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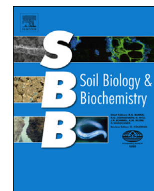

Bacterial community structure in fumigated soil

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A B S T R A C T

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.

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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 toplayer 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

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