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# The bacterial community structure in an alkaline saline soil spiked with anthracene

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## Abstract

**Background:** The application of polycyclic aromatic hydrocarbons (PAHs) will affect the bacterial community structure as some groups will be favoured and others not. An alkaline saline soil with electrolytic conductivity (EC) 56 dS $m^{-1}$ was spiked with anthracene and acetone while their effect on bacterial community structure was investigated.
**Results:** The percentages of Acidobacteria and Actinobacteria decreased over time, while the percentage of Proteobacteria, mostly Xanthomonadales, increased. The percentage of the phylotypes belonging to the *Nocardioides*, *Rhodococcus* and *Streptomyces*, known degraders of PAHs, was larger in the anthracene-amended soil than in the acetone-amended and unamended soil at day 14. The phylotypes belonging to the genera *Sphingomonas*, also a known degrader of PAHs, however, was lower. Weighted and unweighted PCoA with UniFrac indicated that phylotypes were similar in the different treatments at day 0, but changed at day 1. After 14 days, phylotypes in the unamended and acetone-amended soil were similar, but different from those in the anthracene-spiked soil.
**Conclusions:** It was found that incubating the soil and contaminating it with anthracene changed the bacterial community structure, but spiking the soil with acetone had little or no effect on the bacterial community structure compared to the unamended soil.

**Keywords:** C dynamics, N mineralization, phylogenetic analysis, polycyclic aromatic hydrocarbons, principal component analysis, UniFrac.

## INTRODUCTION

Mexico is an important petroleum producing country so contamination during extraction and transport occurs frequently. In Mexico, 27971 ton oil contaminated the environment as a result of leaks and spills in 2010. Polycyclic aromatic hydrocarbons (PAHs) are important components of petroleum. They are resistant to degradation and have been listed as priority pollutants by both the US Environmental Protection Agency and European Union (Doyle et al. 2008).

Consequently PAHs, such as anthracene, have often been used as a model in the study of factors controlling the removal of hydrocarbons from soil (Vázquez-Núñez et al. 2009; Zhang et al. 2011b). Anthracene is a tricyclic aromatic hydrocarbon and has been found in fumes from vehicle exhaust, coal, coal tar, tobacco smoke and at waste sites. Humans exposed to anthracene experienced headaches, nausea, loss of appetite, inflammation or swelling of the stomach and intestines (ATSDR, 1995).

Microorganisms remove PAHs from soil and even complex compounds, such as anthracene with three benzene rings, are dissipated within weeks (Amezcua-Allieri et al. 2012). Numerous bacteria have been described that can degrade PAHs, such as phylotypes belonging to the genus *Sphingomonas* (Jurelevicius et al. 2012), *Achromobacter* (Tiwari et al. 2010), *Acidovorax* (Jurelevicius et al. 2012), *Herbaspirillum* (Louvel et al. 2011), *Methylibium* (Zhang et al. 2012), *Polaromonas* (Jurelevicius et al. 2012) and *Variovorax* (Zhang et al. 2011a), so they will be favoured (Jones et al. 2011). Contaminating soil with PAHs will also inhibit certain microorganisms (Wang et al. 2011). As such, changes in the bacterial community structure will be indicative of the effect of anthracene.

An alkaline saline soil of the former lake Texcoco (Mexico) with electrolytic conductivity (EC) 56 dS $m^{-1}$ and pH 9 was spiked with acetone, contaminated with anthracene dissolved in acetone or left unamended. The C and N dynamics, and anthracene concentrations were monitored in an aerobic incubation while the bacterial community structure was determined after 0, 1 and 14 days. The objective of this study was to investigate how incubation time, acetone and anthracene affected the bacterial community structure in an alkaline saline soil.

## MATERIALS AND METHODS

### Site description and soil sampling

The sampling site is located in the former lake Texcoco in the valley of Mexico City. The soil was sampled at random by augering the 0-10 cm layer of two 0.5 ha plots. The soil from each plot was pooled so that two soil samples were obtained ($n$ = 2). This field-based replication was maintained in the incubation study. The $pH_w$ in the sandy soil (clay 22 g $kg^{-1}$, silt 106 g $kg^{-1}$ and sand 872 g $kg^{-1}$) was 9.0, electrolytic conductivity 56 dS $m^{-1}$, water holding capacity (WHC) 504 g $kg^{-1}$ soil and organic carbon content 12.9 g $kg^{-1}$ soil. Techniques used to characterize the soil are described in Fernández-Luqueño et al. (2008).

### Treatments and aerobic incubation

Four different treatments were applied to the two soil samples ($n$ = 2). First, 20 g sub-samples were amended with 2 mL acetone (acetone-amended soil). Second, 20 g sub-samples were spiked with anthracene dissolved in 2 mL acetone (anthracene-spiked soil). As such, 550 mg anthracene $kg^{-1}$ was added. It was found that this concentration was sufficient to study dynamics of the contaminant in an aerobic incubation experiment. Third, 20 g sub-samples that were sterilized on three consecutive days were spiked with anthracene dissolved in 2 mL acetone under sterile conditions (anthracene-sterile soil). Fourth, 20 g sub-samples were left untreated (unamended soil). All soil samples were mixed and placed under vacuum in a desiccator for 45 min to evaporate the acetone.

The aerobic incubation experiment was conducted in the same way as described in Fernández-Luqueño et al. (2008). Briefly, each soil sample ($n$ = 2) was 5-mm sieved, adjusted to 40% WHC and incubated for 7 days. Sub-samples of 25 g from each soil sample ($n$ = 2) were added separately to 120 ml glass flasks and the earlier mentioned treatments were applied. The glass flasks were placed separately in 1 l jars. The jars contained a 25 ml flask with 20 ml water to avoid desiccation of the soil and a 25 ml flask with 20 ml 1 M NaOH to trap evolved $CO_2$. The soil was incubated aerobically for 56 days, while $CO_2$ emissions, mineral N and anthracene concentrations were monitored at 0, 1, 3, 7, 14, 28 and 56 days. At the same time, a sub-sample of 5 g soil was taken and stored at -80ºC until DNA extraction. The remaining flasks were opened and aired for 10 min to maintain aerobic conditions in the soil.

Anthracene in the soil was determined using an exhaustive ultrasonic extraction method developed by Song et al. (1995). Details of the extraction technique and the setting for the gas chromatograph (GC) can be found in Contreras-Ramos et al. (2008).

**DNA extraction and PCR amplification of bacterial rDNA genes**

The DNA was directly extracted from soil. The technique used was based on the techniques described by Valenzuela-Encinas et al. (2008). Primers 46F (5'GCC TAA CAC ATG CAA GTC 3') and 1540R (5'GGT TAC CTT GTT ACG ACT T 3'), were used for amplification of *ca.* 1500 bp-long 16S rRNA gene segments from the metagenomic DNA (Edwards et al. 1989; Yu and Morrison, 2004). The PCR was done using a Touchgene Gradient thermal cycler (Techne, Cambridge United Kingdom).

**Cloning and sequencing PCR products**

The 1500 bp-long segments were used to clone and construct 16S rRNA gene libraries. The TOPO TA cloning kit with the pCR® II-TOPO® vector (Invitrogen, Carlsbad, CA) was used to clone the PCR products. Details of the cloning procedure can be found in Valenzuela-Encinas et al. (2008). The 16S rRNA gene sequences were obtained with a 3730X DNA Analyzer (Applied Biosystems, Foster City, CA) using M13 primers at the LANGEBIO (Cinvestav, Mexico).

**Phylogenetic and statistical analysis**

A total 2966 of sequences were aligned using the NAST tool from Greengenes (DeSantis et al. 2006) and chimeras were detected using Bellerophon v. 3.0 (Huber et al. 2004). The screened sequences (2898) were classified using the naïve Bayesian rRNA classifier from the Ribosomal Data Project (http://rdp.cme.msu.edu/classifier/classifier.jsp) (Wang et al. 2007).

Reference sequences were obtained from the Ribosomal Database Project 10 website (http://rdp.cme.msu.edu/) using seqmatch (type and non-type strains, isolates, > 1200 bp, good quality) to construct the phylogenetic trees (Cole et al. 2009). Sequences were aligned using the NAST tool from Greengenes (DeSantis et al. 2006). Maximum likelihood phylogenetic trees were constructed with the online program PhyML 3.0 (http://www.atgc-montpellier.fr/phyml/) (Guindon et al. 2010) using the general time reversible model (GTR) (Tavaré, 1986).

Rarefaction, richness and diversity indices were calculated using mothur (Schloss et al. 2009). The input files were in the form of distance matrices generated by using phylip program dnadist (Felsenstein, 1989). The Good's coverage of our libraries was calculated (Good, 1953). Operational taxonomic units (OTUs) for community analysis were 20%, 10%, 5%, 3% distance cut-off (Rosselló-Mora and Amann, 2001).

The effect of the different treatments on the different genera was analyzed by principal component analysis (PCA) using the orthogonal/varimax rotation. Data were transformed using the Hellinger transformation (Ramette, 2007).

Differences among microbial communities were also characterized using UniFrac (Lozupone and Knight, 2005). A neighbour-joining tree was constructed with a maximum-likelihood approximation method using FastTree (Price et al. 2009) with a GTR substitution model (Tavaré, 1986). UniFrac and weighted UniFrac were used to determine the β-diversity of the different treatments at day 0, 1 and 14 (Lozupone et al. 2006). PCoA, abundance weighted and unweighted cluster analysis and lineage specific analyses were used to determine effects of treatment and time on the bacterial community structure. Lineage specific analysis to determine whether the sequences have a different distribution among environments was done with a branch length threshold 0.80 and minimum descendants of six. The abundance weighted Jackknife environment cluster analysis was done with 255 sequences and 1000 permutations.

### Nucleotide sequence accession numbers

The sequences were deposited in the GenBank database and assigned the accession numbers JQ425854-JQ428819.

## RESULTS

### Dynamics of anthracene in soil

The anthracene concentration in the sterilized soil was not significantly different over time (Figure 1). In the unsterilized soil, the anthracene concentration dropped sharply within 14 days, but changes were small thereafter. After 14 days, 64% of the anthracene was removed from soil and 65% after 56 days.

The $CO_2$ emission immediately increased when soil was amended with acetone or anthracene compared to the unamended soil (Figure 2a). After 56 days, the $CO_2$ emission increased 7.5 times when soil was amended with acetone compared to the unamended soil, but only 3.1 times when spiked with anthracene.

The $NH_4^+$ and $NO_2^-$ concentrations were not affected by the application of acetone or anthracene (Figure 2b, Figure 2c). The $NO_3^-$ concentration was significantly lower in the soil amended with acetone or anthracene than in the unamended soil (Figure 2d).

### Bacterial diversity and richness analyses

Large amounts of anthracene were removed from soil after 1 day and no significant changes occurred after 14 days. Consequently, DNA was extracted from soil 0, 1 and 14 days after the anthracene was added.

A marked decrease in the rates of OTUs from rarefaction curves was observed only at the 10% and 20% cut-off, but not at the level of genus (5% cut-off) or species (3% cut-off) (No data shown). This indicated that at the order and phylum level, the major bacterial groups were detected, but not at the level of genus or species.

The Chao1 richness of the three treatments showed different values (from 148 to 673 OTUs), but was generally highest in the acetone-amended soil (from 409 to 673 OTUs) (Table 1). The Shannon index (*H*') ranged from 3.23 to 4.47 in the unamended soil, from 3.32 to 4.51 in acetone-amended soil, and from 3.05 to 4.33 in anthracene-spiked soil. The Simpson index (*D*) in all the studied bacterial communities ranged from 0.02 to 0.16. The lower *D* values were founded at day 0 and the higher ones at day 1 (except in the acetone-amended soil).

### Bacterial community structure

Eleven different phyla were found in the different treatments (Table 2). Bacteria belonging to the Proteobacteria were the most abundant. Their abundance increased in the unamended and acetone-amended soils over time, but not in the anthracene-spiked soil (Table 2). The abundance of the Acidobacteria (contributing between 4.7% and 14.5%) decreased over time in all treatments, while the abundance of the Actinobacteria (contributing between 1.2% and 17.3%) decreased in the unamended and acetone-amended soils, but increased in the anthracene-spiked soil. Of the other phyla detected, only phylotypes belonging to Chloroflexi were found in all soil samples.

The class of the Gammaproteobacteria was the most abundant in all treatments at all sampling times and the Alphaproteobacteria the second most abundant (Table 2). The abundance of the Alphaproteobacteria was larger in the unamended and acetone-amended treatments at day 14 than at day 0, but the opposite was found in the anthracene-spiked soil. The Acidobacteria-Gp6 was the third most abundant class of bacteria, but its abundance decreased over time in all treatments.

Phylotypes belonging to the order of Xanthomonadales (Gammaproteobacteria) were the most abundant. They often represented > 50% of all phylotypes with a maximum of 58.3% in the anthracene-spiked soil at day 1 (Table 2). Sphingomonadales (Alphaproteobacteria) were the second most abundant order. They were more abundant in the unamended and acetone-amended treatments at day 14 than at day 0, but the opposite was found in the anthracene-spiked soil.

Phylotypes belonging to the genus *Lysobacter* (Xanthomonadales, Gammaproteobacteria) were the most abundant in each of the treatments at each of the sampling times (Table 3). They represented 31.26% of all the sequences analyzed (2898). The second most abundant group (12.46%) was the genus *Sphingomonas* (Sphingomonadales, Alphaproteobacteria). Only four more genera of the 87 found, represented ≥ 1% of all phylotypes (*Altererythrobacter* (1.69%), *Steroidobacter* (1.59%), *Gemmatimonas* (1.59%) and *Iamia* (1.00%)), while 38.13% of the phylotypes could not be assigned to a genus.

The abundance of the genus *Iamia* was higher in the acetone and anthracene-amended soil at day 0 and day 1 compared to the unamended soil, but was similar at day 14 (Table 3). The abundance of the genera *Nocardioides*, *Rhodococcus* and *Streptomyces*, known degraders of PAHs, was larger in the anthracene-spiked soil than in the acetone-amended and unamended treatments at day 14. The abundance of the genus *Sphingomonas* was lower in the anthracene-spiked soil than in the acetone-amended and unamended treatments at day 14.

**Principle component analysis**

At day 0, the different treatments showed a large difference in the PC1 value, but not in PC2 (Figure 3). PC1 was loaded mostly by the genera *Acidovorax*, *Bacillariophyta*, *Catellatospora*, *Cellulomonas*, *Corallococcus*, *Enterobacter*, *Ignavibacterium*, *Porticoccus*, *Pseudofulvimonas*, *Skermanella* and *Smaragdicoccus*. After 1 and 14 days, PC1 and PC2 were similar and negative in all treatments, except for the anthracene-amended soil after 14 days, which was characterized by a large positive PC2.

The PCoA based on the absence or presence of sequences (qualitative β-diversity) gave a different picture of the effect of treatment and time on the bacterial community structure (Figure 4). The different treatments at time zero were grouped together in the lower left quadrant and had similar negative values for PC1 and PC2. At day 1 and day 14, the different treatments were grouped together and were characterized by a positive PC1 and a small negative PC2. The acetone-spiked soil at day 1, however, was characterized by a negative PC1 and the anthracene-contaminated soil at day 14 by a large negative PC1 and large positive PC2. The PCoA based on the abundance of sequences (quantitative β-diversity) showed a similar effect of treatment and time on the bacterial community structure as the qualitative β-diversity (No data shown).

Lineage specific analysis with UniFrac indicated that sequences belonging to the genera *Nocardioides* and *Marmoricola* were highly significant ($P < 0.001$) and those belonging to *Arthrobacter* were significant (*P*-value between 0.001 and 0.01) with a branch length threshold of 0.8.

## DISCUSSION

**Removal of anthracene and emissions of $CO_2$**

The amount of anthracene extractable with the exhaustive extraction technique appeared not to be affected by abiotic processes, as the concentration of the contaminant did not change significantly over time in the anthracene-amended sterile soil. In this study approximately 65% of the anthracene was removed from the soil after 56 days. Lower amounts were reported by Fernández-Luqueño et al. (2008) as they found that 52% of the applied anthracene was removed from soil after 56 days. Differences in the amount of anthracene that are removed from soil depend on soil characteristics and the capacity of the soil microorganisms to degrade the pollutant (Silva et al. 2009).

It has been reported that acetone increases the emission of $CO_2$ from soil, while anthracene reduced it (Vázquez-Núñez et al. 2009). Acetone can liberate soil organic C or can serve itself as a C substrate for soil microorganisms thereby increasing emissions of $CO_2$. PAHs can be toxic for microorganisms thereby reducing emissions of $CO_2$, but not always (Silva et al. 2009).

**Bacterial diversity and richness analyses**

The highest values of *H*' (4.47, 4.51 and 4.33) and the lowest of *D* (0.02, 0.02 and 0.03) found at day 0 revealed that no immediate effect was detected in the bacterial community in terms of heterogeneity (*H*') and evenness (*D*). Negative effects on the bacterial diversity of the treatments were observed after day 1. Lower evenness in the soil bacterial communities could be due to the dominancy of *Lysobacter*, *Sphingomonas* and Acidobacteria Gp6, which comprises 40%, 16% and 11% of the clones, respectively. Apparently, contaminating the soil and incubation conditions stimulated the proliferation of these three genera. A recovery in heterogeneity and evenness was found at day 14 in the unamended and anthracene-spiked soils. The highest values for *H'* at a genetic distance of 3% found in the acetone-amended soil confirmed that acetone had little or no effect on bacterial diversity.

**Phylogenetic analysis of the bacterial population**

Phylotypes belonging to the Proteobacteria were the most dominant in the different treatments. Phylotypes belonging to the Proteobacteria are often dominant in soil (Roesch et al. 2007). For instance, Martin et al. (2012) found that phylotypes belonging to the Proteobacteria were dominant (35-66%) in soil amended with phenanthrene and Wang et al. (2011) in an uncontaminated soil amended with anthracene (96.4%).

Gammaproteobacteria were the most abundant class of the Proteobacteria in this study ranging from 27.4 to 61.7%. Zhang et al. (2011a) found similar results in municipal solid waste composting soil amended with anthracene after 15 days and Lors et al. (2010) reported that 73% of the sequences belonged to the Gammaproteobacteria in soil contaminated with PAHs. The order of the Xanthomonadales (20.4% to 54.9%) was the most dominant Gammaproteobacteria and included the most abundant genus, *i.e. Lysobacter* (9.77% to 43.8%). *Lysobacter* spp. are versatile. They have the capacity to degrade chlorophenols (2,4,6-trichlorophenol and pentachlorophenol) (Caliz et al. 2011) and hydrocarbons (Cervantes-González et al. 2008).

Numerous genera belonging to the Gammaproteobacteria found in this study are known to have the capacity to degrade PAHs and anthracene (*e.g. Enterobacter* (Bautista et al. 2009), *Pseudoxanthomonas* (Yutthammo et al. 2010), *Pseudomonas* (Bautista et al. 2009; Yutthammo et al. 2010; González et al. 2011; Jurelevicius et al. 2012) and *Stenotrophomonas* (González et al. 2011)), but the percentage of phylotypes belonging to these genera did not increase in the anthracene-amended soil.

Phylotypes belonging to the Alphaproteobacteria were the second most abundant class of bacteria in soil, with the Sphingomonadales (5.3 ± 4.4% to 20.5 ± 18.6%) and Rhizobiales (2.9% to 7.5%) the most important orders. Numerous genera belonging to the Alphaproteobacteria are capable to metabolize PAHs or are associated with their degradation, *e.g.* all the genera detected in this soil belonging to the Caulobacterales (*Brevundimonas* (Phillips et al. 2008) and *Caulobacter* (Chang et al. 2007) and Sphingomonadales). However, the percentage of phylotypes belonging to the genus *Caulobacter* were not affected by the application of anthracene and the abundance of phylotypes belonging to the genus *Sphingomonas* even decreased, although they are known degraders of anthracene (Jurelevicius et al. 2012). It appears that in this soil anthracene inhibited them. The phylotypes belonging to the Betaproteobacteria (order Burkholderiales), *i.e.* the least abundant class of Proteobacteria, were not affected in this study by the application of anthracene although all the genera detected are known to degrade PAHs (*Achromobacter* (Tiwari et al. 2010), *Acidovorax* (Jurelevicius et al. 2012), *Herbaspirillum* (Louvel et al. 2011), *Methylibium* (Zhang et al. 2012), *Polaromonas* (Jurelevicius et al. 2012) and *Variovorax* (Zhang et al. 2011a)).

Acidobacteria were the second most important phylum in this study. Wang et al. (2011) found the same in a soil spiked with anthracene.

Acidobacteria are characterized as versatile heterotrophs and among the most abundant bacteria in soil (Eichorst et al. 2011). However, the percentage of phylotypes belonging to the Acidobacteria decreased over time in all treatments. This might be due to changes in the soil organic matter as the easily decomposable fraction is mineralized. Sequences belonging to eight subgroups of the Acidobacteria were detected. Phylotypes belonging to Acidobacteria Gp6 were the most abundant while phylotypes belonging to the other groups were represented only sporadically. Only a few of the known Acidobacteria have been isolated until now, although they are so abundant in soil (George et al. 2011), and consequently little is known about their functionality.

Actinobacteria were the third most important phylum in this study. Their importance varies from study to study, independent of the contamination with PAHs. Wang et al. (2011) found the major phylum was Actinobacteria in an uncontaminated soil spiked with anthracene, but they were not detected after 45 days. In contaminated soil amended with phenanthrene, however, they found that the percentage of Actinobacteria was low and varied between 0 and 3%. However, Ros et al. (2010) found that the percentage of Actinobacteria (50%) was similar to that of Proteobacteria in uncontaminated soil, but six times higher (67%) in soils contaminated with PAHs. The percentage of phylotypes belonging to the *Euzebya* (Euzebyales, Actinobacteria) was higher in all treatments at day 0 than day 14.

Application of acetone increased the emission of $CO_2$ 7.5 times, but this increase in microbial activity had little effect on the soil bacterial community structure. However, one group of bacteria appeared to be affected by the application of acetone. The percentage of *Iamia* (Iamiaceae, Acidimicrobiales) increased when acetone was applied to soil (day 0 and 1), and although they have not been reported as acetone degraders, they appear to be favoured by acetone application to soil.

The percentage of phylotypes belonging to three genera of the Actinomycetales (Actinobacteria), *i.e. Nocardioides*, *Rhodococcus* and *Streptomyces*, known degraders of PAHs, increased in the anthracene-spiked soil at day 14 compared to day 0 and 1. These genera have been studied intensively and have been shown to metabolize PAHs or have genes that encode for enzymes involved in the degradation of PAHs (Cébron et al. 2008). The percentage of other known degraders of PAHs, such as *Microbacterium* (Cébron et al. 2011), however, did not increase in the anthracene-spiked soil at day 14.

Chloroflexi were found in all treatments. Ros et al. (2010) reported that phylotypes belonged to Chloroflexi made up 6.7% of bacterial population found in soil contaminated with PAHs. However, little direct evidence exist that they are capable of degrading PAHs. Gemmatimonadetes appeared in all libraries except one. They have been found in coal-tar-contaminated soil (Kumar and Khanna, 2010), but there is no evidence that they degrade PAHs.

Although in this study phylotypes belonging to the Bacteroidetes and Firmicutes made up < 1%, they are sometimes well presented in soil. Petrić et al. (2011) found that phylotypes belonging to the Bacteroidetes were one of the three most abundant phyla in soils contaminated with PCBs. Species belonging to the genus *Bacillus* (*e.g. Bacillus pumilus* and *Bacillus subtilis*) are well known metabolisers of PAHs (Toledo et al. 2006). Additionally, many species belonging to the genus *Bacillus* are acetone tolerant (*e.g. Bacillus aquimaris*, Trivedi et al. 2011), but they were not favoured by the application of acetone to the soil in this study.

Phylotypes belonging to the Chlorobi, Cyanobacteria/Chloroplast, Nitrospira and TM7 represented on average < 1%. These phyla are often considered rare phyla (Militon et al. 2010). Bacteroidetes, TM7 and Cyanobacteria/Chloroplast have been found in municipal solid waste composting soil amended with anthracene (Zhang et al. 2011b). However, none of the genera reported in this study belonging to the Chlorobi, Nitrospira and TM7 are known degraders of PAHs, but it has to be remembered that most of them have not yet been isolated and/or studied in detail.

The PCA using the number of phylotypes belonging to the different genera indicated an immediate effect of spiking the soil with acetone. However, the UniFrac, weighted UniFrac and clustering the environments (Lozupone et al. 2006), which is more robust as it deals with sequences directly and their relationship, did not confirm this result, as the different treatments at day 0 were grouped.

The UniFrac and weighted UniFrac also confirmed the fact that incubating the soil for even just one day had an effect on the bacterial population. This might have been due to the handling of the soil. Mixing the soil is known to liberate organic material that is then used by the soil microorganisms. The effect on the bacterial community structure of applying acetone appeared to be retarded as after 1 day the acetone-amended soil grouped with the different treatments at day 0 (UniFrac and weighted UniFrac), but after 14 days the unamended soil and the soil spiked with acetone were grouped together. The three analyses (PCA, unweighted PCoA and weighted PCoA (UniFrac)) and clustering the environments, confirmed the fact that contaminating the soil with anthracene had a profound effect on the bacterial community structure at day 14, *i.e.* anthracene increased the phylotypes belonging to the Actinomycetales.

## CONCLUDING REMARKS

It is clear from the results obtained that mixing and incubating the soil, and application of anthracene had an effect on the soil bacterial structure, but a possible effect of acetone was less clear. Incubating the soil nearly halved the percentage of sequences belonging to the Acidobacteria after 14 days. The changes in the sequences belonging to the Acidobacteria was mostly due to a decrease in the sequences belonging to the Acidobacteria-Gp6 class. Application of acetone had little effect on the bacterial community structure in this soil, but the percentage of sequences belonging to the genus *Iamia* (Acidomicrobiales) increased at the onset of the incubation. Application of anthracene increased the percentage of Actinobacteria compared to the unamended soil or soil applied with acetone, while it reduced the percentage of Proteobacteria after 14 days. Changes within the Actinobacteria were mostly due to an increase in the percentage of sequences belonging to the genera *Nocardioides*, *Rhodococcus* and *Streptomyces* (Actinomycetales), all known degraders of PAHs. Increases in the percentages of sequences belonging to the Proteobacteria in the unamended and acetone-amended soil were mostly due to increases in sequences belonging to the genus *Lysobacter* (Xanthomonadales, Gammaproteobacteria), while the decrease in the anthracene-spiked soil was mostly due to a decrease in phylotypes belonging to the Rhizobiales (Alphaproteobacteria) and the genus *Sphingomonas* (Sphingomonadales, Alphaproteobacteria).

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# Tables

**Table 1. Comparison of microbial richness and diversity of 16S rRNA gene libraries in the unamended soil, soil amended with acetone or spiked with anthracene as calculated with mothur (Schloss et al. 2009) after 0, 1 or 14 days**.

| Treatment | Time (days) | Number of sequences obtained | Number of different OTUs[a] | Richness estimator | | Diversity index | |
|---|---|---|---|---|---|---|---|
| | | | | Ace[b] | Chao1[c] | H'[d] | D[e] |
| Unamended soil | 0 | 255 | 123 | 165 (158, 174)[f] | 148 (135, 172) | 4.47 (4.34, 4.61) | 0.02 (0.01, 0.02) |
| | 1 | 321 | 89 | 156 (124, 219) | 154 (120, 225) | 3.23 (3.02, 3.44) | 0.14 (0.10, 0.18) |
| | 14 | 284 | 89 | 333 (255, 450) | 200 (144, 311) | 3.31 (3.10, 3.51) | 0.10 (0.08, 0.13) |
| Acetone amended | 0 | 275 | 156 | 1103 (875, 1403) | 673 (441, 1092) | 4.51 (4.35, 4.67) | 0.02 (0.01, 0.03) |
| | 1 | 337 | 157 | 409 (314, 563) | 411 (305, 595) | 4.24 (4.06, 4.42) | 0.05 (0.03, 0.07) |
| | 14 | 426 | 109 | 514 (415, 646) | 264 (189, 411) | 3.32 (3.15, 3.50) | 0.11 (0.08, 0.13) |
| Anthracene spiked | 0 | 301 | 146 | 365 (280, 502) | 356 (266, 514) | 4.33 (4.16, 4.50) | 0.03 (0.02, 0.05) |
| | 1 | 308 | 94 | 262 (183, 411) | 213 (155, 326) | 3.05 (2.83, 3.28) | 0.16 (0.12, 0.19) |
| | 14 | 391 | 146 | 715 (580, 892) | 365 (272, 528) | 3.86 (3.68, 4.04) | 0.07 (0.05, 0.09) |

[a] OTUs defined by using the furthest-neighbour algorithm in mothur at 97% similarity, [b] Ace, abundance based coverage estimator, [c] Chao1, bias corrected Chao1, [d] H': Shannon-Weaver diversity index, [e] D: Simpson diversity index, [f] Confidence intervals (95%) are given between parenthesis.

**Table 2. Percentage of identified clones belonging different phyla, classes and orders based on the ribosomal data project in soil of the former lake Texcoco (CONTROL) amended with acetone (ACETONE) or spiked with anthracene (ANTHRA) incubated aerobically for 14 days.**

| Phylum | CONTROL | | | ACETONE | | | ANTHRACENE | | |
|---|---|---|---|---|---|---|---|---|---|
| Class | | | | | | | | | |
| Order | Day 0 | Day 1 | Day 14 | Day 0 | Day 1 | Day 14 | Day 0 | Day 1 | Day 14 |
| Acidobacteria | 13.8 (6.0)[a] | 9.7 (6.8) | 5.2 (0.2) | 12.4 (0.5) | 13.9 (1.5) | 4.5 (3.3) | 15.0 (5.5) | 7.1 (8.0) | 6.1 (0.4) |
| Acidobacteria-Gp1 | 0.3 (0.5) | 0 | 0 | 0.4 (0.6) | 0.3 (0.4) | 0 | 2.3 (3.3) | 0 | 1.0 (0.1) |
| Acidobacteria-Gp3 | 0 | 0 | 0 | 0 | 0 | 0 | 0.2 (0.4) | 0 | 0.3 (0.4) |
| Acidobacteria-Gp4 | 1.9 (0.2) | 0.6 (0.8) | 0 | 0 | 0.6 (0.9) | 0 | 0 | 0.6 (0.8) | 0 |
| Acidobacteria-Gp5 | 0 | 0.4 (8.8) | 0 | 0.4 (0.6) | 0 | 0 | 0.2 (0.4) | 0 | 0 |
| Acidobacteria-Gp6 | 10.2 (4.7) | 8.8 (5.6) | 4.9 (0.2) | 10.5 (0.5) | 12.6 (0.5) | 3.6 (1.9) | 12.2 (8.1) | 6.3 (6.8) | 4.3 (0.1) |
| Acidobacteria-Gp10 | 0 | 0 | 0 | 0.4 (0.6) | 0 | 0 | 0 | 0 | 0 |
| Acidobacteria-Gp17 | 1.4 (1.9) | 0 | 0 | 0.6 (0.9) | 0.3 (0.5) | 0.7 (1.0) | 0 | 0.3 (0.4) | 0.5 (0.7) |
| Acidobacteria-Gp21 | 0 | 0 | 0.3 (0.5) | 0 | 0 | 0 | 0 | 0 | 0 |
| Actinobacteria | 10.0 (5.2) | 1.2 (0.7) | 2.5 (0.6) | 8.9 (1.8) | 6.0 (3.4) | 2.3 (0.5) | 5.5 (6.8) | 8.0 (2.8) | 17.1 (19.3) |
| Actinobacteria | 10.0 (5.2) | 1.2 (0.7) | 2.5 (0.6) | 8.9 (1.8) | 6.0 (3.4) | 2.3 (0.5) | 5.5 (6.8) | 8.0 (2.8) | 17.1 (19.3) |
| Acidimicrobiales | 0.8 (0.2) | 0.9 (0.3) | 0.7 (1.0) | 3.7 (4.1) | 2.5 (0.8) | 1.4 (0.1) | 3.0 (2.9) | 2.8 (3.2) | 2.0 (0.1) |
| Actinomycetales | 5.1 (7.3) | 0.3 (0.4) | 1.1 (1.5) | 3.1 (2.7) | 2.3 (3.2) | 0.2 (0.3) | 1.2 (1.0) | 0 | 13.5 (19.1) |
| Euzebyales | 3.3 (1.7) | 0 | 0.3 (0.5) | 1.6 (1.1) | 1.0 (1.4) | 0.7 (0.3) | 2.4 (0.6) | 0 | 0.5 (0.7) |
| Nitriliruptorales | 0.3 (0.5) | 0 | 0 | 0.4 (0.6) | 0 | 0 | 0.2 (0.4) | 0 | 0 |
| Solirubrobacterales | 0 | 0 | 0.4 (0.5) | 0 | 0.3 (0.4) | 0 | 0 | 0 | 0.7 (1.0) |
| Bacteroidetes | 0.5 (0.6) | 0 | 0 | 0.8 (1.2) | 1.9 (1.9) | 0 | 0 | 0.3 (0.4) | 0.5 (0.7) |
| Flavobacteria | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.3 (0.4) | 0 |
| Flavobacteriales | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.3 (0.4) | 0 |
| Sphingobacteria | 0.5 (0.6) | 0 | 0 | 0.8 (1.2) | 1.9 (1.9) | 0 | 0 | 0 | 0.5 (0.7) |
| Sphingobacteriales | 0.5 (0.6) | 0 | 0 | 0.8 (1.2) | 1.9 (1.9) | 0 | 0 | 0 | 0.5 (0.7) |

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Chlorobi | 0 | 0 | 0.4 (0.5) | 1.2 (1.7) | 0 | 0 | 0 | 0 | 0.4 (0.5) |
| Ignavibacteria | 0 | 0 | 0.4 (0.5) | 1.2 (1.7) | 0 | 0 | 0 | 0 | 0.4 (0.5) |
| Ignavibacteriales | 0 | 0 | 0.4 (0.5) | 1.2 (1.7) | 0 | 0 | 0 | 0 | 0.4 (0.5) |
| Chloroflexi | 5.0 (4.5) | 0.3 (0.4) | 1.0 (0.4) | 3.2 (0.2) | 4.1 (4.3) | 1.4 (0.5) | 1.7 (1.1) | 1.6 (0.2) | 2.8 (0.5) |
| Anaerolineae | 2.2 (1.8) | 0.3 (0.4) | 1.0 (0.4) | 2.8 (0.3) | 2.5 (2.9) | 0.7 (0.3) | 0.2 (0.4) | 1.3 (0.2) | 1.5 (0.1) |
| Anaerolineales | 2.2 (1.8) | 0.3 (0.4) | 1.0 (0.4) | 2.8 (0.3) | 2.5 (2.9) | 0.7 (0.3) | 0.2 (0.4) | 1.3 (0.2) | 1.5 (0.1) |
| Caldilineae | 1.0 (1.5) | 0 | 0 | 0 | 0.3 (0.4) | 0.2 (0.3) | 1.0 (1.4) | 0.3 (0.4) | 0.5 (0.7) |
| Caldilineales | 1.0 (1.5) | 0 | 0 | 0 | 0.3 (0.4) | 0.2 (0.3) | 1.0 (1.4) | 0.3 (0.4) | 0.5 (0.7) |
| Unclassified | 1.8 (1.3) | 0 | 0 | 0.4 (0.6) | 1.3 (1.8) | 0.5 (0.1) | 0.5 (0.7) | 0 | 0.8 (0.3) |
| Cyanobacteria/ Chloroplast | 0 | 0 | 0 | 0.4 (0.6) | 0 | 0 | 0 | 0 | 0 |
| Chloroplast | 0 | 0 | 0 | 0.4 (0.6) | 0 | 0 | 0 | 0 | 0 |
| Firmicutes | 0 | 0 | 0 | 0 | 0.6 (0.9) | 0 | 0 | 0 | 0 |
| Bacilli | 0 | 0 | 0 | 0 | 0.3 (0.5) | 0 | 0 | 0 | 0 |
| Bacillales | 0 | 0 | 0 | 0 | 0.3 (0.5) | 0 | 0 | 0 | 0 |
| Clostridia | 0 | 0 | 0 | 0 | 0.3 (0.5) | 0 | 0 | 0 | 0 |
| Gemmatimonadetes | 4.2 (0.8) | 0.6 (0.8) | 0 | 1.7 (0.1) | 2.1 (1.6) | 0.2 (0.3) | 1.4 (0.6) | 1.1 (1.5) | 1.3 (0.4) |
| Gemmatimonadetes | 4.2 (0.8) | 0.6 (0.8) | 0 | 1.7 (0.1) | 2.1 (1.6) | 0.2 (0.3) | 1.4 (0.6) | 1.1 (1.5) | 1.3 (0.4) |
| Gemmatimonadales | 4.2 (0.8) | 0.6 (0.8) | 0 | 1.7 (0.1) | 2.1 (1.6) | 0.2 (0.3) | 1.4 (0.6) | 1.1 (1.5) | 1.3 (0.4) |
| Nitrospira | 0.9 (1.3) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nitrospira | 0.9 (1.3) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nitrospirales | 0.9 (1.3) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria | 60.3 (3.9) | 86.5 (9.2) | 89.2 (0.9) | 66.5 (4.6) | 66.2 (13.7) | 91.1 (2.7) | 71.3 (7.9) | 84.1(1.9) | 66.5 (25.3) |
| Alphaproteobacteria | 25.4 (4.9) | 25.0 (0.0) | 29.2 (5.5) | 28.4 (0.2) | 21.9 (2.0) | 33.7 (6.5) | 28.2 (3.3) | 20.4 (0.2) | 14.8 (1.6) |
| Caulobacterales | 0.3 (0.5) | 1.0 (0.6) | 1.0 (1.4) | 1.0 (0.3) | 0.8 (0.3) | 0.5 (0.7) | 1.0 (0.1) | 1.3 (0.2) | 0.5 (0.7) |
| Rhizobiales | 5.7 (7.2) | 5.4 (1.8) | 4.2 (1.8) | 5.6 (2.8) | 3.7 (0.6) | 7.5 (2.6) | 7.0 (2.1) | 4.0 (2.5) | 2.9 (2.7) |
| Rhodobacterales | 0.7 (1.0) | 0.6 (0.1) | 0.4 (0.5) | 1.4 (0.3) | 0.6 (0.9) | 0.9 (0.6) | 1.6 (1.6) | 0.4 (0.5) | 0.3 (0.4) |
| Rhodospirillales | 1.8 (2.5) | 0.3 (0.4) | 0.4 (0.5) | 0.7 (0.2) | 0.3 (0.5) | 0 | 0.5 (0.7) | 0 | 0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Sphingomonadales | 14.0 (4.5) | 17.4 (1.0) | 23.0 (3.8) | 18.9 (3.0) | 14.6 (0.4) | 24.1 (9.0) | 15.8 (2.6) | 14.2 (2.7) | 10.3 (3.4) |
| Unclassified | 2.8 (1.1) | 0.3 (0.5) | 0.4 (0.5) | 0.7 (0.2) | 1.8 (1.1) | 0.7 (0.3) | 2.4 (0.6) | 0.6 (0.8) | 0.8 (1.1) |
| Betaproteobacteria | 1.7 (2.4) | 0 | 0.4 (0.5) | 1.6 (1.4) | 2.5 (2.9) | 0 | 0.2 (0.4) | 0.6 (0.1) | 2.6 (3.0) |
| Burkholderiales | 1.7 (2.4) | 0 | 0.4 (0.5) | 0.7 (0.2) | 1.3 (1.8) | 0 | 0 | 0 | 0.5 (0.7) |
| Unclassified | 0 | 0 | 0 | 0.8 (1.2) | 1.2 (1.0) | 0 | 0.2 (0.4) | 0.6 (0.1) | 2.1 (2.3) |
| Deltaproteobacteria | 5.8 (0.5) | 3.0 (2.7) | 3.4 (2.8) | 2.6 (1.1) | 2.9 (0.5) | 0.5 (0.7) | 3.2 (1.9) | 1.4 (2.0) | 2.6 (1.6) |
| Myxococcales | 4.0 (0.8) | 1.3 (1.1) | 0.7 (1.0) | 2.3 (1.5) | 2.3 (0.4) | 0 | 2.2 (0.4) | 0.6 (0.8) | 2.1 (1.6) |
| Syntrophobacterales | 0 | 0 | 0 | 0.3 (0.5) | 0 | 0 | 0 | 0 | 0 |
| Unclassified | 1.8 (1.3) | 1.7 (1.6) | 2.7 (3.8) | 0.3 (0.4) | 0.3 (0.4) | 0.5 (0.7) | 1.0 (1.4) | 0.8 (1.2) | 0.5 (0.1) |
| Gammaproteobacteria | 27.4 (2.0) | 58.3 (7.0) | 55.7 (8.2) | 33.9 (2.0) | 38.8 (19.1) | 56.7 (2.7) | 39.6 (6.8) | 61.7 (4.0) | 46.3 (31.1) |
| Alteromonadales | 0 | 0.6 (0.8) | 0 | 0.4 (0.6) | 0 | 0 | 0 | 1.1 (1.5) | 0.2 (0.3) |
| Chromatiales | 0 | 0 | 0.7 (0.1) | 0 | 0.3 (0.4) | 0.2 (0.3) | 0 | 0 | 0 |
| Enterobacteriales | 0 | 0 | 0 | 1.7 (2.4) | 0 | 0 | 0 | 0 | 0 |
| Gammaproteobacteria-incertae-sedis | 0.5 (0.6) | 0 | 0 | 1.6 (1.4) | 1.2 (1.0) | 0 | 0 | 0 | 0 |
| Legionellales | 0 | 0 | 0.4 (0.5) | 0.3 (0.4) | 0.3 (0.4) | 0 | 0 | 1.1 (1.5) | 0 |
| Oceanospirillales | 0 | 0.3 (0.5) | 0 | 1.3 (1.8) | 1.0 (1.4) | 0.2 (0.3) | 1.2 (1.8) | 0 | 0 |
| Pseudomonadales | 3.2 (0.6) | 1.7 (1.6) | 0.7 (1.0) | 2.1 (3.0) | 0.9 (0.6) | 1.0 (0.7) | 0.2 (0.4) | 0 | 0.8 (0.4) |
| Xanthomonadales | 20.4 (2.1) | 54.9 (6.9) | 52.9 (6.1) | 21.8 (8.3) | 31.8 (23.9) | 54.8 (1.2) | 34.5 (8.8) | 58.4 (2.5) | 42.5 (30.9) |
| Unclassified | 3.4 (2.9) | 0.8 (1.2) | 1.1 (0.5) | 4.7 (1.6) | 3.4 (2.6) | 0.5 (0.1) | 3.6 (0.2) | 1.1 (1.6) | 2.8 (0.2) |
| Unclassified | 0 | 0.3 (0.4) | 0.4 (0.5) | 0 | 0 | 0.2 (0.3) | 0 | 0 | 0.2 (0.3) |
| TM7 | 0 | 0 | 0 | 0 | 0.3 (0.5) | 0 | 0 | 0 | 0 |
| Unclassified | 5.3 (1.2) | 1.8 (0.6) | 1.7 (1.4) | 4.9 (3.3) | 4.8 (0.4) | 0.5 (0.1) | 3.8 (0.2) | 3.0 (1.9) | 5.2 (3.9) |
| Unclassified | 7.2 (2.5) | 2.1 (1.0) | 2.1 (0.9) | 5.3 (2.7) | 6.5 (1.9) | 1.2 (0.5) | 4.3 (0.5) | 3.0 (1.9) | 6.4 (3.6) |
| Unclassified | 29.4 (6.8) | 14.6 (6.9) | 11.4 (3.9) | 24.7 (0.8) | 27.3 (8.2) | 7.3 (4.2) | 26.5 (6.1) | 13.3 (9.5) | 19.0 (6.8) |

[a] Values between parenthesis are standard deviation of the mean ($n$ = 2).

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**Table 3. Abundance of clones belonging different genera based on the ribosomal data project that represent ≥ 0.1% of the total number of clones in the unamended soil of the former lake Texcoco (CONTROL) or in soil amended with acetone (ACETONE) or spiked with anthracene (ANTHRA) incubated aerobically for 14 days.**

| Genus | CONTROL | | | ACETONE | | | ANTHRACENE | | | Saline [a] | Acetone [b] | PAHs [c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 0 | Day 1 | Day 14 | Day 0 | Day 1 | Day 14 | Day 0 | Day 1 | Day 14 | | | |
| *Lysobacter* | | | | | | | | | | * | | * |
| *Sphingomonas* | | | | | | | | | | * | | * |
| *Altererythrobacter* | | | | | | | | | | * | | * |
| *Steroidobacter* | | | | | | | | | | | | |
| *Gemmatimonas* | | | | | | | | | | * | | (*) |
| *Iamia* | | | | | | | | | | * | | |
| *Euzebya* | | | | | | | | | | * | | |
| *Arthrobacter* | | | | | | | | | | * | * | * |
| *Pseudomonas* | | | | | | | | | | * | * | * |
| *Nocardioides* | | | | | | | | | | * | | * |
| *Rhodococcus* | | | | | | | | | | * | * | * |
| *Pseudoxanthomonas* | | | | | | | | | | * | | * |
| *Brevundimonas* | | | | | | | | | | * | | * |
| *Luteimonas* | | | | | | | | | | * | | |
| *Hyphomicrobium* | | | | | | | | | | * | | (*) |
| *Halomonas* | | | | | | | | | | * | | * |
| *Caldilinea* | | | | | | | | | | | | |
| *Mesorhizobium* | | | | | | | | | | * | | * |
| *Streptomyces* | | | | | | | | | | | * | * |
| *Arenimonas* | | | | | | | | | | | | (*) |
| *Cellvibrio* | | | | | | | | | | | | |
| *Novosphingobium* | | | | | | | | | | * | | * |
| *Azotobacter* | | | | | | | | | | * | | * |
| *Pedomicrobium* | | | | | | | | | | | | |
| *Bauldia* | | | | | | | | | | | | |
| *Aciditerrimonas* | | | | | | | | | | | | |
| *Aquicella* | | | | | | | | | | | | |
| *Conexibacter* | | | | | | | | | | | | |
| *Devosia* | | | | | | | | | | * | | |
| *Gracilimonas* | | | | | | | | | | * | | |
| *Ignavibacterium* | | | | | | | | | | | | |
| *Rhodobacter* | | | | | | | | | | * | * | * |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Agromyces* | | | | | | | | | | * | | (*) |
| *Haliangium* | | | | | | | | | | * | | |
| *Rhodoplanes* | | | | | | | | | | | | |
| *Stenotrophomonas* | | | | | | | | | | * | | * |
| *Aeromicrobium* | | | | | | | | | | * | | * |
| *Caulobacter* | | | | | | | | | | | | * |
| *Methylohalomonas* | | | | | | | | | | * | | |
| *Nitriliruptor* | | | | | | | | | | * | | |
| *Paracoccus* | | | | | | | | | | * | * | * |
| *Pseudofulvimonas* | | | | | | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | >20% | | >10% | | >5% | | >1% | | >0.5% | | <0.5% |

[a] * Bacteria encountered in saline environments.
[b] * Evidence exist that the bacteria belonging to this genus are capable of degrading acetone.

[c] * Evidence exist that the bacteria belonging to this genus are capable of degrading PAHs, (*) Bacteria isolated from hydrocarbon contaminated environment, but no direct evidence that they can degrade PAHs.

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# Figures

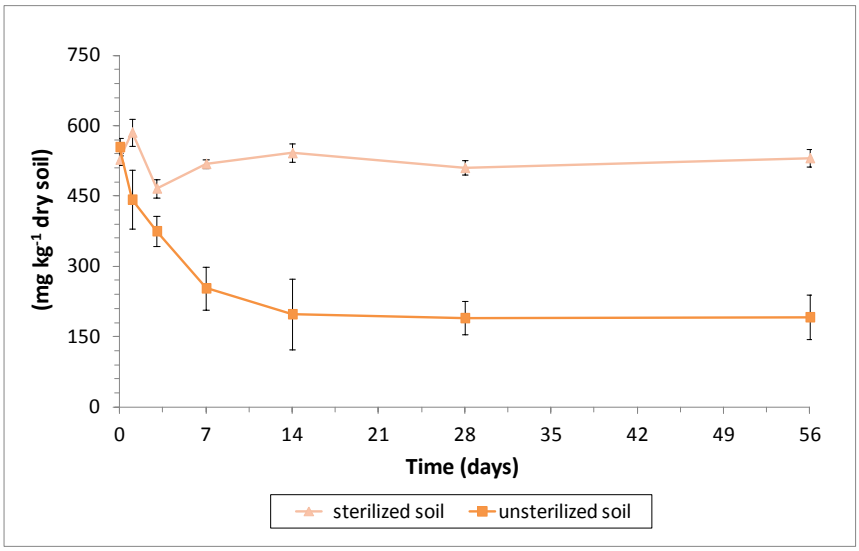


**Fig. 1 Concentration of anthracene (mg $kg^{-1}$ dry soil) in unsterilized or sterilized soil incubated aerobically at 22 ± 2ºC for 56 days.** Bars are ± one standard deviation.

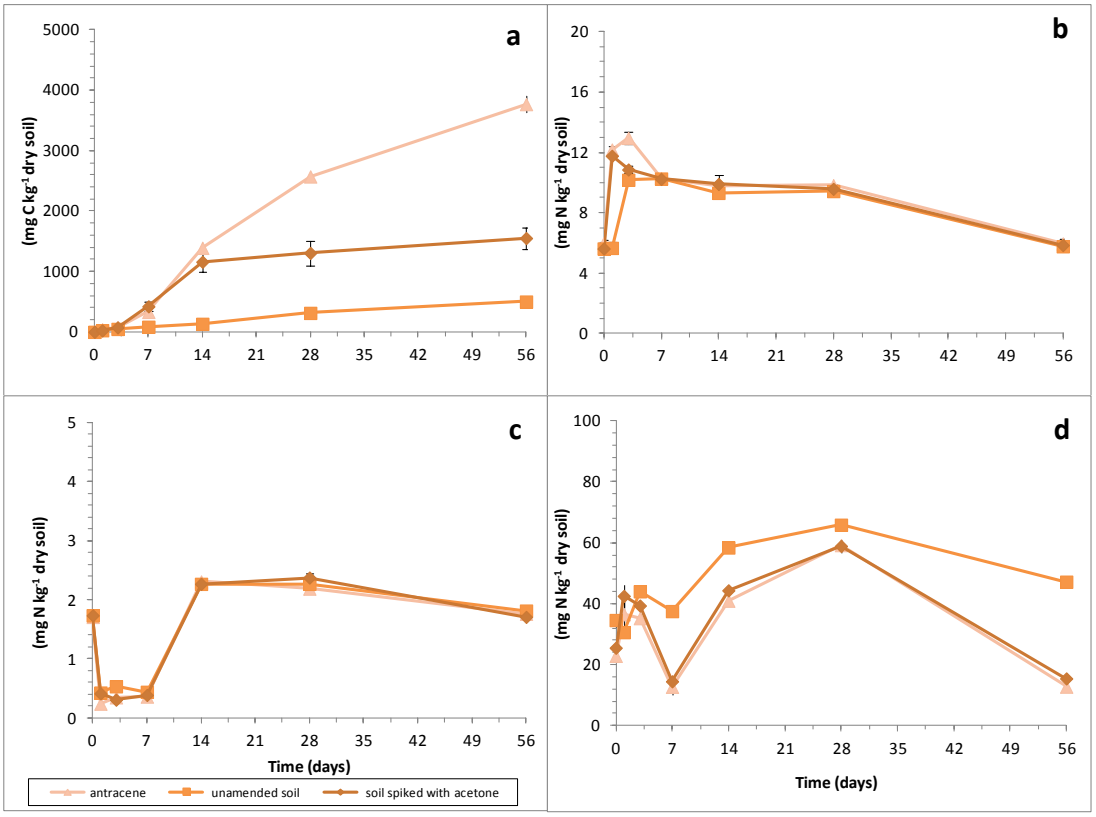


**Fig. 2 (a) Emission of $CO_2$ (mg C $kg^{-1}$ dry soil) and concentration of (b) $NH_4^+$, (c) $NO_2^-$ and (d) $NO_3^-$ (mg N $kg^{-1}$ dry soil) in unamended soil or soil spiked with acetone or anthracene incubated aerobically at 22 ± 2ºC for 56 days.** Bars are ± one standard deviation.

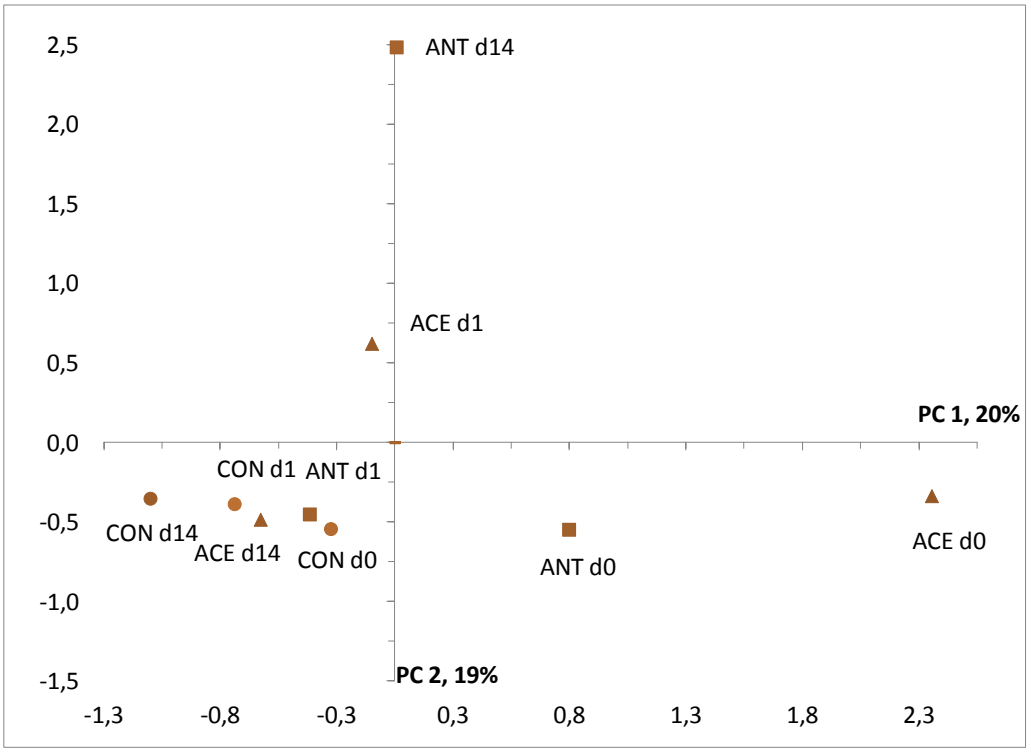


**Fig. 3 Principal component analysis with the different genera found in unamended soil (CON), in the acetone-amended soil (ACE) and spiked with anthracene (ANT) at day 0 (d0), day 1 (d1) and after 14 days (d14) of the aerobic incubation.** Data were transformed using the Hellinger transformation before analysis (Ramette, 2007).

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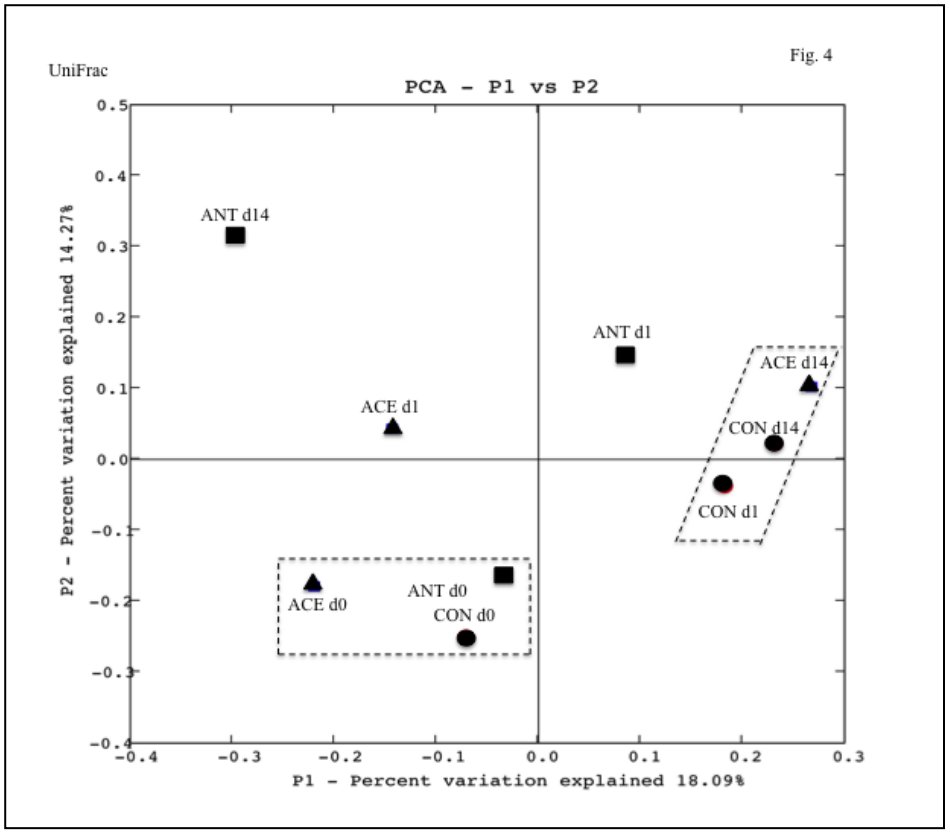


**Fig. 4 Principal coordinate analysis using UniFrac with the different sequences found in unamended soil (CON), in the acetone-amended soil (ACE) and soil spiked with anthracene (ANT) at day 0 (d0), day 1 (d1) and after 14 days (d14) of the aerobic incubation.**