/2 NPK+compost). It is thought that soil management may affect the survival and community structure of bacteria. Table 4 showed the relative abundance of major bacterial genera which accounted for 1.0% or more based on Control treatment. Major dominant genera included *Acidobacteria*, *Pseudolabrys*, *Koribacter*, *Bradyrhizobium*, *Arthrobacter*, *Nitrosospira*, and *Rhodospirillaceae*. *Pseudolabrys* ranged from 3.9 to 6.0%. and It showed at 6.0% in compost treatment, which was the highest. *Acidobacteria*, *Nitrosospira*, *Bradyrhizobium*, *Solibacteres*, and *Koribacter* showed the range of 3.1–5.6%, 1.0–2.3%, 1.4–2.8%, 1.0–2.6%, and 0.6–3.5%, respectively. He *et al.* (2012) reported about bacterial diversities and compositions that less genera were discovered and *Nitrosospira* disappeared in Al-treated soils. However, *Nitrosospira* was similar regardless of exchangeable Al content between treatments. It was affected by high calcium content in soil. Bacterial community structure was more influenced by bulk soil chemistry (Coats *et al.*, 2014). Youssef and Elshahed (2009) have reported that the patterns of diversity and correlations between diversity and relative abundance could significantly vary when examined at class or order taxonomic level.

However, the ratio which cannot be classified in bacterial genus was higher in 3 NPK (73.2%) than in Control (58.9%). It was implied that active aluminum concentration was increased and bacterial community was affected by characteristics of volcanic ash soil. Bacterial beta diversity dendrogram showed that it was affected by fertilization manage-

Fig. 2. Bacterial beta diversity dendrogram by 16 years different fertilization management in citrus orchard soil of volcanic ash in May.

Table 5. Pearson correlation coefficient between physicochemical factors and bacterial richness estimators and diversity indices (N=12)

Factors	Ace	Chao1	Shannon
pH	0.578*	0.586*	0.728**
Al	-0.686*	-0.687*	-0.634*

Duncan's multiful range test: **, $p<0.01$; *, $p<0.05$.

ment practices (Fig. 2). Microbial communities and composition divided into 3 group such as Control, 3 NPK and NPK+compost, NPK, Compost, and 1/2 NPK+compost treatment. Control treatment showed different bacterial community structure between treatments. Rahman *et al.* (2008) reported that microbial community affected by land use and tillage practice in andisol soil and an excess of nitrogen fertilizer reduced microbial biomass, activity and community structure of a particular group on grassland (Clegg, 2006; Fredrik *et al.*, 2008). These results showed soil bacterial community structure changes due to soil nutrient status as affected by long-term chemical fertilizers (Marschner *et al.*, 2003; Coats *et al.*, 2014).

Correlation between soil chemical property and bacterial diversity

Soil physicochemical property affects the bacterial community structure (Coats *et al.*, 2014). Table 5 showed that species richness estimate and diversity index are significantly affected by soil pH and exchangeable aluminum content. Species richness estimates such as Ace, Chao1 ($p<0.05$), Shannon index ($p<0.01$) showed the positive correlation with soil pH. But negative correlation with exchangeable aluminum content ($p<0.05$). The composition of the bacterial communities was closely defined by soil pH (Rousk *et al.*, 2010; Chaudhry *et al.*, 2012; Li *et al.*, 2012). He *et al.* (2012) reported that bacterial community diversity decreased by aluminum stress in acidic red soils. Soil pH affected bacterial diversity (Lauber *et al.*, 2009; Chaudhry *et al.*, 2012). Mercier *et al.* (2013) reported that bacterial community structure was significantly distinct related to soil physicochemical characteristics.

As above results, volcanic ash soil in citrus orchard showed low species richness estimates such as Ace, Chao1 and low diversity due to characteristics of volcanic ash soil (Mercier *et al.*, 2013).

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