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

Marcela Hernández¹*, Marcela Calabi², Ralf Conrad¹ and Marc G. Dumont³

¹Max-Planck-Institute for Terrestrial Microbiology, Marburg, Germany
²Scientific and Technological Bioresource Nucleus, Universidad de La Frontera, Temuco, Chile
³School of Biological Sciences, University of Southampton, Southampton, SO17 1BJ, United Kingdom

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*Corresponding author: M. Hernández, m.t.hernandez-garcia@soton.ac.uk
Present address: M. Hernández: University of Southampton, Southampton, SO17 1BJ, United Kingdom
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 (CH₄), hydrogen sulfide (H₂S), hydrogen (H₂), 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).
studies showed that the class Ktedonobacteria, which belongs to the phylum Chloroflexi, is predominant in non-vegetated volcanic soils (Gomez-Alvarez et al., 2007; Weber and King, 2010b), while Proteobacteria dominate vegetated sites (Weber and King, 2010b). Some of these Ktedonobacteria, among other microbial groups, have been associated with CO and H₂ consumption in both young, organic matter-poor volcanic deposits, and in older more mature deposits with well-developed plant communities (King, 2003a; King and Weber, 2008, Weber and King, 2010b).

The colonization of volcanic deposits by plants will also play an important role in soil formation, as their roots enhance rock weathering and photosynthates contribute to soil organic matter accumulation. Some studies have shown that relatively young volcanic deposits harbor distinct microbial communities when plants are present (Dunfield and King, 2004; Ohta et al., 2003, Guo et al., 2014). The interaction, and potential co-dependence, of plants and microbes in volcanic soils has not been specifically investigated. Plant diversity is known to affect microbial diversity, function, and soil carbon accumulation (Leff et al., 2018).

Also, the soil community, including bacteria, fungi and invertebrates, is also important for the establishment and growth of plants (Philippot et al., 2013; Lange et al., 2015; Wubs et al., 2016). These types of interactions have been demonstrated in some mature soils, but have not been investigated in the early stages of soil formation, for example in volcanic soils.

Llaima Volcano is one of the largest and most active volcanoes in Chile. It has had three detailed lava eruptions at different sites of the volcano and plants have colonized these zones. Previously, we sampled soils from Llaima volcano at sites destroyed by lava in different centuries (1640, 1751 and 1957). We hypothesized that 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 MgCl₂ (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.
**Results**

**Microbial abundance**

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

**Diversity of bacterial communities**

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

For beta-diversity, Non-metric multidimensional scaling (NMDS) analysis showed that the composition of the bacterial 16S rDNA also changed across soil types and environmental factor ($P<0.05$, Fig. 3). The youngest soils (site 1957) cluster separated in comparison with the two older soils (sites 1640 and 1751) (Fig. 3). The recent soil (site 1957) was positive related to iron, ration carbon/nitrogen, total 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 Xanthobacteraceae (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
analysis of variance, $P=0.001$). The results presented here support those already published indicating that as the soil recovers, diversity increase. An early study by King (2003a) indicates that the diversity of substrates utilized in Biolog plate assays increased with soil age (18 to 300 years old), assuming changes in the microbial communities from Hawaiian volcanic deposits. Analyzing the same deposits, Gomez-Alvarez and colleagues (2007) indicated that the diversity is higher in moderately vegetated deposits (Shannon index $H=3.87$ in site 1790) than the youngest soil (Shannon index $H=2.62$ in site 1921). The diversity indexes in Llaima volcano in south of Chile are higher than those in Kilauea volcano in Hawaii, USA. However, it is important to indicate that the study of Gomez-Alvarez and colleagues (2007) are from cloning-based analysis and thus it is probably that not all the phylogeny was covered. The diversity in the vegetated areas of Llaima volcano (in sites 1640 and 1751; Fig. 2b, Table S1) is also higher than those in lava-formed Gotjawal forest soils in Korea (Kim et al., 2018), in which authors found indexes below 5.5. (Fig. S3, Kim et al., 2018) for bacterial 16S rRNA gene based on Illumina sequencing analyses. The diversity was also higher than vegetated areas from Iceland volcanic deposits, in which diversity increases as the area becomes fully vegetated (Byloos et al., 2018).

The microbial communities in the vegetated areas (sites 1640 and 1751) cluster together, and in a separate cluster we can identify members of the unvegetated site (1957) (Fig. 3). This is corroborated with the diversity indexes presenting similar values for the vegetated areas. NMDS analysis showed that pH is mostly the only physico-chemical parameter governing the vegetated areas. On the other hand, Iron and carbon/nitrogen ratio play important role in the establishment of bacterial communities in the youngest unvegetated area (1957) (Fig. 3). It has been reported 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, N₂- and H₂-fixer 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). N₂-fixer members of Proteobacteria and Firmicutes were found in a 300 year-old soil from Kilauea volcano (Nüsslein and Tiedje, 1998). H₂-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* SOSP1-21T (Chang et al., 2011). *K. racemifer* SOSP1-21T 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 (H₂) oxidisers), which provides intriguing evidence that CO and H₂ 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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Figure legends

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

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

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

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

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

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