Analysis of the microbial communities in andisols of different ages from volcanic soils

Marcela Hernández[1*], Marcela Calabi[2], Ralf Conrad[1] and Marc G. Dumont[3]

[1]Max-Planck-Institute for Terrestrial Microbiology, Marburg, Germany

[2] Scientific and Technological Bioresource Nucleus, Universidad de La Frontera, Temuco, Chile

[3]School of Biological Sciences, University of Southampton, Southampton, SO17 1BJ, United Kingdom

Running title: *Microbial communities in volcanic soils*

*Corresponding author: M. Hernández, m.t.hernandez-garcia@soton.ac.uk

Present address: M. Hernández: University of Southampton, Southampton, SO17 1BJ, United Kingdom

Abstract

Volcanism is a primary process of land formation, and provides a model to understand soil-forming processes and the role of potential Bacteria and/or Archaea as early colonizers in new environments. The objective of the present study was to identify the microbial communities involved in soil formation. DNA was extracted from soil samples of Llaima volcano (Chile) at sites destroyed by lava in different centuries (1640, 1751 and 1957). Bacterial- and archaeal-16S rRNA genes were analyzed by quantitative PCR (qPCR) and Illumina MiSeq sequencing. Results showed that the microbial diversity increased with soil age, particularly between the 1751 and 1640 soils. For archaeal communities, Thaumarchaeota was detected in similar abundances in all soils but Euryarchaeota was detected mostly in the older soils. The analysis of bacterial 16S rRNA gene indicated dominant bacterial phyla including high abundances of Chloroflexi (37%), Planctomycetes (18%) and Verrucomicrobia (10%) in the youngest soil. Proteobacteria and Acidobacteria were highly abundant in the oldest soils (16% in 1640 and 15% in 1751 for Acidobacteria; 38% in 1640 and 27% in 1751 for Proteobacteria). The microbial profiles in the youngest soils were unusual. We found a high abundance of bacteria belonging to the order Ktedonobacterales (Chloroflexi) in the 1957 soil (37%) compared with the 1751 (18%) and the 1640 soils (7%). In this study, we show that over the course of centuries there is a gradual establishment of a diverse microbial community in volcanic soils following an eruption. Poorly characterized microbial groups are dominant in the early stages of recovery.

Highlights

- Microbial diversity becomes even as soil starts recovering after a lava eruption

- Specific groups dominate early soils
- Bacteria outnumber Archaea at all stages, but the latter are present at all stages of soil establishment

Keywords: volcanic soils, soil formation, Ktedonobacterales, 16S rRNA gene, high-throughput sequencing

Introduction

Volcanic eruptions provide a model to understand soil-forming processes, including microbiological components. After lava solidifies, microbes begin colonizing the rock surface, presumably by photosynthesis or from the chemical energy in reduced gases such as methane (CH_4), hydrogen sulfide (H_2S), hydrogen (H_2), and carbon monoxide (CO). A number of studies have shown that microorganisms are among the first colonizers on volcanic deposits contributing to early ecosystem development (Fujimura *et al*., 2002; King, 2003; Ohta *et al*., 2003; Sato et al., 2004; Kim *et al*., 2004; Gomez-Alvarez *et al*., 2007; Guo *et al*., 2014; Kim *et al*., 2018). As volcanic environments are widely distributed on Earth (Cockell *et al*., 2011), it is important to understand the microbial communities that play an important role colonizing these extreme environments. Volcanic environments can be used as a model to understand pioneer species colonization.

The pioneer microbes colonizing early volcanic deposits are able to fix nitrogen and carbon from the atmosphere and contribute to the organic matter addition into the deposits (Crews *et al*., 2001; Kurina and Vitousek, 2001; Dunfield and King, 2004; Sato *et al*., 2006; King *et al*., 2008; King and Weber, 2008; Sato *et al*., 2009; Weber and King, 2010b; King and King, 2014; Weber and King, 2017). Previous

76 studies showed that the class Ktedonobacteria, which belongs to the phylum
77 Chloroflexi, is predominant in non-vegetated volcanic soils (Gomez-Alvarez *et al*.,
78 2007; Weber and King, 2010b), while Proteobacteria dominate vegetated sites (Weber
79 and King, 2010b). Some of these Ktdenobacteria, among other microbial groups, have
80 been associated with CO and H_2 consumption in both young, organic matter- poor
81 volcanic deposits, and in older more mature deposits with well-developed plant
82 communities (King, 2003a; King and Weber, 2008, Weber and King, 2010b).
83 The colonization of volcanic deposits by plants will also play an important
84 role in soil formation, as their roots enhance rock weathering and photosynthates
85 contribute to soil organic matter accumulation. Some studies have shown that
86 relatively young volcanic deposits harbor distinct microbial communities when plants
87 are present (Dunfield and King, 2004; Ohta *et al*., 2003, Guo *et al*., 2014). The
88 interaction, and potential co-dependence, of plants and microbes in volcanic soils has
89 not been specifically investigated. Plant diversity is known to affect microbial
90 diversity, function, and soil carbon accumulation (Leff *et al*., 2018).
91 Also, the soil community, including bacteria, fungi and invertebrates, is also
92 important for the establishment and growth of plants (Philippot *et al*., 2013; Lange *et*
93 *al*., 2015; Wubs *et al*., 2016). These types of interactions have been demonstrated in
94 some mature soils, but have not been investigated in the early stages of soil formation,
95 for example in volcanic soils.
96 Llaima Volcano is one of the largest and most active volcanoes in Chile. It has
97 had three detailed lava eruptions at different sites of the volcano and plants have
98 colonized these zones. Previously, we sampled soils from Llaima volcano at sites
99 destroyed by lava in different centuries (1640, 1751 and 1957). We hypothesized that
100 autotrophic microorganisms were important in soil formation and indeed found large

numbers and high activities of autotrophic ammonia-oxidizing bacteria and archaea in those soils, being particularly high in recent soils (Hernández *et al*., 2014). In the present study, we aim to evaluate the structure of total microbial community composition at these three sites with different defined age and soil characteristics, to obtain information regarding recolonization by Bacteria and Archaea after a lava event. We hypothesize that these communities are the fundamental drivers of soil formation.

Material and methods

DNA extraction and qPCR

DNA was extracted from soil samples of the Llaima volcano (Chile). Sampling and DNA extractions have been previously described (Hernández *et al*., 2014), briefly samples were taken from three sites according to the lava eruption (Naranjo & Moreno, 2005), named 1640, 1751 and 1957. Samples were taken by using a triangle pattern (Gomez-Alvarez *et al*., 2007) and triplicates were taken per each point, a total 9 replicates per site were taken. The abundance of bacterial- and archaeal- 16S rRNA genes was performed using an iCycler Instrument (BioRad). For all assays, standards containing known number of DNA copies of the target gene were used. qPCR conditions were based on dual-labeled probes described by Yu *et al*. (2005). Primer sets Bac338F/Bac805R/Bac516P and Arc 787F/Arc1059R/Arc915P were used for bacterial- and archaeal- 16S rRNA genes, respectively. Conditions for both runs are describe as follows: 0.5 µM of each primer, 0.2 µM of the dual-labeled probe, 3 µl template, 4mM $MgCl_2$ (Sigma) and 12.5 µl of JumpStart Ready Mix (Sigma-Aldrich). 1 µl BSA (0.8 µg/µl) was added to archaeal 16S rRNA gene reaction. The program used for both assays: 94 °C for 5 min, 35 cycles of 95 °C for 30 s and 62 °C for 60 s,

extension and signal reading. We obtained efficiencies of 91.2% and R^2 of 0.994 for bacterial 16S rRNA genes and 93.1% and R^2 of 0.994 for archaeal 16S rRNA genes.

Illumina library preparation and sequencing

MySeq Illumina sequencing was performed for total bacterial 16S rRNA gene. PCR primers 515F/806R were used (Bates *et al*., 2011). PCR conditions for bacterial 16S rRNA gene consisted of: 94°C for 5 min, followed by 28 cycles of 94°C for 30 s, 50°C for 30 s, and 68°C for 30 s and a final elongation at 68°C for 10 min (Hernández *et al*., 2015). Individual PCRs contained a 6-bp molecular barcode integrated in the forward primer were used. Amplicons were purified using a PCR cleanup kit (Sigma) and quantified using a Qubit 2.0 fluorometer (Invitrogen). Equal equimolar concentration of the samples were pooled and sequenced on MiSeq using a 2 x 300 bp paired end protocol. Library preparation and sequencing was performed at the Max Planck Genome Centre (MPGC), Cologne, Germany.

Bioinformatics, Data processing

Quality filtering and trimming forward and reverse adaptors from the sequences was carried out using cutadapt (Martin, 2011). Forward and reverse reads were merged using the usearch fastq_mergepairs command (Edgar, 2013). Downstream processing was performed by using UPARSE (Edgar, 2013) and UCHIME pipelines (Edgar *et al*., 2011) and by following the steps detailed in Reim *et al*. (2017).

Taxonomy analysis and data accession

A representative sequence of each operational taxonomic unit (OTU) was aligned against the SILVA-132 16S rRNA gene database using the naïve Bayesian classifier

(bootstrap confidence threshold of 80%) in mothur (Schloss *et al*., 2009). Sequence data were deposited in the NCBI Sequence Read Archive (SRA) under accession number PRJNA496066.

Statistical Analyses

Multivariate statistics for bacterial 16S rRNA Illumina data was mostly done with phyloseq (McMurdie and Holmes, 2013) and vegan (Oksanen *et al*., 2018) packages in R software version 3.5.1. Alpha diversity indexes were carried out using the *estimate_richness* command in the phyloseq packages. Evenness index was carried out using vegan package. Relative abundance was calculated removing taxa not seen more than three times in at least 20% of the samples by using the *filter_taxa* command and figures were constructed by using the ggplot2 package (Wickham, 2016). For beta diversity analyses, non-metric multidimensional scaling (NMDS) was carried out using the *decostand* function for ordination of Hellinger distances in vegan package. The influence of environmental variables (Table S1 in Hernández *et al*., 2014) on the total community was analyzed using *envfit* function also in vegan package (permutations = 999). Heatmaps were constructed with the gplots (Warnes *et al*., 2016), vegetarian (Charney and Record, 2012) and vegan (Oksanen *et al*., 2018) packages. Principal components analysis (PCA) of the Hellinger transformed data was performed using the *prcomp* function. The OTUs explaining most of the differences between samples were defined as the 20 OTUs contributing the largest absolute loadings in the first and second dimensions of the PCA, obtained from the rotation output file (Hernández *et al*., 2017). Hierarchical clustering of the distance matrix used the "ward.D2" method and *hclust* function. The heatmap was constructed using the *heatmap.2* function in gplots package.

176

177 **Results**

178 *Microbial abundance*

179 The abundance of bacterial 16S rRNA genes was more numerous than those of
180 archaeal 16S rRNA genes (Fig. 1). Copy numbers of bacterial presented no
181 differences between the sites fluctuating in the range of 10^8 per gram of soil for all the
182 three sites and no significant differences were found (P=0.184). Copy numbers of
183 archaeal 16S rRNA also presented no significant differences (P=0.479). The site 1957
184 presented the highest abundance being an order of magnitude higher (in the range of
185 10^6 copies per gram of soil) than those for 1640 and 1751.

186

187 *Diversity of bacterial communities*

188 For alpha-diversity, in average, the observed species (*i.e.* count of unique OTUs in
189 each sample recovered after sequence analysis) were 7575 OTUs for site 1640, 7116
190 OTUs for site 1751 and 4593 for site 1957 (Fig. 2a, Table S1). The Shannon diversity
191 indices were originally between 5.21 (youngest soil 1957) and 6.99 (older soils 1640
192 and 1751) (Fig. 2b, Table S1). Species evenness has the same behavior than Shannon
193 indexes, being larger in the older sites (0.71 in site 1640 and 0.76 in site 1751) than in
194 the newest site 1957 (0.62) (Fig. 2c, Table S1).

195 For beta-diversity, Non-metric multidimensional scaling (NMDS) analysis showed
196 that the composition of the bacterial 16S rDNA also changed across soil types and
197 environmental factor (P <0.05, Fig. 3). The youngest soils (site 1957) cluster
198 separated in comparison with the two older soils (sites 1640 and 1751) (Fig. 3). The
199 recent soil (site 1957) was positive related to iron, ration carbon/nitrogen, total
200 nitrogen, organic matter, magnesium and high (elevation above the sea level) and

negatively with pH. On the other hand, the soils of site 1751 were positively related to pH and site 1640 was positively related to silicon dioxide (SiO_2).

Analysis of microbial communities

The archaeal communities encompasses mostly the phylum Thaumarchaeota in all sites, with relative abundances of 82.68%, 95.89% and 97.70% for the sites 1640, 1751 and 1957, respectively. Site 1640 presented highest abundance of the phylum Euryarchaeota (16.48%) when compare with the other sites (data not shown). Within the archaeal classes, Nitrososphaeria and soil group 1.1c dominated mostly sites 1751 and 1957, but also site 1640 (Fig. S1). Site 1640 shows a higher relative abundance of the class Thermoplasmata (12.62%) when compare with the other sites (1751: 2.47% and 1957: 0.40%) (Fig. S1).

The bacterial communities at the different sites consisted of several phyla including Acidobacteria, Actinobacteria, Chloroflexi, Planctomycetes, Proteobacteria, and Verrucomicrobia (Fig. 4). The site 1640 presented a high relative abundance of Acidobacteria (16.27%), Actinobacteria (11.47%), Planctomycetes (12.42%) and Proteobacteria (38.53%). The site 1751 presented similar relative abundance with 1640 for the phyla Acidobacteria, Actinobacteria and Planctomycetes (15.43%, 11.72% and 13.24% respectively). For this site (1751), high abundances of Chloroflexi (18.09%) and lower abundances of Proteobacteria (27.49%) when compare with site 1640 were found. On the other hand, the site 1957 presented the highest relative abundance of the phyla Chloroflexi (37.84%), Planctomycetes (18.36%) and Verrucomicrobia (10.79%) when compare with the sites 1640 and 1957. Within the phylum Chloroflexi, the Class Ktedonobacteria was higher in site 1957 (97.05%) compare with the other sites (1640: 23.95% and 1751: 55.92%) (Fig. 5).

Phylogenetic analysis of bacterial communities

Clustering analyses show distinct differences in bacterial 16S rRNA among the sites, with less difference between the oldest soils (1640 and 1751) (Fig. 6). Many OTUs were highly abundant in the youngest soils (site 1957) and belong mostly to the family of Ktedonobacteraceae in the phylum Chloroflexi (specially OTU-62, OTU-176, OTU-370, OTU-478, OTU-84, OTU-549, OTU-385, OTU-697) and two genera from the phylum Acidobacteria: *Bryobacter* (OTU-175) and *Acidipila* (OTU-893). Several OTUs belong to Ktedonobacteraceae (specially OTU-597 and OTU-2070) were also abundant in the soil 1751. The site 1751 also presented a high abundance of Actinobacteria. The heatmap also show that some OTUs were abundant in all soils, especially OTUs belong to the phylum Proteobacteria, such as the OTU-150 which belongs to Xanthobacteracea (Alpha-proteobacteria). Proteobacteria was, in general, more abundant in the old site 1640. Finally, we also identified OTUs belong to the phylum Verrumomicrobia, being abundant also in all the sites (Fig. 6).

Discussion

Microbial abundance

The abundance of bacterial 16S rRNA gene copies per gram of soil was higher in all the sites sampled with respect to those of archaeal (Fig. 1). Interestingly as the soil recovers, the abundance of bacterial 16S rRNA gene maintains stable. This may probably be because as the soil recovers and plants start colonizing, the competition among bacteria, fungi and eukaryota might increase in order to colonise new niches. On the other hand, the results indicate that the abundance of archaeal 16S rRNA gene decreases as the soil recovers, although not significance differences were found. To

our knowledge, this is the first study that incorporates bacterial- and archaeal- 16S rRNA gene abundances for andisol soils at Llaima volcano. Our previous study reported abundance of ammonia-monoxigenase gene (*amoA*) for archaeal and bacterial in the same sites (Hernández *et al*., 2014) and showed higher archaeal *amoA* genes in all sites, being higher in the youngest soil, when compare to the bacterial *amoA*. Notably, the archaeal *amoA* genes reported previously were in the same range with those of archaeal 16S rRNA reported in the present study. The bacterial 16S rRNA abundance in the range of 10^8 copies per gram of soil is very similar to earlier studies from volcanic deposits. Weber and King (2010a) reported $2.6x10^8$ cells per gram of dry soil of bacterial 16S rRNA gene in unvegetated patches on a 1959 Hawaiian volcanic deposits in Kilauea volcano. In another study, by doing total direct microscopic counts of bacteria, abundances of bacteria in the range of 1-4 $x10^8$ cells per gram of dry soil were found at volcanic deposits in Miyake-jima volcano in Japan, which was affected by the 2000 lava eruption (Guo *et al*., 2014). Similar cell numbers (in the range of 10^7 to 10^8) were found in a poorly to fully vegetated volcanic deposit of different ages (32-, 35- and 39-year old) in Iceland (Byloos *et al*., 2018).

Diversity of bacterial microbial communities

We found higher number of OTUs in the older soils (7575 OTUs in site 1640 and 7116 OTUs in site 1751) when compare with the youngest soil site 1957 (4593 OTUs) (Fig. 2a). In general, Shannon diversity and evenness indexes were larger at older sites (1640 and 1751) (Fig. 2b-c). Significance differences were found within soil sites for Shannon indexes (one-way analysis of variance, P=0.015 in 1640; P=0.028 in 1751 and P=0.01 in 1857). For evenness indexes, significance differences were found in the youngest (one-way analysis of variance, P=0.009) and oldest soils (one-way

276 analysis of variance, P=0.001). The results presented here support those already
277 published indicating that as the soil recovers, diversity increase. An early study by
278 King (2003a) indicates that the diversity of substrates utilized in Biolog plate assays
279 increased with soil age (18 to 300 years old), assuming changes in the microbial
280 communities from Hawaiian volcanic deposits. Analyzing the same deposits, Gomez-
281 Alvarez and colleagues (2007) indicated that the diversity is higher in moderately
282 vegetated deposits (Shannon index H=3.87 in site 1790) than the youngest soil
283 (Shannon index H=2.62 in site 1921). The diversity indexes in Llaima volcano in
284 south of Chile are higher than those in Kilauea volcano in Hawaii, USA. However, it
285 is important to indicate that the study of Gomez-Alvarez and colleagues (2007) are
286 from cloning-based analysis and thus it is probably that not all the phylogeny was
287 covered. The diversity in the vegetated areas of Llaima volcano (in sites 1640 and
288 1751; Fig. 2b, Table S1) is also higher than those in lava-formed Gotjawal forest soils
289 in Korea (Kim *et al*., 2018), in which authors found indexes below 5.5. (Fig. S3, Kim
290 et al., 2018) for bacterial 16S rRNA gene based on Illumina sequencing analyses. The
291 diversity was also higher than vegetated areas from Iceland volcanic deposits, in
292 which diversity increases as the area becomes fully vegetated (Byloos *et al*., 2018).
293 The microbial communities in the vegetated areas (sites 1640 and 1751)
294 cluster together, and in a separate cluster we can identify members of the unvegetated
295 site (1957) (Fig. 3). This is corroborated with the diversity indexes presenting similar
296 values for the vegetated areas. NMDS analysis showed that pH is mostly the only
297 physico-chemical parameter governing the vegetated areas. On the other hand, Iron
298 and carbon/nitrogen ratio play important role in the establishment of bacterial
299 communities in the youngest unvegetated area (1957) (Fig. 3). It has been reported
300 that acidophilic iron-oxidizers bacteria are pioneer microbes in young deposits of

volcanic ash from Miyake-jima, Japan (Fujimura *et al*., 2012), but this certainty needs to be further investigated for Llaima volcano.

Phylogenetic analysis of archaea communities

We found that members of the ammonia-oxidizing Thaumarchaeota group dominated all the sites (Fig. S1), being highly abundant in the youngest soil (1957= 97.7%) and the middle site 1751 soil (95.89%) compare with the oldest vegetated site (1640= 82.68%). Our study also shows that members of the phylum Euryarchaeota were abundant in the vegetated site (1640= 16.48%). Very little is known about the colonization of archea in volcanic deposits. The high abundance of Thaumarchaeota is in accordance with our previous study in which we show that *amoA* genes were higher in the youngest soils than in the older soils (Hernández *et al*., 2014). A previous study from a lava-formed forest soils in Korea shows that archaeal clones affiliated to the Thaumarchaeota group were more abundant (96.2%) than the clones affiliated to the Euryarchaeota group (3.8%) (Kim *et al*., 2014).

Phylogenetic analysis of bacterial communities

The abundance of Acidobacteria, Actinobacteria and Proteobacteria increased with soil age (Fig. 4). Our study found that Acidobacteria and Actinobacteria are stable in the vegetated sites 1640 and 1751. Acidobacteria and Actinobacteria, among others, have been found to be abundant in soils from Kilauea volcano (sites 1959 and 1790, Gomez-Alavarez *et al*., 2007). Actinobacteria has been identified as an abundant phylum from Icelandic volcanic rocks of different compositions (Cockell *et al*., 2013). Within the Proteobacteria, a study looking at the microbial communities from a lava-formed Gotjawal forest soils in Korea indicated that members of the class

Rhizobiales are abundant in these sites (Kim *et al*., 2015). Proteobacteria was also found to be the most abundant phylum in young deposits of volcanic ash from Miyake-jima in Japan (Guo *et al*., 2014), from Icelandic volcanic deposits of different ages and vegetation (Byloos *et al*., 2018) and from vegetated sites (site 1959) deposits in Kilauea volcano (Weber and King, 2010b). In our study, within alpha-proteobacteria, the abundance of Rhizobiales were very high in the vegetated old sites (76% and 68%) compared with the youngest soil (42%) (Fig. S2). Members of the class Rhizobiales, known for their ability to fix nitrogen, have been identified in old volcanic deposits in Kilauea Volcano (Nüsslein and Tiedje, 1998).

CO-oxidizers, N_2- and H_2-fixers are present in volcanic deposits of different ages and vegetation conditions. For example, *coxL* genes, which encode the large subunit of carbon monoxide dehydrogenase, were found in members of Proteobacteria in vegetated sites in Kilauea volcano, USA (Gomez-Alvarez *et al*., 2007; Weber and King, 2010b) and Miyake-jima, Japan (King *et al*., 2008). *Burkholderia* and *Paraburkholderia* (Weber and King, 2017) and *Burkholderia*, *Stenotrophomonas* and *Pseudomonas*, among others presented *coxL* genes responsible for CO-oxidation (King, 2003b). N_2-fixer members of Proteobacteria and Firmicutes were found in a 300 year-old soil from Kilauea volcano (Nüsslein and Tiedje, 1998). H_2-oxidizers members of Proteobacteria were isolated from volcanic mudflow deposits from Mt. Pinatubo, Philippines (Sato *et al*., 2006).

In our study, we found that the abundance of bacteria belonging to the order Ktedonobacterales (Chloroflexi) decreased with soil age (Fig. 4). Being highly abundant in the 1957 unvegetated soil (37%) compared with the 1751 (18%) and the 1640 soils (7%) (Fig. 5, Fig. 6). Similar results were reported from Kilauea volcano, in which Ktedonobacteria was found to be more abundant in unvegetated soils from a

1959 cinder deposit (Weber and King, 2010b). The capacity of Ktedonobacteria (Chloroflexi) for CO uptake has been poorly studied. Only about 14 strains able to either consume CO and/or harbor *coxL* genes, has been characterized (King and King, 2014). So far, the class Ktedonobacteria contains only six species (Yabe *et al*., 2017). The order Ktedonobacterales contains the species *Ktedonobacter racemifer* SOSP1-21^T (Chang *et al*., 2011). *K. racemifer* SOSP1-21^T presents the larger genome ever reported, it contains a *cox* operon conferring potential for CO oxidation (King and King, 2014). Future studies will be significantly important in order to establish the importance of this group of bacteria as pioneer organisms, probably utilizing atmospheric trace gases as energy substrates for the colonization of new environments in volcanic deposits.

Conclusions

In the present study, we show that there is a gradual reestablishment of the microbial community in volcanic soils following an eruption and that specific microbial groups play a role in the early stages of recovery. Some Ktedonobacterales are carboxydotrophs and hydrogenotrophs (i.e. carbon monoxide (CO) and hydrogen (H_2) oxidisers), which provides intriguing evidence that CO and H_2 might be important energy sources for the microbial community during the reestablishment of this soil. This study is the first to analyze the total bacterial communities in Llaima volcano, and further work is necessary to fully elucidate the composition of the bacterial community and the functions of these soils. For example, DNA high-throughput-sequence analysis will need to be required to establish the importance of this poorly characterized group of Ktedonobacteria in extreme environments.

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526

527 **Figure legends**

528 Figure 1. Copy numbers of bacterial and archaeal 16S rRNA genes for soils from
529 different sites sampled. The error bars are standard deviations of n=9.

530 Figure 2. Alpha diversity indices as indicators of microbial community biodiversity in
531 Llaima volcanic soils. Three estimators of alpha diversity are shown: (A) observed
532 number of OTUs, (B) Shannon index, and (C) Evenness index. For each estimator,
533 values for each sample are plotted by points.

534 Figure 3. NMDS indicating changes in bacterial community structures between soil
535 recovered after the lava eruption in 1640 (red), 1751 (green) and 1957 (blue) with
536 environmental variables that explained most of the variability. O.M.: organic
537 matter; S: sulphur; Mn: manganese; N: nitrogen; K: potassium; P: phosphorous;
538 Fe: iron. The arrows indicate the direction at which the environmental vectors fit
539 the best (using the envfit function) onto the NMDS ordination space.

540 Figure 4. Mean relative abundance of the most abundant bacterial phyla. Taxa not
541 seen more than 3 times in at least 20% of the samples were removed using
542 phyloseq package on R.

543 Figure 5. Mean relative abundance of classes (A) orders (B) of the phylum
544 Chloroflexi. Taxa not seen more than 3 times in at least 20% of the samples were
545 removed using phyloseq package on R.

546 Figure 6. Heatmap of the most relevant OTUs derived from bacterial 16S rRNA
547 genes. The samples and OTUs were clustered according to Euclidean distances
548 between all Hellinger transformed data. The colored scale gives the percentage
549 abundance of OTUs.

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