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Effect of pH on soil bacterial diversity

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Abstract

Background: In order to evaluate the effect of pH, known as a critical factor for shaping the biogeographical microbial patterns in the studies by others, on the bacterial diversity, we selected two sites in a similar geographical location (site 1; north latitude 35.3, longitude 127.8, site 2; north latitude 35.2, longitude 129.2) and compared their soil bacterial diversity between them. The mountain soil at site 1 (Jiri National Park) represented naturally acidic but almost pollution free (pH 5.2) and that at site 2 was neutral but exposed to the pollutants due to the suburban location of a big city (pH 7.7).

Methods: Metagenomic DNAs from soil bacteria were extracted and amplified by PCR with 27F/518R primers and pyrosequenced using Roche 454 GS FLX Titanium.

Results: Bacterial phyla retrieved from the soil at site 1 were more diverse than those at site 2, and their bacterial compositions were quite different: Almost half of the phyla at site 1 were Proteobacteria (49 %), and the remaining phyla were attributed to 10 other phyla. By contrast, in the soil at site 2, four main phyla (Actinobacteria, Bacteroidetes, Proteobacteria, and Cyanobacteria) composed 94 %; the remainder was attributed to two other phyla. Furthermore, when bacterial composition was examined on the order level, only two Burkholderiales and Rhizobiales were found at both sites. So depending on pH, the bacterial community in soil at site 1 differed from that at site 2, and although the acidic soil of site 1 represented a non-optimal pH for bacterial growth, the bacterial diversity, evenness, and richness at this site were higher than those found in the neutral pH soil at site 2.

Conclusions: These results and the indices regarding diversity, richness, and evenness examined in this study indicate that pH alone might not play a main role for bacterial diversity in soil.

Keywords: Pyrosequencing, pH, Bacterial diversity, Biodiversity indices

Background

Soil environment, such as the water/sediment environment, are the most complex of all microbial habitats that include water and air (Horner-Devine et al. 2004). The first attempt at quantifying microbial diversity was undertaken in 1990 (Amann et al. 2001). However, questions regarding microbial diversity and geographic distribution have yet to be completely answered (Green et al. 2008; Maier and Pepper 2009; Madigan et al. 2010). One of the main difficulties associated with obtaining overviews of microbial diversity is the method of analysis used. Traditionally, for over a century, microbes have been cultured using media in microbial diversity studies, but it was realized comparatively recently that microbial diversities determined using a cultivation-based method due to the restriction of culture condition and media accounted for only 0.1 to 1 % of total bacterial communities (Torsvik et al. 1990). For example, the diversity of DNA extracted from bacteria in 1 g of soil is about 200 times that in same samples analyzed using a cultivation-based method (Torsvik et al. 1990), which substantially reduces reported numbers and diversities of microorganisms (Smit et al. 2001; Torsvik and Øvreås 2002). Accordingly, it is believed that results obtained using cultivation-based methods do not reflect the true diversities and compositions of bacterial communities (Smit et al. 2001; Sandaa et al. 2001). To solve this problem, ribosomal RNA (rRNA) (ribosomal DNA (rDNA)) molecules with highly conserved sequence region which is in all of organisms have been introduced (Amann et al. 2001; DeLong et al. 1989; Ludwig and Klenk 2001), and their use has increased rapidly in parallel with the development of molecular techniques, such as fluorescent in situ hybridization (FISH), polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), and bar-coded pyrosequencing for the study of microbial diversity in

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various environments (Amann et al. 2001; Torsvik and Øvreås 2002; DeLong et al. 1989; Ludwig and Klenk 2001; Schramm et al. 1998; Liang et al. 2011; Li et al. 2012).

In addition, recent studies have shown relations between microbes and environmental factors, such as geographical location (Fierer and Jackson 2006; Lauber et al. 2009), soil texture (Sessitsch et al. 2001), land use (Smit et al. 2001; Cookson et al. 2007; Brons and van Elsas 2008; Hartman et al. 2008; Will et al. 2001), pH (Fierer and Jackson 2006; Lauber et al. 2009; Hartman et al. 2008), nutrients (Cookson et al. 2007; Will et al. 2001; Han et al. 2008), contaminants like oil (Liang et al. 2011), and heavy metals (Sandaa et al. 2001; Kelly et al. 1999; Roane and Pepper 2000; Lee et al. 2008). Of the abovementioned factors, pH is regarded one of the most important in terms of shaping biogeographical patterns (Fierer and Jackson 2006; Lauber et al. 2009). Lauber et al. (2009) analyzed soils collected from 88 sites across North and South America that represented a wide range of ecosystems on a continental scale by pyrosequencing and postulated that five dominant bacterial groups, that is, Acidobacteria, Actinobacteria, Bacteroidetes, α-Proteobacteria, and β·γ-Proteobacteria dominated, and their abundances were independent of geographical location but dependent on pH, which was found to be significantly correlated with bacterial diversity at 66 sites. These authors concluded pH could be considered a predictor of biogeographical microbial patterns.

If pH is so critical to microbial community and its growth, the following question could be raised. How would microbial community structure differ between a site with naturally acidic conifer forest soil (low pH) that is ecologically stable and a (sub-)urban forest with neutral pH but probably loaded by several contaminants?

Furthermore, between ecological stability achieved in pollution-free environment and pH, which factor has the greater effect on microbial community dynamics? To answer these questions, we selected two sites for study.

Therefore, the purpose of this study was to compare bacterial community structure in natural acidic forest soil collected from the lodging area of Mt. Jiri, a Korean national park, with neutral mountain soil obtained in a suburban area using bar-coded pyrosequencing. In addition, to better understand the soil properties in the study areas, geochemical parameters, such as pH, concentration of ions, cation exchange capacity (CEC), and heavy metal concentration, were measured concurrently.

Methods

Site description and sampling

Soil samples were collected to determine the bacterial community structures of soils obtained from two mountains with different pHs and ecological stability. The first sample was collected from a lodging area of Mt. Jiri (site 1), a Korean national park, representative of acidic pH due to its dominant conifer vegetation (Korea Forest Service: http://www.forest.go.kr. Accessed 24 June 2015). The second sample was obtained from Mt. Jang (site 2), representing a less stable ecosystem and located close to the city of Busan (Fig. 1). At each site, about 200 g of minimally disturbed soil with high litter contents was obtained from a depth of 10 cm at 5 to 10 randomly selected locations within an area of 100 m^2. Soil samples were composited and stored in an ice box at 4 °C for transportation. On receipt, samples were sieved and mixed by passing them through a 2-mm mesh (10 mesh). Homogenized soils were frozen at −80 °C until subjected to microbial DNA extraction.

Geochemical property of soils

The pH values of air dried soil were measured using a pH Meter (Horiba, Japan) after mixing them with distilled water in a ratio of 1:5 (*w*:*v*) for 30 min. To measure water-soluble anion (NO_3^-, Cl^-, SO_4^{2-}, F^-) concentrations, 50 g soil samples was mixed with 500 mL of deionized water under continuous agitation for 12 h at 200 rpm on a reciprocal shaker (Ahn et al. 2007), and filtered through an 8-μm-pore-size filter (Whatman 40). Concentrations of anions in filtrate were determined by IC (Dionex ICS-3000, USA). Concentrations of exchangeable cations were measured by ICP (Varian 720-ES, USA) after extracting the filtrate with 1 M NH_4OAc and expressed in milliequivalents of negative charge per 100 g of soil (meq/100 g).

Heavy metals

The concentrations of heavy metals, such as Pb, Zn, Cd, Cu, As, Ni, Cr^{6+}, and Hg, were measured using the Korean standard method for soil pollution (Environment standard methods 2009). Briefly, 3 g of air dried soil was suspended in a Teflon vessel containing a mixture of 7 mL of 0.7 M nitric acid and 21 mL of 0.36 M hydrochloric acid. The solution was then adjusted to a final volume of 100 mL with 0.5 M nitric acid. The vessel was heated to 200 °C for 2 h on a heating mantle (Echofree, Korea) to ensure a complete digestion of the sediment. Concentrations of heavy metals were measured by ICP (Varian 720-ES, USA) and results are expressed as milligrams per kilogram.

Microbial analysis of soils

Enumeration of heterotrophic bacteria and fungi using culture media

To get a glimpse of the biodegradability of organic matter present at each study site, numbers of cultivable fungi and bacteria, that is, the so-called heterotrophic plate count (HPC), were enumerated by three replicates

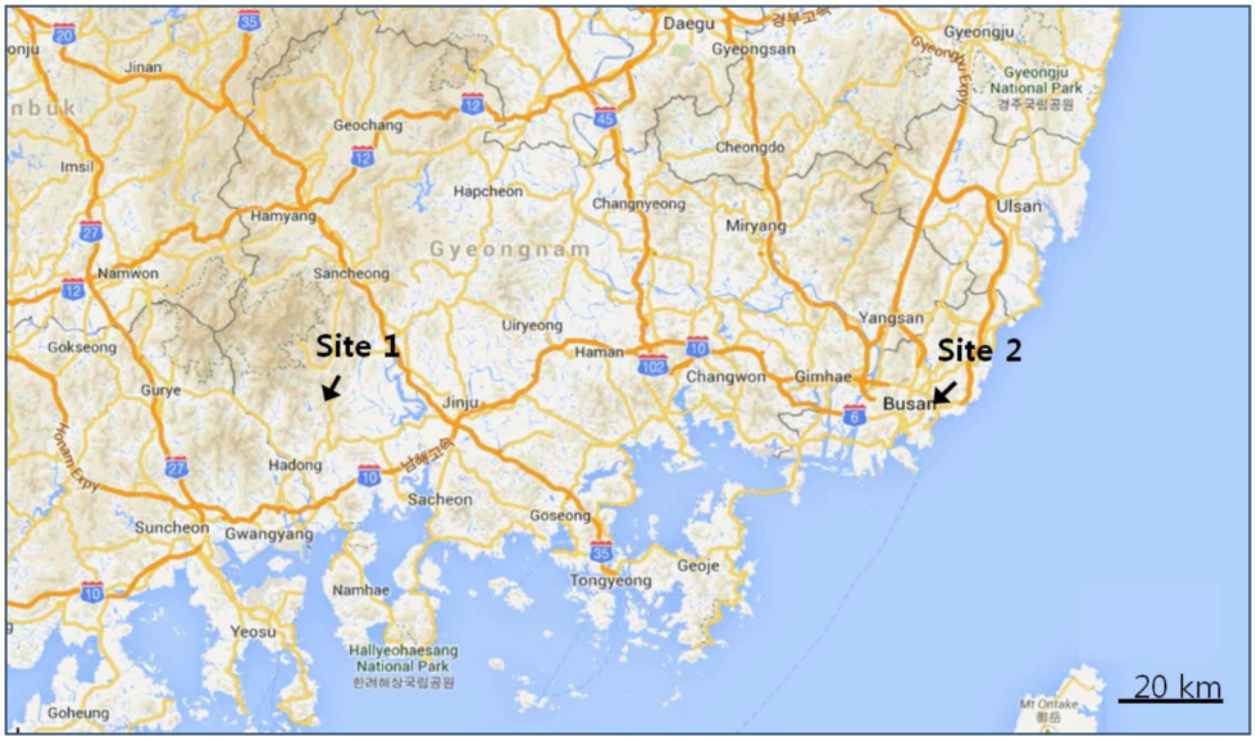

Fig. 1 This map of South Korea shows the two sampling sites. Site 1 which is the lodging area at Mt. Jiri is located southwest (north latitude 35.3; longitude 127.8) and site 2 which is Mt. Jang is located near the metropolitan city of Busan (north latitude 35.2; longitude 129.2)

using conventional plating techniques from soil slurries decimally diluted with sterile distilled water. Bacteria and fungi were cultured by Nutrient Agar (Difco, Baltimore, MD) or Potato Dextrose Agar (Difco, Baltimore, MD), respectively, and incubated at 30 °C for 48 h. Counting revealed 30 to 300 colonies per plate, and mean values were expressed in colony-forming unit (CFU) per gram of soil (dry weight).

Analysis of 16S rDNA for bacterial diversity by pyrosequencing

DNA extraction and bar-coded pyrosequencing

Metagenomic DNAs were extracted from 0.5 g soil samples using a FastDNA Spin Kit (MP, USA), with an additional 2 min of bead beating step to limit DNA shearing. Eluted DNAs were stored at −20 °C before a pyrosequencing procedure. A 20 ng aliquot of the 16S rDNA gene from the extracted DNA samples was amplified using universal primer sets, 27F (5′-GAGTTTGATCMTGGCTGG-3′) primer with a Roche 454 A pyrosequencing adapter and 518 R (5′-GTATTACCGCGGCTGCTGG-3′) with a Roche 454 B sequencing adapter. The targeted gene region is believed as the most reliable for the accurate taxonomic classification of bacterial sequences (Liu et al. 2007). Pyrosequencing library was prepared using PCR products according to the GS FLX titanium library prep guide.

Fast Start High Fidelity PCR System (Roche) was used for PCR under the following conditions: 94 °C for 3 min followed by 35 cycles of 94 °C for 15 s; 55 °C for 45 s; 72 °C for 60 s; and a final elongation step at 72 °C for 8 min. After PCR, the products were purified using AMPure beads (Beckman-Coulter, USA). Sequencing was performed on a Roche 454 GS FLX Titanium from Macrogen Ltd. (Seoul, Korea).

Processing of pyrosequencing data

After sequencing, reads were sorted by tag sequence (bar-coded sequence). Then the sequences were trimmed to remove any non-16S bacterial reads such as barcodes, primers, chimeras, and sequences less than 350 bp by the in-house software of Macrogen (Korea). Extracted sequences were clustered by OTU (operational taxonomic unit) sequences which were obtained by calculating at a 97 % similarity cut-off (Hwang et al. 2014). OTU clustering was performed using software of mothur (v.1.27.0) and CD-HIT-OUT (OTU clustering, mothur (version 1.27.0). (http://www.mothur.org), CD-HIT-OTU (http://weizhong-lab.ucsd.edu/cd-hit-otu/). Accessed 29 Aug 2013). Representative sequences from each phylotype were blasted, and the taxonomic identity of each phylotype was determined using the Silva rRNA database (Silva rRNA database (http://www.arb-silva.de/). Accessed 29 Aug 2013). Results were calculated by

expressing same reads as percentages of total classifiable reads.

Diversity indices

To assess bacterial diversities, we calculated Shannon-Weaver indices (*H′*), Simpson indices, evenness indices (*E*) developed by Pielou, and richness by Margalef were calculated with the extracted OTU sequences as described by Kennedy and Smith (1995). To unify the measurement criterion regarding diversity indices of Shannon-Weaver and Simpson, the reciprocal of the Simpson index was used because Shannon-Weaver index is a high number for high diversity, but Simpson index is a low number for high diversity.

Results and discussion

Geochemical properties of soils in the studied area

Anions and exchangeable cation concentrations, pH values, and CEC at both sites are summarized in Table 1. The soil from site 1 (lodging area of Jiri National Park) had an acidic pH of 5.2, while that from site 2, which was located close to the metropolitan city of Busan, was almost neutral (pH 7.7), which is in the optimal range (pH 6–8) required for microbial growth (Maier and Pepper 2009). The pH value of soil affects the solubilities of chemicals by influencing ionization degrees (Maier and Pepper 2009). It should be added that the pH values at the two sited mentioned above are integrated results due to numerous interactions between cations and anions in the soil solution (Fierer and Jackson 2006). Just the large difference in pH values at the two sites implies that the geochemical environment of both sites differed. As has been reported by others (Fierer and Jackson 2006; Lauber et al. 2009), we presumed that pH played a definite role on the diversities and compositions of bacterial community.

The total concentration of anions at site 1 was greater than at site 2. In particular, the concentration of NO_3^-, which can be utilized immediately by microbes and plants, was much higher at site 1 (12.7 cmol/kg) than at site 2 (0.04 cmol/kg). For total exchangeable cations (Ca^{2+}, Mg^{2+}, K^+, Na^+), their summed concentration at site 1 was almost the same as that at site 2 (Table 1). CEC, a measure of the capacity of soils and organic colloids to remove cations from solution, varies depending on the type of soil, and its value increases in line with the decomposition rate of organic matter by microorganisms (Alexander 1977). At the time of the sampling in November of 2012, the sites were already densely covered by litter to be degraded by microbes; determined values of CEC to an extent reflect the decomposing of organic matter (leaves).

The CEC of 30.7 meq/100 g determined for soil from site 1 indicates relatively better conditions for microbial growth than at site 2 (13.0 meq/100 g). According to Maier and Pepper (2009), the average of CEC of soils range from 15 to 20 meq/100 g, and that CEC values of <15 meq/100 g leads to low nutrient levels in soil because of a reduced capacity to retain cations and essential nutrients, such as NO_3^- and PO_4^{3-}. Other factors, such as soil particle size, water, and nutrient availability not investigated in this study, might also have influenced the soil environment in various, but not fully explored, ways, as reported by others (Cookson et al. 2007; Brons and van Elsas 2008; Hartman et al. 2008; Will et al. 2001; Han et al. 2008). Particle size might change chemical properties by changing adsorption affinities (Maier and Pepper 2009). For example, small particle soil (silt and clay) allow more diverse microbial inhabitants than large particle. In terms of dominant bacterial groups related to particle size, bacteria belonging to the phylum Acidobacteria and the genus *Prosthecobacter* sp. (phylum Verrucomicrobia) were found to be more diverse in soils with small particles, whereas α-Proteobacteria dominated in large particle soils (Sessitsch et al. 2001). In addition, the availabilities of nutrients and organic matter also strongly influence bacterial abundances and diversities (Smit et al. 2001; Hartman et al. 2008; Will et al. 2001; Han et al. 2008).

Heavy metals

Of the eight heavy metals detected in this study, Cr^{6+} and Hg were almost undetectable (less than 0.0001 mg/kg) at both sites. Addressing the remaining six metals in decreasing order, Zn was detected at the highest concentration at both sites, although its value at site 1 (47.26 mg/kg) was lower than at site 2 (58.43 mg/kg). Pb and Ni were present at site 1 at slightly higher concentrations (19.43 and 13.27 mg/kg vs. 13.04 and 11.44 mg/kg), which was unexpected. Cu had concentrations of 11.67 and 14.25 mg/kg at

Table 1 Chemical properties of the soil analyzed

Sample	pH	CEC (meq/100 g)	Anion (cmol/kg DW)					Cation (cmol/kg DW)				
			F^-	Cl^-	NO_3^-	SO_4^{2-}	Total	Ca^{2+}	K^+	Mg^{2+}	Na^+	Total
Site 1	5.2	30.7	0.03	0.51	1.27	1.18	2.99	10.0	0.75	0.99	0.19	13.90
Site 2	7.7	13.0	0.12	0.46	0.04	0.99	1.61	12.1	0.18	0.63	0.16	13.00

DW (dry weight)

sites 1 and 2, respectively. As had concentrations of 4.64 and 5.11 mg/kg, respectively, and Cd had concentrations of 0.40 and 1.35 mg/kg, respectively. So the concentrations of heavy metals, with the exception of Pb and Ni, were higher at site 2 than in soil from the Korean National Park (site 1), as depicted in Fig. 2. It has been reported that heavy metals can not only inhibit microbial growth and activity but also shift bacterial populations from heavy metal non-resistant to resistant populations over time (Kelly et al. 1999; Roane and Pepper 2000). Kelly and coworkers (1999), in a laboratory investigation on the effects of a Zn smelter on microbes, added 6000 mg/kg of Zn to soil, and 15 days later found Zn level in soil had reduced to 4660 mg/kg, which was ascribed to adsorption on the surfaces both of soil and microbes (Lee et al. 2008), and that cultured bacteria (isolates) had reduced by 87 %. However, over the course of the experiment, it was found that the bacterial composition had changed from a non-resistant to a resistant population. In addition, contamination by Cd or Pb at concentrations of 5–55 mg/kg and 75–1660 mg/kg, respectively, reduced of bacterial numbers by up to 1 %. Accordingly, it would appear the relatively low concentration of heavy metals found at both sites was insufficient to have affected microbial growth. However, the slightly higher heavy metal levels at site 2 might have had a negative effect on the dynamics of the bacterial community.

Moreover, in accord with the abovementioned results concerning the geochemical property of soils, the number of heterotrophic bacteria/fungi was higher at site 1 than at site 2 (Table 2). In the site 1 soil sample, numbers of heterotrophic bacteria (HPC) which is considered as an indicator of easily degradable organic compounds (Maier and Pepper 2009) were more than ten times greater than that at site 2.

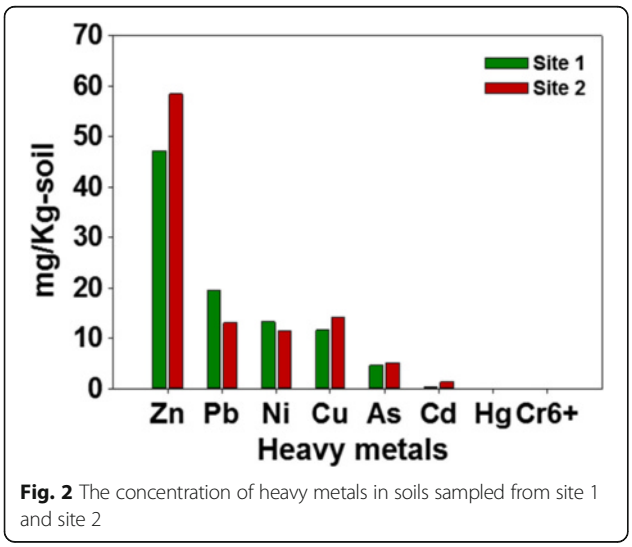

Fig. 2 The concentration of heavy metals in soils sampled from site 1 and site 2

Table 2 Microbiological characteristics evaluated by culturing bacteria and fungi

Sample	HPC (bacteria, CFU/g DW)	Fungi (CFU/g DW)
Site 1	9.2×10^7	3.6×10^3
Site 2	8.0×10^6	6.0×10^2

HPC heterotrophic plate count, *CFU* colony-forming unit, *DW* dry weight

Taken all together, the geochemical environment at site 1 seems to be more favorable for microbial growth than that at site 2. Because of the greater heterogeneity of soil per se that was revealed from millimeters of a micro scale, the so-called micro-environment (Madigan et al. 2010; Schramm et al. 1998; Hartman et al. 2008), maybe up to a continental scale, soil investigations are very time-demanding and costly, as compared with similar investigation on water or air. Nevertheless, overall but precise knowledge regarding the physico-geochemical properties of soil gathered through sophisticated methods is an important prerequisite to the understanding of its microbiological characteristics.

Comparison of bacterial compositions at the two sites

From the 7614 retrieved sequences, 344 chimeric sequences were removed using ChimeraSlayer (Haas et al. 2011) to avoid misreading, and the remaining 7270 classifiable sequences were analyzed by pyrosequencing (Table 3). For checking the validity of data used in this study, rarefaction curves, which were generated using the relationship between the OTU numbers and the sequence reads, were created using mothur output (data not shown) (OTU clustering, mothur (version 1.27.0). (http://www.mothur.org), CD-HIT-OTU (http: //weizhong-lab.ucsd.edu/cd-hit-otu/). Accessed 29 Aug 2013). Based on the rarefaction curve, the numbers of reads obtained were sufficient to assess the bacterial diversity at both sites.

Bacteria at both sites were affiliated with 12 phyla across the entire data set. Regarding the bacterial diversity observed on the phylum level in Table 4, with the exception of unclassified (1.4 %), the 1428 classifiable sequences retrieved from site 1 were distributed widely to 11 different phyla, while those for site 2 belonged to only six phyla. Three phyla, Proteobacteria (site 1; 49.2 %, site 2; 21.8 %), Actinobacteria (site 1; 21.8 %, site 2; 29.8 %), and Cyanobacteria (site 1; 9.8 %, site 2; 17.5 %) dominated both sites. The next most abundant phylum, Planctomycetes (7.2 %), at site 1 was not observed at site 2, whereas Bacteroidetes, which is regarded as a typical inhabitant of soil (Madigan et al. 2010; Lauber et al. 2009; Will et al. 2001), was much more abundant at site 2 (site 1; 0.5 %, site 2; 24.3 %,). Acidobacteria, known as bacteria occurring frequently in

Table 3 Data sets used for pyrosequencing and bacterial diversity indices

Samples	No. of reads		No. of OTUs	Richness (Margalef)	Evenness (Pielou)	Diversity index	
	Raw	Chimera removed				Simpson ($D = 1/\lambda$)[a]	Shannon-Weaver (H')
Site 1	1465	1428	165	22.58	0.93	0.99	4.76
Site 2	6149	5842	66	7.49	0.64	0.85	2.65

[a]Reciprocal of Simpson index and Shannon-Weaver index, higher numbers represent greater diversity

not only acidic soil but in all kinds of soil (Smit et al. 2001; Lauber et al. 2009; Will et al. 2001; Han et al. 2008), accounted only for a small proportion at both sites (site 1; 2.7 %, site 2; 1.1 %). On the other hand, somewhat unexpectedly, Cyanobacteria made up high proportion (site 1; 9.8 %, site 2; 17.5 %) at both sites. In a study on the bacterial diversity based on 16S rDNA clone from Korean acidic pine (pH 4.1) and oak wood (pH 5.3) soil, Proteobacteria was found to be the most dominant, followed by Firmicutes, Acidobacteria, Actinobacteria, Bacteroidetes, Verrucomicrobia, and Planctomycetes, but not Cyanobacteria (Han et al. 2008). In a loamy sand soil with a little acidic pH (5.5–6.5), Brons and van Elsas (2008) observed the bacterial community by clone analysis and found Cyanobacteria in a minor proportion.

In agreement with studies by others (Madigan et al. 2010; Brons and van Elsas 2008; Han et al. 2008), Proteobacteria, the most abundant phylum in soil, composed almost half of total bacteria at site 1, and of the classes of this phylum, Alpha(α)·Beta(β)·Gamma(γ)··Delta(δ)·Zeta(ζ)·Epsilon(ε)-Proteobacteria, the first three classes α·β·γ-Proteobacteria dominated with proportions of 28.7, 5.3, and 13.6 % at site 1, while those at site 2 accounted for 18.3, 5.2, and 2.0 %, respectively (Fig. 3). In a comprehensive study, bacterial composition based on the analysis of 287,933 sequences obtained from soil across the large spatial scale revealed that the phyla Proteobacteria, Acidobacteria, Bacteroidetes, Verrucomicrobia, and Planctomycetes dominated, but in different proportions depending on soil characteristics and geographical location (Madigan et al. 2010).

Agricultural use of land also causes changes in bacterial diversity and composition (Smit et al. 2001; Will et al. 2001). Smit et al. (2001) analyzed the bacterial community in a wheat field using a cultivation-based method and by analyzing 16S rDNA clone sequences and found that high GC Gram-positive bacteria were mainly detected by a cultivation-based method and not by clone analysis. Instead, based on clone analysis, Acidobacteria Proteobacteria, Nitrospira, Cyanobacteria, and green sulfur bacteria dominated, and they were found to be more evenly distributed. This result demonstrates the limitation of the cultivation-based method. Moreover, the abundance of γ-Proteobacteria, which is regarded as a fast growing bacteria in nutrient-rich environment like *Pseudomonas* sp. (Smit et al. 2001), was unexpectedly greater at site 1 than at site 2 (Fig. 4), which was probably due to litter decomposition. It was reported that plant root showed a selective effect towards γ-Proteobacteria (Marilley and Aragno 1999), which comprise the majority of fast growing decomposer for easily degradable substrates (Madigan et al. 2010). In addition, the phylum Elusimicrobia, previously known as "Termite Group 1" and occurring in various environments

Table 4 Comparison of soil bacterial composition between two sites on the phylum level

Phylum	Site 1 (%)	Site 2 (%)
Proteobacteria	49.2,	21.8
Actinobacteria	21.8	29.8
Cyanobacteria	9.8	17.5
Bacteriodetes	0.5	24.3
Planctomycetes	7.2	0
Acidobacteria	2.7	1.1
Firmicutes	1.3	5.5
Chloroflexi	2.5	0
Verrucomicrobia	2.1	0
Elusimicrobia	1.2	0
Gemmatimonadates	0.3	0
Unidentified	1.4	0.1

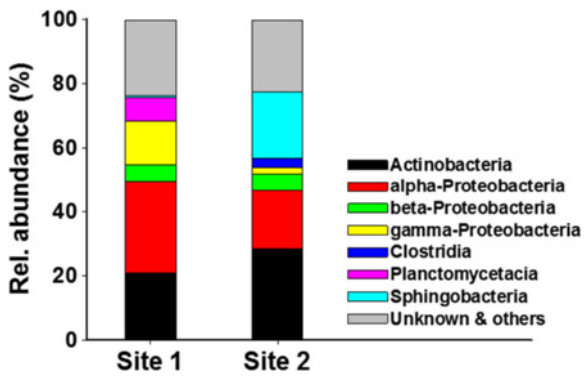

Fig. 3 Difference in bacterial composition on class level obtained from soils at site 1 and site 2. Their relative abundance was assessed by grouping the OTUs derived from 16S rDNA retrieved from each soil. Based on classifiable sequences, the OTUs were determined from the mothur clustering. Others are the sum of minor classes which individually show a relative abundance of less than 3 %

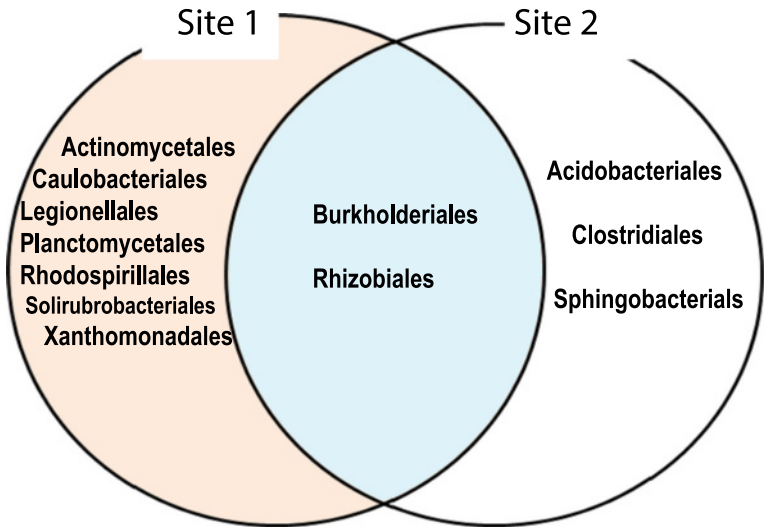

Fig. 4 A Venn diagram showing the distribution of phylotypes identified on order level by 16S rDNA pyrosequencing from soils at site 1 and site 2. There were only two orders, Burkholderiales and Rhizobiales, belonging to β-Proteobacteria and α-Proteobacteria, respectively, at both sites

(Herlemann et al. 2007), was present in a lower proportion at site 1 (1.2 %), but not at site 2 (Table 4).

Differences between the bacterial compositions at the two sites became clearer when the distribution of phylotypes were examined on the order level using a Venn diagram, as shown in Fig. 4. To make the Venn diagram, orders which represented less than 3 % of relative abundance were discarded. Only two orders, Burkholderiales and Rhizobiales, were found at both sites. The common order Burkholderiales belongs to β-Proteobacteria and was found in similar proportions at both sites (Fig. 4). The other order Rhizobiales belongs to α-Proteobacteria and includes genus *Rhizobium* that is able to fix nitrogen and is associated with the roots of legumes (Madigan et al. 2010). The genus *Rhizobium* is a typical soil inhabitant in the rhizosphere, which is considered a nutrient-rich niche (Marilley and Aragno 1999). Rhizobiales constituted a high proportion at both sites [site 1; 18.2 %, site 2; 10.3 % (Fig. 4)]. At site 2, bacteria belonged to three different orders, Acidobacteriales, Clostridiales, and Sphingomonadales, whereas at site 1, seven different orders were identified (Actinomycetales, Caulobacterales, Legionellales, Planctomycetales, Rhodospirillales, Solirubrobacterales, Xanthomonadales). Based on our assessment of the bacterial community, it could be concluded that the soil ecosystem at site 1 may have a higher degree of bacterial diversity than at site 2. Of course, the degree of variability resulted from phylogenetic assemblages does not reflect degree of functional or ecological diversity. Therefore, more detailed studies, including its functional capability, are needed to determine why these bacteria are present in given soil.

Status of bacterial communities as evaluated using different indices

Originally, the diversity index was developed to assess diversity and stability of plant and animal communities (Kennedy and Smith 1995), but nowadays it is also used in bacterial community (Smit et al. 2001; Liang et al. 2011; Han et al. 2008; Hwang et al. 2014). In the present study, the Shannon-Weaver index and the reciprocal of Simpson index were used to assess diversity, and the Margalef and Pielou indices were used to assess richness and evenness (Kennedy and Smith 1995). As shown in Table 3, the index of diversity refers to the number of different phylotypes, the index of richness refers to the abundance of the same phylotype, and the index of evenness refers to how close in numbers phylotypes are to each other in the bacterial community. The numbers were much higher for site 1, even showing an acidic pH (5.2).

This implies that at site 1 bacterial communities, which act primarily as decomposers, were more diverse than at site 2. Accordingly, it would appear that nutrient cycling, which is achieved by the interactions between many types of microbes, would be smoother at site 1 than at site 2 (Cookson et al. 2007; Hartman et al. 2008; Will et al. 2001). As compared with the results obtained for bacterial diversities in Korean mountain pine and oak woods, for which the Shannon-Weaver index was estimated to fall in a range of from 3.42 to 3.63 (Han et al. 2008), our results (site 1; 4.76, site 2; 2.65) show that in terms of bacterial biodiversity, the soil ecosystem at site 1 was more stable than that at site 2.

Conclusions

In order to determine the effect of pH on bacterial diversity, we compared the bacterial communities of acidic soil (pH 5.2) from Jiri National Park's lodging area (site 1) with neutral mountain soil (pH 7.7, site 2) by bar-coded pyrosequencing of 16S rDNA.

Regarding the question, which factor has the greater effect on microbial community dynamics either pH or ecological stability of studying site, pH may have an effect on bacterial diversity. So depending on pH, the bacterial community in soil at site 1 was found to differ from that at site 2, and although the acidic soil of site 1 represented a non-optimal pH for bacterial growth, the bacterial diversity, evenness, and richness at this site were higher than those found in the neutral pH soil at site 2. Accordingly, these results imply that pH might not be a critical factor for shaping bacterial diversity and its stability. However, this study was performed over a short period without regard of the effects of other environmental factors, such as precipitation, oxygen, and some other primary nutrients such as N and P. So nothing could be considered as conclusive proof. Therefore, we suggest more advanced detailed studies in a long term should be conducted to identify the environmental factors responsible for the establishment of particular bacterial community structure.

Abbreviations

CEC: Cation exchange capacity; CFU: Colony-forming unit; DW: Dry weight; FISH: Fluorescent in situ hybridization; OTU: Operational taxonomic unit; PCR-DGGE: Polymerase chain reaction-denaturing gradient gel electrophoresis

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Availability of data and materials

Data sharing is not applicable to this article as no data sets were generated or analyzed during the current study.

Authors' contributions

SJC and YOL carried out in the design of the study and the molecular genetic studies, participated in the sequence alignment, and drafted the manuscript. MHK analyzed the chemical properties of the sampled soil. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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