Elevational Gradients in Microbial Diversity:
A case study of Mt. Fuji and Mt. Halla

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A thesis submitted for the Partial Fulfillment

of the Degree of Doctor of Philosophy

February 2013

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ABSTRACT

Little is known of how microbial diversity and community ecology behaves along elevational gradients. We chose to study Mount Fuji of Japan as a geologically and topographically uniform mountain system along with Mt. Halla of Jeju Island, South Korea, a massive shield volcano, consisting of a mosaic of slightly different volcanic types (mainly trachybasalt and basalt) of different ages.

PCR-amplified soil DNA for the archaeal and bacterial 16S rRNA gene was pyrosequenced and taxonomically classified against EzTaxon-e microbial database for a wide range of elevational zones on both the mountains. Previous studies on soil bacteria/archaea have variously found either a diversity decline, or no trend. However most of these studies did not control for confounding geological factors. Here we studied how microbial diversity and community composition varies in relation to elevation.

There was a significant "peak" in total bacterial/archaeal diversity at certain elevations for both the mountain ranges except for the Yeongsil transect on Mt. Halla which had a "hollow" (U-shaped) trend with elevation, rarely observed in elevation studies in nature. Individual bacterial/archaeal phyla show distinct trends with elevation—increase, decrease, or a mid-elevational "bulge" in diversity.

Elevation, together with the closely related parameters of mean annual temperature and mean annual precipitation, was clearly the best predictor of variation in community composition on both the mountains. These variables exceeded the explanatory power of all other measured variables such as pH, organic C, N and P on Mt. Halla whereas on Mt. Fuji microbial soil communities were also highly responsive to soil environmental gradients, in terms of both their diversity and community composition. Distinct communities of archaea and bacteria specific to each elevational zone on Mt. Fuji suggest that many microbes may be quite finely niche-adapted within the range of soil environments. A further interesting finding is the presence of a mesophilic component of archaea at high altitudes on a mountain that is not volcanically active. This emphasizes the importance of microclimate – in this case solar heating of the black volcanic ash surface for the ecology of soil archaea.

A "hollow" trend that has not been found before in studies of microbial diversity on mountains, and set alongside with the other diversity trends we found here on Mt. Halla,

emphasizes that no simple rule can be generalized for the world's mountain systems. Apart from elevational soil chemistry and climatic factors, stochastic processes involving complex environmental mosaics may also be playing a role in shaping the displayed patterns observed on both the mountains.

Keywords: microbes, soil, pyrosequencing, elevation, Mt. Fuji, Mt. Halla, volcanic.

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CHAPTER 1. GENERAL INTRODUCTION

1.1 ELEVATIONAL GRADIENTS IN NATURE

The best documented pattern in macroecology (Brown and Maurer 1989) after the latitudinal gradient in species diversity is the elevational gradient (Rahbek 1995, Rahbek 2005). Species richness along elevational gradients was previously assumed to increase universally from cool highlands to warm lowlands, mirroring the latitudinal increase in species richness from cool to warm latitudes (MacArthur 1972, Rohde 1992, Rahbek 1995). However, a recent quantitative analysis of elevational species richness gradients including 204 data sets demonstrated that about 50% of the elevational patterns were hump-backed, about 25% showed a monotonically decreasing pattern, and about 25% followed other distributions(Rahbek 2005).

Elevational gradients offer many characteristics that make them perhaps more suitable for uncovering the underlying cause(s) of spatial variation in diversity than latitudinal gradients.

Firstly, there are many replicates of elevational diversity gradients – essentially each mountain or mountain range is a replicate, so it is possible to test for the generality of the underlying cause(s). This approach of examining the generality of elevational diversity gradients by focusing on several replicate elevational gradients within the same region has been used in many recent studies, so that species occurring along the gradient might come from the same regional species pool and share similar evolutionary histories (Sanders 2002, Grytnes 2003, Wang et al. 2009).

Secondly, field data can be collected more readily along elevational gradients than along latitudinal gradients, simply because the spatial extent of elevational gradients is small relative to latitudinal gradients.

Thirdly, although of less relevance in this particular study, it is readily possible to carry out manipulative experiments along elevational gradients as in case of reciprocal transplants done by Angert and Schemske (2005) to estimate the potential elevational ranges of Monkey flowers in the Sierra Nevada of California.

Additionally, altitudinal gradients are highly suitable for the study of the influences of contemporary climate, history and stochastic factors, as these vary along the altitudinal gradient itself and with the geographical (latitudinal) position of the gradient.

Given the ease of studying elevational gradients relative to latitudinal gradients, it seems clear that they can be useful tools for understanding the underlying cause(s) of diversity gradients. And, in fact, there is a growing appreciation of the utility of elevational gradients as tools to uncover the mechanisms that shape both patterns of biodiversity and the functioning of ecosystems (Fukami and Wardle 2005, Nogues-Bravo et al. 2008).

Although study of elevational patterns of macroscopic taxa has a history going back two centuries (Rohde 1992, Rosenzweig 1995, Willig et al. 2003), until recently such patterns had not been reported for microorganisms and remained poorly understood. As a result, it is unclear whether microbes exhibit elevational gradients in diversity that parallel those reported for macroorganisms. This represents a key gap in ecological knowledge, especially given the ubiquity, abundance, and functional importance of microbes. One of the most important reasons for this could be the un-culturability or as yet un-cultivability (used to describe microorganisms that have yet to be grown on artificial media in vitro) of microorganisms. Approximately 1% of bacteria on Earth can be readily cultivated in vitro – the so called 'great plate count anomaly', based on the observation that microscopic counts are considerably larger than the equivalent total viable counts (Staley and Konopka 1985, Amann et al. 1995, Hugenholtz et al. 1998). There are currently estimated to be 61 distinct bacterial phyla, of which 31 have no culturable representatives (Hugenholtz et al. 2009). The topology of the archaeal phylogenetic tree remains uncertain, but it is clear that the 54 species of Archaea cultured to date represent only a fraction of the total diversity, with 49 major lineages mostly uncultured (Auguet et al. 2010). Until recently, representative elevational studies were impossible for microorganisms, arguably the most diverse and abundant group of organisms on Earth. The study of microbial ecology has been revolutionized by metagenomics, the culture-independent cloning and analysis of microbial DNA extracted directly from an environmental sample. The age of inexpensive, high throughput pyrosequencing allows the assessment of the full taxonomic diversity of microbes in soil for the first time (Roesch et al. 2007).

In this study, we examined two elevational gradients, one being the Mt. Fuji (35°21′28.8″N 138°43′51.6″E) in the eastern Japan from 1000 to 3700masl. This is a volcanic cinder cone covered by moderately silica rich volcanic ash of nearly uniform composition and the other being Mt. Halla (33°12'42" N 126°31'45" E), a shield volcano, consisting of a mosaic of slightly different volcanic types (mainly trachybasalt and basalt) of different ages. We used a bar-coded pyrosequencing procedure to compare diversity levels and the composition of the microbial communities in each of the habitats across the elevation gradient. Previous work has demonstrated that this molecular method can effectively detect pronounced gradients in bacterial diversity that corresponds to changes in environmental factors (Lauber et al. 2009, Rousk et al. 2010). Additionally, we tested several of the proposed factors potentially underlying global biodiversity trends. Climate, vegetation and soil chemistry were studied to determine if microbes exhibit trends in biodiversity that differ significantly from those commonly observed with plants and animals.

1.2 METAGENOMICS APPROACH

1.2.1 Sequencing metagenomics and Pyrosequencing

The term metagenomics (Handelsman et al. 1998), refers to culture-independent studies of the collective set of genomes of mixed microbial communities and applies to explorations of all microbial genomes in consortia that reside in environmental niches, such as in soils, in water, in plants, or in animal hosts.

Metagenomics and its associated meta-strategies have arrived at the forefront of biology primarily because of two major developments. The deployment of next-generation DNA-sequencing technologies in many centers has greatly enhanced capabilities for sequencing large meta–data sets(DeLong 2009 and the references within). The second key development is an emerging appreciation for the importance of complex microbial communities in mammalian biology and in human health and disease. The Human Microbiome Project was approved in May 2007 as one of 2 major components (in addition to the human epigenomics program) of Road Map version 1.5 of the US NIH (Turnbaugh et al. 2007). The "metagenome" of microbial communities that occupy various sites in the body is estimated to be approximately 100-fold greater in terms of gene content than the human genome.

Most of the data gathered up to the middle of the 2000s had been compiled using Sanger (dideoxy) sequencing platforms, but most recent studies have focused on emerging parallel DNA-sequencing technologies based on pyrosequencing. Such next-generation sequencing (NGS) systems introduce possibilities for deeply sequenced data collections and strategies aimed at microbial identification via single genetic targets or whole-genome methodologies.

Several important issues have recently emerged with respect to metagenomics and microbes. One issue is that the science of metagenomics, in contrast to individual microbial or animal genomes, is ultra-complex and challenged by the existence of vast unknowns, often known as knowledge "deserts."

Several studies published in the 1990s indicated that sequencing of 16S rRNA genes could be useful for pathogen discovery and identification (Relman et al. 1991, Kolbert et al.

2004). Prior studies of bacterial evolution and phylogenetics provided the foundation for subsequent applications of sequencing based on 16S rRNA genes (or 16S rDNA) for microbial identification (Winker and Woese 1991). The initial studies were based on Sanger-sequencing strategies that included targeted sequencing of 16S rRNA genes (approximately 1.5 kb of target sequence). However, with the advent of NGS, sequence-based identification could be established with a reasonable amount of confidence from relatively long reads and with the aid of sequence-classifier algorithms that included most of the 16S rDNA coding sequence. It is now known that less than half of the coding sequence (approximately 500 bp, including several hypervariable regions), may be sufficient for genus and species level pathogen identification via Sanger sequencing (Kolbert and Persing 1999, Kolbert et al. 2004). As sequence targets for microbial identification have become more precisely defined, the introduction of pyrosequencing has provided a user-friendly approach for the clinical laboratory that has enabled more extensive sampling of microbial diversity with improved labor efficiencies (Luna et al. 2007).

DNA pyrosequencing, or sequencing by synthesis, was developed in the mid-1990s as a fundamentally different approach to DNA sequencing (Ronaghi et al. 1996). Sequencing by synthesis occurs by a DNA polymerase–driven generation of inorganic pyrophosphate, with the formation of ATP and ATP-dependent conversion of luciferin to oxyluciferin (Fig. 1). The generation of oxyluciferin causes the emission of light pulses, and the amplitude of each signal is directly related to the presence of one or more nucleosides. One important limitation of pyrosequencing is its relative inability to sequence longer stretches of DNA. Sequences rarely exceeded 100–200 bases with first- and second-generation pyrosequencing chemistries but after the launch of GS FLX titanium reagents in 2008, sequences with 400-500base pair read lengths can be obtained.

The term 454 sequencing refers to high-throughput sequencing platforms (e.g., Roche/454 Life Sciences) for metagenomics that are based on pyrosequencing chemistry. 454 Life Sciences (now a subsidiary of Roche Diagnostics) was the one company that commercially developed pyrosequencing for metagenomics; hence the term “454 sequencing”.

The 454 instruments are the most widely deployed next-generation sequencing systems currently in the scientific community, and these pyrosequencing-based platforms preceded other high-throughput platforms, such as the Illumina/Solexa and SOLiD

technologies. Each 454 platform uses a modern adaptation of DNA-pyrosequencing chemistry (Bentley 2006, Margulies et al. 2006).

Generally, the sequencing community regards the 454 technology as advantageous because of the technical robustness of the chemistry. The relatively long reads generated by 454 sequencing allow more frequent unambiguous mapping to complex targets than the products of the other next-generation technologies, which feature shorter reads. During the past decade, sequencing read lengths have improved because of refinements in pyrosequencing biochemistry, such as the addition of recombinant enzymes including single-stranded binding protein (Ronaghi 2000, Mashayekhi and Ronaghi 2007).

Additionally, the large numbers of reads per run that are possible with 454 technology, delivering much greater depth of coverage for metagenomic sequencing than Sanger sequencing.

DNA pyrosequencing has been successfully applied to microbial identification by combining informative target selection (e.g., hypervariable regions within the 16S rRNA gene) and signature-sequence matching (Jonasson et al. 2002, Tarnberg et al. 2002).

Because of the relatively short read lengths, DNA-pyrosequencing applications for microbial identification have focused attention on hypervariable regions within small ribosomal-subunit RNA genes, especially 16S rRNA genes. Specific hypervariable regions have preferentially been used to identify different groups of bacteria via pyrosequencing (Monstein et al. 2001, Tarnberg et al. 2002).

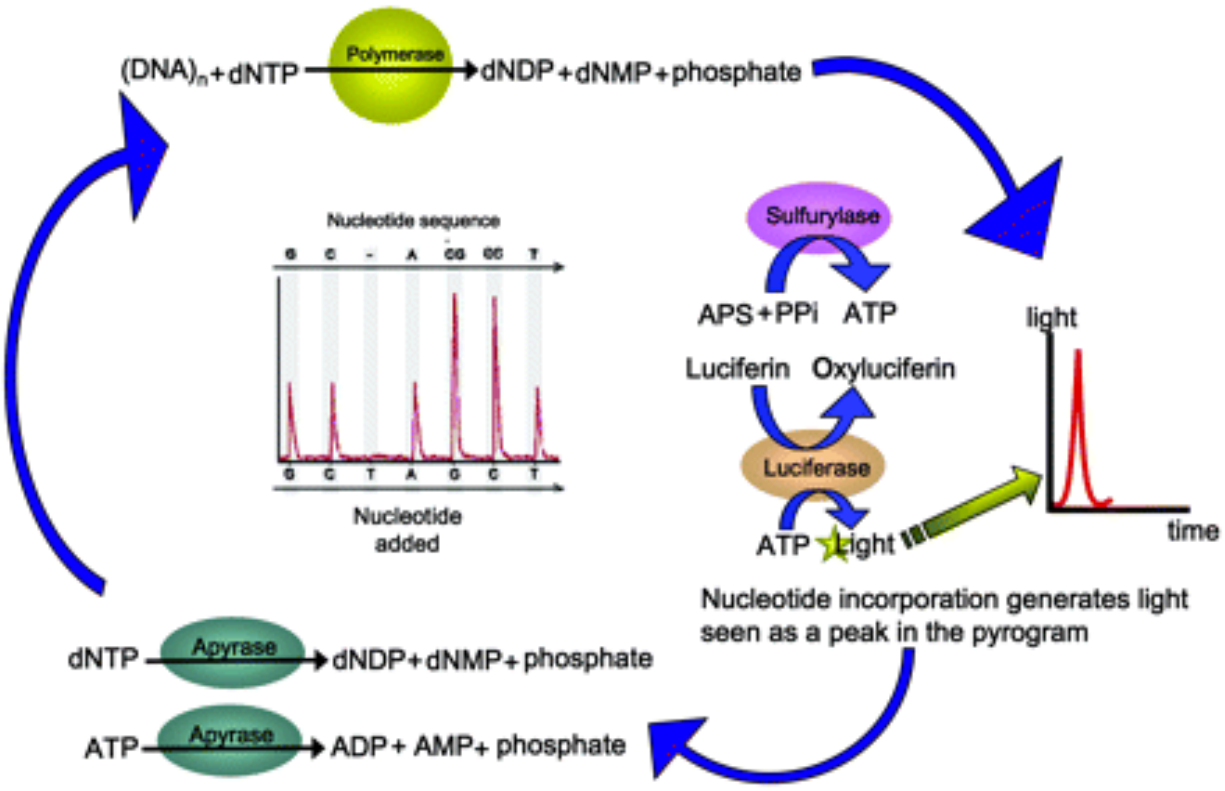

Fig. 1. Pyrosequencing Chemistry: biochemical reactions and enzymes involved in the generation of light signals by DNA pyrosequencing (Marsh 2007)

1.2.2. Microbial Identification Strategies

Metagenomic strategies may be directed at examining microbial composition of simple cultures, or the more challenging task of tackling phylogenomic diversity of highly complex microbial populations. In the case of complex communities, one basic approach is to exploit universal and conserved targets, such as rRNA genes. By amplifying selected target regions within 16S rRNA genes (Fig. 2), microbes (specifically bacteria and archaea) can be identified by the effective combination of conserved primer-binding sites and intervening variable sequences that facilitate genus and species identification. With 16S rRNA gene sequence data, genera and species are typically distinguished at levels of 95% and 97% pairwise sequence identities, respectively (Peterson et al. 2008). The 16S rRNA gene in bacteria consists of conserved sequences interspersed with variable sequences that include 9 hypervariable regions (V1–V9, Fig. 2). The lengths of these hypervariable regions range from approximately 50 bases to 100 bases, and the sequences differ with respect to variation and in their corresponding utility for universal microbial identification. A recent study documented that the longest stretch of totally conserved bases in 16S rDNA was only 11 bases but that the longest strings of absolutely conserved bases were only 1–4 bases in most areas of this gene (Baker et al. 2003). This stark reality for a highly conserved gene highlights the enormous challenge with any metagenomics strategy. Different hypervariable regions demonstrated different efficacies with respect to species calls in different genera, and the V2 and V3 regions were most effective for universal genus identification (Chakravorty et al. 2007). In a separate study, parallel analysis of 3 different hypervariable regions of 16S rDNA sequence (V2–V3, V4–V5, and V6–V8 regions) was effective in determining the composition of bacterial consortia in maize rhizospheres (Schmalenberger et al. 2001). As a universal approach to the identification of bacterial pathogens, a 2-region approach yielded bacterial-genus identifications in approximately 90% of isolates not amenable to biochemical identification (Luna et al. 2007). These studies highlight the degree of variability in the representation of operational taxonomic units (OTUs), which depends on the hypervariable region used for the analysis.

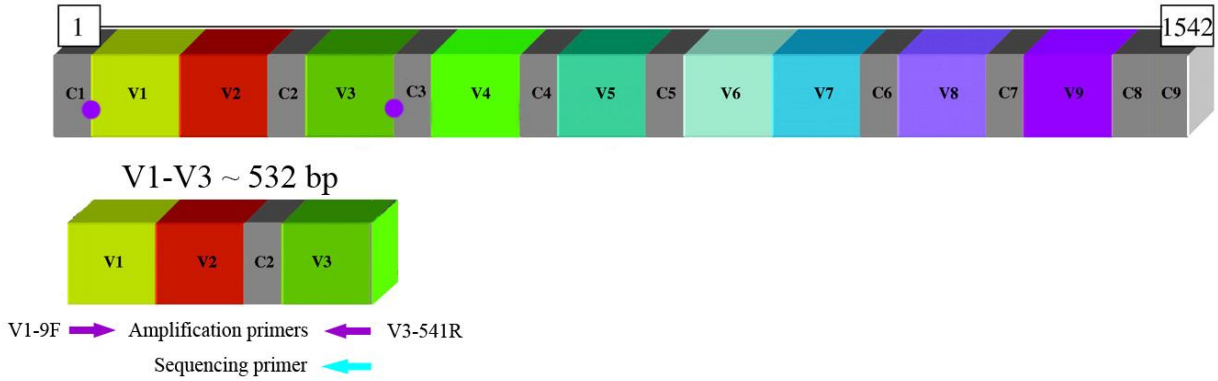

Fig. 2. Conserved and hypervariable regions in the 16S rRNA gene.

The interspersed conserved regions (C1-C9) are shown in gray, and the hypervariable regions (V1-V9) are depicted in different colors. Also illustrated is an example of bacterial primer, which I used in my study for DNA amplification and sequencing based microbial identification. V1-V3 sub-region with violet circles and arrows represents primer binding sites.

Multiple online databases have been developed on the basis of different taxonomic schemes which provide convenient access to large rRNA sequences for clinical laboratories and research teams. The most prominent databases include the Ribosomal Database Project (RDP) (http://rdp.cme.msu.edu/) (Cole et al. 2009); Greengenes (http://greengenes.lbl.gov) (DeSantis et al. 2006); ARB-SILVA (Pruesse et al. 2007), and EzTaxon-e (Kim et al. 2012b). RDP II is based on Bergey's taxonomy, which contains a relatively small number of phyla (divisions). Greengenes includes multiple taxonomic schemes, allowing the results of queries made with different classification schemes to be compared. The ARB-SILVA database also offers a choice of microbial taxonomies, although it is more limited in its flexibility than Greengenes. EzTaxon-e covers not only prokaryotic species with validly published names (www.bacterio.net) but also species with non-validly published names and Candidatus taxa (Murray and Stackebrandt 1995), as well as representatives of yet uncultured phylotypes.

Online rRNA databases also include a variety of software tools for sequence classification and multiple sequence alignments for facilitating microbial identification. These software environments (Greengenes, RDP, and ARB-SILVA) contain sequence-query tools (only this tool in EzTaxon-e), sequence-alignment programs, and sequence editors. Apart from these Mothur (Schloss et al. 2009) and QIIME (Caporaso et al. 2010) are two other open source, software platforms for comparison and analysis of microbial communities, primarily based on high-throughput amplicon based data (such as SSU rRNA) generated on a variety of platform like 454 or Illumina.

After pyrosequencing, we begin with sequence collection and verification; algorithms must be in place to trim sequences and to vet the quality of individual reads via various strategies. Furthermore, most of the amplicon based protocols include 'barcode' sequences in the adaptor that can be used to identify the source of the sequence read in multiplex samples. So, analysis begins with removal of adaptor and barcode sequences, and poor-quality sequence reads elimination, by utilizing sequence trimming which with the help of various algorithms, removes primer and low-quality sequence data before sequence assembly. The next step in the bioinformatics analysis is alignment against a reference genome sequence (Huse et al. 2007).

Another problem is that the PCR may generate sequence chimeras because of errors that couple disparate DNA sequences during the amplification process. Chimera-checking

software has been developed so that amplicons can be vetted for the presence of "sequence hybrids" with tools such as Bellerophon (DeSantis et al. 2006), Pintail (Ashelford et al. 2005), Chimera Slayer (Haas et al. 2011), and UCHIME (Edgar et al. 2011). The remaining reads after removing erroneous sequences are then clustered against an average algorithm and taxonomic classification of each Operational Taxonomic Unit (OTU) (clustered at 97% sequence similarity) are obtained by classifying alignments against any reference taxonomy and non-redundant nucleotide databases files.

1.3 OBJECTIVES OF THIS STUDY

In this study we set out to answer the following questions:

1. What trends in diversity are seen in soil bacteria and archaea with elevation, and what identifiable environmental parameters seem to control diversity? Do the two mountains show the same trends? How do trends seen on Fuji/Halla compare to those seen on other mountain systems?
2. How closely does community composition on Mt. Fuji and Mt. Halla relate to elevation and the associated climate trends? Are there distinct communities of soil microbes associated with each elevational zone?
3. Are particular vegetation types on Halla and Fuji associated with differences in community composition and diversity?

CHAPTER2. MT. FUJI: A GEOLOGICALLY AND TOPOGRAPHICALLY SIMPLE MOUNTAIN SYSTEM, JAPAN.

2.1 INTRODUCTION

Patterns in the diversity of life have long been a source of fascination for ecologists (Gatson 2000). Although the major trends in diversity of vertebrates, larger invertebrates, and higher plants are well documented, the diversity patterns of many other groups of small organisms such as prokaryotes, protozoa, microfungi, and nematodes are still largely unknown (Currie and Paquin 1987, Brown 2001, Lomolino 2001, McCain 2005, Renaud et al. 2009). The metagenomic approach, extracting DNA from bulk environmental samples, allows rapid cataloging of diversity of known and unknown forms of such organisms.

Metagenomic study of microbes, concentrating mainly on the 16S rRNA gene, has already identified at least some tentative geographical trends in microbial diversity in soils (Lozupone and Knight 2007, Lauber et al. 2009, Auguet et al. 2010, Bates et al. 2011, Nemergut et al. 2011). Although, there has been some study of bacterial diversity trends with elevation to the best of our knowledge, only a single study by Zhang et al. (2009) has looked at archaeal diversity along an elevational gradient.

An incidental analysis of samples from varying elevations in a broad-scale geographical study of the Americas concentrating on bacteria (Lauber et al. 2009), consisting of 88 sites scattered across Argentina, Canada, Ecuador, Peru, Puerto Rico, and USA, Fierer and Jackson(2006) suggested no trend with elevation. A more localized systematic study in mountains of the SW USA suggested a decline in bacterial diversity towards higher elevations (Bryant et al. 2008). However, these previous studies suffer from certain basic limitations that make it unclear how general their findings may be. The geographical study by Lauber et al. (2009) mixes different latitudes, climate zones, and geologies, which might disguise any trend if one exists. The study by Bryant et al. (2008) in the western USA mixes different rock types and also a complex trend in rainfall with elevation (increasing from desert lowlands to moist mid and upper elevations). Furthermore, Bryant et al. (2008) concentrated only on one particular phylum of bacteria, the acidobacteria. It is by no means clear a priori that the full diversity of bacterial life would show such a trend or whether it also occurs in other bacterial phyla. A more comprehensive study by Fierer et al. (2011) in the eastern Andes sampled

bacteria in the phyllosphere, soil organic layer, and soil mineral layer, and did not find any elevational diversity trend in any of these. However, it also sampled across a range of geologies, complicating the picture.

Other broad scale studies have sampled soil prokaryotic communities across a range of climates, soil and vegetation types irrespective of elevation. Concentrating on archaea, Auguet et al. (2010) provided valuable insight into broad-scale ecological patterns exhibited by the archaeal domain in general, using around 2,000 archaeal 16S rRNA environmental sequences available online from a large set of environments and utilizing this information to extract general macroecological patterns found among archaeal communities along global environmental gradients. Similarly, Bates et al. (2011) sought the environmental factors which regulate the diversity and abundance of archaeal communities in soil with 146 samples from the Americas and Antarctica. These two major studies and other recent investigations suggest that archaeal communities can be influenced by salinity and pH, (Nicol et al. 2008, Auguet et al. 2010, Cao et al. 2012), elevation, (Zhang et al. 2009), climate and vegetation cover (Angel et al. 2010) or C/N ratio (Bates et al. 2011). The only study which looked at archaeal diversity along an elevational gradient showed that the abundance of ammonia oxidizing archaea (AOA) was negatively correlated with altitude (Zhang et al. 2009). This study concentrated only on ammonia oxidizing microbes at Mount Everest (12 soils at altitudes of 4000-6500 masl), However, the taxonomic and environmental sampling range studied here is very narrow.

Given the few examples of studies to date, it is important to add other studies of mountain systems. Especially desirable for study are mountain systems which offer more uniform geology and simpler climate gradients. It is only when more observations of actual patterns have been made that a theoretical framework for bacterial diversity on mountains can be formulated and discussed in terms of its broader implications for understanding biological diversity patterns.

The present study aims to provide a clearer picture by focusing on a single mountain that is geologically relatively uniform: Mount Fuji of Japan. The mountain has mesic environments of uniform age from its base to summit. In this study, we analyzed all groups of bacteria together and the four most abundant phyla separately along with total archaea with the most abundant phylum Thaumarchaeota separately in the next section.

2.2 SITE DESCRIPTION AND SAMPLING

Geological Background

Mount Fuji (35°21′28.8″ N 138°43′51.6″ E) is a volcanic cinder cone covered by moderately silica-rich volcanic ash of nearly uniform composition. Over the main part of the volcano, including the northeastern slope sampled here, the surface ash layer is around 7,000–10,000 years old. There is no volcanic outgassing from the crater or the slopes of Fuji. The only historical volcanic eruption (in 1703) occurred from the Hoei crater halfway down the east slope (whereas this study sampled only the north slope), and its ash was uniformly deposited downhill on the east side and eastwards across the coastal plain (Available from: Mt. Fuji Volcano Disaster Management Conference (2002) http://www.bousai.go.jp/fujisan-kyougikai/.). Hence, there is no reason to believe that any part of our transect has been affected by volcanic activity in the last few thousand years.

Climate/Vegetation zones and Sampling

Reaching 3,776 meters above sea level (masl), Fuji has a wide range of climate and vegetation types. The mountain has experienced a warming trend of about 0.8°C over the last 30 years, but currently the mean annual temperature (MAT) around its base at 992masl is around 9.9°C, while its summit has a MAT of around −5.3°C (a total MAT gradient of around 15.4°C). The climate of Fuji is mesic at all elevations, with a mean annual precipitation at its base of around 2,100 mm concentrated in summer. The higher elevations mainly above 3600masl along with crater were covered sporadically by small patchy snowfields. The higher elevations, above 2,500 m, are frequently shrouded in cloud providing additional moisture. There is little detailed information available on how the amount of precipitation and cloud varies with elevation on Fuji. Fujimura (1971) reported that more precipitation was observed in 2,000–2,500masl in August 1961–1965; but during that period, the peak shifted upslope to 3,000–3,500masl in September illustrating the temporal complexity of the elevational gradient.

The vegetation and landscape of Fuji is protected as a national park. At the base of Fuji, the forest is temperate mixed deciduous, with such species as *Fagus crenata*, *Quercus crispula*, various *Acer* species, and a considerable variety of other tree species (Ohsawa 1984). Above 1,600 m is a subalpine forest belt dominated by *Abies veitchii*, *Tsuga diversifolia*, *Larix leptolepis*, and *Betula ermanii*. As is usually the case on mountains, there is

a marked decrease in tree species richness with increasing elevation. The forest gives way to an alpine zone above 2,500 m with scattered herbs and shrubs such as *Polygonum cuspidatum*, *Salix reinii*, and *Alnus maximowiczii* (Ohsawa 1984). With elevation, the herbaceous cover becomes sparser; and above 3,500 m, there is essentially no vascular plant cover nor any evident lichen or moss cover with the surface consisting of fine clinker mixed with sand–grade volcanic ash. Although snow covers the upper slopes for most of the year, there is no permanent snow cover anywhere on Fuji; and by July, even the uppermost slopes are essentially free of snow.

Sampling took place at the peak of the summer warm season, the last week in July 2010—a time when bacterial activity at all elevations can be expected to be at its maximum. Sampling took place during the same week along a broad transect on the north face of Fuji that paralleled the main Subaru road and hiking trail system (Fig. 3 and Fig. 4). At each altitudinal sampling band, five samples were taken in a line paralleling the contours at 100 m intervals horizontally. Each individual sample consisted of a 10 m square. Individual samples were approximately 100 g, consisting of the top 5 cm of organic/mineral soil (B horizon) taken at each corner of the square and in the center, and mixed together into the same bag. Biological replicates from the same elevation points were stored separately in sample bags in drinks coolers at ambient to minimize temperature changes on the way down the mountain before they could be deposited in the freezer. Samples were frozen at −80°C within 24 h of sampling, and stored there until filtering and extraction could take place.

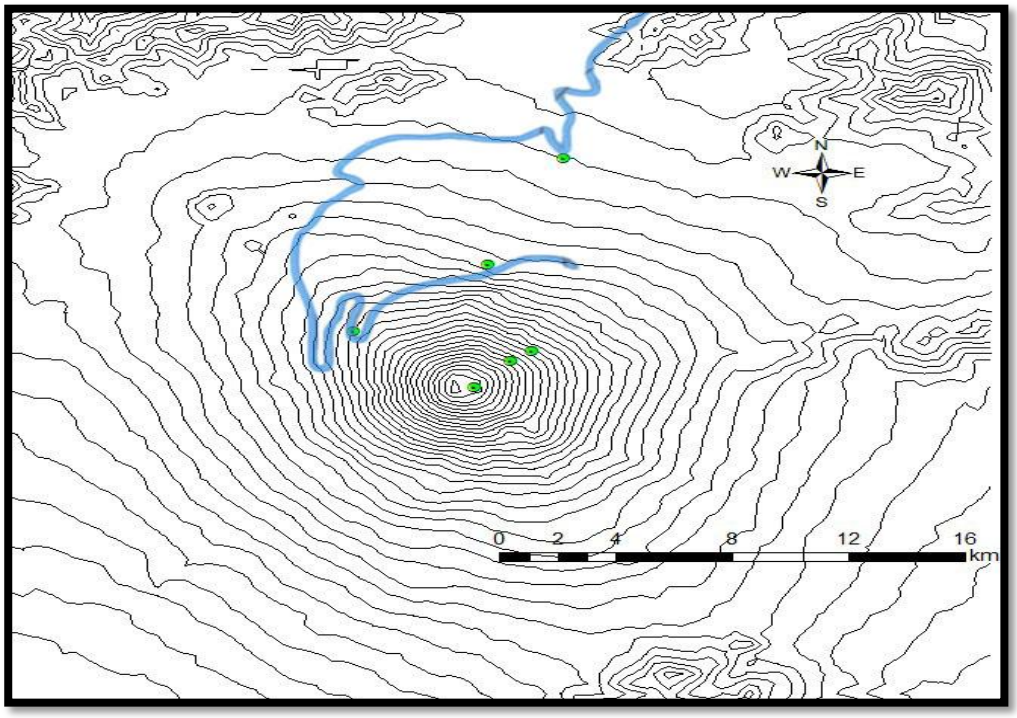

Fig. 3. Contour map view of Mt. Fuji.

Sampling locations are located at 500m intervals along transect and are shown as green dots. Contour interval is 100m and the Subaru hiking trail is depicted in blue.

Fig. 4. Soil sampling at 3000masl on Mt. Fuji

2.3 BACTERIAL DIVERSITY ON MT. FUJI

2.3.1 Materials and Methods

2.3.1.1 DNA extraction, Soil Analysis and PCR amplification

The samples were crushed and sieved while still frozen through a 3-mm sieve. The isolation and extraction procedure for soil bacteria is as follows: DNA was extracted from each of the collected sieved regolith/soil samples using the MOBIO Power Soil DNA extraction kit (MOBIO Laboratories, Carlsbad, CA, USA) as directed by the manufacturer. Isolated DNA was stored at −80°C. DNAs isolated from each sample were amplified using primers targeting the V1–V3 regions of the bacterial 16S rRNA and polymerase chain reaction reactions were carried out as described previously (Chun et al. 2010). The DNA sequencing was performed by Macrogen Incorporation (Seoul, Korea) using 454 GS FLX Titanium Sequencing System (Roche) according to the manufacturer's instructions.

Soil nutrient content was analyzed for each soil sample. Nutrient was extracted by distilled water after drying of soil in a natural condition. Total nitrogen and carbon were measured by using an elemental analyzer (Flash EA 1112, Thermo Quest Ltd., USA). pH was measured in distilled water (Kalra et al. 1995). Concentration of NO_3^-, NH_4^+, PO_4^-, and K^+ was determined by using a reflectometer (Merck Ltd., Germany). Concentrations of NO_3^-, NH_4^+, and PO_4^- were converted to that of NO_3-N, NH_4-N, and P_2O_5, respectively.

2.3.1.2 Processing of Pyrosequencing Data and Taxonomic Analysis

Sequences were processed and analyzed following the bioinformatic procedures described previously by Unno et al. (2010) with an additional step of removing chimeric sequences. Putative chimeric sequences were detected and screened using a similarity-based approach, which splits each query sequence into two even length fragments and then assigns each fragment to a taxon using BLAST search against EzTaxon-extended database (http://www.eztaxon-e.org); (Kim et al. 2012b) followed by removal of the sequences when two fragments differ at the order level or percent identities are greater than 95% for both fragments despite assigned to different taxonomies. A similar approach has been used in the QIIME package (Caporaso et al. 2010) and Mothur software (Schloss et al. 2009).

2.3.1.3 Statistical Processing and Analysis of Results

To check relationships between soil bacterial species richness/diversity and elevation as well as edaphic factors such as pH, nutrients, and vegetation characteristics, operational taxonomic units (OTUs), and other diversity indices were calculated using the Mothur platform (Schloss et al. 2009) for subsamples standardized to 800 reads as diversity is directly correlated with the number of sequences collected. To compare community-level bacterial diversity, we used the non-parametric Shannon index as well as Faith's phylogenetic diversity (PD) because Shannon and other diversity indices such as Ace or Chao do not describe the evolutionary history or phylogeography of each bacterial community. The diversity metrics described above were also applied to four most abundant lineages of bacteria (Proteobacteria, Acidobacteria, Actinobacteria, and Bacteroidetes) by extracting sequences for these specific lineages from a subset of 800 reads.

In the pyrosequencing results, some samples did not give a sufficient number of 800 reads for the subsampling analysis to represent the bacterial community. For instance from the 3,000 m elevation sampling level, three out the five samples gave too few reads for analysis, including one that gave no reads at all (note that the two samples with low reads were used in calculating the relative average abundance: refer to Table 1 and Table AT1 in appendix).

Even with these omissions, regression analyses gave statistically significant trends, leading to a total of 27 samples which were used in the final analysis. We followed the Costello analysis example on the Mothur platform to calculate diversity indices from the high quality reads using EzTaxon alignment bacterial database as a template. To assign sequences into OTUs, a neighbor clustering algorithm ("furthest neighbor") was employed using the cluster command, with 97% sequence similarity as the designated cut off. The assigned OTUs were then used to calculate coverage, richness, diversity, and rarefaction values for each sample. Rarefaction curve, heatmap, and regression analysis against pH, elevation, C/N ratio, and other parameters were drawn using R software package 2.10.1.

We performed a principal co-ordinates analysis (PCoA) in the Fast UniFrac web interface [14] for determining whether our different elevational samples cluster together or form their unique clusters using the EzTaxon tree, an ID mapping file and category mapping file describing additional relationships between samples and sub categories for visualizations.

Table 1: Site/sample characteristics and dominant vegetation types on Mt. Fuji.

Sample	Elevation	Co-ordinate	pH	Total N (%)	Total C (%)	K (mg/kg)	$P_2 0_5$ (mg/kg)	NO_3-N (mg/kg)	MAT (^oC)	PPT (mm)	Vegetation†
F10	1000	35°27'36.0"N, 138°45'41.6"E	5.16	1.40	31.8	1.256	1.43	3.53	9.9	2100	*Quercus crispula*, *Acer* spp., *Fagus crenata*
F15	1500	35°24'52.7"N, 138°44'18"E	5.51	1.48	23.2	1.408	0.79	17.27	6.6	NA	*Fagus crenata*, *Abies homolepis*, *Quercus crispula*
F20	2000	35°23'10.2"N, 138°41'48.1"E	5.98	0.63	13.9	1.344	0.54	5.42	3.2	NA	*Tsuga diversifolia*, *Abies veitchii*, *Betula ermanii*, *Larix leptolepis*
F25	2500	35°22'39.7"N, 138°45'06.6"E	6.22	0.06	0.80	0.948	0.27	5.02	0.8	NA	*Polygonum cuspidatum*, *P. weyrichii* var. *aplinum*, *Salix reinii*, *Alnus maximowiczii*
F30	3000	35°22'23.4"N, 138°44'42.9"E	6.24	0.04	0.44	0.632	0.27	3.35	-1.6	NA	Mostly lacking plant cover. A few scattered *P. cuspidatum*, *P. weyrichii* var. *aplinum*, *Salix reinii*, *Alnus maximowiczii*
F37	3760	35°21'35.6"N, 138°43'56.6"E	6.17	0.01	0.09	0.632	0.21	3.07	-5.3	NA	no plant cover

Elevation is measured here as meters above sea level; †taken from Ohsawa (1984).

2.3.2 Results

In total, 117,967 quality sequences were classified into 3,843 OTUs at 97% similarity level, distributed across all 27 samples. On average, 1,069 species were found in each sample but due to differences in the total number of reads per sample, these raw numbers would not be comparable. Instead, we randomly took 800 reads per sample and measured bacterial species richness and diversity from this utilizing around 18% of the total sequences available, for further processing as previously mentioned in materials and method (see also Table AT2 in appendix). Similarly, we only used 19.6%, 17.2%, 17.9%, and 18.8% of the total sequences found under Proteobacteria, Acidobacteria, Actinobacteria, and Bacteroidetes after subsampling for 800 reads.

Rarefaction curve (drawn at the 97% sequence identity level of taxonomic resolution) reveal that samples from the summit were the least diverse ones and appear to reach an asymptote; whereas for other samples, it appears that more sampling would be needed to cover the full extent of taxonomic diversity (Fig. 5).

The results show a significant difference in bacterial diversity with elevation. There is a "bulge" seen in diversity indices in the upper mid-elevations at around 2,500masl with a curve showing the best fit based on adjusted R^2 and residual standard mean error. Minimum richness was observed in the samples from the summit which reached about 79% of the number of OTUs observed at the 2,500 masl where the richness was at its maximum (unique OTUs for both the elevational points were not taken into account while calculating the percentage). Richness at the summit was somewhat similar (being a little less) than the samples at the lowest sampled elevation point at 1,000 m. For the overall community (Fig. 6A), among all the soil and site characteristics considered, only elevation was significantly correlated ($P < 0.05$) with either OTU richness ($R^2 = 0.33$) or diversity (Shannon index, $R^2 = 0.18$; Faith's PD, $R^2 = 0.25$).

Again, when we further examined four most dominant phyla individually, richness and the phylogenetic structure of these groups correlated most strongly with altitude as supported by the adjusted R^2 values. Focusing on particular subgroups of bacteria, the Acidobacteria show a striking decrease in richness/diversity with elevation (Fig. 6B) whereas Proteobacteria (Fig. 6C) as well as Bacteroidetes (Fig. 6D) followed a humpback curve ($P < 0.05$). Actinobacteria (Fig. 6E)

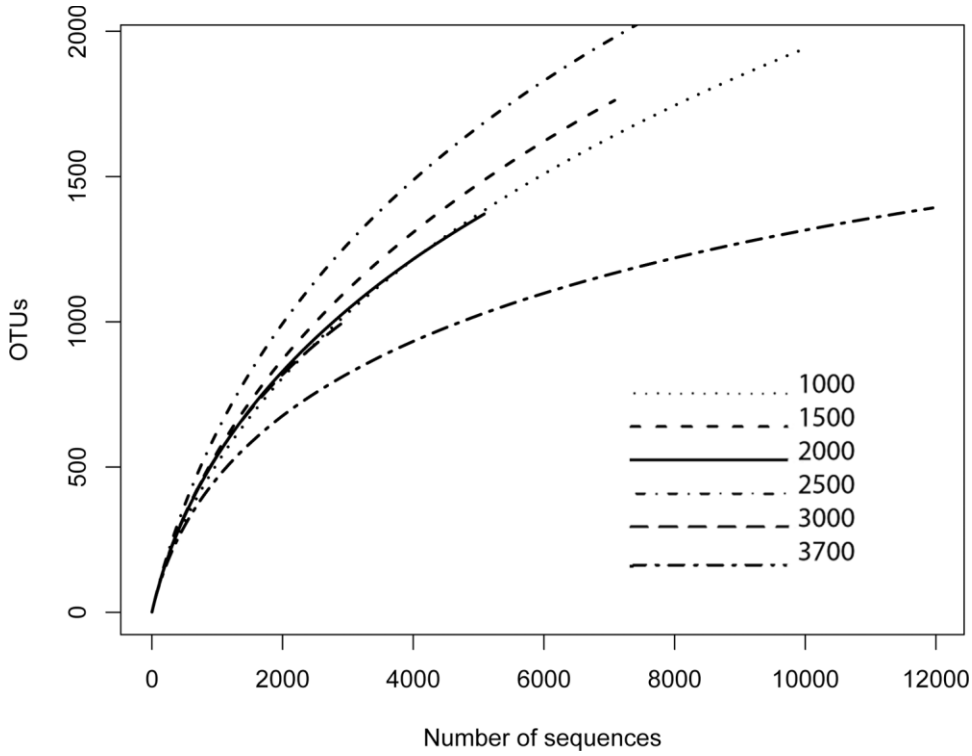

Fig. 5. Rarefaction analysis of one example from each elevational set of samples calculated for the 0.3 OTU definition based on pairwise distance (Mt. Fuji, bacteria). The numbers written against the dotted lines in the legend denotes the elevation from which the samples were collected and are measured as meters above sea level.

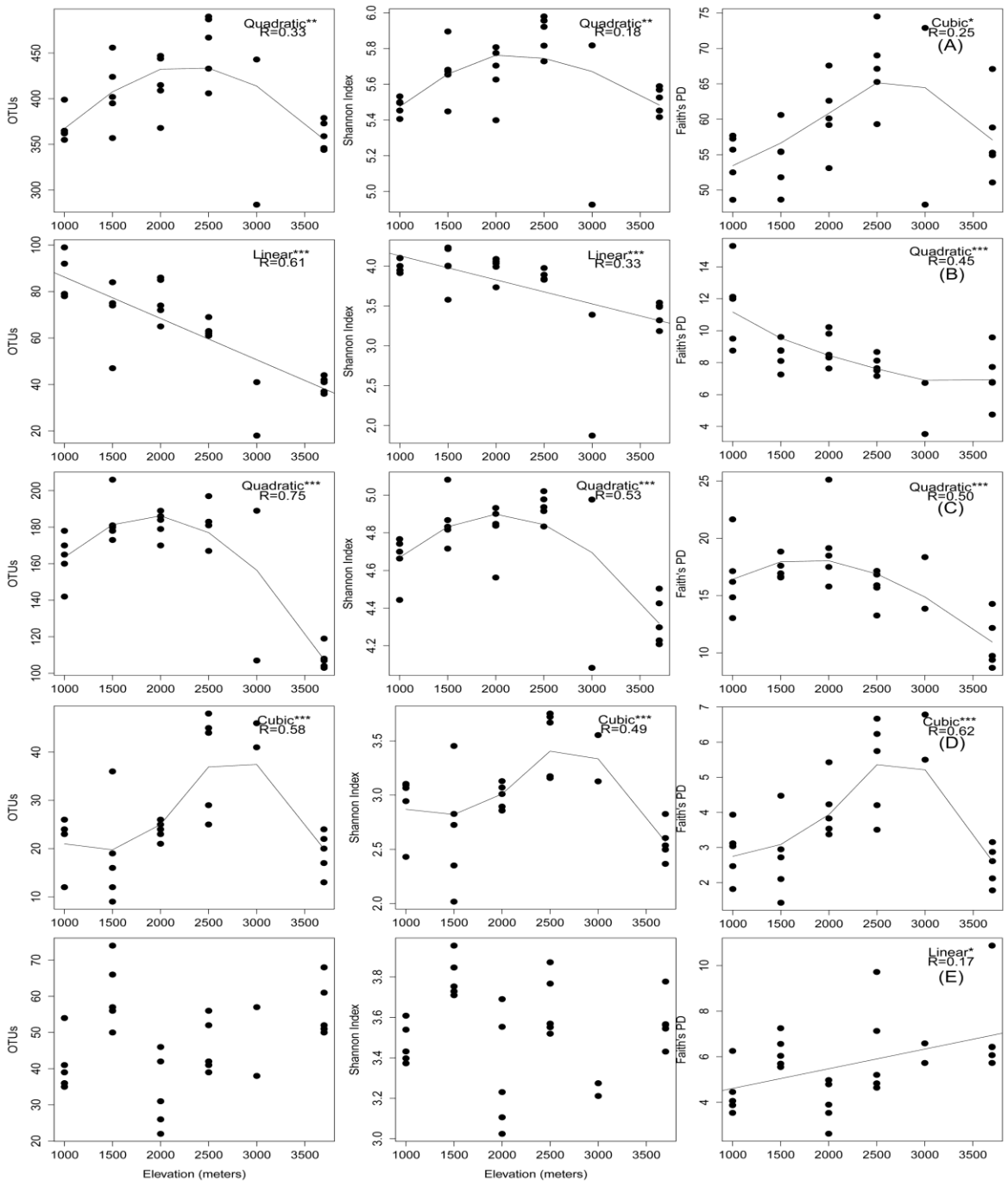

Fig. 6. Relationship between OTUs (left), Shannon index (middle), and Faith's PD (right) on Mt. Fuji (bacteria). We tested 3 models (linear, quadratic, & cubic) to describe the relationships and model selection was carried out based on adjusted R^2 and root mean square error (value not shown). Significance level was shown with***P<0.001, **P<0.01, and P<0.05. A Overall community, B Acidobacteria, C Proteobacteria, D Bacteroidetes, E Actinobacteria.

was the only abundant bacterial phylum that did not show any pattern with elevation (a pattern was observed only in case of Faith's PD with elevation). Soil pH plotted against phylotype diversity (Faith's PD; Fig. AF1 in appendix) showed a linear relationship. No relationship could be found between C/N ratio and richness or diversity (figure not shown). Proteobacteria was by far the most abundant phylum (38.5%) out of the 38 phyla identified across the entire sample set, followed by Acidobacteria (20.6%), Actinobacteria (11.7%), Chloroflexi (5.2%), and Bacteroidetes (5.1%; Fig. 3, left and Table AT1 in appendix). Alphaproteobacteria, the most abundant subphylum (18.5%) of Proteobacteria was also almost as abundant as the second most abundant phylum Acidobacteria and both decreased in relative abundance towards summit.

Other dominant groups which decreased in abundance towards the summit were Planctomycetes and Gammaproteobacteria—whereas Actinobacteria, Betaproteobacteria, Chloroflexi, and Gemmatimonadetes showed a reverse trend. In contrast, the relative abundance of Bacteroidetes increased with elevation with a maximum at 3,000 m asl, then decreasing again near the mountain summit while Deltaproteobacteria remained almost constant throughout the different elevational gradient. These trends can be seen in Fig. 7A. Several of the phyla considered rare in an earlier study by Lauber et al. (Lauber et al. 2009) (having a total relative abundance of $< 0.5\%$ in that study) were not so rare here, most importantly Chloroflexi (5.2%) and Planctomycetes (3.03%). Others such as AD3 (2.6%), OP10 (1.3%), Nitrospira (1.1%), and several others were much more abundant than phyla such as Verrucomicrobia and Firmicutes (each 0.92%) which are generally more abundant in soil samples. Phyla level abundance is discussed further in Table AT1 in appendix.

The most abundant single phylotype across the entire sample was classified under genus Afipia (Alphaproteobacteria) represented by a total of 2,579 sequences accounting for approximately 2.19% of total classifiable sequences. This particular OTU was found throughout the elevation gradient with an increase in abundance at mid-elevations, finally decreasing to its lowest abundance at the mountain summit. We attempted to visualize the comparison between the elevational abundance of different phylotypes at the OTU level by drawing a heat map of 60 abundant OTUs classified at least to genus level (including only the acidobacterial, TM7, and AD3 OTUs classified up to phylum or subphyla level), chosen specifically for those showing a definite pattern along the elevation gradient, out of the 200 most abundant OTUs (Fig. 7B).

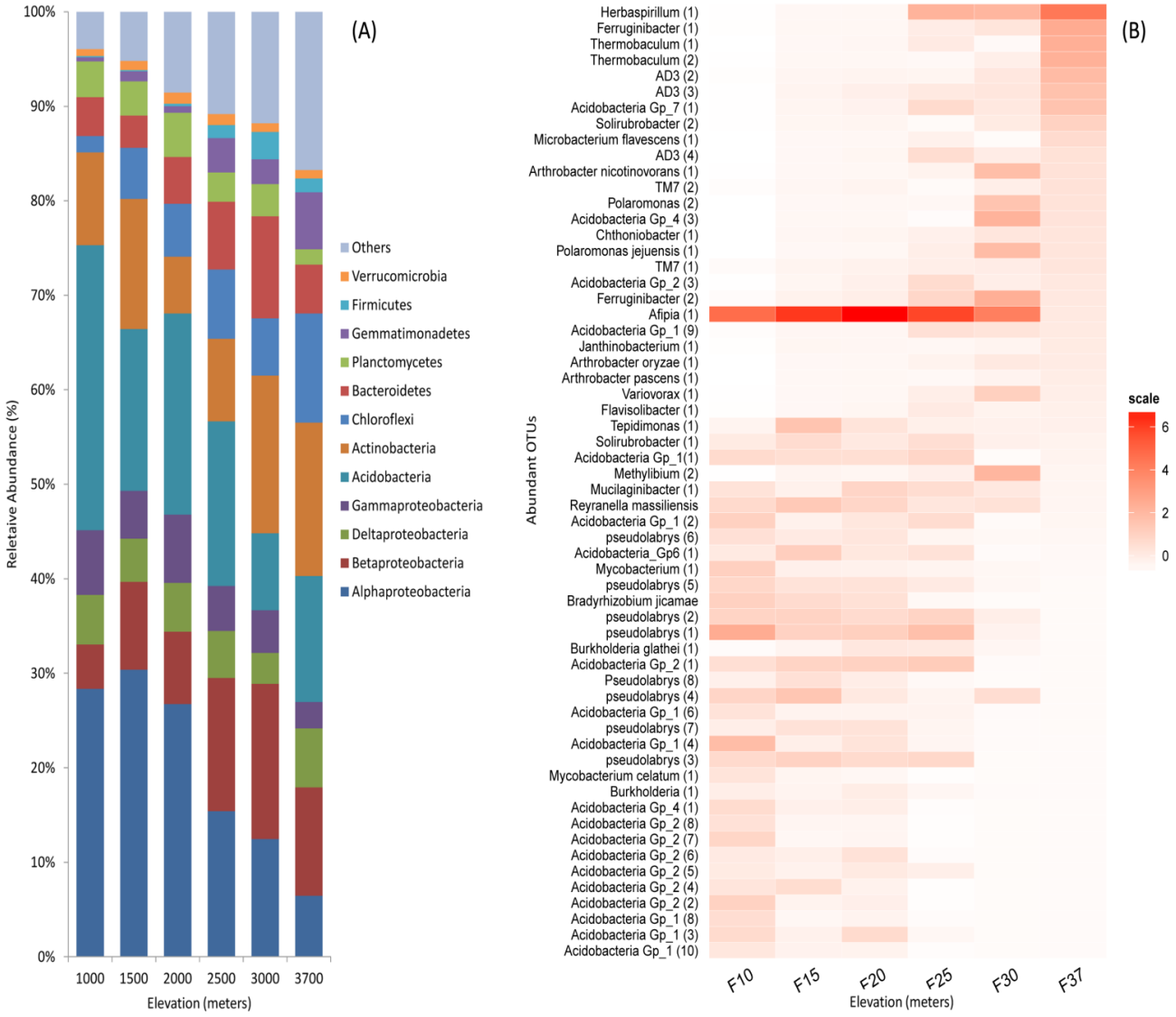

Fig. 7. (A): relative average abundances of dominant bacterial taxa at different elevational sampling points. (B): the heat map shows the relative percentages of the given 60 abundant phylotypes at different elevational sampling points with a color legend and scale provided (Mt. Fuji). All the phylotypes were classified up to genus level except for the Acidobacteria, AD3 and TM7 due to the taxonomic restrictions. The numbers written in brackets in front of the taxon names on Y-axis indicates their comparative abundance with other similar phylotypes, such as "(1)" in "Herbaspirillum (1)" denotes that it is the most abundant OTU classified up to genus Herbaspirillum.

Most of the abundant acidobacterial OTUs were present in their greatest numbers at lower altitudes whereas a few proteobacterial sequences were abundant at lower altitudes and others at higher altitudes.

PCoA performed on the pairwise UniFrac distances calculated for the total community showed obvious affinities within the samples taken from the same elevational sampling points and indicated significant variability among different elevation's bacterial community. The UniFrac method by utilizing phylogenetic information about the environmental samples has been shown to be able to successfully characterize and compare many microbial communities simultaneously using clustering and ordination techniques such as the PCoA used here Lozupone and Knight (2005). Strong clustering could be seen within samples from the same elevational points along the principal coordinate 1 (PC1) with samples from lower elevation clustered into independent groups on the left hand side of the figure and vice versa (Fig. 8A). Although PC1 and PC2 were singlehandedly able to distinguish the samples, we checked it with PC3 also. PC1 vs. PC3 also yielded the same results as previous (Fig. 8B). These results showed that samples from same elevational level harbored similar bacterial communities and thus clustered together, and once again reinforcing the point made in Fig. 6, that elevation is the major predictor of bacterial diversity/richness on Fuji.

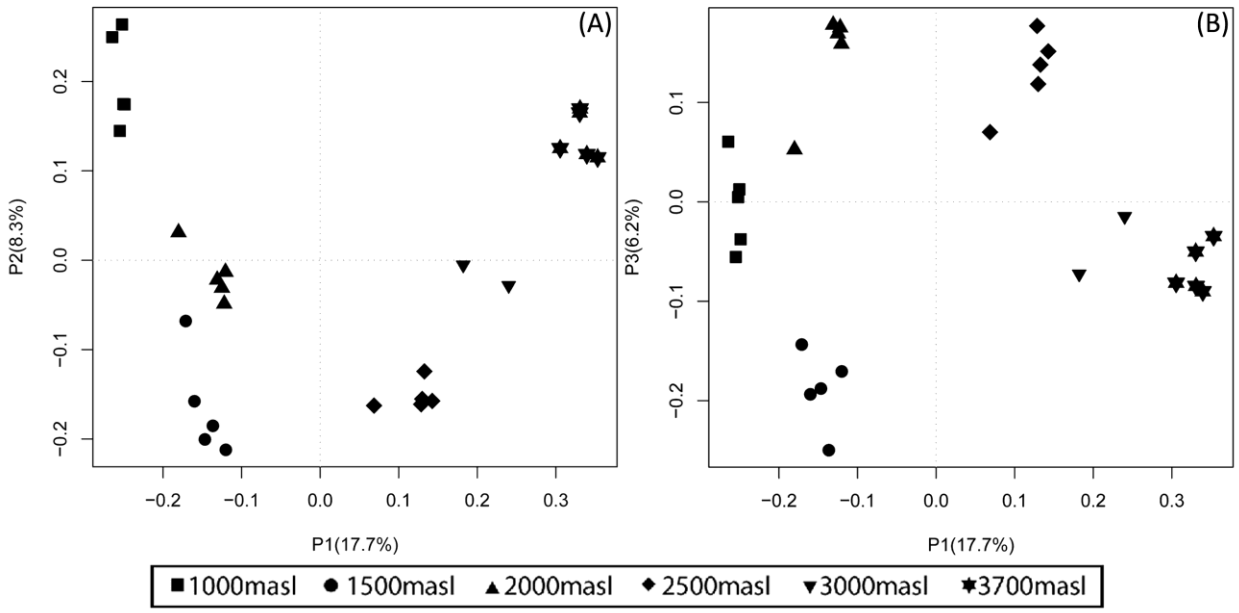

Fig. 8. PCoA analysis results with a UniFrac distance matrix (bacterial community) comparing all 27 samples from six different elevational points from Mt. Fuji. (summarized in Table 1.) (A): the scatter plot is of principal co-ordinate 1 (P1) vs. principal co-ordinate 2 (P2). Axes indicate the percent variation in the samples described by plotted principal co-ordinates. (B): scatter plots between P1 vs. P3

2.3.3 Discussion

2.3.3.1 Broad Taxonomic features/patterns of soil bacterial communities

It is important to bear in mind that "bacteria" as a whole are a vast and diverse category and that there may be patterns of interest in the individual phyla of bacteria. In our data, some phyla (Proteobacteria, Bacteroidetes; Fig. 6) show a "humpbacked" trend with elevation similar to that seen for the bacteria in general. By contrast, some show no discernible trend (Actinobacteria; Fig. 6E). Acidobacteria show a strong decreasing trend in diversity (Fig. 6B) with elevation, which agrees with the earlier study by Bryant et al. (Bryant et al. 2008) that focused only on this group.

It is not surprising that different groups of bacteria show quite distinct trends for they are often genetically as distinct from one another as (for instance) plants are from birds or even more so. The decline in Acidobacteria with elevation may be explained by their well-known ecological preference for acidic soils with high C/N ratios—for with elevation on Fuji the pH increases slightly and C/N ratio decreases. The "rare phyla" may be benefitting from the lack of competition from otherwise abundant phyla such as Acidobacteria and this may emphasize their ecological position as "stress tolerators" {sensu (Grime 2002)}.

The "humpback" curve seen in selected groups such as Proteobacteria suggests that the "intermediate disturbance" or "intermediate productivity" mechanism may be at work in controlling the diversity of these groups in particular. Intermediate disturbance or productivity hypothesis simply states that local species diversity or productivity of a community is maximized when ecological disturbance is neither too rare nor too frequent (Dial and Roughgarden 1998).

Overall, the humpback diversity pattern seen across the entire assemblage of phyla seems to be a complex result of the differing ecologies of many individual groups. As such, it might be said that there is no one ecological mechanism that explains the humpback trend in diversity seen here. However, the same could probably be said with fine enough taxonomic dissection of any group of organisms along a classic humpbacked diversity-disturbance/diversity-productivity gradient, for example if one were to focus on individual families of angiosperms or corals, each showing their own unique trend but contributing to the overall humpbacked curve. Thus, it seems

that focusing on the overall emergent pattern of a humpback trend across all bacterial groups might still be a valid, ecologically meaningful approach.

2.3.3.2 Explaining Variance in Bacterial Community Diversity

The pattern found across all bacteria combined in this study is for a mid-elevation "bulge" that increases bacterial OTU richness by about 25%. This trend differs from that which is typical of many groups of organisms; for example, trees and birds where there is typically a decline in diversity with elevation or a "bulge" only in lower elevations (Huston 1994, Lomolino 2001, McCain 2005, Adams 2009). It differs in particular from the trend of decreasing tree and herbaceous plant species richness with elevation on Fuji (Ohsawa 1984). The pattern seen in soil bacteria is clearly in contradiction with the idea that habitat area is key to explaining trends in species richness with elevation (Lomolino 2001). Fuji tapers progressively towards its peak, but the lower slopes are not the most diverse in terms of bacteria as would be expected. However, given that scaling in bacterial communities (with their vast population sizes even in small areas) is very different from that in larger organisms, it is unsurprising that bacteria are not affected by habitat area on this scale.

A mid-elevation diversity bulge is more typical of the patterns found in some other groups; for example amphibians and ferns and, in some cases, birds (Terborgh 1977, Huston 1994, McCain 2005). It also differs from the main finding of the study by Bryant et al. (2008) although this is not surprising in that the taxonomic range studied here is very different their study which concentrated only on the phylum Acidobacteria.

The study by Fierer et al. (2011) suggested no trend with elevation across bacteria in general even though it reached up to similar elevations to our own study. It is not clear why there may be a difference in our study, although it is possible that the "bulge" shows up because the higher latitude position of Fuji allows sampling of an alpine fellfield zone not sampled in the Andean transect. Another possible reason for the lack of a trend in the study by Fierer et al. (2011) is that the mountain landscapes they studied were geologically and topographically much more complex that the monotonous cinder cone of Fuji.

Given the general decrease in species richness of vascular plants, both trees and herbaceous plants, with elevation on Fuji, one might expect a decrease in bacterial richness because the overall complexity of the community that ultimately supplies food to the bacterial community is decreasing. This should restrict the range of potential hosts for mutualism and parasitism, and the range of litter types for saprotrophy. However, the species richness and diversity of the soil bacterial community actually shows a very different trend, increasing with elevation from 1,000 to 2,500 m. This lack of correspondence between bacterial richness and other groups of organisms is also the case in the study by Fierer et al. (2011) in the Andes, which shows decreasing richness of larger organisms but no trend among bacteria, and in the study by Bryant et al. (2008) where the phylum Acidobacteria does not parallel the mid-elevation bulge in vascular plant diversity found in the western USA.

What could explain this mid-elevation maximum in diversity of bacteria that we find on Fuji? A possible explanation may lie with the "intermediate disturbance" hypothesis (Huston 1994). We suggest that just beyond the tree and vegetation line, the more extreme temperature fluctuations, stronger UV, lack of food supply (based on what is known of other fellfield alpine systems, most primary production is by Cyanobacteria, not larger plants: (Körner 2003) and more frequent disturbance of the loose substrate of these slopes (e.g., by freeze–thaw) allows less competition and greater species diversity due to "lottery" recruitment. However, at the highest elevations, the physiological challenges may be so extreme that fewer bacterial species are capable of surviving, due to this having been a relatively restricted and unstable environment through evolutionary time that has thus not accumulated species (essentially the argument used for vascular plants by (Grime 2002). Thus, bacteria may perhaps be seen as joining plants and corals in showing a humpback curve along disturbance frequency gradients or physiological gradients (Huston 1994).

The peak of August precipitation with elevation reported by Fujimura (1971) coincides roughly with that of the diversity of soil bacteria but it is not clear how stable this peak is with respect to time or why it would affect bacterial diversity. If the precipitation maximum is indeed around 2,500 m, it is possible that plentiful precipitation is important because it reduces UV and prevents drying of soil above the timberline thus, increasing the diversity of soil bacteria. However, there have been no previous reports of increasing soil moisture increasing bacterial

diversity in mesic climates and it is to be expected that the upwards shift of precipitation by September might affect the longer-term average bacterial diversity in soil.

Another possible explanation may be that the mid-elevations, just above the altitude of closed vegetation cover and below the open alpine fellfield/alpine desert zone, offer a mix of these two distinct environments that allows different sets of bacteria to occur in a close mosaic in the same sample of soil. The presence of scattered plants and their roots, with open patches of bare volcanic ash, may thus offer a small scale mosaic that increases species richness within the soil. This resembles an explanation put forward by Lomolino (Lomolino 2001) for mid-elevation diversity bulges in larger organisms.

It is interesting that in this study, pH—usually the best predictor of variation in microbial diversity—is a weaker predictor than elevation. Partly this may be because the pH range was not very great (4.76–6.42; see Table.1 and Fig. AF1 in appendix) but it illustrates that interesting patterns may emerge despite pH variation at least when pH variation is relatively low.

PCoA clustering (Fig. 8), also shows that the combinations of bacterial species living at particular elevational levels are specialized according to some aspect or aspects of the environment which vary with elevation. This seems to emphasize broad-scale predictability in the total bacterial community, in relation to environment, even if lottery recruitment plays an important role is coexistence on the smallest scale within the soil.

2.4 ARCHAEAL DIVERSITY ON MT. FUJI

2.4.1 Materials and Methods

2.4.1.1 Processing of Pyrosequencing Data and Taxonomic Analysis

The sequence data obtained after pyrosequencing were processed using Mothur (Schloss et al. 2009) except for the step of removing chimeric sequences. To begin with, sequences shorter than 150 nt with homo-polymers longer than 8 nt and all reads containing ambiguous base calls or incorrect primer sequences were removed. Next, the sequences were aligned against the EzTaxon-e database and then trimmed, so that subsequent analyses were constrained to the same portion of the 16 S rRNA gene (V1–V3 region). Putative chimeric sequences were detected and screened using a similarity-based approach, which splits each query sequence into two even length fragments and then assigns each fragment to a taxon using BLAST search against EzTaxon-extended database (http://eztaxon-e.ezbiocloud.net/; (Kim et al. 2012b) followed by removal of the sequences when two fragments differ at the order level or percent identities are greater than 95% for both fragments despite assigned to different taxonomies. The remaining reads were pre-clustered using the pre-cluster command (http://www.mothur.org/wiki/Pre.cluster) to remove erroneous sequences derived from sequencing errors and then clustered using Mothur's average algorithm. Taxonomic classification of each OTU (clustered at 97% sequence similarity) was obtained by classifying alignments against EzTaxon-e reference archaeal taxonomy and non-redundant nucleotide archaeal databases files using the classify command at 80% Bayesian bootstrap cutoff with number of iterations as 1000. DNA pyrosequences are available under the following GenBank SRA Accession No. SRA050374.1.

2.4.1.2 Statistical Processing and Analysis of Results

Operational taxonomic units (OTUs) (at ≥97% similarity) and other diversity units such as Shannon, Faith's PD etc., and rarefaction values were calculated using the Mothur platform (Schloss et al. 2009) on a subset standardized to 309 reads per sample using the sub.sample command (http://www.mothur.org/wiki/Sub.sample) in Mothur. This subset was used to assess the relationships between OTUs and diversity indices with elevation and other edaphic factors by correlation analysis. Best fitting modeling of correlations were performed in

SigmaPlot, using linear, polynomial (quadratic) and power (cubic) law functions. To evaluate if 309 reads per sample are representative of the patterns observed, we repeated the regression analyses using a subsampling size of 1000 reads (available only for 22 samples). OTUs and other diversity metrics were also calculated for the largest phylum Thaumarchaeota and analyzed in the same way as for the whole community.

Community similarity matrices for analysis were built using the Bray–Curtis similarity coefficient (Magurran 2004) and the UniFrac metric (Lozupone and Knight 2005). UniFrac is a phylogenetic metric which measures the distance between communities based on the lineages they contain. UniFrac distances were calculated based on a phylogenetic tree of randomly chosen subsets (n = 309reads/subset) of 30 samples. Sequences aligned using Mothur software were used to infer a maximum likelihood (ML) tree using RAxML (Stamatakis 2006). RAxML (v.7.2.7) with GTR + CAT model was done on CIPRES Portal 2. Non-metric multidimensional scaling (NMDS) plots as implemented in PRIMER v6 (Clarke and Gorley 2006) for visualizing archaeal community at the different elevational scales were generated using Bray-Curtis Index.

We used a multiple regression on matrices (MRM) approach to look at the relative importance of each of the environmental factors on community similarity (Legendre et al. 1994). Before applying MRM to the dataset, we looked for redundant edaphic factors using the VARCLUS procedure (Sarle 1990) in the Hmisc R package. Mean annual temperature (MAT) (Spearman's $\rho^2 = 1.00$), total carbon and nitrogen (Spearman's $\rho^2 = 0.84$ & 0.85 respectively), extractable ammonium (Spearman's $\rho^2 = 0.74$) were highly correlated with elevation (Fig. AF2 in appendix), and thus we removed them from the MRM analysis. With the 5 environmental variables left (on the basis of VARCLUS results), we estimated an environmental distance (Euclidean distance) matrix using Primer v6 (Clarke and Gorley 2006) and performed MRM using this environmental distance matrix and genetic matrices calculated as specified above (i.e., UniFrac and Bray-Curtis). Non-significant factors were removed sequentially and the MRM analysis was repeated until only significant factors were left in the model. Significance was tested by permutations (9999 permutations) and P-values of two-tailed tests are reported for this analysis.

Rarefaction curve, heatmap, regression analysis, VARCLUS and MRM procedures were performed using R software package 2.10.1. A neighbor-joining phylogenetic tree for inferring phylogeny for our large dataset was constructed after aligning representative phylotypes with reference sequences (J-PHYDIT software) downloaded from NCBI and EMBL in the MEGA 4 software package(Tamura et al. 2007).

2.4.2 Results

Community Composition

Based on the results of Kan et al. (2011) and our supplementary analysis (see Fig. AF3 in appendix), we decided to use EzTaxon-e to assign the taxonomy to our recovered sequences, as a better database for this task. A total of 89672 quality archaeal sequences (with an average length of 444 bp) were obtained from the 30 samples, with an average of 2989 sequences per soil sample and with coverage ranging from 398 to 8488 reads per sample. Even with this level of coverage, the lack of asymptotes in the rarefaction curves (Fig. 9) suggests that much archaeal diversity remains un-sampled. Out of a total 89016 sequences that remained after the trimming, aligning and screening processes, around 99.9% sequences could be classified up to phylum level with a total 1478 phylotypes (defined at ≥97% sequence similarity level) (Table. AT3 in appendix). Thaumarchaeota emerged as the most abundant archaeal phylum on Mt. Fuji with 85840sequences, (96.4%) of the total across all elevations, although it was more abundant at higher elevations. Euryarchaeota was the only other phylum present on Mt. Fuji (3515sequences, 3.9%) with a trend opposite to that found for the Thaumarchaeota: a higher relative abundance at lower elevations which progressively decreased to an almost negligible presence at the summit (Fig. 10).

The most abundant single phylotype across the entire sample was classified under the order Nitrososphaerales (soil cluster I.1b, Thaumarchaeota) represented by a total of 41,433 sequences accounting for approximately 46.5% of total classifiable sequences. This particular OTU increased in relative abundance with increasing elevation. It was almost absent at the lowest elevation, but reached around 20% to 70% at the mid elevation sites, and then dominated at high elevations where it represented nearly all of the sequences recovered at 3000 and 3750masl. Of the 10 most abundant OTUs, (94.5% of the total sequences; Fig. 10) only 3 belonged to Euryarchaeota, all of them within the class Thermoplasmata. Most of the abundant thaumarchaeotal and euryarchaeotal OTUs were present in their greatest numbers at lower/mid altitudes except for the most abundant phylotype DFT1(Dominant Fuji Thaumarchaeota 1) which was more abundant at higher altitudes (Fig. 11).

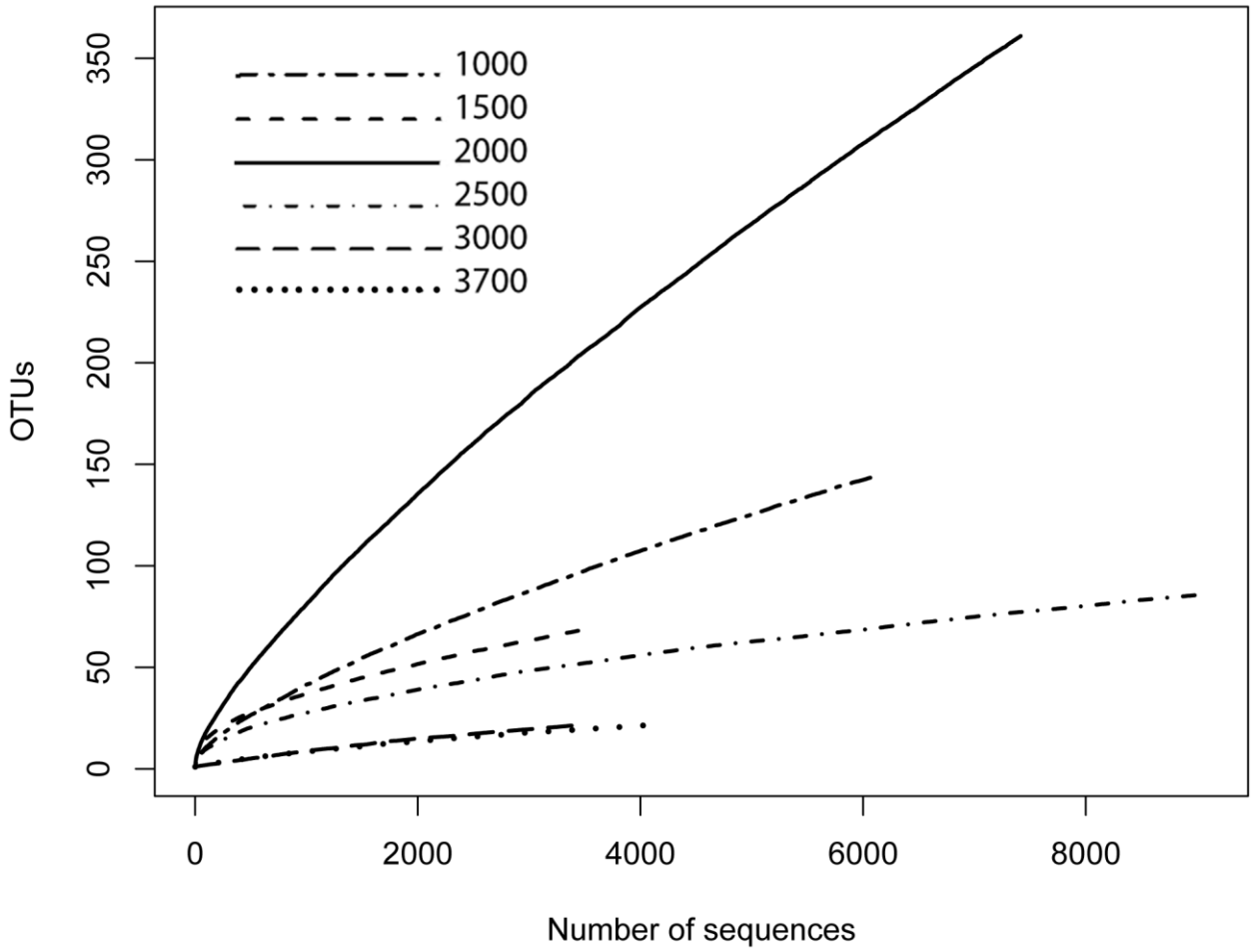

Fig. 9. Rarefaction analysis of one example from each elevational set of samples calculated for the 0.3 OTU definition based on pairwise distance (Mt. Fuji, archaea). The numbers written against the dotted lines in the legend denotes the elevation from which the samples were collected and are measured as meters above sea level.

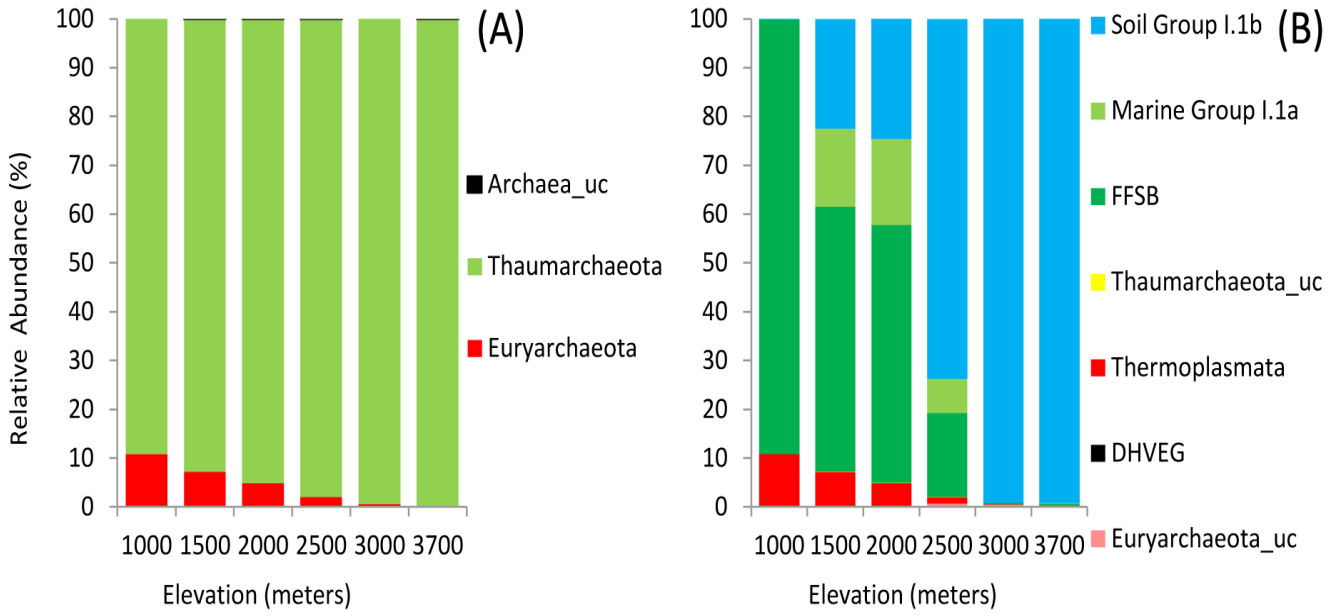

Fig. 10. Relative average abundances of archaeal taxa on Mt. Fuji at different elevational sampling points at the phylum level (A) and at the sub-phylum level (B). (Abbreviations: FFSB-Finnish Forest Soil archaea type B; _uc-unclassified)

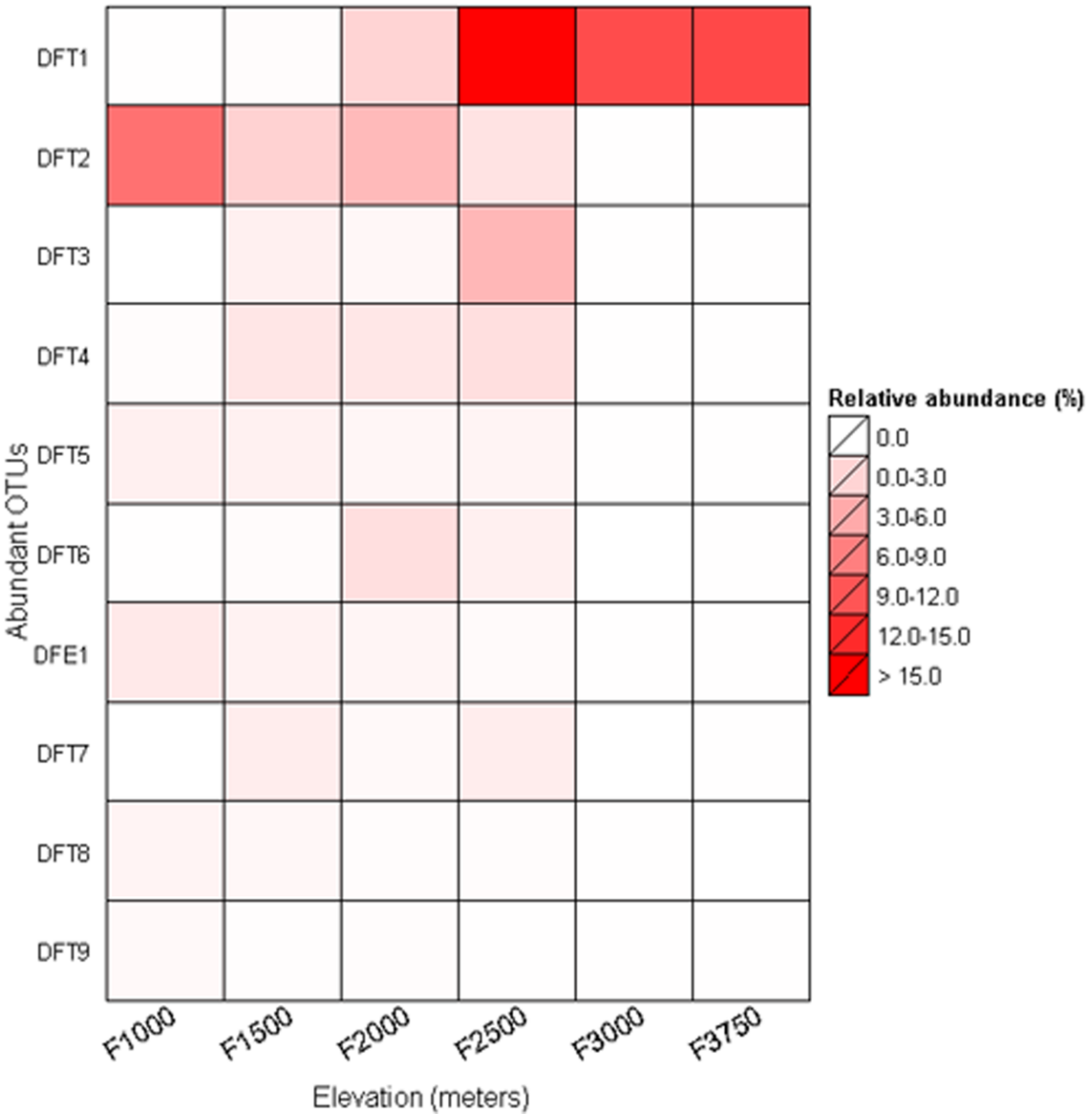

Fig. 11. Heat map showing the percent relative abundance of the 10 most abundant archaeal phylotypes at different elevational sampling points present on Mt. Fuji, with a color legend and scale provided. DFT here abbreviates for Dominant Fuji Thaumarchaeota and DFE for Dominant Fuji Euryarchaeota. The number written against them denotes their abundance e.g., DFT1 stands for the most abundant thaumarchaeotal phylotype present on Mt. Fuji.

Archaeal Diversity along the Elevational Gradient

The archaeal communities rarified to the same level of subsampling (309 reads per sample), showed significant differences in diversity and richness in relation to elevation (Fig. 12, see Table. AT4 in appendix). There was a "peak" in diversity/richness in the lower mid-elevations at around 1,500masl with a curve showing the best fit based on adjusted R^2 and residual standard mean error. Maximum richness with approximately 79% of OTUs was observed at 1500masl whereas minimum richness was observed at 3000masl. Richness at the summit was lower than that observed at the lowest elevation 1000masl, with only 92.4% as many OTU's present at the summit.

Among all the site characteristics examined, elevation was most significantly correlated ($P<0.05$) with both OTU richness ($R^2 = 0.36$) and diversity (Shannon index, $R^2 = 0.89$; Faith's PD, $R^2 = 0.50$) (Fig. 12). The same analysis at a subsampling level of 1000 reads with only 22 samples showed results with similar values (results not shown). When we further examined the most dominant phylum Thaumarchaeota, richness and the phylogenetic structure once again correlated most strongly with elevation as supported by the adjusted R^2values and corrected Bonferroni P values (Fig. 12). Among all edaphic variables, extractable ammonium, nitrate and potassium ion concentration also showed a positive correlation with both richness and diversity (Table. 2). No relationship was found between C/N ratio and richness but C/N ratio was correlated to both diversity measures; soil pH was only correlated with Shannon diversity index (Table. 2).

Elevation acted as a strong structuring factor of the archaeal assemblages showing that samples belonging to different elevational zones harbored distinct communities, based on the Bray-Curtis index (Fig. 13).

We assessed the relative importance of environmental variables in explaining their contributions to the correlation using MRM. Only 5 out of the 9 environmental variables were used for this analysis (see Methods and Fig. AF2). For the whole community (both UniFrac and Bray-Curtis matrices), elevation alone was able to predict more than 38% of the total variability (Table. 3). Apart from elevation, potassium ion concentration was also able to explain a smaller portion (around 18%) of the variation.

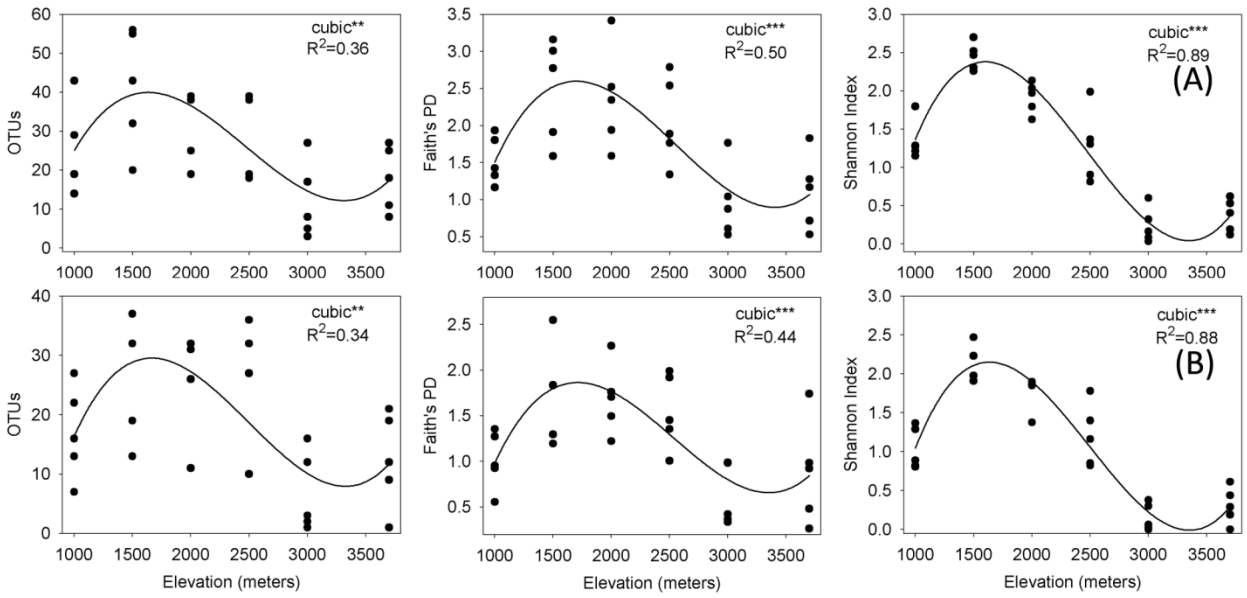

Fig. 12**.** Relationship between elevation and phylotype richness (left), phylogenetic diversity (middle), phylotype diversity (right) for the whole community (A) and Thaumarchaeota (B) on Mt. Fuji. We tested three models (linear, quadratic, and cubic) to describe the relationships and model selection was carried out based on adjusted R^2 and RMSE (root mean square error; value not shown). Significance level is shown with***P<0.001; **P<0.01; and P<0.05.

Table. 2. Relationship between soil parameters and phylotype richness, phylogenetic diversity and phylotype diversity for the whole archaeal community and Thaumarchaeota on Mt. Fuji.

Variables	OTUs	Faith's PD	Shannon Index
Whole community			
CN ratio	-	0.13(l*)	0.24(l**)
pH	-	-	0.35(q**)
Total Carbon	0.18(l*)	0.17(l*)	0.58(q***)
Ammonia	0.28(l**)	0.21(l**)	0.56(q***)
Nitrate	0.27(l**)	0.27(l**)	0.34(q**)
Phosphorus	-	0.21(q*)	0.44(q***)
Elevation	0.36(c**)	0.50(c***)	0.89(c***)
Potassium	0.29(l**)	0.35(q**)	0.70(q***)
Thaumarchaeota			
CN ratio	-	0.13(l*)	0.21(l**)
pH	-	-	0.23(q*)
Total Carbon	0.15(q*)	-	0.46(q***)
Ammonia	0.15(l*)	0.17(l**)	0.52(q***)
Nitrate	0.12(l*)	0.29(l**)	0.34(q**)
Phosphorus	-	0.18(q*)	0.40(q***)
Elevation	0.34(c**)	0.44(c***)	0.88(c***)
Potassium	0.22(l**)	0.30(q**)	0.64(q***)

We tested three models (linear-l, quadratic-q, and cubic-c) to describe the relatioships; model selection was carried out based on adjusted R^2 and RMSE (root mean square error; value not shown). Significance level was shown with***P<0.001; **P<0.01; and P<0.05; only relationships which were significant are shown in table.

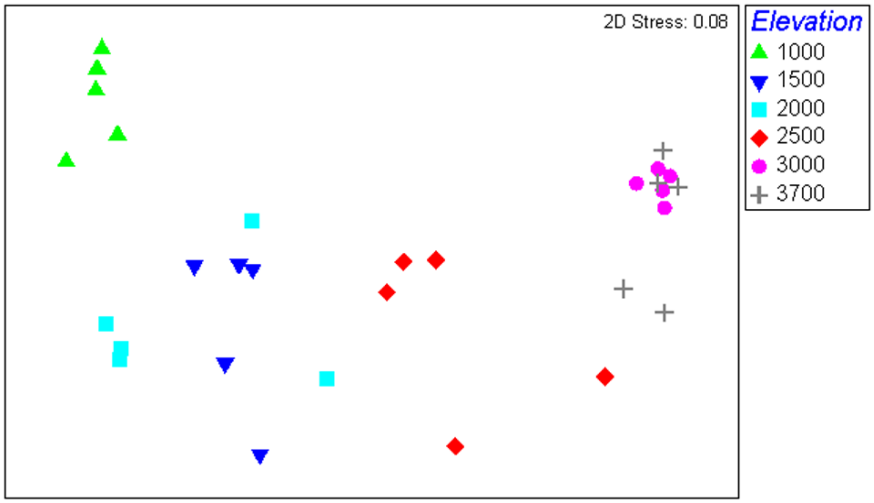

Fig. 13. NMDS analysis results with a Bray Curtis similarity matrix comparing all 30 samples from 6 different elevational points from Mt. Fuji.

Table. 3. Results of the multiple regression on matrices analysis for the whole archaeal community on Mt. Fuji.

Environmental Variables	**Whole community**	
	Bray-Curtis (R^2= 0.63b)	UniFrac (R^2= 0.38b)
pH	-	-
Sqr (Elevation)	-14.8***	0.032***
Ln (P)	-	-0.012*
Sqr (K)	-4.5**	0.013*
NO_3^-	-	-

The variation (R^2; both values are significant at $P \leq 0.0001$) of community distance that is explained by the remaining variables and the partial regression coefficients (b) of the final model is reported. Partial regression coefficients are reported for only significant values (*$P \leq 0.0100$, **$P \leq 0.0010$, and ***$P \leq 0.0001$).

2.4.3 Discussion

2.4.3.1 Broad Taxonomic Features/Patterns of Soil Archaeal Communities

Archaeal diversity on Fuji, as with bacteria (Singh et al. 2012b), shows a mid-elevation "peak", although at a lower elevation of 1500masl than in the case with bacteria. This 'humpback' trend contrasts with the trend in vascular plant species richness on Mt. Fuji, where both tree and herbaceous plant richness steadily declines with increasing elevation (Ohsawa 1984). A greater variety of plant species might be expected to provide more diverse environments for soil microbes, but since archaea do not tend to be involved in litter decomposition in contrast to bacteria or fungi (Manerkar et al. 2008, Buee et al. 2009); it is not surprising that they show an independent trend. The only comparable study of archaea with elevation has been that by Zhang et al. (2009) on Mount Everest (12 soils at altitudes of 4000–6500masl). That study concentrated only on ammonia oxidizing microbes, showing a significantly negative correlation with altitude, with a maximum in abundance at the lowest altitudes. Our results differ from those of Zhang et al. (2009). However, the taxonomic and environmental sampling range studied here is very different; they also examined only a limited number of sites over each elevation range, with almost no replicates except three at the lowest sampling site at 4000masl.

A humpback trend in diversity with elevation is quite commonly found in groups of animals and plants in mountains around the world (Lomolino 2001, McCain 2005), most often towards the lower altitudes. However, a monotonous decline in diversity is also very common for a wide range of groups (Lomolino 2001, McCain 2005). A range of hypotheses have been put forward for such trends (Lomolino 2001), including intermediate disturbance intensity, a 'mid domain effect', and the effects of combining the communities of two relatively distinct environments (from upper and lower slopes) in the intermediate elevations (Singh et al. 2012b).

As in our previous study of bacterial diversity on Fuji (Singh et al. 2012b), the mid elevation 'bulge' in diversity might be explicable in terms of several different factors/processes (Huston 1994). It is possible that a more physically stable soil environment in the lowermost forest zone of Fuji allows out-competition between archaeal species with overlapping niches, reducing overall diversity. The very unstable upper slopes of Fuji, with bare

alpine ash/clinker fields subject to frost heave, landslips and avalanches, may provide the opposite extreme of an environment in which few species can maintain viable populations (or in which few niches are viable due to frequent population reductions) – hence the lower diversity of the upper elevations (Huston 1994).

Another possibility is that the mid-altitudes of Fuji in effect combine a small-scale mosaic of two environments: the upper slope unstable environment of the ash/clinker fields, and the lower slope stable forest soil environment. This is a variant of the hypothesis of Lomolino (2001). The combination of two distinct environments, and their associated archaeal communities, on a micro-scale in the mid-altitudes of Fuji could increase diversity by adding together two sets of species. This demands further investigation through fieldwork observations, experiments, and microcosm studies.

Potentially very important however, are the observations of relationships between diversity and soil parameters. These may hint at other mechanisms that control diversity at the level of resource availability, perhaps mediated by competition or by the availability of extra niches. Potassium, ammonium and nitrate concentrations are all significantly correlated with diversity, although none as strongly as elevation itself. Since potassium and ammonium concentrations co-vary, their relative importance is difficult to discern, and they might all perhaps be correlated with some unknown factor (also related to elevation, such as disturbance) which could be in fact the most important in controlling the diversity trend. Again, further studies are necessary to elucidate this.

2.4.3.2 Explaining Variance in Archaeal Community Diversity

Elevation was significantly correlated with both the composition of the whole community and the relative abundance of subgroups within the major phylum Thaumarchaeota (Table. 2 & 3; Fig. 12 & 13). All of the statistical analyses emphasize the overwhelming predictive power of elevation as a principal driving force in the soil archaeal community on Mt. Fuji. The explanation to this strong correlation with elevation may be the strong co-variation of different soil edaphic variables with elevation (VARCLUS results, Fig. AF2 in appendix). Among these elevation-dependent variables, extractable potassium ion concentration also has a particularly strong influence (Table. 3) on the community structure and phylogeny. Interestingly, the archaeal diversity bulge at 1500masl coincides with the maximum values of most of the soil variables we studied, except pH and total carbon (soil and site characteristics previously described in Singh et al (2012b). Earlier studies on soil archaeal communities from elsewhere found a negative correlation between soil archaeal abundance/diversity and pH (He et al. 2007, Nicol et al. 2008, Jia and Conrad 2009, Lehtovirta et al. 2009) but in this study pH was generally not found to be significant. However, pH range was quite narrow on Mt. Fuji, with a general pH gradient from lower elevations to higher ones (4.8 to 6.4).

Two other studies that have concentrated on broad scale differences in soil archaeal communities concluded that salinity (Auguet et al. 2010) and C/N ratio (Bates et al. 2011) is the principal driving force behind archaeal taxonomic distribution at global scales. Auguet et al. (2010) collected c.2000 sequences of the archaeal 16S rRNA gene from 67 globally distributed studies with samples ranging from hydrothermal vents to chemical reactors including water and sediments samples from freshwater and marine environments, and soil samples. Their study focused on how ecology relates to the community structure and therefore it may not be directly comparable with the soil gradient we explore here. Bates et al. (2011) on the other hand, collected 146 soil samples from North and South America and Antarctica resulting in a total of 2500 sequences corresponding to archaea. They primarily examined the influence of environmental factors on archaeal abundance relative to that of soil bacteria. Although both studies took a global perspective, the total number of sequences taken in for consideration at 2000 and 2500 reads, were rather few. In contrast, our study recovered around 80,000 archaeal sequences from 30 samples examined here, which allowed for a more comprehensive assessment

of archaeal diversity in these soils. Also, it is interesting to note that despite the greater number of sequences, the soils in our study were still dominated by very few archaeal taxa as has been observed before (Leininger et al. 2006, Oline et al. 2006, Auguet et al. 2010, Bates et al. 2011).

At lower elevations, the dominance of FFSB (I.1c gp) of Thaumarchaeota cannot be ignored. It has been seen in many previous studies that archaeal communities in acidic forests are dominated by the FFSB group (Oline et al. 2006, Lehtovirta et al. 2009). Soil pH is a major determinant of the abundance of FFSB group with lower abundance at neutral/higher pH values and vice versa. Lehtovirta et al. (2009) sampled across pH manipulated plots in the range of 4.5 to 7.5 (maintained at 0.5 pH unit intervals) to study whether soil pH is a major driver of FFSB group and found that FFSB could be detected only in soils at pH 4.5 to 6.0 with highest abundance at the lowest pH accompanied with a steady decline as pH increased. This may explain the dominance of FFSB at lower elevations, where the pH is comparatively lower as compared to higher elevations (Singh et al. 2012b).

We found an overall shift away from Euryarchaeota towards Thaumarchaeota abundance (relative abundance, Fig. 10) with increasing elevation. The Euryarchaeota assemblage present on Mt. Fuji was almost entirely composed of sequences that could be classified into class Thermoplasmata. Thermoplasmata is a large class consisting of thermoacidphiles (pH optima 0.7 to 3 and optimum temperatures above 50°C – based upon cultured specimens) which are aerobic or microaerophilic heterotrophs (Angelov and Liebl 2006). Increase in Thaumarchaeota towards upper elevations was mostly due to increasing prevalence of the thaumarchaeotal soil cluster I.1b (see Fig. 10B). Looking at a finer taxonomical scale, this increase was largely due to a single but most abundant (53.7%) OTU cluster DFT1 (designated ‘Dominant Fuji Thaumarchaeota 1′) classified under thaumarchaeotal soil group I.1b. Our results are in accordance with previous soil studies, where the majority of the archaeal phylotypes were contained within the same lineage of Thaumarchaeota (i.e., soil I.1b clade, earlier classified under Crenarchaeota) (Jurgens et al. 2000, Ochsenreiter et al. 2003, Bates et al. 2011).

Interestingly, while our samples at 2500 masl and above were overwhelmingly dominated by sequences belonging to soil group I.1b (see Fig. 10), these were present at lower elevations in only minimal numbers. Bates et al. (2011) had earlier suggested that soil group I.1b could be an

AOA as it formed a tight clade with the uncultured soil clone ‘54d9’, a large genomic fragment obtained from a soil fosmid library that included the entire 16S/23S rRNA gene,(Ochsenreiter et al. 2003, Treusch et al. 2005) as this clone was shown to contain genes encoding ammonia monooxygenase (Amo)-related proteins (Treusch et al. 2005). A phylogenetic tree (Fig. 14) incorporating the 15 most abundant phylotypes including ‘DFT1’ on Mt. Fuji with sequences for uncultured clone 54d9 and other fellow AOA from the soil cluster I.1b like *Nitrososphaera gargensis* (GU797786), *N. viennensis* (FR773157), and *Cenarchaeum symbiosum* (DP000238) revealed DFT1 within a tight clade with the other AOA and the uncultured clone 54d9. This suggests that DFT1 could be a possible member of the AOA clade and that the substantial increase in the soil cluster I.1b at higher elevations on Mt. Fuji could be due to an increase in abundance of AOA.

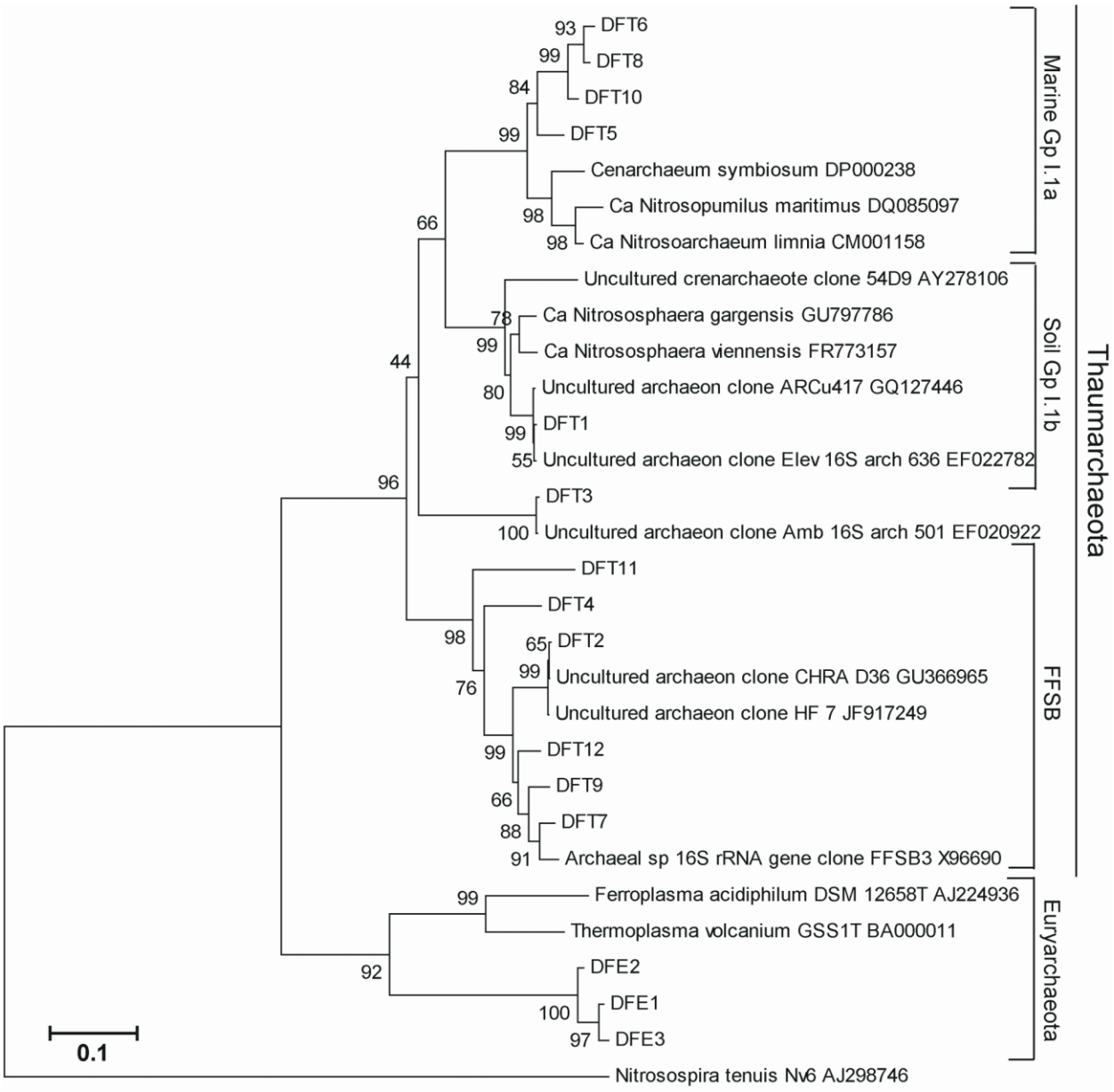

Fig. 14. Neighbor joining tree based on the alignment of 16S rRNA gene sequences (~400bp long) showing the relationship between archaeal phylotypes (DFT: Dominant Fuji Thaumarchaeota and DFE: Dominant Fuji Euryarchaeota) recovered from Mt. Fuji by pyrosequencing. The dominant soil thaumarchaeote DFT1 is indicated along representative archaeal isolates and clone 54d9. The tree is rooted with an AOB from bacterial phylum Proteobacteria.

Dominance of Nitrososphaera-like AOA at higher elevations where the mean annual temperature (MAT) is much lower than 5°C, contrasts with findings of culture studies of other AOA belonging to group I.1b such as *N. gargensis* {optimum temperature 46°C; (Hatzenpichler et al. 2008)}, *N. viennensis* {optimum temperature 35°C; (Tourna et al. 2011)} and strain JG1 {optimum temperature 35–40°C; (Kim et al. 2012a)}. This point could be explained by the fact that even though the atmospheric temperature is much lower than required for the cultivated group I.1b AOA, the temperature of a soil surface exposed to strong solar radiation is much warmer than the atmospheric temperature (Masuzawa and Nishitani 1991). According to this report by Masuzawa and Nishitani (1991), on partly vegetated black volcanic soil at the timberline, located at around 2500masl on Mt. Fuji, the maximum soil temperatures exceeded 50°C for several days in July and August, and were regularly above 40°C. We sampled at the same time of year – late July 2010. Keeping such observations in mind, and given the black-colored volcanic ash/scoria present on most of the upper half of Mt. Fuji, almost free of vegetation, one may expect soil temperatures as high or even higher than Masuzawa reported, as soil temperatures tend to remain closer to mean air temperatures under trees than under treeless vegetation or no vegetation (Winiger 1981, Korner et al. 1986, Miehe and Miehe 1994). Direct solar heating of the open volcanic ash soils may thus provide suitable temperatures for Nitrososphaera-like AOA at higher elevations.

In earlier reports, substrate uptake assays have shown that the affinity of AOA for ammonia was much higher than ammonia oxidizing bacteria (AOB) (Stehr et al. 1995, Park and Noguera 2007, Martens-Habbena et al. 2009, Jung et al. 2011, Kim et al. 2012a) which indicates that AOA may be physiologically adapted to ammonia oxidation in environments with low concentrations of ammonia. Clearly, it is likely that a variety of environmental variables determine the relative contribution of AOA to soil nitrification, although it is generally agreed that AOA have a competitive advantage at low concentrations of ammonia/ammonium (Di et al. 2010, Verhamme et al. 2011). Strain JG1{pH range:6–8, optimum temperature:35–40°C, ammonia tolerance up to 20 mM, (Kim et al. 2012a)}, Ca. *N. viennensis* {optimum pH:7.5, optimum temperature:35°C, ammonia tolerance up to 15 mM, (Tourna et al. 2011)} and Ca. *N. gargensis* {optimum pH:7.4, optimum temperature:46°C, ammonia tolerance up to 3.08 mM, (Hatzenpichler et al. 2008)} are the only described archaea affiliated with thaumarchaeotal

gp I.1b suggesting that the archaea affiliated to this group are generally mesophiles (20°C–50°C) which prefer near neutral pH conditions and have been found to tolerate ammonia/ammonium up to a concentration of 20 mM. This might explain why there is a surge in abundance of the soil group I.1b (OTU DFT1) at higher altitudes above 2000 masl: soil ammonium concentrations rapidly decrease above 2000 masl to reach concentrations of around 20 mM, combined with high soil temperatures (often 40–50°C) and suitable pH (6.2 at higher altitudes) on Mt. Fuji. It is also important to note that group I.1b AOA are consistently predominant over group I.1a AOA (Ochsenreiter et al. 2003, Hansel et al. 2008, Auguet et al. 2010) and AOB (Leininger et al. 2006, He et al. 2007, Chen et al. 2008, Herrmann et al. 2008) in terrestrial environments and such high occurrence of DFT1 here on Mt. Fuji higher altitudes (where the environmental conditions are quite optimum for a group I.1b AOA) could be a just a norm. Although our results and this explanation above are not definitive proof that archaeal community composition in higher altitude soils is dominated by AOA, they suggest that DFT1 could be an AOA.

In conclusion, our results have revealed a humpback diversity pattern for archaea along an elevational gradient. The most important findings of this study are: 1) Soil archaeal communities and their diversity are strongly responsive to environmental gradients on the scale of a single mountain. The humpback trend may be a consequence of the various environmental parameters which co-vary with elevation on Mt. Fuji, including temperature, vegetation type or soil nutrients such as ammonium and potassium. The finding of relatively discrete communities of archaea specific to each elevational zone suggests that many archaea may be quite finely niche-adapted within the range of soil environments. 2) A further interesting finding is the presence of a thermophilic component of archaea at the soil surface at high altitudes on a mountain that is not volcanically active. This emphasizes the importance of microclimate – in this case solar heating of the black volcanic ash surface – for the ecology of soil archaea.

This study also revealed an elevational gradient in relative abundance of Thaumarchaeota vs. Euryarchaeota, and amongst the various classes of the Thaumarchaeota. Groups of Thaumarchaeota which are likely to contain ammonium oxidizers become relatively more abundant towards the summit of Fuji. Further work is needed to understand the underlying causes of these patterns, including both additional observational studies along gradients, and experiments involving manipulation of soil conditions. Soil manipulation experiments to better

understand the controls on archaeal community structure and diversity should focus on: 1) artificial opening and disturbance of the vegetation below the tree line to simulate the hypothesized role of disturbance in producing the observed patterns; 2) transplantation of small quantities of soil between various elevations to understand the role of temperature in controlling the characteristic microbial communities found in each elevational zone, and 3) shading experiments to understand the importance of soil direct heating by the sun in producing the thermophilic community found on the upper parts of Fuji.

CHAPTER3. MT. HALLA: A MASSIVE SHIELD VOLCANO ON JEJU ISLAND, SOUTH KOREA

3.1 INTRODUCTION

Understanding of how the bacterial communities are distributed at the landscape scales still remains rudimentary (Dequiedt et al. 2009). Although some encouraging progress has recently been made on the horizontal distribution of microbial communities recently (Lozupone and Knight 2007, Lauber et al. 2009, Chu et al. 2010, Rousk et al. 2010, Griffiths et al. 2011, Nemergut et al. 2011), only few investigations have looked into the microbial distribution in relation to elevation (Bryant et al. 2008, Zhang et al. 2009, Fierer et al. 2011, Wang et al. 2012, Singh et al. 2012a, Singh et al. 2012b) with a broad range of diversity patterns emerging. For instance, an incidental analysis of specimens from varying elevations in a broad scale geographical study of the Americas (Lauber et al. 2009), consisting of 88 sites scattered across Argentina, Canada, Ecuador, Peru, Puerto Rico and USA (Fierer and Jackson 2006) suggested no trend with elevation, while a more localized systematic study in mountains of the SW USA suggested a decline in bacterial diversity towards higher elevations (Bryant et al. 2008). However, the geographical study by Lauber et al. (2009) mixes different latitudes, climate zones and geologies, whilst the study by Bryant et al. (2008) in the western USA also mixes different rock types, and also a complex trend in rainfall with elevation (increasing from desert lowlands to moist mid and upper elevations). These confounding factors might disguise any trend if one exists making it unclear how general their findings may be.

A more comprehensive study by Fierer et al. (2011) in the eastern Andes which analyzed bacteria in the phyllosphere, soil organic layer and soil mineral layer, did not find any diversity trend in any of these. However, they it also sampled across a range of geologies, complicating the picture. Two different studies on geologically standardized sites offer contradictory results: the study by Shen et al. (2012) on Changbai Mts., China, observed that soil bacterial diversity exhibits no apparent elevational gradient, neither monotonous nor unimodal, whereas Singh et al. (2012a, 2012b) on Mount Fuji, a high mountain of uniform composition, found a mid-elevation diversity 'bulge' for both bacteria (Singh et al. 2012b) and archaea (Singh et al. 2012a). Given the few examples of studies to date, with contradictory results, it is important to add other studies of mountain systems needed for a better understanding of underlying mechanism of microbial

patterns across the elevational gradients. Especially desirable for study are mountain systems which offer fairly uniform geology and simpler climate gradients suitable for studies of biodiversity and biogeography (Lomolino 2001, Rahbek 2005, Reche et al. 2005). It is only when more observations of actual patterns have been made that a theoretical framework for bacterial diversity on mountains can be formulated and discussed in terms of its broader implications for understanding biological diversity patterns.

In this study we set out to answer the following questions:

1. To explore the elevational diversity gradient of bacterial communities on Mt. Halla.
2. To determine key factors controlling the distribution of bacterial communities.
3. To compare the differences of bacterial communities on Gwaneum and Yeongsil trails.

3.2 SITE DESCRIPTION AND SAMPLING

Geological Background

Jeju Island is a volcanic island (between 33' 12" ~ 33' 34" N and 126' 10" ~ 126' 58" E) dominated by a large volcanic cone, Mount Halla (1,950m). This is a shield volcano, consisting of alkaline lavas: basalts and trachytes (Park et al. 1998, Park et al. 2000aa, Park et al. 2000bb). The island is pocked by some 360 pyroclastic cones with most of them being scoria cones and about 20 are tuff cones and rings (Sohn 1996). The main cone of Mount Halla is of Quaternary age (last 2 million years), but the present day covering of volcanic rocks is much younger. The extensive trachybasalt and the other eruptive products that make up the surface layers of Mount Halla are of late Quaternary age, deposited around 25,000 years ago (Sun et al. 2005) from separate feeder pipes. Four small flank eruptions have taken place in the last 1000 years, but these have not had a significant effect on the surface covering/lithology of the island (Cheong et al. 2007).

Climate and Vegetation Zones

The whole island has a moist climate, with the smallest annual temperature range in South Korea. The annual mean rainfall averaged from station across Jeju Island is 1,975mm, however the annual mean rainfall recorded in the southern area is almost double as that observed in western area due to orographic and local effects. Similarly, more rainfall occurs at higher elevations, and thus rainfall received in central mountainous part of the island is almost double to that observed in coastal areas (Lee et al. 1999, Won et al. 2006). According to the climate classification system of Koeppen, the climate of the lowlands of Jeju Island is Cfa, a subtropical moist climate. The mean annual temperature is 14.7^{o}C with lowest and highest temperatures in January (5.1^{o}C) and August (25.4^{o}C), respectively. By contrast the annual mean air temperature of the summit of Mt. Halla is only 3.7℃, with much colder winters (Lee et al. 1999, Won et al. 2006). The peak normally regains its winter snow cover during mid to late October.

Vegetation cover on Mt. Halla is almost complete and varies in composition with altitude due to differences in climate, but it also differs locally depending on the underlying geology. The vegetation of Jeju Island can be categorized into six groups: coastal vegetation, pasture/croplands, evergreen broadleaf forest, deciduous forest, coniferous forest and shrub. Coastal vegetation at

the foot of Halla includes many rocky cliffs with chasmophytes. Evergreen broadleaf forest, dominated by *Castanopsis cuspidata* var. *sieboldii* and *Quercus salicina* - *Quercus glauca* community, is the main natural vegetation in the lowlands of Jeju, and remains widespread where not cleared for agriculture. The lower parts of Mt. Halla, itself has a long history of human land use but its slopes above about 800m are in a semi-natural state, now protected as a national park. Deciduous forest is distributed below and within the park, around 600m~1,400m in an altitudinal zone between evergreen broadleaf forest and coniferous forest. The deciduous *Quercus serrata* forest also contains *Acer palmatum*, *Prunus sargentii*, *Quercus mongolica*, and *Carpinus laxiflora*. Coniferous subalpine forest dominated by *Abies koreana* is densely concentrated around 1,400masl, on the northern face of Mt. Halla. A shrub zone is concentrated around 1,600masl on the northern side, and 1,500masl on the southern side with species such as *Juniperus chinensis* var. *sargentii*, *Empetrum nigrum* var. *japonicum*, *Ilex crenata*, and *Ligustrum obtusifolium* (Lee et al. 2010). Hallasan National Park has been designated a World Heritage Site (2007; http://www.hallasan.go.kr/english/content.php?page=0102) and an UNESCO Biosphere Reserve (2002; http://www.hallasan.go.kr/english/content.php?page=0102) for its high level of endemism and unique distribution of vegetation.

As large sections of Jeju are of fairly uniform volcanic composition, it is possible to study a cross section of altitudes with slightly different climates, yet with most of the complicating factor substrate geological variation removed. Despite the uniform geology of large areas, Jeju island/Mt. Halla also consists of a mosaic of slightly different volcanic types of differing ages. This provides a range of different substrate ages and geologies which allows comparison of the effects of different volcanic types under similar climate.

Sampling

In early September 2010, we took an altitudinal transect which roughly followed the Yeongsil Hiking Trail (Lee 2012) along the south-west slope of Mount Halla, and down into the more gently sloping surrounding lowland areas leading down to sea level towards the south coast of Halla. This transect was mainly covered in trachybasalt lava deposits.

A second altitudinal transect, which followed the Gwaneumsa Hiking trail (Lee 2012) was also taken to compare basalts on the north-eastern side of Halla and down into the more gently sloping lowlands. For both transects, Yeongsil – ranging from 150 to 1700masl- and

Gwaneumsa – ranging from 500 to 1700masl, we avoided sampling in the immediate vicinity (within 50m) of intensively used trails to avoid the various possible human influences. We sampled 9 and 7 altitudinal bands at Yeongsil and Gwaneumsa transects respectively, as closely as possible to 200m intervals (determined by GPS) within the prevalent vegetation types. Finally, 4 more samples were taken at the summit encircling the edges of the Crater Lake located at 1950masl. These four samples acted as the common summit samples for both transects. The use of two separate transects provides a replicate to determine if the trend with altitude seen in one transect is spurious or not, and at the same time allows to study the effect of different lava types on the bacterial community (Fig. 15).

In each transect the aim was to compare the effects of altitude on soil microflora, with variation in vegetation and climate type. The two transects are also on slightly different geologies, one being the more porous trachybasalt (Yeongsil), allowing some understanding of possible influences of rock type on soil prokaryotic flora.

'Sample areas' were all 0.1 hectare (a 10m by 10m square), located at least 100m apart from one another, except where access difficulties or the tapering width of the summit prevented this (in which case they were located at least 50m apart). Each individual subsample consisted of 5 equal scoops (50g) of soil from the top 5cm underneath the litter layer. Five subsamples, one taken at each corner and one at the center, were gathered from each 0.1 ha area, and mixed into a single sample bag (Fulthorpe et al. 2008). Four replicates were taken at each elevational point adding a total of 68 samples from both transects and the summit (Table. 4a & 4b).

GPS coordinates taken during the field survey were used to construct a spatial point layer from which Mean Annual Temperature (MAT) and Mean Annual Precipitation (MAP) values were extracted for each altitudinal band using digital climate maps produced by the Korea Meteorological Administration (KMA) and the National Center for Agrometeorology (NCA). MAT data is based on the data observed from 1971 to 2008, and precipitation amount is based on data from 1981 to 2008. The spatial resolution of the raster data is 30m for temperature and 270m for precipitation.

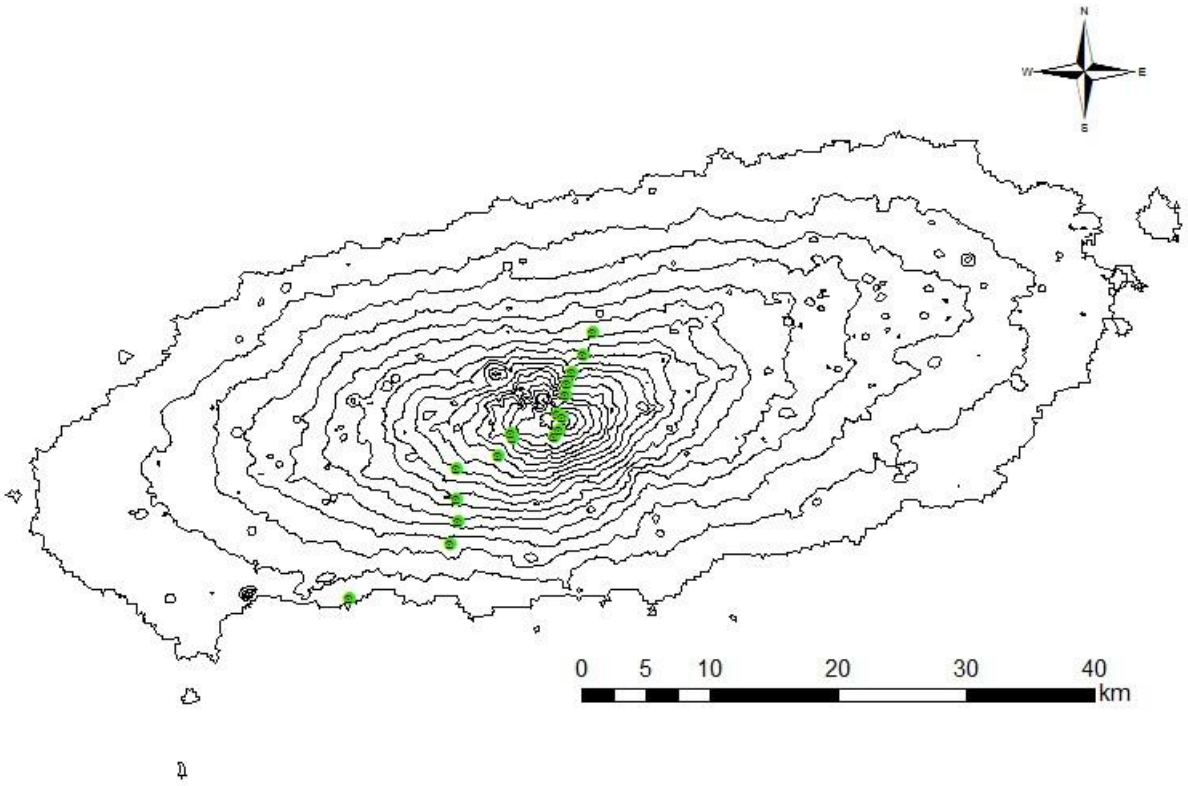

Fig. 15. Contour map view of Mt. Halla. Sampling locations are located at 200m intervals along transect and are shown as green dots with Yeongsil transect is located in south eastern portion of Jeju map and Gwaneum transect in north western portion. Contour interval is 100m.

3.3 BACTERIAL DIVERSITY ON MT. HALLA

3.3.1 Materials and Methods

3.3.1.1 DNA Extraction and PCR Amplification

Samples were processed as discussed earlier under section 2.3.1.1.

3.3.1.2 Processing Pyrosequencing Data and Taxonomic Analysis

Data obtained after pyrosequencing was processed using Mothur (Schloss et al. 2009) except for the step of removing chimeric sequences. Briefly, sequences shorter than 150nt with homo-polymers longer than 8nt and all reads containing ambiguous base calls or incorrect primer sequences were removed. Next, the sequences were aligned against the EzTaxon-extended database (http://eztaxon-e.ezbiocloud.net/; (Kim et al. 2012b) and then trimmed, so that subsequent analyses were constrained to the same portion of the 16S rRNA gene (V1-V3 region). Putative chimeric sequences were detected and screened using a similarity-based approach, which splits each query sequence into two even length fragments and then assigns each fragment to a taxon using BLAST search against EzTaxon-e database followed by removal of the sequences when two fragments differ at the order level or percent identities are greater than 95% for both fragments despite being assigned to different taxonomies. The remaining reads were pre-clustered using the pre-cluster command (http://www.mothur.org/wiki/Pre.cluster) to remove erroneous sequences derived from sequencing errors and then clustered using Mothur's average algorithm. Taxonomic classification of each OTU (clustered at $\geq$ 97% sequence similarity) was obtained by classifying alignments against EzTaxon-e reference bacterial taxonomy and non-redundant nucleotide bacterial databases files using the classify command at 50% Bayesian bootstrap cutoff with 1000 iterations.

Table. 4a: Site and sample characteristics of Gwaneumsa transect, Mt. Halla with vegetation types.

Sample	Coordinates	Elevation (masl)	pH	Total Organic Carbon (%)	Total Nitrogen (%)	Total Phosphorus (mg/kg)	Salinity (%)	MAT (^{o}C)	MAP (mm)	Vegetation
GES500	N 33, 25, 29.8 E 126, 33, 24.3	500	4.72	16.363	1.119	1377.415	0.125	12.849	2585.5	Evergreen deciduous with dwarf bamboo
GES700	N 33, 24, 33.0 E 126, 33, 01.2	700	4.25	26.430	1.161	771.168	0.075	11.395	2958.25	Deciduous with dwarf bamboo
GES900	N 33, 23, 48.5 E 126, 32, 30.8	900	4.05	18.155	1.006	732.000	0.070	10.222	3308.75	Deciduous with dwarf bamboo
GES1100	N 33, 23, 20.6 E 126, 32, 21.4	1100	4.18	20.395	1.140	806.918	0.065	9.181	3206.5	Deciduous with dwarf bamboo
GES1300	N 33, 22, 52.9 E 126, 32, 15.8	1300	3.78	16.368	1.229	810.618	0.123	8.170	3520.5	Deciduous with dwarf bamboo
GES1500	N 33, 22, 52.9 E 126, 32, 15.8	1500	4.34	7.438	0.437	1003.330	0.075	7.026	3742.75	Coniferous with dwarf bamboo
GES1700	N 33, 21, 56.2 E 126, 32, 09.5	1700	4.40	13.805	0.664	1464.500	0.103	5.387	3668	Coniferous with dwarf bamboo
GES1950	N 33, 21, 22.6 E 126, 32, 00.3	1950	4.40	4.280	0.273	402.008	0.035	6.992	3700.5	Shrubland

masl: meters above sea level, MAT: Mean Annual Temperature, MAP: Mean Annual Precipitation.

Table. 4b. Site and sample characteristics of Yeongsil transect, Mt. Halla with vegetation types.

Sample	Coordinates	Elevation (masl)	pH	Total Organic Carbon (%)	Total Nitrogen (%)	Total Phosphorus (mg/kg)	Salinity (%)	MAT (^{o}C)	MAP (mm)	Vegetation
YS150	N 33, 14, 17.6 E 126, 23, 06.9	150	5.52	8.54	0.552	2728.085	0.057	15.387	1712.8	Pine with evergreen deciduous
YS300	N 33, 16, 34.2 E 126, 27, 23.2	300	5.52	17.192	0.948	1032.833	0.067	14.433	2077	Pine with evergreen deciduous
YS500	N 33, 17, 32.8 E 126, 27, 43.6	500	4.74	14.435	0.983	849.737	0.07	13.839	2309	Evergreen deciduous with dwarf bamboo
YS700	N 33, 18, 28.1 E 126, 27, 38.6	700	4.56	28.137	1.539	919.96	0.052	12.823	2425.5	Evergreen deciduous with dwarf bamboo
YS900	N 33, 19, 48.0 E 126, 27, 39.9	900	4.53	26.657	1.562	892.637	0.067	11.360	2686.8	Deciduous with dwarf bamboo
YS1100	N 33, 20, 16.9 E 126, 29, 24.2	1100	4.34	24.677	1.137	739.685	0.062	10.378	2923.3	Deciduous with dwarf bamboo
YS1300	N 33, 21, 02.9 E 126, 30, 03.5	1300	4.16	13.06	0.978	812.495	0.107	8.594	3353.3	Coniferous with dwarf bamboo
YS1500	N 33, 21, 16.2 E 126, 29, 57.6	1500	4.29	13.12	0.922	1146.858	0.057	9.128	3288.3	Shrubland
YS1700	N 33, 21, 08.2 E 126, 31, 49.4	1700	4.29	6.873	0.469	315.87	0.036	7.112	3738	Shrubland
YS1950	N 33, 21, 22.6 E 126, 32, 00.3	1950	4.40	4.28	0.272	402.007	0.035	6.991	3700.5	Shrubland

masl: meters above sea level, MAT: Mean Annual Temperature, MAP: Mean Annual Precipitation.

3.3.1.3 Statistical Processing and Analysis of Results

To assess the relationship between soil bacterial species richness/diversity, and elevation as well as with edaphic factors such as pH, soil nutrients and environmental parameters, OTUs and other diversity indices were calculated using the Mothur platform (Schloss et al. 2009) for samples standardized to 566 reads (size decided by default, set to the size of smallest sample size) as diversity is directly correlated with the number of sequences collected (Table. AT5a and AT5b in appendix). One sample from the Yeongsil transect at 1700masl was removed due to low number of reads. To compare community-level bacterial diversity, we used the non-parametric Shannon index and the Faith's PD as this index describes the evolutionary history or phylogeography of each bacterial community. Regression analysis using OTUs, Shannon Index and Faith's PD in relation to elevation, pH and other environmental parameters were performed using SigmaPlot (version 10.0). To assess whether if the relative abundance of the nine most abundant phyla differed between the two transects we performed linear models for normal data, or generalized linear models for not normal data, with site, elevation and their interaction as factors. Non-significant interactions and terms were removed sequentially from the model. Analyses were performed with R version 2.15.1.

We performed a Non-metric Multi-Dimensional Scaling (NMDS) using Primer v6 (Clarke and Gorley 2006) to explore patterns in species composition using a Bray Curtis similarity matrix. Because total number of reads was used for this analysis, abundance data was square root transformed and then standardized by sample total.

Mantel tests (Legendre and Legendre 1998) were performed between Bray-Curtis and UniFrac dissimilarity matrices (obtained using Mothur − (Schloss et al. 2009) and Euclidean distance matrices of each environmental variable calculated on an averaged normalized data using PRIMER-6 (Clarke and Gorley 2006) to look at the variation explained by environmental variables. Finally to tease apart the relative importance of the environmental variables on the bacterial community similarity and phylogenetic structure, we used Multiple Regression on Matrices (MRM) approach (Legendre et al. 1994). Before applying MRM to the dataset, we looked for redundant edaphic factors using the VARCLUS procedure in the Hmisc package (Sarle 1990). The highest correlation was between elevation and MAT/MAP (Spearman's $\rho 2 \geq 0.82$; on both the trails). Total organic carbon and total nitrogen were the next most correlated

(Spearman $\rho 2 \geq 0.54$; on both the trails). Therefore, we removed MAT, MAP and total nitrogen and the remaining six environmental variables, namely pH, total phosphorus, C:N ratio, elevation, and total organic carbon were taken for the final MRM analysis for both trails (Fig. AF4 in appendix). Non-significant factors were removed sequentially and the MRM analysis was repeated until only significant factors were left in the model. Significance was tested by permutations (9999) and P-values of the two tailed tests are reported for this analysis. Both Mantel and MRM analysis were performed in the ecodist R package (Goslee and Urban 2007).

To investigate whether there is an effect of vegetation type regardless of elevation, we performed an ANOVA on phylogenetic diversity, chao Index and number of OTUs taking into account only the five main types of vegetation (i.e. coniferous forest, deciduous forest, evergreen forest, pine forest and shrub land). Post-hoc Tukey's HSD test was used for pairwise comparisons when the effect of vegetation was significant. All variables were checked for normality or transformed to meet a normal distribution before analysis. This analysis was performed using R version 2.15.1.

3.3.2 Results

Broad taxonomic features of bacterial community on Mt. Halla

A total of 189,409 quality sequences were classified into 5, 256 OTUs at ≥ 97% similarity level, distributed across all 67 samples. On average, 355 OTUs were found in each sample standardized at 566 reads. Proteobacteria was by far the most abundant phylum accounting for approximately 34% of the total sequences obtained, followed closely by Acidobacteria with around 32.5% of the total sequences (Fig.16, Table AT6a & AT6b). The most abundant single phylotype across the entire sample was classified under genus Acidobacteria Gp2 with a total of 4563 sequences (around 2.4%). The second most abundant phylotype was from family Sinobacteraceae of Proteobacteria with a total of 2640 sequences (1.4%).

Comparison of two transects

The two transects were quite different in terms of community composition of the dominant phyla. On the Gwaneumsa transect, Acidobacteria was the most dominant phyla (31819seq, 35.14%) followed by Proteobacteria (28960seq, 31.98%; Fig. 16A), whereas the opposite was found at the Yeongsil transect (Proteobacteria, 37460seq, 35.47%; Acidobacteria, 30719seq, 28.95%; Fig. 16B). Interestingly, the most abundant single phylotype (belonging to Acidobacteria Gp_2) on Mt. Halla is the most abundant phylotype on both transects, although with a higher abundance on Gwaneumsa (2716seq, 3.0%) than on Yeongsil transect (1847seq, 1.74%). Comparison of the relative abundance for the nine most abundant phyla between the two transects reveals striking and consistent differences (Fig. AF5 in appendix). On the Yeongsil transect, Planctomycetes ($F_{1, 48} = 49.88$, $P < 0.0001$) was more abundant than on the Gwaneumsa transect. Gemmatimonadetes followed the same trend, but it was marginally non-significant ($F_{6, 42} = 5.55$, $P = 0.08$). The interaction between transect and elevation was significant for Acidobacteria ($F_{6, 42} = 7.87$, $P < 0.0001$), Proteobacteria ($F_{6, 42} = 4.63$, $P<0.001$) and TM7 ($F_{6, 42} = 2.36$, $P = 0.05$).

Results show a significant difference in bacterial diversity/richness with elevation, although on the two transects diversity tends to vary differently along the elevational gradient (Fig. 17). On the Gwaneumsa transect, there was a “peak” in diversity/richness in higher

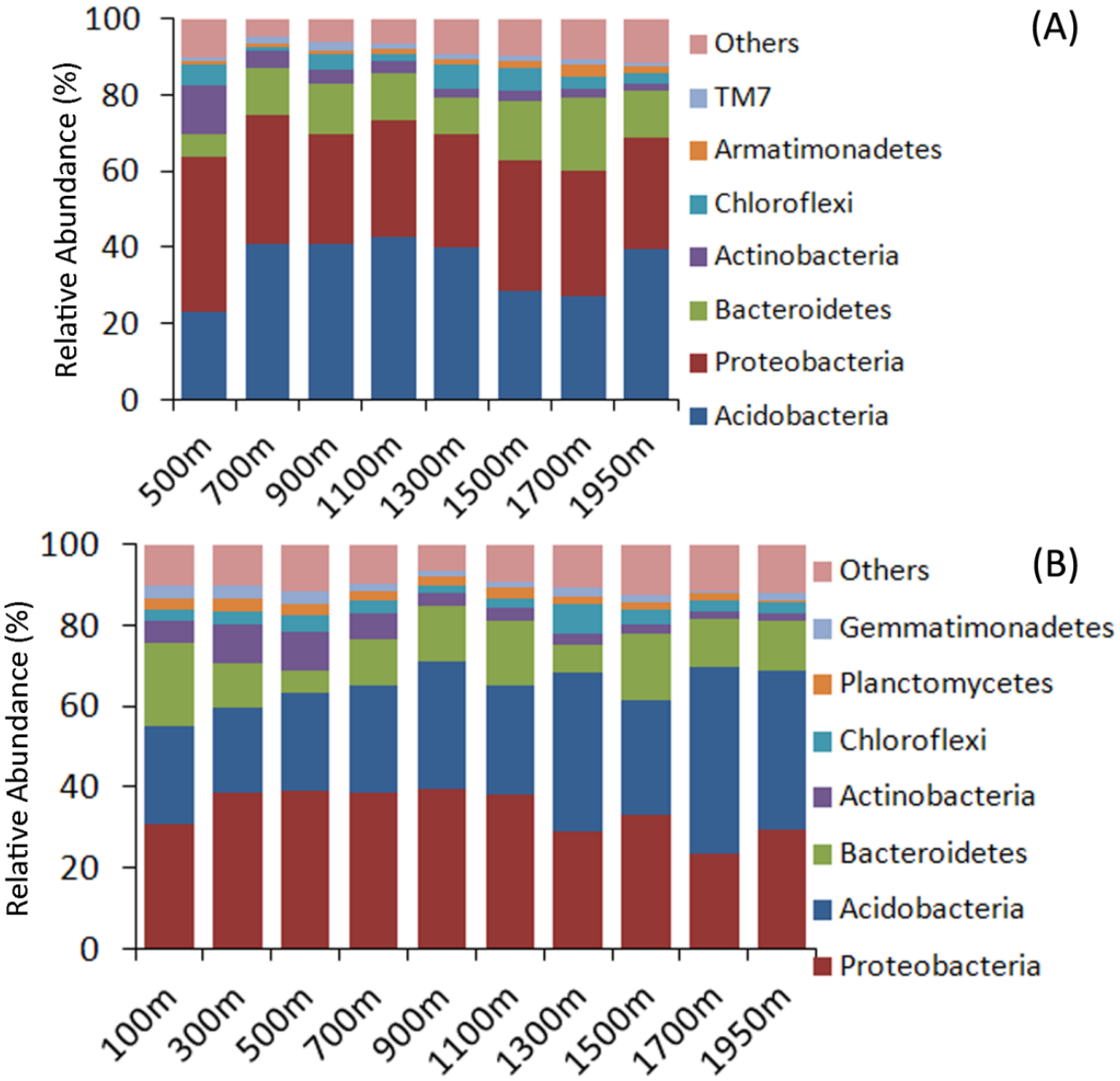

Fig. 16. Phyla breakdown (bacterial community) for the Gwaneumsa (A) and Yeongsil transects (B) of Mt. Halla.

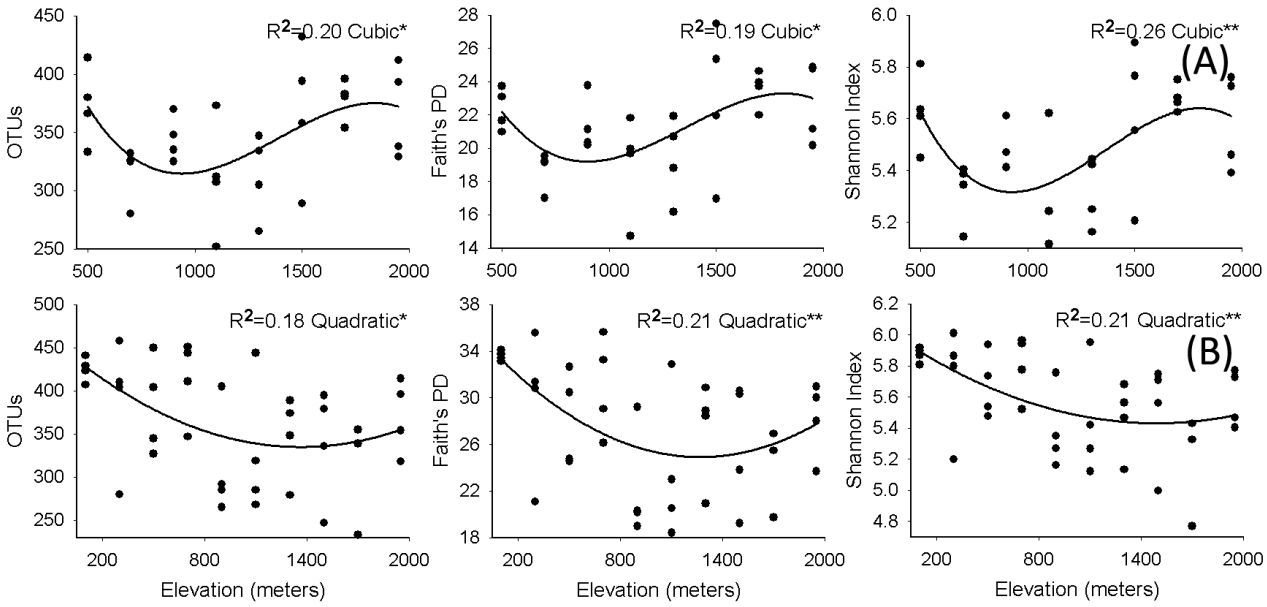

Fig. 17. Relationship between elevation and phylotype richness (left), phylogenetic diversity (middle), phylotype diversity (right) for the Gwaneumsa (A) and Yeongsil transects (B) on Mt. Halla. We tested three models (linear, quadratic, and cubic) to describe the relationships and model selection was carried out based on adjusted R^2 and RMSE (root mean square error; value not shown). Significance level is shown with***P<0.001; **P<0.01; and P<0.05.

elevations at around 1700masl. Maximum richness with approximately 56% of OTUs was observed at 500masl whereas minimum richness was observed at 1300masl (only around 19% of total OTUs) (see Fig.17). On the other hand, Yeongsil transect showed a "hollow" pattern in diversity/richness towards higher altitudes with a maximum richness observed at 700masl (57% of total OTUs observed) while minimum richness was observed at 1700masl (20% of total OTUs observed). These results show that the bacterial communities were sharply different among the different elevations on Mt. Halla. To confirm whether there is no role of sampling in presence of different patterns on both transects, we removed the 100 and 300masl sampling points from the Yeongsil transect to make it more comparable to the Gwaneumsa transect and reanalyzed the data. This resulted in no significant relationship between richness/diversity with elevation at the Yeongsil transect when data from 100 and 300masl were removed (results not shown). There was also a significant correlation between bacterial richness/diversity and pH, soil phosphorus content, MAP and MAT on both transects but not as strong as with the elevation (Table. 5).

When we further examined the % relative abundance of the five most abundant phyla individually, most of them were significantly correlated with elevation on at least one of the two transects. Specifically, the relative abundance of Acidobacteria and Alpha-proteobacteria was significantly correlated with elevation on both transects (Fig. AF6 in appendix). Soil pH also showed significant correlation with the relative abundance of Acidobacteria and Beta-proteobacteria on both transects (results not shown). In addition, soil total phosphorus was also significantly correlated with the relative abundance of different dominant phyla (acidobacterial abundance correlated with total phosphorus on both transects; Beta proteobacteria only on Gwaneumsa and Bacteroidetes only on Yeongsil were correlated with total phosphorus) (results not shown).

Both richness and diversity indices are useful in describing community characteristics by a single vector, but they provide no evaluation of important compositional features of biodiversity relating to the abundances of shared taxa. An NMDS performed on the Bray-Curtis similarity matrix calculated from the total community, specifically assesses changes in bacterial community composition (incorporating both taxon abundance and identity). NMDS showed that composition of the bacterial communities was highly variable across the soils represented by different elevations (Fig. 18). Both transects showed significant variability across the elevational

Table. 5. Relationship between elevation and soil parameters with phylotype richness, phylogenetic diversity and phylotype diversity for the whole bacterial community on two transects at Mt. Halla. (Adjusted R^2 shown)

Variables	OTUs	Faith's PD	Shannon Index
Gwaneumsa Trail			
Elevation (masl)	0.20 (c*)	0.19 (c*)	0.26 (c**)
pH	0.11 (l*)	-	0.14 (l*)
Phosphorus (mg/kg)	0.11 (l*)	-	0.10 (l*)
Total Nitrogen (%)	-	0.10 (l*)	0.10 (l*)
MAT (^{o}C)	0.20(q*)	0.20(q*)	0.28(q**)
Precipitation (mm)	0.25(q**)	0.25(q**)	0.30(q**)
Yeongsil Trail			
Elevation	0.18 (q*)	0.21 (q**)	0.21 (q**)
pH	0.10 (l*)	0.12 (l*)	0.16 (l*)
Phosphorus	0.10 (l*)	0.15 (l**)	0.15 (l*)
Total Nitrogen	-	-	-
MAT (^{o}C)	0.18(q*)	0.22(q**)	0.21(q**)
Precipitation (mm)	0.17(q*)	0.21(q**)	0.21(l**)

We tested three models (linear-l, quadratic-q, and cubic-c) to describe the relationships: model selection was carried out based on adjusted R^2 and RMSE (root mean square error). Significance level is shown as ***P≤0.0001, **P≤0.001, and *P≤0.05. Only values for significant relationships are shown.

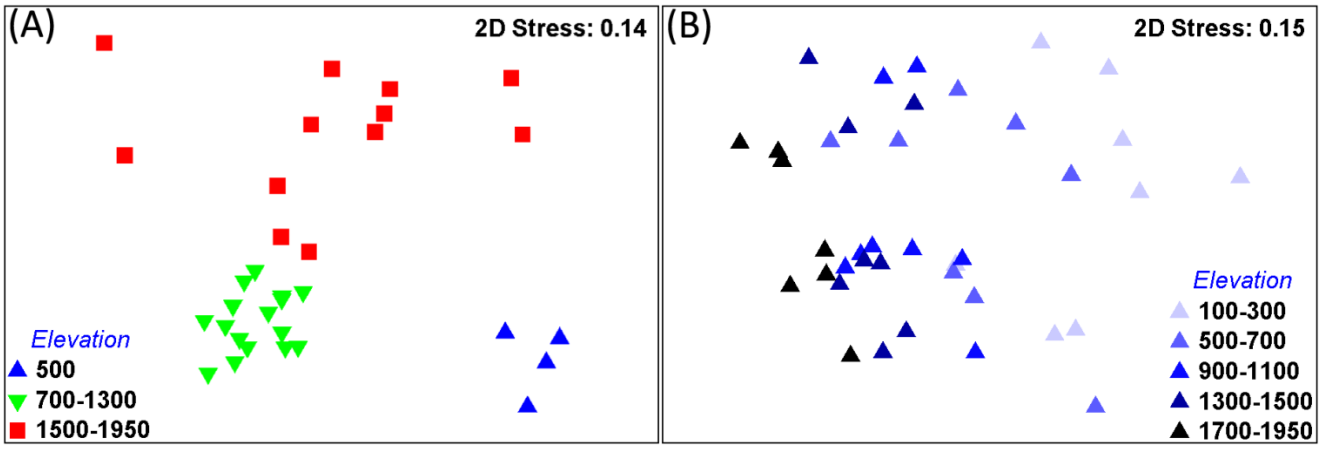

Fig. 18. NMDS of Bray-Curtis similarity of overall community composition (bacteria) in relation to elevation for the Gwaneumsa (A) and Yeongsil (B) transects of Mt. Halla.

gradient, however the clustering pattern observed on the two transects was different. Gwaneumsa formed three separate clusters according to the sample elevation range, showing that the samples belonging to the different elevational zones harbored distinct communities. Yeongsil, on the other hand had samples arranged in a pattern on the NMDS plot from left to right in order of their decreasing elevation. Bray-Curtis similarity index on Yeongsil shows minimal overlap between communities that differ by more than 400masl.

A Mantel test was used to determine the environmental factors that significantly correlated with the community composition (Bray-Curtis and UniFrac). There was a significant correlation with elevation and MAT with community composition on both transects, but MAP and pH were correlated with community composition only on the Yeongsil transect. Total phosphorus was significantly correlated with both UniFrac and Bray-Curtis distances on Gwaneumsa but was correlated only marginally with UniFrac on Yeongsil (Table. 6).

To further assess the relative importance of 6 independent environmental variables (see Fig. AF4 in appendix) contributing to these correlations, we used a MRM which was able to explain a significant portion of the variability in bacterial community composition on both the Gwaneumsa (Bray-Curtis: R^2=59%, P < 0.0006; UniFrac: R^2 = 64%, P < 0.0002) and the Yeongsil (Bray-Curtis: R^2= 74%, P < 0.0001; UniFrac: R^2=55%, P < 0.0008) transects. Elevation was the only variable which was able to explain a significant proportion of variability on both transects for both Bray-Curtis and UniFrac dissimilarities (Table. 7). pH had a significant effect on community similarity only on the Yeongsil transect, but not on the Gwaneumsa one. Notably, total phosphorus and elevation explained the same proportion of variation at Gwaneumsa transect, whereas at the Yeongsil transect total phosphorus was only significant for Bray-Curtis index (Table. 6).

Effect of vegetation type

An ANOVA showed that, irrespective of elevation, vegetation had a significant effect on phylogenetic diversity ($F_{4, 65}$=10.25, P<0.0001; Fig. 19), Shannon index ($F_{4, 65}$=6.26, P=0.0002; Fig. 19) and number of OTUs ($F_{4, 65}$=6.23, P<0.0003; Fig. 19).

Table. 6. Correlation between Bray-Curtis and unweighted UniFrac dissimilarity (bacterial community) with environmental and edaphic factors (Spearman rank correlations estimated using Mantel tests) on Mt. Halla

	Variable	**Bray-Curtis**	**UniFrac**
Gwaneumsa	Elevation	0.562**	0.595**
	MAT	0.600*	0.558*
	MAP	-	-
	pH	-	-
	Total Phosphorus	0.642*	0.654*
Yeongsil	Elevation	0.688**	0.648**
	MAT	0.729**	0.702**
	MAP	0.765**	0.733
	pH	0.773**	0.670
	Total Phosphorus	-	0.574[a]

Significance level is shown as ***P≤0.001, **P≤0.01, *P≤0.05, and [a]P=0.054 (Bonferroni corrected P values). Only values for significant relationships are shown.

Table. 7. Results of the multiple regression on matrices analysis for the whole bacterial community on Mt. Halla.

	Variables	**Whole community**	
Gwaneumsa		Bray-Curtis (R^2= 0.594a)	UniFrac (R^2= 0.637a)
	pH	-	-
	Elevation	0.035**	0.014**
	Total Phosphorus	0.038*	0.014*
Yeongsil		Bray-Curtis (R^2= 0.743a)	UniFrac (R^2= 0.551a)
	pH	0.022*	0.008*
	Elevation	0.019*	0.009*
	Total Phosphorus	0.012*	-

The variation in community composition (both R^2 values are significant at $P \leq 0.001$) explained by only significant variables. Partial regression coefficients (a) of the final models are reported for only significant values***$P \leq 0.0001$, **$P \leq 0.001$, *$P \leq 0.01$).

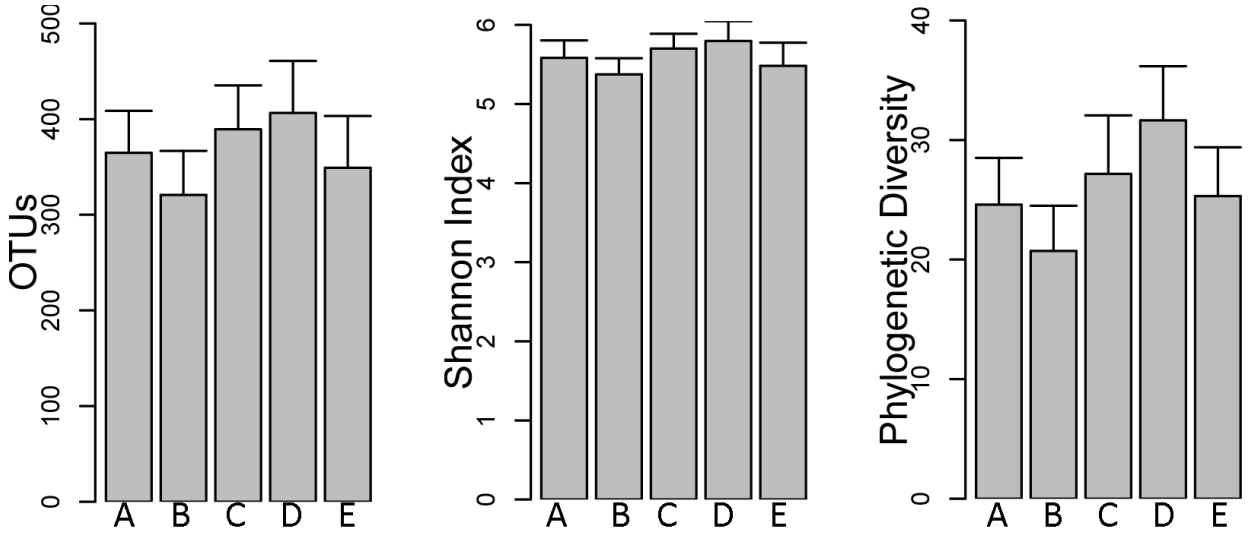

Fig. 19. Bacterial Richness (OTUs), Diversity (Shannon Index) and Phylogenetic Diversity (Faith's PD) for the five main vegetation types sampled at Mt. Halla. Mean ± SD. Abbreviations: A – Coniferous with dwarf bamboo, B – Deciduous with dwarf bamboo, C – Evergreen Deciduous with dwarf bamboo, D – Pine with evergreen deciduous and E – Shrubland.

3.3.3 Discussion

In this study, we examined the elevational diversity gradient and community composition along two geologically distinct transects located on opposite faces of Mt. Halla, to elucidate the factors which might affect variation in bacterial community structure on the mountain. We found clear elevational patterns in taxonomic richness, phylotype diversity, phylogenetic diversity and community composition. All microbial parameters were strongly related to elevation and soil total phosphorus. Soil bacterial diversity/richness exhibited different patterns with elevation on different transects of Mt. Halla; however none of these patterns was unimodal or monotonous as observed in other studies (Bryant et al. 2008, Fierer et al. 2011, Singh et al. 2012b). This suggests that a unique set of descriptors (biotic and abiotic) could be responsible for a specific pattern observed between bacterial diversity and elevation on different sampled landscapes.

3.3.3.1 Diversity/Richness patterns with elevation

A trend in diversity with altitude is found for both transects, with a 'dip' in diversity which reaches its low point at around 1100m elevation. This dip was then followed by subtle "peak" on Gwaneumsa whereas on Yeongsil the patterns of both bacterial species richness and diversity were hollow towards higher elevations (Fig. 17). This hollow elevational pattern has rarely been observed in nature (Rahbek 2005), although (Wang et al. 2012) reported it for species richness and phylogenetic diversity on biofilm bacterial communities along an elevational gradient from 1820 to 4050m in China, for the whole community as well as for the phylum Proteobacteria It is not clear, however, what exactly has caused this elevation-diversity trend. One possibility is that it represents some undetected gradient in soil chemistry or texture, produced by either the physico-chemical weathering conditions of the underlying lava rock, or by variation in the chemistry or texture of the lava rock itself. Another possibility is that the observed trend is product of a gradient in disturbance rates in the soil. Disturbance gradients may have a role in the elevational gradient in soil bacterial diversity on Fuji found by our group (Singh et al. 2012b). However, there is no obvious quantifiable disturbance factor available for Jeju to test this hypothesis. Unlike Fuji, with its unstable volcanic ash fields from mid-elevations upwards, Mt Halla is mainly vegetated at all altitudes. Thus it is unlikely that soil movement or frost heave of surface stones is a significant factor on Halla as it may be on Fuji. Therefore, the

generality of these observations for microbes still needs to be addressed by more extensive studies for specific habitats, as well as across habitats.

3.3.3.2 Consistent differences in phyla breakdown between the two transects

The consistent abundance of phyla Planctomycetes and Gemmatimonadetes on the Yeongsil transect is a particularly intriguing result (Fig. AF5 in appendix). One such study, reported that the Planctomycetes abundance in soil was correlated with the soil nitrate (NO_3N) levels suggesting that the diversity of the Planctomycetes community may be a function of either heterogeneity in the NO_3N levels themselves or heterogeneity in soil processes or characteristics correlated with soil NO_3N levels (Buckley et al. 2006). To test this hypothesis, we randomly chose single soil replicates from an elevation range of 500 -1700masl on both transects and measured their NO_3N contents. Taking % relative abundance of Planctomycetes as response variable and soil NO_3N as a predictor variable, while incorporating data from both transects, showed that the abundance of phylum Planctomycetes was indeed correlated to the soil NO_3N content.

Gemmatimonadetes abundance on the other hand, has been suggested to be inversely correlated to soil moisture (DeBruyn et al. 2011) with many phylotypes being reported from semiarid and arid environments (Chanal et al. 2006, Acosta-Martinez et al. 2008, Kim et al. 2008, Mendez et al. 2008, Costello et al. 2009, Cary et al. 2010). We used Mean Annual and Mean Monthly (for the month of September) precipitation (Tables. 4a & 4b) values as a proxy for soil moisture, and we compared these against abundance of Gemmatimonadetes. We found that indeed low precipitation areas of Mt. Halla had increased abundance of this phylum (Spearman correlation: MAP, ρ = -0.38, P= 0.002; mean monthly temperature for September, ρ = -0.44; P=0.0002).

3.3.3.3 Explaining variance in bacterial diversity and community composition

We used NMDS ordination to overcome the shortcomings of using a single richness or diversity index which are unable to provide a whole picture of important compositional features of biodiversity relating to the abundance of different taxa encompassing the total community. The NMDS provided different clustering patterns for microbial community on both transects (Fig. 18). These patterns could be explained in terms of environmental filtering mainly due to MAT and MAP, as these two climatic variables co-vary very strongly with elevation (Varclus

results; see Fig. AF4 in appendix). In fact, an NMDS plot on the Euclidean distances from MAT and MAP values looked very similar to the patterns observed on both transects (graph not shown). Climatic factors have already been shown in many studies as the strongest positive forces in shaping the elevational diversity patterns (Hawkins et al. 2003, Currie et al. 2004, von Storch et al. 2004, Forister et al. 2010, Griffiths et al. 2011). This correlation between the community composition and MAT/MAP can be seen again, when we see the results of the Mantel tests. MAT along with elevation was the only other variable which was significantly correlated with the community composition (Bray-Curtis) and phylogenetic structure of the community (UniFrac) on both transects (Table. 2). Temperature has earlier been shown to control bacterial communities in natural environments (Hall et al. 2008, Miller et al. 2009, Adams et al. 2010). Moreover, water temperature was the strongest environmental filter for phylogenetic structure in one study on elevational gradient in a biofilm bacterial community (Wang et al. 2012). MRM results further emphasize this point where elevation (MAT and MAP removed due to high co-variance with elevation) was the only variable which constantly explained a significant portion of variability observed for community composition and phylogenetic structure on both transects (Table. 9). Overall, these results indicate that ecological processes possibly related to temperature and rainfall, may play a dominant role in structuring bacterial biodiversity along the elevational gradient as was recently found in a study across a range of ecosystems in Britain (Griffiths et al. 2011).

This study has confirmed the generality of certain patterns in soil bacterial diversity seen in other parts of the world. Soil pH, generally found to be the most important identifiable factor controlling soil bacterial diversity has recently been found to be driving the spatial distribution of bacterial community along an elevational transect on Mt. Changbai (Shen et al. 2012). Interestingly, soil pH was a key determinant explaining bacterial community composition and phylogenetic structure only on Yeongsil transect. The reason for the absence of soil pH as a determinant factor on Gwaneumsa could be the presence of a very small pH range on the Gwaneumsa transect, ranging from 3.67 to 4.95.

Diversity and vegetation cover

Vegetation cover (broadleaved evergreen forest, deciduous forest, pine forest, scrub, and grassland) was significantly correlated with bacterial phylotype diversity (Chao), phylogenetic

diversity (Faith's PD) and richness (OTUs) on Mt Halla. Comparison of data points at the same altitude on Jeju but with different vegetation cover does reveal statistically significant differences in diversity, but this pattern is not consistent across different altitudinal zones. It has been suggested earlier that local scale variation in dominant microbial communities can be explained by plant identity and substrate hotspots (Bezemer et al. 2010, Orwin et al. 2010, Thomson et al. 2010, Chu et al. 2011). In a recent study on forest refugia at Mt. Fuji, elevation was one of the main drivers of the vegetation patterns in the forests (Dolezal et al. 2012). Thus, we might infer that vegetation type may affect indirectly bacterial distribution along elevation through altering the soil composition, mainly the C and N status.

It is clear, comparing our results with those of other published studies, that no unifying pattern can be expected in terms of soil bacterial diversity trends amongst the world's mountain systems. Even different transects on the same mountain may show different diversity and community compositional trends. However, what is clear is that in the local context of geology, there are strong community composition trends with elevational gradients that follow climate rather than soil chemistry, suggesting the importance of climate adaptation in bacterial niches.

CONCLUSIONS

Our study, replicated across two geologically different volcanic mountains, adds to the growing body of evidence that microbes do weakly follow the well-established biogeographical patterns that are commonly exhibited by plants and animals in general (Rahbek 1995, Rahbek 2005). Except for the case of bacteria on Yeongsil transect of Mt. Halla, the finding that skewed or hump-shaped elevational patterns are present for microbes on both the mountains indicates that several factors may work in concert to determine patterns in species richness and diversity. In these studies, the patterns of diversity with elevation were similar, but the underlying mechanisms differed among mountain and domain types studied.

Our results suggest that a range of factors, including abiotic factors such as MAT, MAP, pH and soil phosphorus, and also biotic factors such as vegetation, operate together to explain variation in soil microbial community distribution along elevational gradients. Dominant amongst these seem to be elevation, a proxy for climate, suggesting that soil bacteria are finely adjusted in their niches to the prevailing climate. On both Fuji and Halla, variation in soil chemistry (e.g. pH) is not broad, and it is likely that holding soil chemistry relatively constant allows the importance of climate to be revealed. It is particularly clear that temperature and possibly precipitation are key to variation in microbial community structure and diversity, with secondary roles for pH, P and vegetation cover type. The importance of climate suggests that many bacterial species are quite finely adjusted to prevailing climate conditions.

Microbial lineages especially archaea on Mt. Fuji exhibited a spatially structured pattern across the gradient and different elevational zone harbored distinct microbial communities. Notably, the overwhelming contrasting presence of FFSB group and soil Gp1.1b of phylum Thaumarchaeota on lower and higher altitudes on Mt. Fuji emphasizes the importance of niche-adaptations and microclimates. Given the parsimonious hypothesis that closely related taxa are more ecologically (or functionally) similar, our observations suggest that microbial lineages harbor increasingly disparate ecological features (or functions) at increased elevational distances as a probable consequence of abiotic filtering. These findings highlight the utility of gathering information on phylogenetic relationships between communities in montane regions as a means of quantifying the potential consequences of selectively trimming evolutionary lineages under the scenario of mountaintop extinctions in response to global warming.

There were consistent differences between the two mountains, but the soils of both were dominated by Acidobacteria and Proteobacteria and the relative abundances of these and other major taxa differed between the lower and higher elevational zones.

Comparing our results with those of other published studies, and the range of different results seen in those studies, it is clear that no unifying pattern can be expected in terms of soil bacterial diversity trends amongst the world's mountain systems. Even different transects on the same mountain may show different diversity and community compositional trends as seen in the case of Mt. Halla. Of course, such transects are still far from perfect, as many other environmental variables co-vary with elevation (e.g. cloudiness, atmospheric density, absolute O_2 or CO_2 concentrations, UV radiation), but with careful interpretation such transects do have the potential to generate new insights into temperature controls on ecosystem structure and function. Although purely correlative, our findings help us to sharpen our hypotheses on the distribution of microbes, and provide a set of potential indicators for predicting microbial community composition in montane soils. Further studies such as experimentally manipulating plant communities and/or the environment and studies with a higher taxonomic resolution will provide even better insights into the underlying mechanisms involved in the spatial distribution of soil microbial communities. Much is still to be learned about this topic, for now, it is necessary to accept the unsatisfactory conclusion that we do not know whether a general relationship exists between species richness and elevation, or whether a universal explanation or model can be given.

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APPENDIX

List of Appendix

Table. AT6a: Relative average abundances of all 35 bacterial phyla classified against EzTaxone aligned database (Kim et al., 2012) across all 32 soil replicates of 8 elevational points on Mt. Halla Gwaneumsa transect.

Table. AT6b: Relative average abundances of all 39 bacterial phyla classified against EzTaxone aligned database (Kim et al., 2012) across all 40 soil replicates of 10 elevational points on Mt. Halla Yeongsil transect.

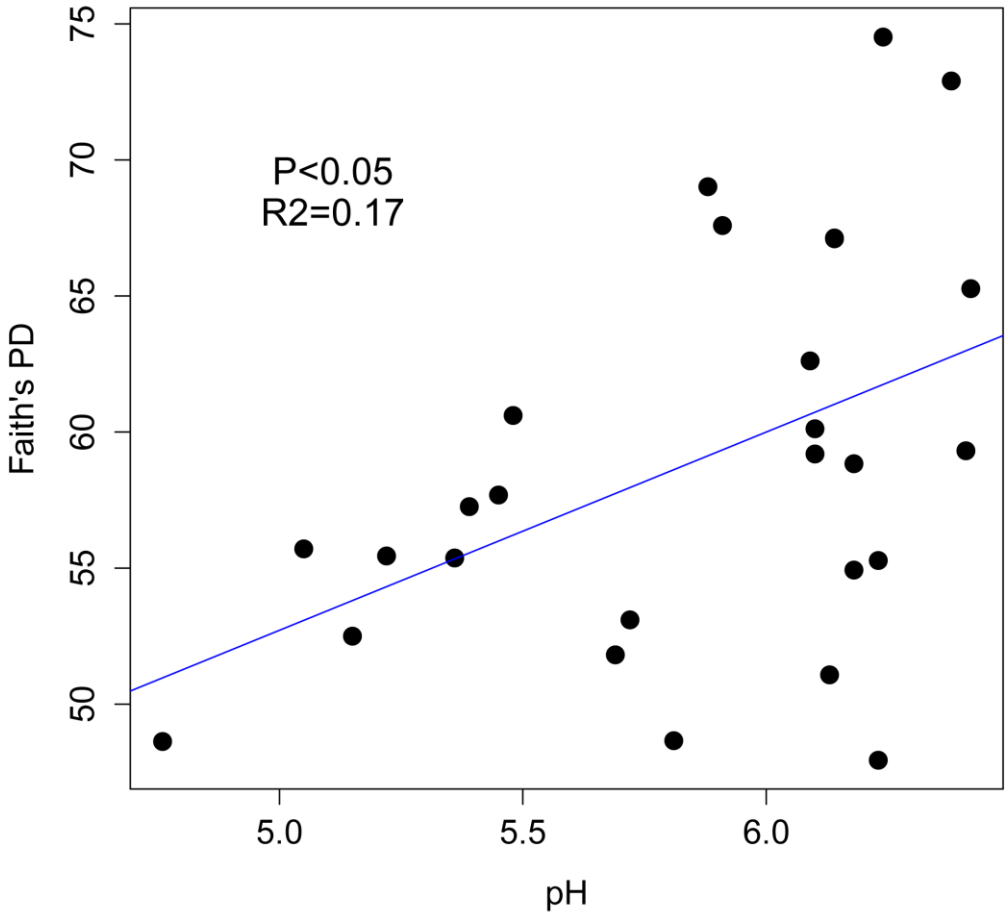

Figure. AF1: Relationship between soil pH and bacterial diversity on Mt. Fuji (bacteria)

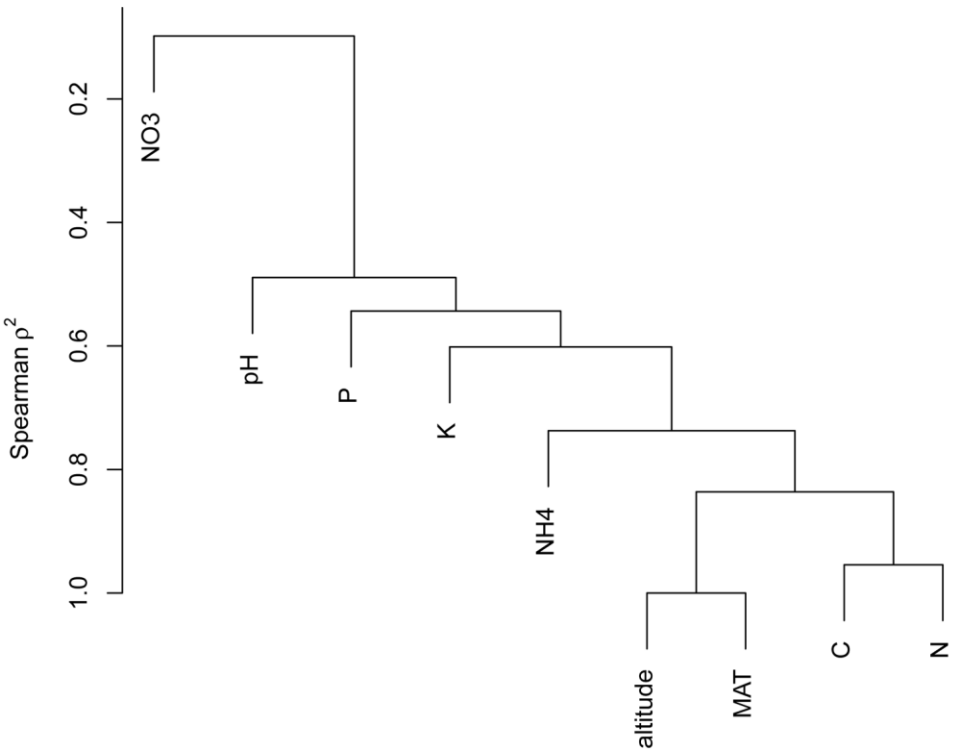

Figure. AF2: Cluster analysis of all the 9 measured environmental variables on Mt. Fuji.

Figure. AF3: Doughnut chart used to compare 3 different archaeal databases using our archaeal dataset from Mt. Fuji.

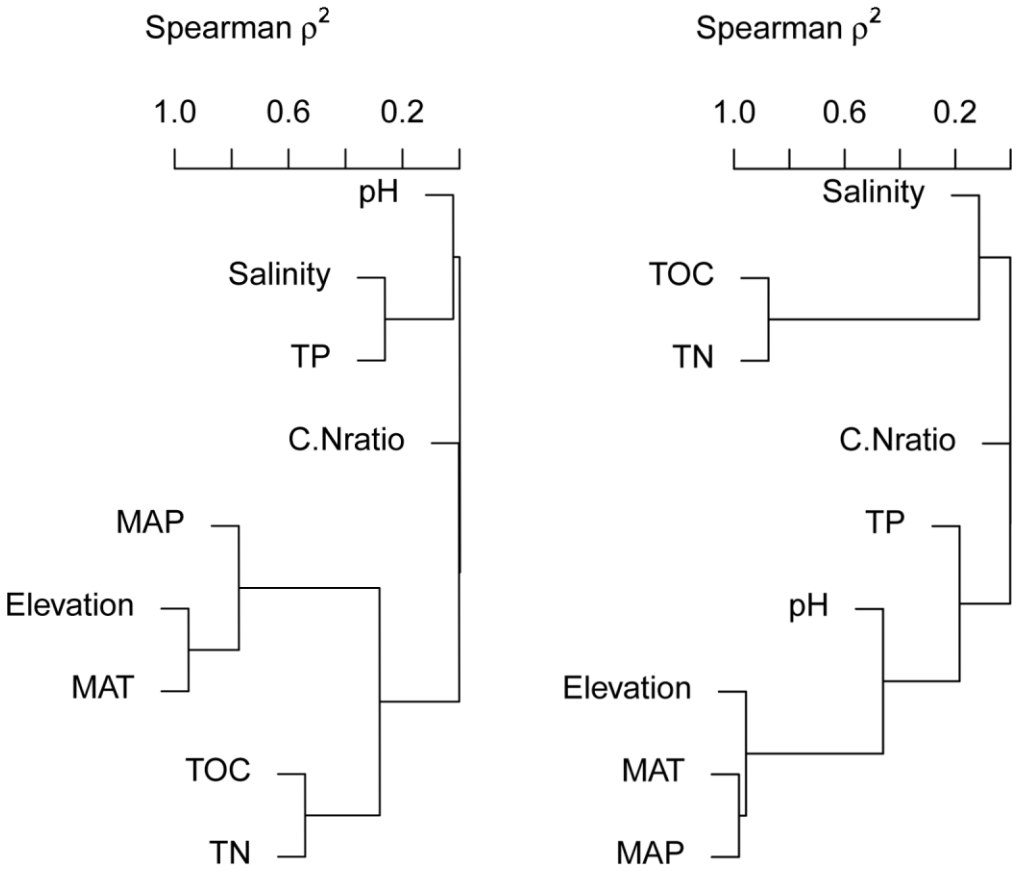

Figure. AF4: Cluster analysis of all the 9 measured environmental variables on Mt. Halla with left as Gwaneumsa and right as Yeongsil transects. Abbreviations; TP: Total Phosphorus, C.N ratio: Carbon/Nitrogen ratio, MAP Mean Annual Precipitation, MAT: Mean Annual temperature, TOC: Total Organic Carbon, TN: Total Nitrogen.

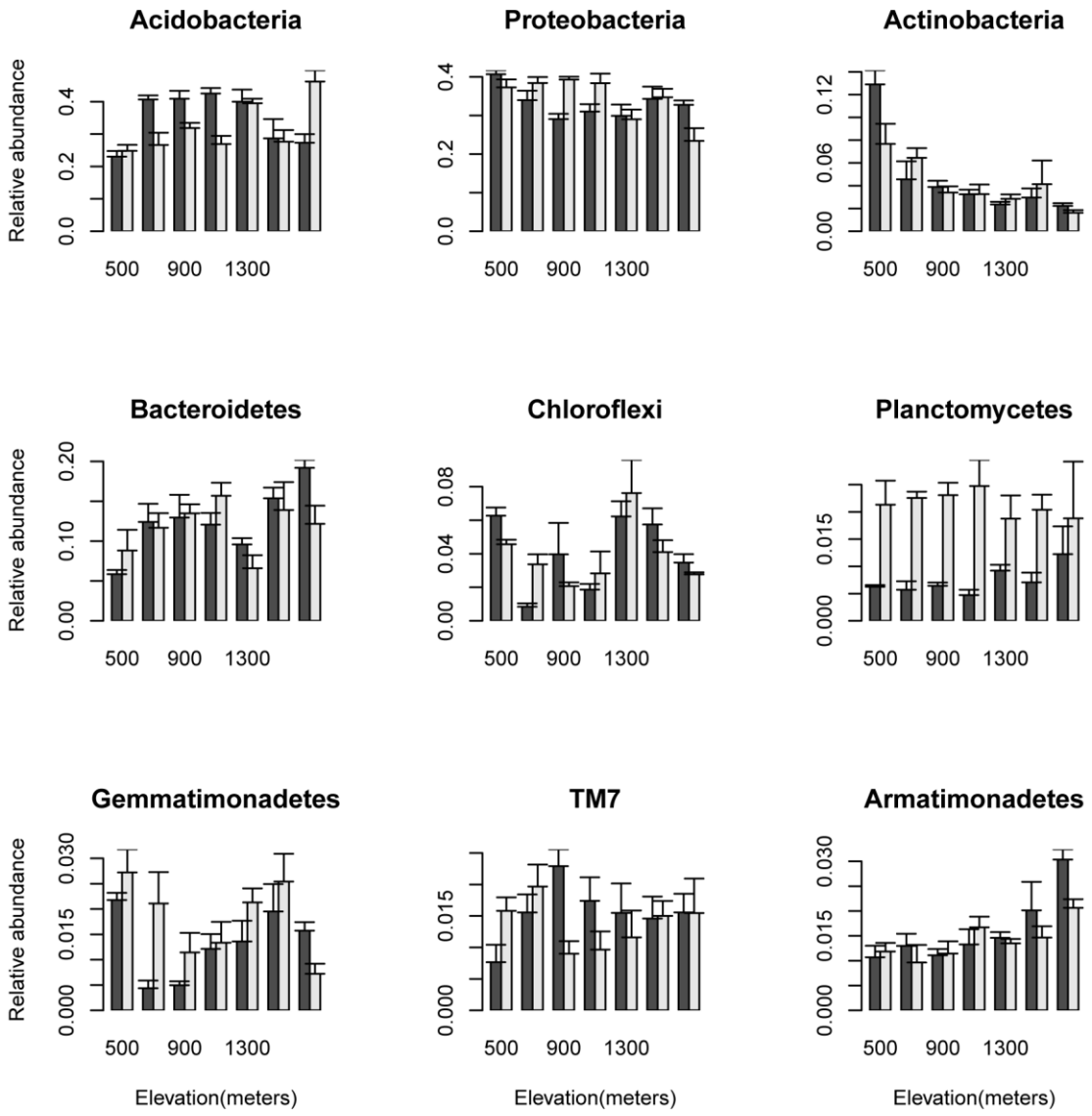

Figure. AF5: Relative abundance of the nine most abundant phyla on Mt. Halla at Gwaneumsa (black bars) and Yeongsil (grey bars) transects (Mean ± SE) in relation to elevation.

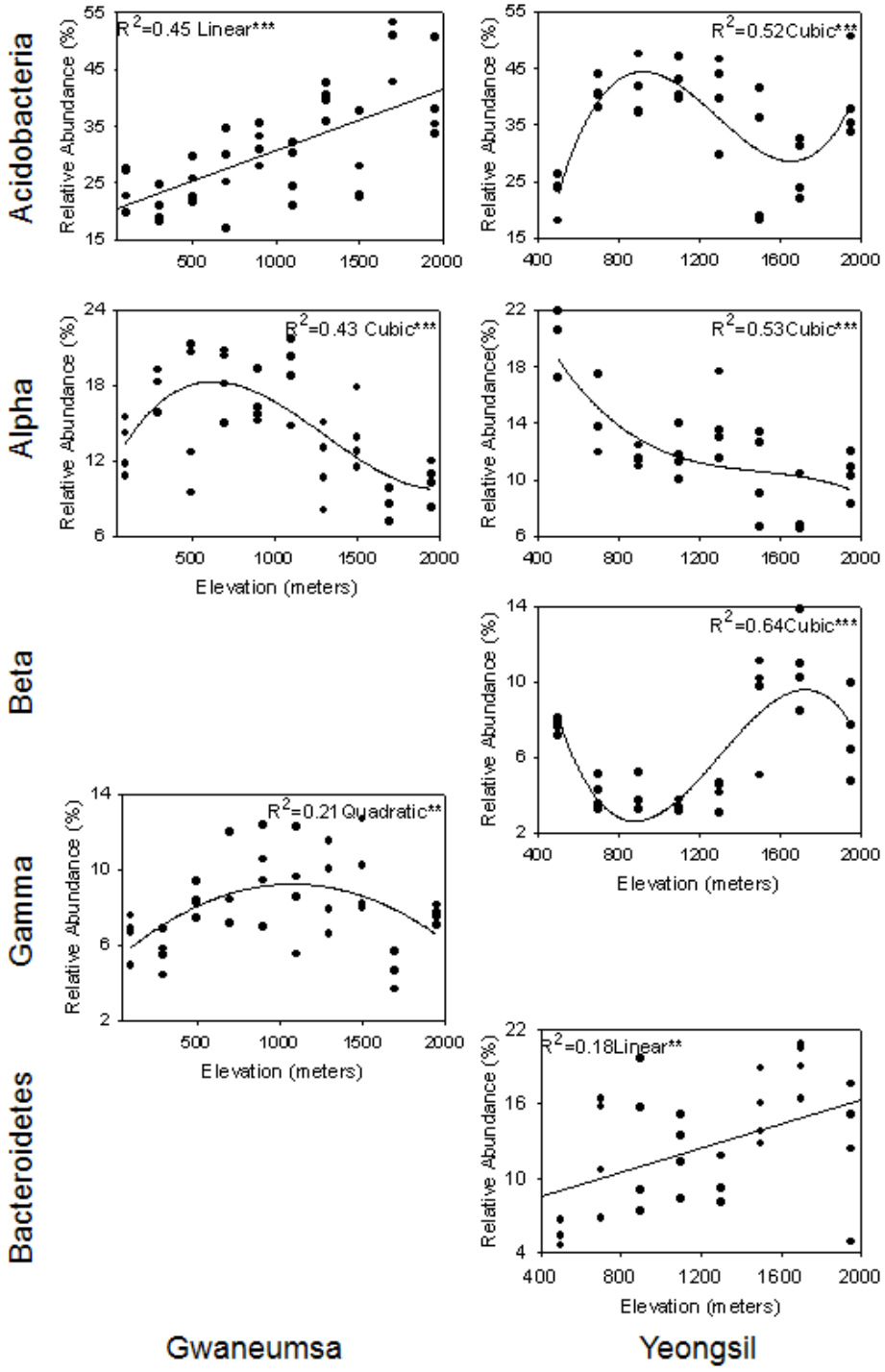

Figure. AF6: Relative abundance of the five most dominant bacterial taxa in two transects at Mt. Halla in relation to elevation. Significance level was shown with***P≤0.001 and **P≤0.01.

Table. AT1: Relative average abundances of all dominant bacterial phyla classified against EzTaxon aligned bacterial database (Chun et al. 2007) across all 27 soil replicates of 6 elevational points on Mt. Fuji.

Phylum	Sub-Phyla	Relative Average abundance
Proteobacteria		38.555
	Alphaproteobacteria	18.504
	Betaproteobacteria	9.619
	Deltaproteobacteria	5.266
	Gammaproteobacteria	5.120
Acidobacteria		20.610
Actinobacteria		11.652
Chloroflexi		5.161
Bacteroidetes		5.132
Planctomycetes		3.028
Gemmatimonadetes		2.782
AD3		2.616
Cyanobacteria		1.734
OP10		1.288
Nitrospirae		1.075
TM7		1.064
Verrucomicrobia		0.930
Firmicutes		0.927
Others		3.335
Bacteria*		0.111

Values represent % of total non-redundant sequences (n=118141) with the taxonomic identity of each sequence determined based on an extension of the EzTaxon-e database.

Table. AT2: Phylotype richness (OTUs) and Diversity indices calculated for subsamples standardized for 800 reads using Mothur platform at (Schloss et al. 2009).

Label	Sample	nseqs	OTUs	Coverage	Shannon	Simpson	Chao	Ace	Phylogenetic Diversity
0.03	F10A	789	355	0.703	5.406	0.006	947.630	1517.939	57.262
0.03	F10B	793	399	0.657	5.533	0.006	984.016	1531.926	52.503
0.03	F10C	789	365	0.700	5.453	0.006	864.393	1471.761	48.631
0.03	F10D	785	363	0.729	5.500	0.006	685.543	963.512	55.711
0.03	F10E	780	362	0.712	5.496	0.005	775.115	1097.381	57.690
0.03	F15A	783	357	0.718	5.449	0.006	824.500	1125.266	48.661
0.03	F15B	773	395	0.688	5.674	0.004	791.164	1096.967	51.816
0.03	F15C	784	456	0.624	5.895	0.002	937.833	1456.039	55.375
0.03	F15D	785	402	0.682	5.654	0.004	791.063	844.885	55.450
0.03	F15E	784	424	0.625	5.682	0.005	1141.850	1738.942	60.613
0.03	F20A	791	368	0.707	5.399	0.010	786.688	1242.880	53.101
0.03	F20B	785	415	0.665	5.705	0.004	874.373	903.051	59.198
0.03	F20C	778	444	0.612	5.808	0.003	1084.155	1634.161	62.619
0.03	F20D	778	447	0.600	5.775	0.004	1089.733	1854.904	67.593
0.03	F20E	780	409	0.656	5.627	0.005	867.692	1381.612	60.123
0.03	F25A	775	433	0.639	5.816	0.003	933.769	981.584	65.274
0.03	F25B	771	467	0.590	5.922	0.002	1089.125	1078.802	67.135
0.03	F25C	763	490	0.540	5.980	0.002	1239.085	2059.030	74.520
0.03	F25D	773	406	0.671	5.729	0.003	840.203	1202.621	59.316
0.03	F25E	776	487	0.550	5.956	0.002	1265.538	1980.706	69.019
0.03	F30C	790	284	0.791	4.927	0.016	539.283	563.857	47.943
0.03	F30E	777	443	0.611	5.818	0.003	1121.373	1640.606	72.905
0.03	F37A	773	373	0.700	5.590	0.004	908.920	1098.372	58.840
0.03	F37B	774	379	0.705	5.569	0.005	743.479	779.790	67.106
0.03	F37C	779	344	0.734	5.417	0.006	724.732	1093.076	55.283
0.03	F37D	782	346	0.746	5.453	0.006	640.045	903.800	51.080
0.03	F37E	782	359	0.728	5.526	0.005	695.985	1071.567	54.932

Samples have been named according to their elevational sampling points on the mountain and A, B, C, D and E represent the biological replicates of the particular sample.

Table. AT3: Relative abundances of archaeal phyla classified against EzTaxon-e database (Kim et al. 2012) across 6 elevational points on Mt. Fuji.

Phylum	Class	1000	1500	2000	2500	3000	Summit
Euryarchaeota		10.805	7.188	4.898	2.015	0.561	0.292
	Thermoplasmata	10.805	7.024	4.748	1.326	0.112	0.163
Thaumarchaeota		89.195	92.786	95.079	97.939	99.439	99.691
	FFSB	89.107	54.312	52.779	17.140	0.294	0.352
	Marine GroupI.1a	0.008	16.002	17.606	7.039	0.000	0.189
	Soil GroupI.1b	0.079	22.446	24.604	73.692	99.145	99.150
Archaea_uc		0.000	0.026	0.023	0.046	0.000	0.017

Table. AT4: Phylotype richness (OTUs) and Diversity indices calculated for subsamples standardized for 309 reads using Mothur platform for Mt. Fuji, Archaea. (Schloss et al. 2009)

Label	Sample*	nseqs	OTUs	Coverage	Shannon	Simpson	chao	ace	Phylogenetic Diversity
0.03	F10A	309	13	0.977	0.820	0.663	23.5	43.548	1.331
0.03	F10B	309	16	0.977	0.890	0.644	20.2	32.202	1.427
0.03	F10C	309	22	0.955	1.289	0.469	113	111.247	1.935
0.03	F10D	309	7	0.994	0.808	0.620	8	9.043	1.166
0.03	F10E	309	27	0.945	1.367	0.437	72.333	94.878	1.805
0.03	F15A	309	37	0.926	2.472	0.130	163.5	286.625	3.007
0.03	F15B	309	13	0.987	1.911	0.178	19	19.841	1.911
0.03	F15C	309	32	0.939	2.234	0.166	117.5	118.223	3.162
0.03	F15D	309	19	0.968	1.977	0.172	34	49.000	1.588
0.03	F15E	309	37	0.932	2.231	0.190	72	149.229	2.774
0.03	F20A	309	32	0.926	1.849	0.261	158.5	352.962	3.416
0.03	F20B	309	26	0.951	1.856	0.223	52.25	88.460	1.939
0.03	F20C	309	11	0.987	1.377	0.379	17	29.991	1.590
0.03	F20D	309	31	0.942	1.859	0.258	61.6	107.476	2.522
0.03	F20E	309	31	0.939	1.900	0.237	65.2	117.784	2.345
0.03	F25A	309	36	0.919	1.401	0.503	86	250.857	2.539
0.03	F25B	309	27	0.948	1.781	0.310	87	153.495	2.788
0.03	F25C	309	10	0.994	1.163	0.504	11	13.504	1.767
0.03	F25D	309	32	0.916	0.852	0.741	140.333	390.132	1.888
0.03	F25E	309	10	0.990	0.823	0.670	13	12.630	1.339
0.03	F30A	309	1	1.000	0.000	1.000	1	0.000	0.877
0.03	F30B	309	3	0.997	0.061	0.981	3	4.000	0.530
0.03	F30C	309	2	0.997	0.022	0.994	2	0.000	0.612
0.03	F30D	309	16	0.961	0.377	0.887	32.5	46.000	1.767
0.03	F30E	309	12	0.971	0.303	0.905	30	75.375	1.042
0.03	FS1	309	21	0.958	0.613	0.809	36.6	39.591	1.830
0.03	FS2	309	1	1.000	0.000	1.000	1	0.000	0.531
0.03	FS3	309	19	0.945	0.437	0.869	155	634.124	1.277
0.03	FS4	309	9	0.977	0.191	0.943	19.5	37.000	0.718
0.03	FS5	309	12	0.971	0.289	0.911	30	40.717	1.169

*Samples have been named according to their elevational sampling points on the mountain and A, B, C, D and E represent the biological replicates of the particular sample. *Samples have been named according to their elevational sampling points on the mountain and A, B, C, D and E represent the biological replicates of the particular sample.

Table. AT5a: Phylotype richness and diversity indices calculated for subsamples standardized for 566 reads using Mothur platform (Mt. Halla, Gwaneumsa Transect; Bacteria), (Schloss et al. 2009).

Label	Sample	nseqs	OTUs	Coverage	Shannon	Simpson	chao	ace	Phylogenetic Diversity
0.03	GES5.1	566	333	0.558	5.448	0.006	1024.7	2337.5	20.992
0.03	GES5.2	566	380	0.452	5.610	0.006	1640.4	2797.2	23.098
0.03	GES5.3	566	366	0.496	5.636	0.004	1353.1	2640.9	21.655
0.03	GES5.4	566	414	0.392	5.812	0.003	1754.8	3721.3	23.724
0.03	GES7.1	566	280	0.655	5.143	0.009	709.89	1396.9	17.007
0.03	GES7.2	566	325	0.578	5.405	0.006	957.02	1868.8	19.130
0.03	GES7.3	566	332	0.551	5.345	0.010	1224.5	1797.5	19.217
0.03	GES7.4	566	326	0.565	5.385	0.007	1098.7	1916.8	19.527
0.03	GES9.1	566	335	0.549	5.410	0.007	1088.1	2514.8	20.193
0.03	GES9.2	566	348	0.519	5.470	0.007	1317.9	2785.3	21.139
0.03	GES9.3	566	325	0.574	5.412	0.006	982.27	2174.9	20.345
0.03	GES9.5	566	370	0.479	5.611	0.004	1454.1	3116	23.783
0.03	GES11.1	566	312	0.569	5.241	0.010	1370.8	2994.3	19.956
0.03	GES11.2	566	307	0.595	5.242	0.010	959.65	2203.1	19.686
0.03	GES11.3	566	252	0.724	5.115	0.007	526.77	846.22	14.729
0.03	GES11.4	566	373	0.473	5.620	0.004	1452.3	3020.8	21.828
0.03	GES13.1	566	347	0.514	5.422	0.008	1365.2	2850.4	21.918
0.03	GES13.2	566	305	0.608	5.250	0.009	862.52	1478.1	18.798
0.03	GES13.3	566	334	0.565	5.444	0.006	975.17	2005.9	20.709
0.03	GES13.4	566	265	0.701	5.163	0.008	587.64	901.6	16.177
0.03	GES15.1	566	432	0.357	5.893	0.002	1868.2	4825.2	27.487
0.03	GES15.2	566	289	0.640	5.206	0.009	819.92	1508.1	16.960
0.03	GES15.4	566	394	0.452	5.765	0.003	1315.1	2569.4	25.366
0.03	GES15.5	566	358	0.512	5.555	0.005	1102.1	2708.9	21.959
0.03	GES17.2	566	381	0.468	5.682	0.004	1302.4	2588.4	23.726
0.03	GES17.3	566	354	0.551	5.625	0.003	938.2	963.27	22.001
0.03	GES17.4	566	396	0.442	5.751	0.003	1502	2506	24.625
0.03	GES17.5	566	383	0.458	5.663	0.004	1450.5	2367.7	23.956
0.03	BRD.1	566	329	0.590	5.459	0.006	807.5	1474.4	20.168
0.03	BRD.2	566	338	0.534	5.391	0.008	1145.3	2469.4	21.162
0.03	BRD.3	566	393	0.445	5.725	0.004	1375.8	2973.9	24.779
0.03	BRD.5	566	412	0.369	5.759	0.004	2856.1	4741.7	24.878

Samples have been named according to their elevational sampling points on the mountain. Samples with name starting with BRD are from summit (1950 masl).

Table. AT5b: Phylotype richness and diversity indices calculated for subsamples standardized for 566 reads using Mothur platform (Mt. Halla, Yeongsil Transect; Bacteria), (Schloss et al. 2009).

Label	Sample	nseqs	OTUs	Coverage	Shannon	Simpson	chao	ace	Phylogenetic Diversity
0.03	YS1.1	566	429	0.369	5.899	0.002	1841.1	4344.8	33.411
0.03	YS1.2	566	423	0.382	5.870	0.002	1780.2	3217.1	33.749
0.03	YS1.4	566	407	0.417	5.808	0.002	1514.9	3379.7	33.114
0.03	YS1.5	566	441	0.323	5.920	0.002	2657.8	6431.7	34.080
0.03	YS3.1	566	410	0.442	5.865	0.002	1164.1	2051.8	30.789
0.03	YS3.2	566	458	0.302	6.012	0.001	2355.9	4313	35.567
0.03	YS3.3	566	404	0.433	5.799	0.003	1305.1	2771.2	31.380
0.03	YS3.4	566	280	0.664	5.199	0.008	740.38	1218.2	21.066
0.03	YS5.1	566	404	0.412	5.736	0.004	1532.1	3592.7	30.455
0.03	YS5.2	566	327	0.597	5.476	0.005	815.26	1364.6	24.749
0.03	YS5.4	566	450	0.302	5.937	0.002	2497.8	6203.6	32.663
0.03	YS5.5	566	345	0.539	5.538	0.004	1193.3	2288.3	24.550
0.03	YS7.1	566	347	0.544	5.520	0.006	1052.4	1581.1	26.127
0.03	YS7.2	566	411	0.385	5.775	0.003	2240.6	3406.6	29.041
0.03	YS7.4	566	444	0.343	5.967	0.001	1881.6	3666.4	33.250
0.03	YS7.5	566	451	0.295	5.942	0.002	2932.3	9037.1	35.633
0.03	YS9.1	566	292	0.677	5.350	0.005	569.55	939.6	20.275
0.03	YS9.2	566	265	0.705	5.161	0.008	553.77	963.68	18.955
0.03	YS9.3	566	405	0.401	5.756	0.003	1874	5008.5	29.189
0.03	YS9.4	566	285	0.673	5.268	0.007	612.31	989.63	20.161
0.03	YS11.1	566	268	0.678	5.122	0.009	738.6	1115.7	18.392
0.03	YS11.2	566	319	0.613	5.421	0.006	769.4	826.05	22.976
0.03	YS11.3	566	285	0.661	5.267	0.006	683.61	1299.3	20.511
0.03	YS11.5	566	444	0.330	5.952	0.002	2191.1	5017.8	32.883
0.03	YS13.2	566	374	0.451	5.564	0.006	1751.3	5309.2	30.855
0.03	YS13.3	566	389	0.442	5.681	0.004	1546.4	3251.3	28.899
0.03	YS13.4	566	348	0.516	5.468	0.007	1386.9	2340.9	28.416
0.03	YS13.5	566	279	0.652	5.134	0.010	774.03	1611	20.921
0.03	YS15.2	566	395	0.435	5.747	0.003	1582	3414.6	30.299
0.03	YS15.3	566	379	0.477	5.710	0.003	1418.5	2321.3	30.567
0.03	YS15.4	566	247	0.728	4.997	0.011	492.44	837.36	19.217
0.03	YS15.5	566	336	0.597	5.562	0.004	753.39	1121.5	23.818
0.03	YS17.1	566	339	0.560	5.430	0.007	871.34	1012.9	26.907
0.03	YS17.2	566	233	0.726	4.768	0.017	555.57	948.08	19.736
0.03	YS17.3	566	355	0.454	5.326	0.012	2859.5	6781.4	25.456
0.03	BRD.1	566	318	0.601	5.404	0.006	806.94	1570.6	23.670

0.03	BRD.2	566	354	0.484	5.467	0.007	1819	3544.4	27.995
0.03	BRD.3	566	396	0.429	5.730	0.003	1664.4	2869.2	30.023
0.03	BRD.5	566	414	0.375	5.771	0.003	2251.7	4552.9	30.963

Samples have been named according to their elevational sampling points on the mountain. Samples with name starting with BRD are from summit (1950 masl).

Table. AT6a: Relative average abundances of all 35 bacterial phyla classified against EzTaxon-e aligned database (Kim et al., 2012) across all 32 soil replicates of 8 elevational points on Mt. Halla Gwaneumsa transect.

Phylum	Total Sequences	Percent abundance
Acidobacteria	31819	35.141
Proteobacteria	28960	31.984
Bacteroidetes	11640	12.855
Actinobacteria	4370	4.826
Chloroflexi	3331	3.679
TM7	1559	1.722
Armatimonadetes	1318	1.456
Cyanobacteria	1219	1.346
Gemmatimonadetes	1063	1.174
Planctomycetes	820	0.906
Verrucomicrobia	743	0.821
Elusimicrobia	648	0.716
Nitrospirae	626	0.691
WS5	367	0.405
AD3	324	0.358
Chlorobi	283	0.313
Firmicutes	276	0.305
TM6	206	0.228
OP3	179	0.198
Thermobaculum	169	0.187
WS3	120	0.133
SM2F11	119	0.131
GN02	58	0.064
CS	48	0.053
OD1	46	0.051
Spirochaetes	35	0.039
Fibrobacteres	24	0.027
OP11	21	0.023
OMAN	19	0.021
Tenericutes	9	0.010
NKB19	7	0.008
BRC1	4	0.004
SAR202	3	0.003
Fusobacteria	1	0.001
SR1	1	0.001
Bacteria*	111	0.123

Table. AT6b: Relative average abundances of all 39 bacterial phyla classified against EzTaxon-e aligned database (Kim et al., 2012) across all 40 soil replicates of 10 elevational points on Mt. Halla Yeongsil transect.

Phylum	Total Sequences	Percent abundance
Proteobacteria	37640	35.473
Acidobacteria	30719	28.950
Bacteroidetes	12411	11.696
Actinobacteria	5722	5.393
Chloroflexi	3811	3.592
Planctomycetes	2473	2.331
Gemmatimonadetes	2369	2.233
TM7	1491	1.405
Nitrospirae	1309	1.234
Armatimonadetes	1244	1.172
Cyanobacteria	1164	1.097
Verrucomicrobia	1066	1.005
Elusimicrobia	977	0.921
AD3	613	0.578
WS3	471	0.444
Chlorobi	414	0.390
Thermobaculum	356	0.336
WS5	345	0.325
OP3	310	0.292
Firmicutes	262	0.247
TM6	172	0.162
SM2F11	168	0.158
OD1	116	0.109
Spirochaetes	64	0.060
CS	60	0.057
OMAN	55	0.052
Fibrobacteres	54	0.051
GN02	47	0.044
OP11	29	0.027
BRC1	27	0.025
NKB19	20	0.019
Tenericutes	10	0.009
GN04	10	0.009
SR1	7	0.007
SAR202	6	0.006
Lentisphaerae	4	0.004

CHS	2	0.002
Fusobacteria	1	0.001
WS6	1	0.001
Bacteria*	89	0.084

ACKNOWLEDGEMENTS

This PhD thesis is the result of a challenging journey, during which time many people have contributed and given their support. Undoubtedly, the most influential person in my graduate career has been my advisor Dr. Jonathan Adams. Dr. Adams's passion, guidance, and discipline has been indispensable to my growth as a scientist and personal development over the past three and a half years. I have neither heard of or experienced a professor's dedication and determination to ensure that students are prepared for the many challenges that often arise in their life as researchers and to help make the journey an enjoyable and pleasurable one . I could not have imagined having a better advisor and mentor for my Ph.D studies.

I am especially indebted to Dr. Koichi Takahashi from Shinshu University, Japan who made me feel so welcome in in his laboratory and made my first sampling trip on the beautiful Mt. Fuji, an unforgettable experience. His help with the soil analysis together with his excellent suggestions was a invaluable source of inspiration for conducting my research project and manuscript writing.

I take this opportunity to sincerely acknowledge the Ministry of Education, Science and Technology (MEST) Korea for providing financial assistance in the form of Korean Government Scholarship Program award which enabled me to perform my work comfortably.

Many thanks to my labmates at SNU whose friendship and support have made my stay in Korea more than just a scientific experience. Larisa provided valuable feedback at both the initial and closing moments of the thesis, Dorsaf offered constant support just by being a good friend who always lent her ear to me, even for my jibber jabbers. Binu for being a loving junior who offered constant support amid talks of Indian politics and lab work. Jung Ok for sportingly bearing mine and Dorsaf's daily jokes and taunts. Mincheol, just for being there and helping in solving my problems with a constant smile on his face and food in his hands. WooSung for handling the lab's administrative tasks single-handedly and for providing a helping hand in his own amicable manner when needed. How can I forget, Yoon Myung, our future Green Party president and our lab-wizard, who was able to solve all of our problems, even the imaginary ones.

I would especially like to thank, my friend Atul Kr. Singh, with whom I started my beautiful journey to Korea. His decisive and energetic support during the entire doctoral course cleared the path towards the completion in his solution-oriented way.

How can I forget Varsha and Sanjeev Sonkaria, the world's best couple who provided this hungry soul with food and encouragement, whenever I needed it.

I am thankful to my South Korean friends, Young Uk, Jin Woo and many others for all the laughter, enjoyment, mutual encouragement that I adored in Korea. Many thanks to their families, who always welcomed me with their open arms and warm hearts and made me, feel at home during my stay in Korea.

I am forever grateful to my beloved parents and grandparents, especially my father Ram Asare Singh, whose foresight and values paved the way for a privileged education, and to my sister Trisindhu, who gently offered counseling and unconditional support at each turn of the road.