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Bacterial community structure in fumigated soil

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ABSTRACT

Soil microbial biomass has been determined since the mid 1970's by the chloroform fumigation incubation technique as proposed by Jenkinson and Powlson (1976). The microbial biomass C can be determined by subtracting the CO_2 emitted from an unfumigated soil (mineralization of soil organic matter) from that emitted from a chloroform fumigated inoculated soil (mineralization of soil organic matter and killed soil microorganisms) and dividing the difference by a proportionality factor ($k_C = 0.45$). The question remained which microorganisms recolonized a fumigated soil. An arable soil was fumigated for one day with ethanol-free chloroform or left unfumigated and incubated aerobically after removal of the chloroform for 10 days. The bacterial population structures were determined in the fumigated and unfumigated soil after 0, 1, 5 and 10 days by means of 454 pyrosequencing of the 16S rRNA gene. Fumigating the arable soil reduced significantly the relative abundance of phylotypes belonging to different groups, but increased the relative abundance of only four genera belonging to two phyla (Actinobacteria and Firmicutes) and two orders (Actinomycetales and Bacillales). The relative abundance of phylotypes belonging to the *Micromonospora* (Micromonosporaceae) increased significantly from 0.2% in the unfumigated soil to 6.7% in the fumigated soil and that of *Bacillus* (Bacillaceae) from 3.6% to 40.8%, *Cohnella* (Paenibacillaceae) from undetectable amounts to 0.6% and *Paenibacillus* (Paenibacillaceae) from 0.3% to 4.2%. The relative percentage of phylotypes belonging to the Acidobacteria, Bacteroidetes, Chloroflexi, Gemmatimonadetes and Proteobacteria (α- β-, δ- and γ-Proteobacteria) were significantly lower in the fumigated than in the unfumigated soil and in most of them the relative abundance of different bacterial orders (i.e. Gp3, Gp4, Gp6, Sphingobacteriales, Gemmatimonadales, Rhodospirillales, Burkholderiales, Xanthomonadales) was reduced strongly ($P < 0.001$). It was found that the relative abundance of a wide range of bacteria was reduced shortly after fumigating an arable soil, but only a limited group of bacteria increased in a fumigated arable soil indicating a capacity to metabolize the killed soil microorganisms or recolonize a fumigated soil.

1. Introduction

Soil microorganisms are involved in most of the processes that occur in soil. However, it has always been difficult to have an accurate estimation of the total soil microbial population (Kirchman, 2012). In a first attempt to measure the soil microbial population, microorganisms were stained and counted. Later soil bacteria were enumerated by culturing techniques in nutrient rich or specific media. It was hoped that the determination of the amount of microorganisms in a soil would allow to estimate the magnitude of certain processes, such as C and N mineralization (Fierer et al., 2009). As such, it would be possible to predict the organic matter dynamics and the amount of mineral N that would be available for growing crops (Abera et al., 2012). However, it became clear that these measurements did not often relate well with microbial activity and soil processes.

In 1976, Jenkinson and Powlson wrote a seminal paper describing a method to measure the soil microbial biomass carbon. They proposed to fumigate a soil and measure the amount of CO_2 emitted within a given period, i.e. 10 days. They assumed that the killed biomass would be mineralized and a certain part of the biomass C would be emitted as CO_2 that could be determined easily.

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At the same time, the unfumigated soil would serve as control and allow to measure the soil organic matter that apart from the killed biomass would be mineralized in the fumigated soil. As such, the microbial biomass could be calculated as the CO_2 emitted from the fumigated soil minus the CO_2 emitted from the unfumigated soil divided by a factor K_C considering the amount of biomass C mineralized to CO_2. The K_C value reported by Jenkinson and Powlson (1976) was 0.45.

It has always been questioned which microorganisms would recolonize the fumigated soil and mineralize the killed soil microbial population. Kemmit et al. (2008) using phospholipid fatty acids analysis showed that fumigation induced changes in the biomass structure. The PLFA technique has given a first insight into which microorganisms colonize a fumigated soil, but the identification of the bacterial groups remains limited (Kemmit et al., 2008; Rousk et al., 2009; Dungait et al., 2013). New molecular and metagenomic techniques do allow to identify the microorganisms in soil in great detail now (Fierer et al., 2009). An arable soil was fumigated with ethanol-free chloroform for 1 day, inoculated with 0.01 g unfumigated soil as in the original technique and incubated together with unfumigated soil for 10 days. At the onset of the incubation and after 1, 5 and 10 days the emission of CO_2 was measured and the microbial population monitored. Our objective was to determine the bacterial community structure in both a fumigated and unfumigated soil by means of 454 pyrosequencing of the 16S rRNA gene.

2. Materials and methods

2.1. Soil sampling

The sampling site is located in Otumba. Details of the sampling site can be found in Aguilar-Chávez et al. (2012). The soil was classified as Typic Fragiudepts. The site was cultivated mainly with maize for >20 y and the cultivated crops were not irrigated. Soil was collected by augering randomly 20 times the 0–15 cm top-layer of three plots of approximately 400 m^2 spatially separated. The soil samples from each plot ($n = 20$) were pooled so that three soil samples were obtained ($n = 3$) and characterized. The sandy loam soil had an organic C content of 6.7 g C kg^{-1} soil and total N content of 0.73 g N kg^{-1} soil. The water holding capacity (WHC) was 665 g kg^{-1} dry soil, pH 7.7 and electrolytic conductivity (EC) 1.23 dS m^{-1}. Details of techniques used to characterize the soil can be found in Ruíz-Valdiviezo et al. (2010).

2.2. Fumigation incubation

Eight sub-samples of 25 g fresh weight soil of each plot ($n = 3$) were added separately to 120 ml glass flasks. Halve of the flasks were placed in a desiccator and fumigated with ethanol-free chloroform (Mueller et al., 1992) for one day (i.e. the fumigated soil samples) as described by Jenkinson and Powlson (1976), while the other half were placed separately in 1 l glass jars containing a vessel with 20 ml 1 M NaOH to trap the CO_2 evolved and a vessel with distilled water to avoid desiccation of the soil samples (i.e. the unfumigated soil samples). The glass jars were closed airtight and incubated in the dark at 25 ± 2 °C for 10 days. After one day of fumigation, the desiccator was aired for 1 h and the headspace was vacuum evacuated until all chloroform was removed. The fumigated soil samples were inoculated with 0.01 g unfumigated soil, mixed, placed separately in a 1 l glass jar and incubated as described for the unfumigated soil samples.

After 0, 1, 5 and 10 days, a jar with fumigated and unfumigated soil from each plot ($n = 3$) was selected at random, opened, the vessel with 1 M NaOH stoppered and the soil removed from the glass flask for extraction of DNA. The 1 M NaOH was titrated with 0.1 M HCl to determine the CO_2 trapped (Jenkinson and Powlson, 1976). The soil microbial biomass was calculated as the [(CO_2 emitted from the fumigated soil − CO_2 emitted from the unfumigated soil)/0.45] (Jenkinson and Powlson, 1976).

2.3. DNA extraction and PCR amplification of bacterial 16S rRNA genes

Each soil sample was washed first with 0.15 M sodium pyrophosphate and 0.15 M pH 8 phosphate buffer to eliminate the soil organic material (Ceja-Navarro et al., 2010). The DNA was extracted from the washed soil as described by Ceja-Navarro et al. (2010) and consisted in a chemical and thermal shock for cell lysis. DNA was extracted four times from 0.5 g soil (a total 2 g soil) and pooled. A total of 2 g soil was extracted for DNA per plot so overall 6 g soil was extracted. The precipitation of the proteins and purification of the DNA are described in Ceja-Navarro et al. (2010).

The V1–V6 region of the 16S rRNA bacterial genes was amplified with 10-pb barcoded primers 8-F (5′-AGA GTT TGA TCI TGG CTC A-3′) and 949-R (5′-CCG TCW ATT KCT TTG AGT T-3′) and containing the A and B 454 FLX adapters (Navarro-Noya et al., 2013). The PCR reactions were done as previously described by Navarro-Noya et al. (2013). The product of five reactions of each metagenomic DNA sample was pooled to minimize PCR bias and constituted a single library (Acinas et al., 2004). All the pyrosequencing libraries were purified using the DNA Clean & Concentrator purification kit as recommended by the manufacturer (Zymo Research, Irvine, CA, USA), and quantified using the PicoGreen® dsDNA assay (Invitrogen, Carlsbad, Ca, USA) and the NanoDrop™ 3300 Fluorospectrometer (Thermo Scientific NanoDrop). Sequencing was done by Macrogen Inc. (DNA Sequencing Service, Seoul, Korea) by using a Roche 454 GS-FLX Plus System pyrosequencer (Roche, Mannheim, Germany). The 24 pyrosequencing–derived 16S rRNA gene sequence datasets were submitted to the NCBI Sequence Read Archive (SRA) under accession number SRA108903.

2.4. Analysis of pyrosequencing data

The QIIME version 1.5.0 software pipeline was used to analyze the pyrosequencing data (Caporaso et al., 2010b). Firstly, the poor quality reads were eliminated from the data sets, i.e. quality score <25, containing homopolymers >6, length <400 nt, and containing errors in primers and barcodes. Operational taxonomic units (OTU) were determined at 97% similarity level with UCLUST algorithm (Edgar, 2010). Chimeras were detected and removed from the data sets using the Chimera Slayer (Haas et al., 2011). Sequence alignments were done against the Greengenes core set and using representative sequences of each OTU using PyNAST, and filtered at a threshold of 75% (Caporaso et al., 2010a).

2.5. Phylogenetic and statistical analysis

The taxonomic distribution estimates at different levels was done using the taxonomy assignation at a confidence threshold of 80% by the naïve Bayesian rRNA classifier from the Ribosomal Data Project (http://rdp.cme.msu.edu/classifier/classifier.jsp) (Wang et al., 2007).

The beta diversity analysis was done using phylogenetic information (UniFrac analysis) with Fast UniFrac (Hamady et al., 2010) using a phylogenetic tree computed with FastTree (Price et al., 2009) and a rarefied 'biom' table at 250 reads as inputs. Also, abundance of the bacterial groups at different taxonomic levels (phylum, order, genus) was separately explored with a PCA using PROC FACTOR (SAS Institute, 1989). Significant difference between

the relative abundance of the different bacterial groups as a result of fumigation was determined by analysis of variance (ANOVA) and based on the least significant difference using the general linear model procedure (PROC GLM, SAS Institute, 1989).

3. Results

3.1. Microbial biomass: fumigation incubation technique

The emission of CO_2 was larger from the fumigated soil than from the unfumigated soil and that from the first day onwards (Fig. S1). Afterward, however, emissions of CO_2 were similar in the fumigated and unfumigated soil. The microbial biomass C was 116.4 mg C kg^{-1} soil or 1.74% of the organic soil C content.

3.2. The bacterial community structure in the arable soil

Phylotypes belonged to 13 different phyla in the unfumigated arable soil (Fig. 1). The relative abundance of phylotypes belonging to the Proteobacteria was the highest (41.7%), followed by Acidobacteria (14.6%) and the Bacteroidetes (14.6%). The relative abundance of phylotypes belonging to the Firmicutes was (5.8%) and three phyla could be described as rare (<1%). Changes in the relative abundance of the different groups on the phylum, class and order level in the unfumigated soil were generally small over time (No data shown).

Fumigating the arable soil reduced significantly the relative abundance of phylotypes belonging to different groups, but increased the relative abundance of only a small proportion of the soil bacteria (Table 1, Fig. 2a and b). The relative abundance of phylotypes belonging to only two phyla (Actinobacteria and Firmicutes), two orders (Actinomycetales and Bacillales) and four genera significantly increased after fumigation. The relative abundance of phylotypes belonging to the *Micromonospora* (Actinomycetales, Actinobacteria) increased significantly from 0.2% in the unfumigated soil to 6.7% in the fumigated soil and that of *Bacillus* from 3.6% to 40.8%, that of *Cohnella* from undetectable amounts to 0.6% and *Paenibacillus* from 0.3% to 4.2% (Fig. 3).

Fumigating the soil strongly reduced the relative abundance of a wide range of phylotypes belonging to different bacterial groups; at the level of genus approximately 30 different groups were affected. The relative percentage of phylotypes belonging to the phyla

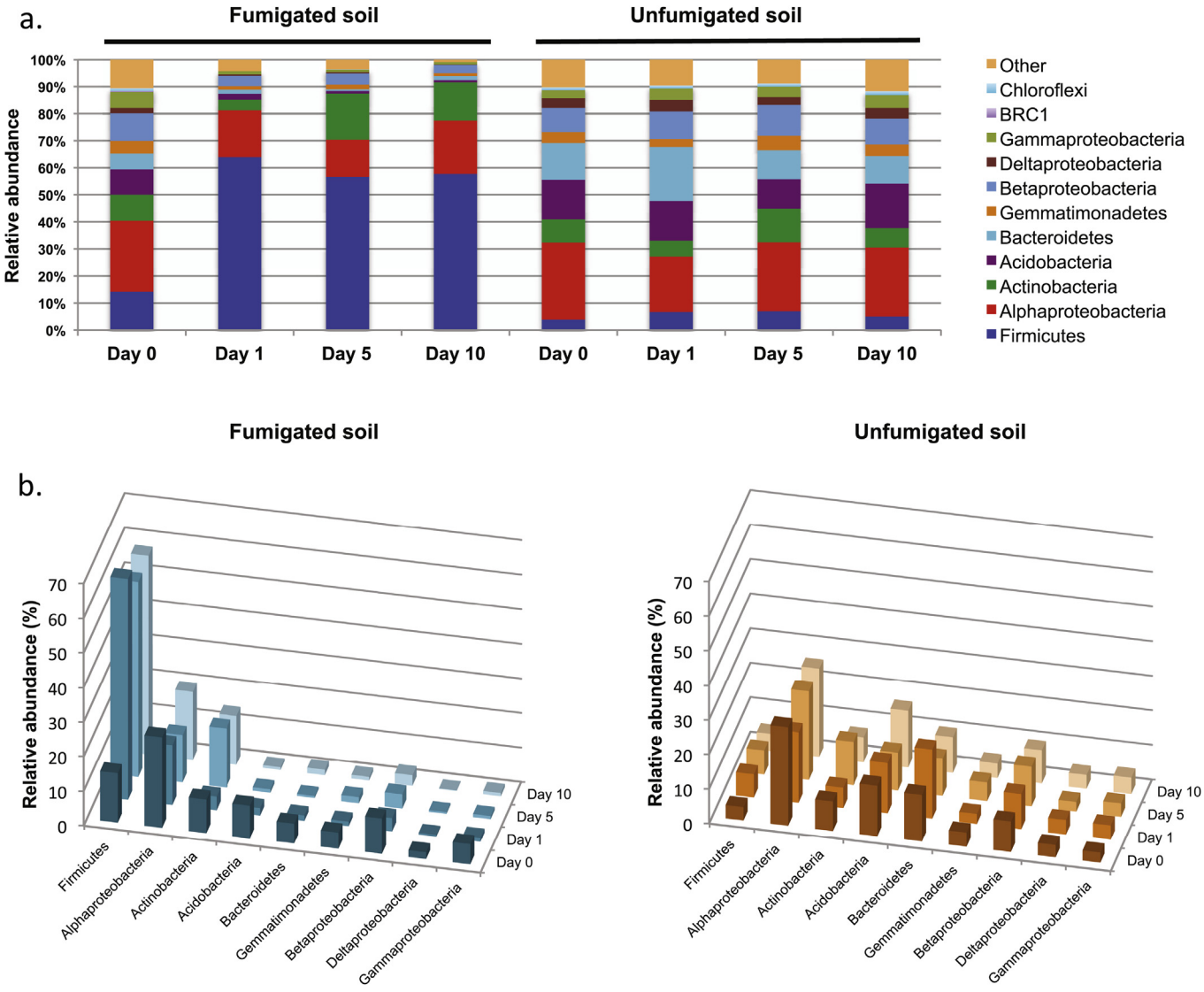

Fig. 1. Relative abundance of the different phyla and Proteobacteria classes found in the fumigated and unfumigated soil incubated aerobically for 10 days.

Table 1
The relative abundance of different bacterial groups (level of phylum, class, order and genus) in an unfumigated or fumigated arable soil incubated aerobically for 10 days. Bacterial groups were affected significantly by fumigation at the $P < 0.05$ level.

Phylum/Class	The relative abundance of the bacterial group was larger in unfumigated soil than in the fumigated soil.
Acidobacteria	Acidobacteria***, Gp16*, Gp3***, Gp4***, Gp6***, Gp7
Actinobacteria	*Marmoricola* (Actinomycetales; Nocardioidaceae)
Bacteroidetes	Bacteroidetes***, Sphingobacteria***, Sphingobacteriales***, *Flavisolibacter*** (Chitinophagaceae; Sphingobacteriales), *Adhaeribacter****, *Sporocytophaga** (Cytophagaceae; Sphingobacteriales)
Chloroflexi	Chloroflexi***, Anaerolineae, Chloroflexi, Thermomicrobia*, Anaerolineales, Sphaerobacterales, *Sphaerobacter* (Sphaerobacterales; Sphaerobacteraceae)
Gemmatimonadetes	Gemmatimonadetes**, Gemmatimonadales**, *Gemmatimonas***
Proteobacteria	Proteobacteria***
α-Proteobacteria	α-Proteobacteria, Caulobacterales, Rhodospirillales**, *Skermanella*** (Rhodospirillaceae; Rhodospirillales), *Porphyrobacter* (Erythrobacteraceae; Sphingomonadales)
β-Proteobacteria	β-Proteobacteria***, Burkholderiales** Nitrosomonadales, *Azohydromonas* (Alcaligenaceae; Burkholderiales) *Methylibium** (Burkholderiales_incertae_sedis; Burkholderiales), *Variovorax** (Comamonadaceae; Burkholderiales), *Herbaspirillum* (Oxalobacteraceae; Burkholderiales), *Nitrosospira* (Nitrosomonadaceae; Nitrosomonadales)
δ-Proteobacteria	δ-Proteobacteria***, Myxococcales
γ-Proteobacteria	γ-Proteobacteria***, Xanthomonadales***, *Steroidobacter* (Sinobacteraceae; Xanthomonadales), *Lysobacter** (Xanthomonadaceae; Xanthomonadales)
	The relative abundance of the bacterial group was larger in fumigated soil than in the unfumigated soil.
Actinobacteria	*Micromonospora*** (Micromonosporaceae; Actinomycetales)
Firmicutes	Firmicutes***, Bacillaceae***, Bacillales***, *Bacillus**** (Bacillaceae; Bacillales), *Paenibacillus*** and *Cohnella* (Paenibacillaceae; Bacillales)

* Significant at the $P < 0.01$, ** Significant at the $P < 0.001$, *** Significant at the $P < 0.0001$.

Acidobacteria, Bacteroidetes, Chloroflexi, Gemmatimonadetes and Proteobacteria were significantly lower in the fumigated than in the unfumigated soil and in most of them the relative abundance of different bacterial groups were reduced (Table 1, Fig. 1). The relative abundance of phylotypes of the Acidobacteria showed the largest drop from an average of 14.6% in the unfumigated soil to 1.4% in the fumigated soil at day 10 and within the Acidobacteria, the largest decrease in relative abundance was found in the class Gp4 (unfumigated soil, 7.3%; fumigated soil, 0.04%).

3.3. Changes in the bacterial community structure

Bacterial community structure in the fumigated and unfumigated soil was compared using taxonomic and phylogenetic information. PCA analysis considering the relative abundance at different taxonomic levels (phyla, orders or genera) clearly separated the fumigated soil from the unfumigated soil. For instance, considering the relative abundance of the different orders, the fumigated soil samples were found in the lower left quadrant (a negative PC1 and PC2) or upper left quadrant (one fumigated soil sample at day 0), while the unfumigated soil samples were found in the lower or upper right quadrant together with the fumigated soil samples at day 0 (a positive PC1) (Fig. 4). The fumigated soil samples were characterized by a higher relative abundance of Actinomycetales and Bacillales, while the unfumigated soil samples by a higher relative abundance of a different orders, e.g. Gp4, Gp6, Gemmatimonadales, Burkholderiales, Rhodospiralles, Sphingobacteriales and Xanthomonadales. The UniFrac analysis also separated clearly the communities of the fumigated from the unfumigated soils (Fig. S2).

4. Discussion

4.1. Microbial biomass: fumigation incubation technique

The emission of CO_2 in the fumigated and unfumigated arable soil followed a pattern described already by Jenkinson and Powlson (1976). The difference in emitted CO_2 between the fumigated and unfumigated soil was evident from day 1 onwards, but afterward the emission of CO_2 was similar in the fumigated and unfumigated soil (Kemmit et al., 2008).

4.2. The bacterial community structure in the arable soil

Fumigating the arable soil, inoculating it with a small soil sample and incubating it aerobically for 10 days, increased the relative abundance of a small number of bacterial groups. The relative abundance of only four genera belonging to only two phyla (Actinobacteria, Firmicutes) and two orders (Actinomycetales, Bacillales), known to be involved in degradation of organic material and composting (Partanen et al., 2010; Martins et al., 2013), increased in the incubated soil after fumigation, i.e. phylotypes belonging to genera *Micromonospora* (Micromonosporaceae), *Bacillus* (Bacillaceae), *Paenibacillus* and *Cohnella* (Paenibacillaceae). Consequently, phylotypes belonging to these genera are early colonizers of fumigated soil and involved in the early stages of the mineralization of decomposable organic material derived from the killed soil microorganisms. Strains belonging to the genera *Bacillus*, *Paenibacillus*, and *Micromonospora* are metabolic versatile, while less is known about strains belonging to *Cohnella*. Although the fumigated soil was inoculated with unfumigated soil and mixed thoroughly, the capacity of phylotypes belonging to the Bacillales (Kolter et al., 1993; Cano and Borucki, 1995; García-Fraile et al., 2008) and *Micromonospora* to form spores (Hardisson and Suarez, 1979) will have favored them in recolonizing the fumigated soil.

Strains belonging to the genus *Bacillus* are extremely versatile. They are well known degraders of organic residue, such as soybean and maize (España et al., 2011), show cellulolytic activity (Cucurachi et al., 2013), have been found during various phases of composting (He et al., 2013) and are known to produce proteases, chitinases and cellulases (Pathma and Sakthivel, 2013). A *Bacillus* sp. strain LD003 showed growth on high-molecular and low-molecular weight lignin fractions and utilization of lignin-associated aromatic monomers and degradation of ligninolytic indicator dyes, e.g. Azure B, Methylene Blue and Toluidene Blue O (Bandounas et al., 2011).

Several members of the genus *Paenibacillus* produces cellulases (Pathma and Sakthivel, 2013 http://apps.webofknowledge.com/OneClickSearch.do?product=WOS&search_mode=OneClickSearch&excludeEventConfig=ExcludeIfFromFullRecPage&colName=WOS&SID=3BxDSAvvo3aH9QEXnWj&field=AU&value=Sakthivel,%20N), phosphatases (Kim et al., 2009), xylanases (Ko et al., 2010a), enzymes which can specifically decompose xylans the main constituent of hemicelluloses, have shown cellulolytic and

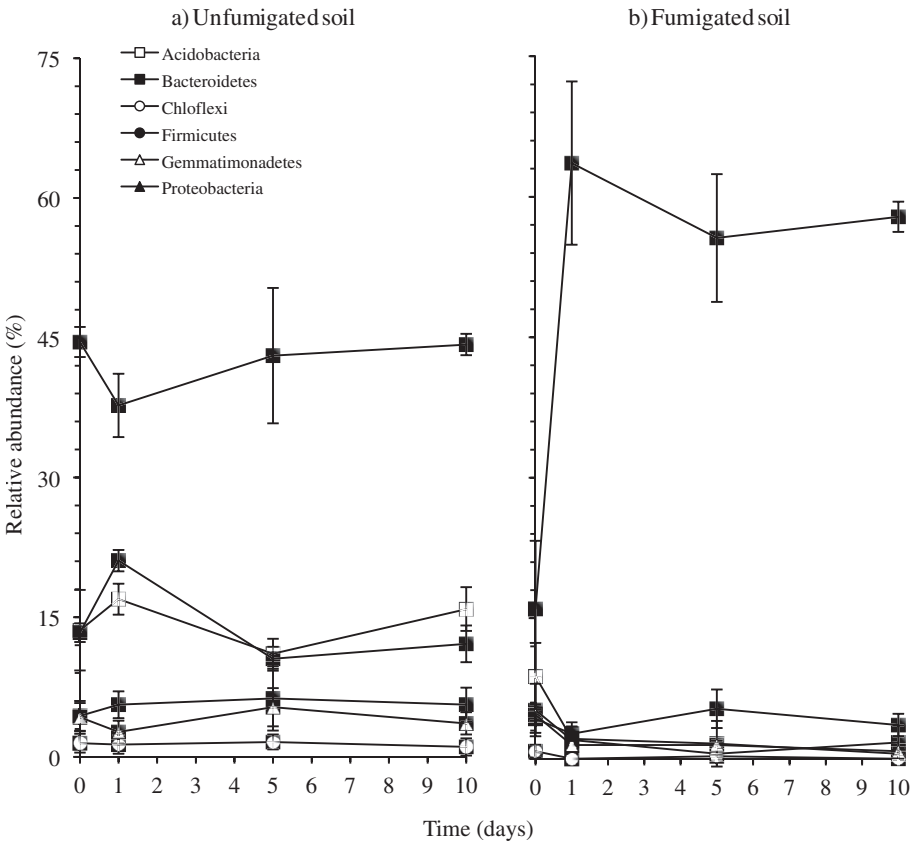

Fig. 2. Relative abundance of the bacterial phyla in a) unfumigated soil and b) soil that was fumigated with ethanol free chloroform, inoculated with 0.01 g unfumigated soil incubated soil aerobically for 10 days. Bars are ± one STD of three soil samples ($n = 3$).

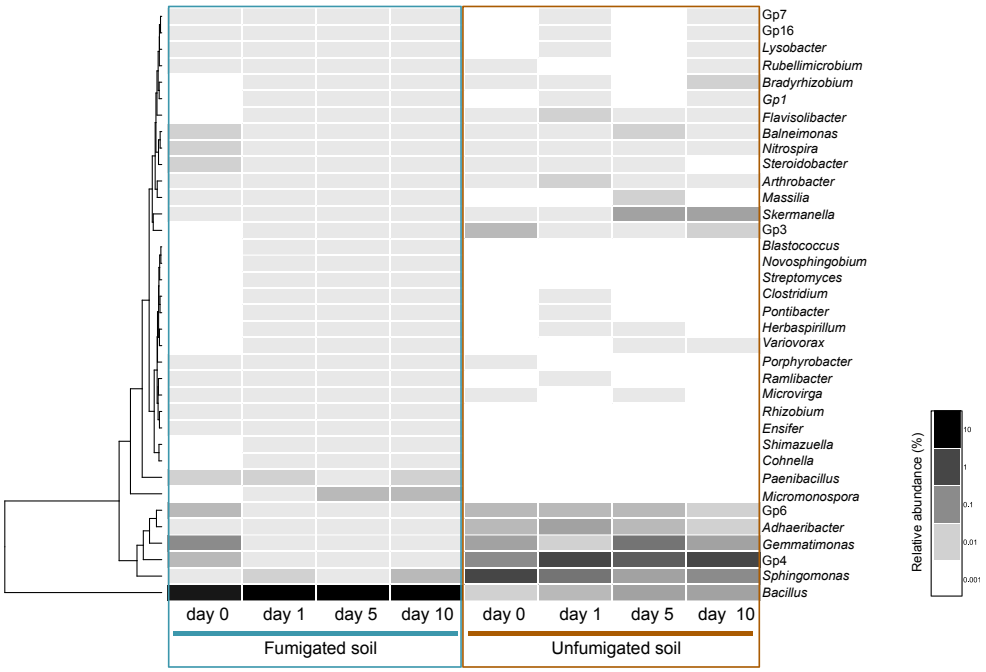

Fig. 3. Heat map of the most important genera and Acidobacteria groups (Gp) in the fumigated and unfumigated soil incubated aerobically for 10 days. Values are the mean of three soil samples ($n = 3$).

hemicellulolytic activity (Hatayama et al., 2006; Ko et al., 2010b; Eida et al., 2012). Additionally, *Paenibacillus* can degrade kraft lignin (Chandra et al., 2007), they consisted 5.1% of the phylotypes in a lignin degradation bacterial consortium (Wang et al., 2013) and identification of genes for lignocellulose processing and metabolic reconstructions suggested *Paenibacillus* a key group in decomposition of lignocellulosic material (D'haeseleer et al., 2013). By genome mining, seven chitinase and four 1,3-beta glucanase encoding genes were identified in the *Paenibacillus* vortex genome (Sirota-Madi et al., 2010); these enzymes are involved in degradation of polysaccharide components of the fungal cell wall (El-Katatny et al., 2001), while the genome of a *Paenibacillus lautus* strain is enriched in GH18 chitinases, GH28 polygalacturonases, GH88 unsaturated glucuronyl hydrolases, GH105 unsaturated rhamnogalacturonyl hydrolases and pectate lyase (PL) family enzymes (Mead et al., 2012).

Micromonospora has been described as one of the most important genus of the soil actinobacterias (Genilloud, 2012). However, it is frequently reported as endophyte and nodulating bacteria or in aquatic ecosystems (de Menezes et al., 2012). Micromonosporas are well known for their ability to degrade complex recalcitrant materials, such as cellulose and chitin (Jendrossek et al., 1997; Wohl and McArthur, 2001; Talia et al., 2012) and produce acid and alkaline phosphatases (El-Tarabily et al., 2008).

Strains belonging to the genus *Cohnella* were found during various phases of composting (He et al., 2013). *Cohnella* strains isolated from coffee residue compost contributed to an efficient cellulolytic and hemicellulolytic processes during composting, and they consisted 2.2% in a lignin degradation bacterial consortium mentioned earlier (Wang et al., 2013).

Although the abundance of phylotypes belonging to the genus *Micromonospora* increased >50-times in the fumigated soil compared to the unfumigated soil, the relative abundance of other phylotypes belonging to Actinobacteria decreased significantly, i.e. *Marmoricola* ($P < 0.05$), while the relative abundance of 44 other groups belonging to the Actinobacteria were not affected by the fumigation process. It is thus clear that not all phylotypes belonging to the same group respond in the same way to fumigation. For instance, the relative abundance of phylotypes belonging to the genus *Marmoricola* was so low in the fumigated soil that they were not detectable, while they consisted of 0.3% of all sequences in the unfumigated soil.

Strains belonging to the phyla Bacteroidetes and Proteobacteria are generally described as responding easily to labile carbon sources (Fierer et al., 2007). Fumigating the soil had a large effect on phylotypes belonging to the Bacteroidetes. Bacteroidetes were the second most abundant phylum in the arable soil (14.6%), but their relative abundance dropped to 1.4% in the fumigated soil. Strains belonging to the α- and β-Proteobacteria are well known to respond to easily decomposable organic material and are generally considered to be r-strategist, or weedy fast-growing microbiota whose populations fluctuate opportunistically (Fierer et al., 2007). In this study, however, they did not colonize the fumigated arable soil or metabolize the liberated microbial organic material as the relative abundance of phylotypes belonging to the Proteobacteria, the most abundant phylum in the unfumigated arable soil (41.7%), dropped to 22.6% in the fumigated soil. The fumigation reduced each of the different classes of Proteobacteria, except for the α-Proteobacteria. The relative abundance of the β-Proteobacteria dropped to 3.8% in the fumigated soil compared to 10.4% in the unfumigated soil. The relative abundance of the Burkholderiales (β-Proteobacteria) increases normally in response to labile substrates (Goldfarb et al., 2011), but they were not favored by the liberation of organic material in the fumigated soil as their relative abundance dropped from 8.9% in the unfumigated soil to 3.3% in the fumigated soil. Mature compost was found to be preferred by δ-Proteobacteria, so the decrease in their relative abundance from 3.0% in the unfumigated soil to 0.5% in the fumigated soil can be expected. γ-Proteobacteria, however, were found in the earlier stages of composting (de Gannes et al., 2013), but in this study they were not involved in metabolizing cell material or

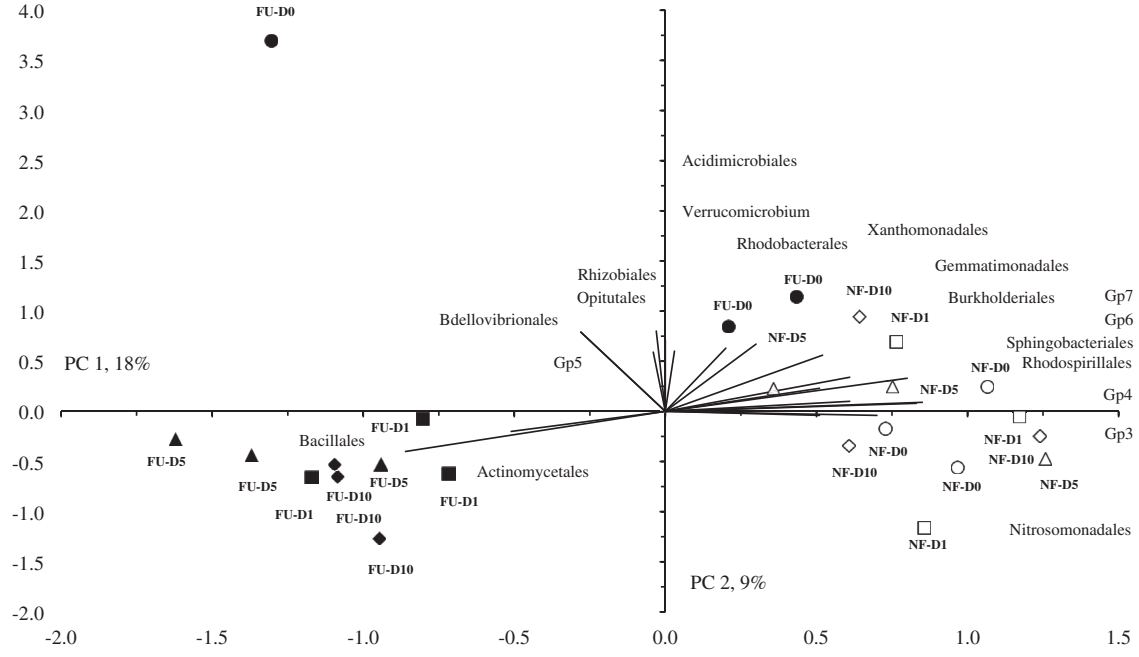

Fig. 4. Principal component analysis considering the percentages of the different orders in the fumigated and unfumigated soil incubated aerobically for 10 days. Unfumigated soil incubated for 0 (○), 1 (□), 5 (△) or 10 days (◇) and fumigated soil incubated for 0 (●), 1 (■), 5 (▲) or 10 days (◆).

colonizing the fumigated soil as their relative abundance dropped from 4.0% in the unfumigated soil to 0.9% in the fumigated soil.

Although the above mentioned Proteobacteria and Bacteroidetes are favored generally by the application of easily decomposable material, their relative abundance did not increase after fumigating the soil. It might be that they lack the necessary enzymes to degrade the killed microorganisms, e.g. chitinase and 1,3-beta glucanase to degrade polysaccharide components of the fungal cell, but most likely they are not the first degraders of these components released after fumigation of the microbial biomass or the available easily decomposable soil organic material; they grow slower than phylotypes belonging to the genera *Micromonospora*, *Bacillus*, *Paenibacillus* and *Cohnella* under the given conditions. It has to be remembered that the increase in the relative abundance of the Firmicutes occurred within a day, so the augmentation of the phylotypes belonging to the Proteobacteria, Bacteroidetes and other groups was slower and they will only later on, i.e. after 10 days, replace the fast growing Firmicutes. The graph showing the changes in relative abundance of the different groups affected significantly by fumigation already indicates this as the relative abundance of the Proteobacteria starts to increase at day 10 compared to day 5.

5. Conclusion

It was found that fumigating an arable soil, reduced the relative abundance of phylotypes belonging to different bacterial groups, but increased the relative abundance of only four genera. The relative abundance of phylotypes belonging to the *Micromonospora* (Actinomycetales, Actinobacteria) increased significantly from 0.2% in the unfumigated soil to 6.7% in the fumigated soil and that of *Bacillus* from 3.6% to 40.8%, *Cohnella* from undetectable amounts to 0.6% and *Paenibacillus* from 0.3% to 4.2%. The genera *Bacillus*, *Paenibacillus* and *Micromonospora* are metabolic versatile with different phylotypes producing proteases, phosphatases, chitinases, cellulases and showing lignolytic activity. This gives them the metabolic capacity to degrade the killed microorganisms and recolonize the fumigated soil. Less is known about the metabolic characteristics of the genus *Cohnella*, although strains belonging to this genus have shown cellulolytic and hemicellulolytic activity, and degrade lignin.

The relative abundance of phylotypes belonging to the Acidobacteria, Bacteroidetes, Chloroflexi, Gemmatimonadetes and Proteobacteria (α-, β-, δ- and γ-Proteobacteria) were significantly lower in the fumigated than in the unfumigated soil and in most of them the relative abundance of different bacterial groups (i.e. Gp3, Gp4, Gp6, Sphingobacteriales, Gemmatimonadales, Rhodospirillales, Burkholderiales, Xanthomonadales) was reduced strongly. It might be that they lack the necessary enzyme to degrade the organic material released after lysis of the microbial cells, but most likely their growth is slower than that of phylotypes belonging to the genera *Bacillus*, *Paenibacillus*, *Micromonospora* and *Cohnella* in the arable soil under the given conditions.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2014.02.012.

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