Biological functioning of PAH-polluted and thermal desorption-treated soils assessed by fauna and microbial bioindicators

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Abstract

A large number of soil bioindicators were used to assess biological diversity and activity in soil polluted with polycyclic aromatic hydrocarbons (PAHs) and the same soil after thermal desorption (TD) treatment. Abundance and biodiversity of bacteria, fungi, protozoa, nematodes and microarthropods, as well as functional parameters such as enzymatic activities and soil respiration, were assessed during a two year period of in situ monitoring. We investigated the influence of vegetation (spontaneous vegetation and Medicago sativa) and TD treatment on biological functioning. Multivariate analysis was performed to analyze the whole data set. A principal response curve (PRC) technique was used to evaluate the different treatments (various vegetation and contaminated vs. TD soil) contrasted with control (bare) soil over time. Our results indicated the value of using a number of complementary bioindicators, describing both diversity and functions, to assess the influence of vegetation on soil and discriminate polluted from thermal desorption (TD)-treated soil. Plants had an influence on the abundance and activity of all organisms examined in our study, favoring the whole trophic chain development. However, although TD-treated soil had a high abundance and diversity of microorganisms and fauna, enzymatic activities were weak because of the strong physical and chemical modifications of this soil.

Keywords: Microorganisms; Enzyme activities; Fauna; Soil; Plant; PAH

1. Introduction

Wasteland soils resulting from 20th century intensive industrialization (i.e., coal tar or coking production or wood preservative plants) are now in great need of remediation because of high levels of multi-pollution. Polycyclic aromatic hydrocarbons (PAHs) and heavy metals (HMs) have been demonstrated to be highly toxic for life and can alter biodiversity (Cerniglia, 1992; Cortet et al., 1999). The organisms on the first level of the trophic chain are often the most vulnerable to these contaminants (Dawson et al., 2007). Therefore, if bacterial, fungal, micro- and meso-faunal community structures and functions are affected, the proper functioning of the entire soil could be disturbed.

To date, different approaches have been developed to remediate these soils. In situ degradation is often a slow process due to environmental constraints and low availability of PAHs in aged polluted soils (Wilson and Jones, 1993). Consequently, industrial thermal desorption (TD) treatments have been used to remove PAH compounds, but this type of treatment does not affect HM content. After such drastic treatment, soil characteristics are modified and most of the biological diversity and functions are likely to be altered (Cébron et al., 2009; Ouvrard et al. in press). It thus appears...
essential to understand whether these soil populations can once again recover and colonize following TD treatment (Cébron et al., 2009). Other complementary remediation methods can be achieved through plant use via phytoremediation or natural attenuation. It has been shown that plants that can grow on contaminated soils could increase microbial density and activity in the rhizosphere (Smalla et al., 2001) and interactions among arbuscular mycorrhizal fungi and plant roots are known to promote PAH dissipation (Joner and Leyval, 2003). Furthermore, the plant root system has been recognized as a hotspot of biodiversity involving many interactions between plants, microorganisms and fauna (Bonkowski et al., 2009).

Thus far, the notion of soil quality has been mainly confined to agricultural soils and refers to the impact of perturbation on soil functioning. Whereas soil “quality” assesses soil functionality, soil “health” is more concerned with conditions required for promoting plant, animal and human health and sustainability (Doran et al., 2002; Gil-Sotres et al. 2005). However, polluted and remediated soils may never resemble native soils in terms of quality and health criteria because of the diverse nature, origin and environmental impact of contaminants and treatments. Therefore, it is crucial to assess the ecology and functioning of these soils under various environmental conditions.

In soils, carbon and nutrient cycles are driven by bacteria (i.e. carbon mineralization, nitrification; Nannipieri et al., 2003), archaea (i.e. ammonia oxidation, C1-compound production; Timonen and Bomberg, 2009) and fungi (i.e. lignin and cellulose degradation; Rabinovich et al., 2004). The literature describes a balance between bacterial and fungal biomass in soil, depending on the content and status of carbon and nutrients. Indeed, Moore and Hunt (1988) reported that soil with recalcitrant C substrate is dominated by fungi, while soil with labile C is dominated by bacteria. This is the base of the trophic chain, and upper biological compartments (micro- and meso-fauna) are thus directly influenced by soil characteristics and the microbial community biomass and diversity (Neher, 1999). To assess the biological soil quality and impact of remediation treatments, various bioindicators referring to these first trophic levels, and recently reviewed (Bispo et al., 2010), have been proposed based either on measured diversity or functional parameters. Microorganisms (mainly bacteria and fungi) are reported to be efficient bioindicators, as they are ubiquitous and respond rapidly to physical and chemical changes in the soil (Kennedy and Smith, 1995). The abundance and diversity of bacteria and fungi can be rapidly assessed using molecular biological tools, including real-time quantitative PCR and fingerprinting techniques (Cébron et al., 2008, 2009). Global microbial activity and specific functions in C, N, P and S cycles can be determined by measurements of respiration and enzyme activity (Floch et al., 2009). The abundance and diversity of micro- and meso-fauna, including nematodes, protozoa and microarthropods, can be measured through direct observation and identified with morphological criteria (Griffiths et al., 2005; Pernin et al., 2006b). However, for any bioindicator, species abundance, diversity and functions acquired during the time series are multivariate and thus difficult to interpret. Because the time vector is often not a straight line in multivariate diagrams (i.e., PCA, MDS), Van Den Brink and Ter Braak (1998) developed the principal response curve (PRC) method. PRC is multivariate analysis that evaluates time series data resulting from experiments in which several treatments are contrasted with a control. This statistical analysis has been employed with success for biomonitoring of water quality (Van den Brink et al., 2009). In this study, we applied PRC as a tool to visualize and compare soil treatments during time and to evaluate the pertinence of each bioindicator in assessing soil biological functioning.

A field study was set up in 2005 to study in situ the natural (spontaneous vegetation) and plant-assisted (Medicago sativa) attenuation of a multi-contaminated soil and the same soil after TD treatment (Cébron et al., 2009; Ouvrard et al., in press). Two years after set-up and plant colonization, various biological parameters (fauna and microbial indicators) were monitored for a further period of two years (2008–2009). In the present study, we evaluated the influence of remediation treatment (vegetation and thermal desorption) on biological diversity and activity in contaminated soil using a large range of soil bioindicators, including biodiversity of organisms (bacteria, fungi, protozoa, nematodes, microarthropods) and functional parameters (enzymatic activities and soil respiration). The large range of bioindicators aimed to evaluate the impact of soil modification on the trophic chain and possible redundancy between bioindicators.

2. Materials and methods

2.1. Experimental methods

2.1.1. Plot device

The in situ plot devices, consisting of 24 stainless steel tanks (2 × 3 × 0.4 m, length × width × height) previously described in Cébron et al. (2009) and Ouvrard et al. (in press) and located at Homécourt (Meurthe et Moselle, France), were established in September 2005. Twenty plots were filled with contaminated soil (NM) from a former coking plant site (Neuves-Maisons, Meurthe et Moselle, France). Five different treatments with four replicates were tested, including bare soil (BS) prevented from growth of vegetation by hand, bare soil that had been filled in March 2008 (BST, previously called NC in Ouvrard et al., in press), soil sown with M. sativa L. cv. Europe (alfalfa) (Ms), soil sown with alfalfa and inoculated with two mycorrhizal fungal strains (Glomus intraradices and Glomus mosseae, Msm) and soil allowed to be colonized by spontaneous vegetation (SV). Four other plots were filled with the same soil treated by TD. TD consisted of heating the excavated soil to 500 °C, where mainly PAHs were transferred to gas phase. These plots were sown with alfalfa and inoculated with both mycorrhizal fungal strains as described above (Msm-TD). Contrary to spontaneous vegetation that was not disturbed, M. sativa plant biomass was harvested each year in September after the soil sampling.

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2.1.2. Soil characteristics

Soil characteristics, previously described in detail by Ouvrard et al. (in press), are shown in Table 1 without distinction between plot treatments, since no significant differences were observed due to vegetation. The NM and TD soils had approximately the same texture: 12.1% and 10.4% clay, 22.1% and 19.5% silt and 65.8% and 70.1% sand, respectively. The main differences observed were: drastically reduced PAH levels in TD compared to NM soil and higher pH levels and C/N ratios (i.e. lower nitrogen content) in TD compared to NM soil (Table 1).

2.1.3. Soil sampling

Soil samples were collected two and a half years after stabilization of the plot devices. Sampling was performed twice a year in April 2008, September 2008, April 2009 and September 2009.

For bacteria, fungi and enzyme assessment, six subsamples per plot were collected with a hand auger and mixed into one mean sample per plot. Soil samples were directly sieved to <5 mm; a portion was stored at -20 °C until DNA extraction and fresh soil was kept at 4 °C for measurement of microbial activity.

For nematodes and protozoa, two separate composite soil samples were collected from the top 15 cm of each plot by randomly collecting six cores with a gouge auger (Eijkelkamp, The Netherlands). The soil was sieved through a 4 mm diameter mesh and stored in sterile 50 mL plastic tubes at 4 °C for transport to the laboratory.

For microarthropods (Collembola and Acari), three intact soil cores (5 cm depth, 5 cm diameter) were taken at each sampling date from the center of each plot to avoid a border effect.

2.2. Abundance and diversity of bacteria and fungi

2.2.1. DNA extraction

Total nucleic acids were extracted from 0.5 g of soil using a bead-beating procedure (Cébron et al., 2008) modified by addition of 200 mg of ion exchanged Dowex resin (Sigma—Aldrich) and equilibrated with a 5 M LiCl solution to exchange Li+ for Ca2+ ions from the soil solution and to prevent co-precipitation of insoluble CaSO4 with DNA.

2.2.2. Abundance of total bacteria, fungi and PAH degraders

Real-time PCR was performed according to the procedure described in Cébron et al. (2008). Fungi, bacteria and two groups of PAH degraders (Gram-positive and Gram-negative) were quantified by targeting 18S rRNA, 16S rRNA and PAH dioxygenase genes using the primer sets FF390R/Fung5F (Luenders et al., 2004), 968F/1401R (Felske et al., 1998) and PAH-RHDX GP and GN (Cébron et al., 2008), respectively. Quantification was performed in triplicate using an iCycler iQ apparatus combined with an iCycler Optical System (Bio-Rad) and a standard plasmid dilution series from 104 to 101 gene copies µL−1. Amplification reactions were carried out in a 20 µL volume using the iQ SYBR green SuperMix (Bio-Rad) as described previously in Cébron et al. (2008).

2.2.3. Community structure and diversity indices

Fungal 18S rDNA and bacterial 16S rDNA fragments were amplified using the same universal primer set described above but with addition of GC clamps on the reverse and forward primer, respectively. PCR was performed using an iCycler (Bio-Rad) in a 50 µL volume with Taq DNA polymerase (Invitrogen). The temperature profile was chosen according to Cébron et al. (2009) for 16S rDNA amplification and a traditional three-step program with 35 hybridization cycles at 50 °C was used for 18S rDNA amplification. PCR products (500 ng) from all samples were loaded onto a temporal thermal gradient gel electrophoresis (TTGE) DCode system (Bio-Rad). TTGE was performed as previously described in Cébron et al. (2009) for 16S rDNA and with slight modification for 18S rDNA (5.5 M urea, running at 145 V with temperature increasing from 51.5 to 60.5 °C at a 1.5 h−1 rate of increase). After migration, gels were stained with SYBR Gold (1/10,000 final, Molecular Probes) and analyzed on a GelDoc transilluminator (bio-rad). QuantityOne 4.0.1 software (Bio-Rad) was used for image and band pattern analysis. Shannon diversity indexes H' = −Σ pi ln pi, where pi is the proportion of the ith band based on its relative intensity against all the bands found in one sample lane, were calculated for all samples.

2.3. Abundance and diversity of micro- and mesofauna

2.3.1. Nematodes and protozoa

Nematodes were extracted from fresh soil using the Whitehead and Hemming tray technique (Whitehead and Hemming, 1965). Soil (ca. 20 g) was placed in a tissue-lined mesh-based (90 µm) plastic sieve and then into a Baermann funnel filled with enough water to cover the soil, but still contained within the sieve (Brown and Boag, 1988). After 48 h, nematodes were collected in a 50 mL tube, heat-killed in Table 1

<table>
<thead>
<tr>
<th>Date</th>
<th>pH</th>
<th>N (g/kg)</th>
<th>C/N</th>
<th>TOC (g/kg)</th>
<th>P2O5 (g/kg)</th>
<th>Cu tot (mg/kg)</th>
<th>Pb tot (mg/kg)</th>
<th>Zn tot (mg/kg)</th>
<th>Σ 16 PAH (US-EPA) (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>April 2008</td>
<td>6.88 ± 0.14</td>
<td>2.52 ± 0.22</td>
<td>25.1 ± 1.3</td>
<td>63.3 ± 5.4</td>
<td>0.072 ± 0.008</td>
<td>135.7 ± 8.1</td>
<td>523.0 ± 127.0</td>
<td>2102 ± 320</td>
<td>1427.3 ± 114.9</td>
</tr>
<tr>
<td>Sept. 2008</td>
<td>7.41 ± 0.09</td>
<td>2.54 ± 0.26</td>
<td>25.6 ± 1.6</td>
<td>64.9 ± 5.2</td>
<td>0.064 ± 0.008</td>
<td>137.5 ± 8.0</td>
<td>489.6 ± 38.1</td>
<td>2248 ± 171</td>
<td>894.5 ± 86.2</td>
</tr>
<tr>
<td>TD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>April 2008</td>
<td>8.39 ± 0.20</td>
<td>1.09 ± 0.07</td>
<td>57.0 ± 3.0</td>
<td>62.0 ± 5.6</td>
<td>0.086 ± 0.008</td>
<td>114.7 ± 9.2</td>
<td>674.5 ± 70.9</td>
<td>2700 ± 285</td>
<td>62.4 ± 4.2</td>
</tr>
<tr>
<td>Sept. 2008</td>
<td>7.69 ± 0.03</td>
<td>1.08 ± 0.08</td>
<td>57.3 ± 5.7</td>
<td>61.2 ± 2.1</td>
<td>0.070 ± 0.002</td>
<td>109.2 ± 2.6</td>
<td>677.7 ± 27.8</td>
<td>2775 ± 95</td>
<td>28.7 ± 3.8</td>
</tr>
</tbody>
</table>

Data for NM soil and TD soil are mean values from 20 to 4 plot analyses, respectively.

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a water bath for 4 min at 65 °C and preserved in a 1% formaldehyde solution. The total number of nematodes was counted under a low power microscope.

The number of protozoa was estimated using a most probable number technique (Darbyshire et al., 1974) and the biomass was calculated using appropriate weight (Griffiths and Caul, 1993). A known weight (ca. 10 g) of soil was dispersed in 40 mL of Neff’s modified amoeba saline medium (NMAS) (Page, 1976) by rolling at 20 °C for 1 h. Four 100 μL aliquots were placed in the first column of a sterile 96-well flat-bottomed microtiter plate. The remaining wells were filled with 100 μL of 1/10 nutrient broth (Oxoid) in NMAS, and a threefold dilution was carried out to the last column. To prevent desiccation, the dilution plates were wrapped in a polyethylene bag containing damp filter paper and incubated at 16 °C. The plates were examined for the presence of amoebae, ciliates and flagellates under an inverted microscope after 7, 14 and 21 d of incubation.

2.3.2. Microarthropods

Microarthropods were extracted from each soil sample using the Macfadyen method for a period of 1 week (Cortet et al., 2007) and identified and sorted using a dissecting stereomicroscope. Adult mites were identified to the suborder level (Actinedida, Gamasida, Oribatida, Acaridida) (Krantz, 1978). When possible, all collembolans were identified at the species level using different available keys (Gisin, 1960; Bretfeld, 1999; Potapov, 2001). For some analyses (see results), Collembola species were grouped into three morphological groups describing their habitat preferences combined with morphological parameters, including (i) epipod, very mobile and living mainly in litter and topsoil; and (ii) eu-edaphic, poorly mobile and living in soil macro pores; and (iii) hemi-edaphic, an intermediate group (Gisin, 1943). All other microarthropods (such as pseudoscorpions and small millipedes) were grouped together. The abundance of each taxon was estimated per m².

2.4. Microbial activities

2.4.1. Respiration

Substrate-induced respiration was measured using an automated Oxytop apparatus (Oxytop® Model OC 110, WTW Wissenschaftlich-Technische Werkstätten GmbH, Munich, Germany). Glucose (100 mg) was added to soil samples (20 g) at water holding capacity (WHC), and O₂ consumption was continuously monitored over 72 h at 20 °C. Substrate-induced respiration was expressed in mg O₂ g⁻¹ dry weight and per day (mg O₂ g⁻¹ DW d⁻¹).

2.4.2. Enzymatic activities

The enzymatic activity measured in this study included the following: arylamidase, arylsulfatase, cellulase, fluorescein diacetate hydrolase (FDase), lipase, alkaline and acid phosphomonoesterases and urease. These enzymes were chosen because of their involvement in the main biogeochemical cycles (C, N, P, and S) and degradation of major biopolymers potentially present in soils. Table 2 gives the corresponding methods used to measure their respective activities. Enzyme activity was calculated in units, defined as μmole of substrate hydrolyzed min⁻¹ and per g of soil dry weight (U g⁻¹ DW).

2.5. Multivariate statistical analysis

The effects of treatment on the soil community and functional parameters were analyzed by the principal response curve (PRC) method (Van den Brink, 1999) using CANOCO software (Ter Braak and Smilauer, 2002). The PRC method is multivariate analysis designed by Van Den Brink and Ter Braak (1998) for analysis of data from microcosm or mesocosm experiments to optimally show the effect of different treatments over time. This method, used by several authors in ecotoxicology (Pernin et al., 2006a) and field studies (Frampton et al., 2000; Pernin et al., 2006b), focuses on differences in taxonomic or biochemical composition between treatments and the control at each particular time point. The PRC results in a diagram showing the sampling date on the x-axis and the first or second principal component of the treatment effect on the y-axis. This yields a diagram representing deviations in time of treatment compared to the control. The bioindicator component weights, which appear on the right side of the figure, can be interpreted as the weight of each species or component with the response given in the diagram. The statistical significance of observed treatment effects at the community level and differences between treatments were also tested using Monte Carlo’s permutation tests (Ter Braak and Smilauer, 2002) with 499 permutations. The same test was used to analyze the observed differences between treatments.

Three different PRCs were calculated on log-transformed, centered and standardized data to obtain similar weight in the analysis; one analysis was performed only on microbiological data (microbial activities, bacteria and fungi abundance, and

Table 2

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>Substrate</th>
<th>Catalytic reaction</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arylsulfatase</td>
<td>p-nitrophenyl sulfate</td>
<td>Sulfoester hydrolisis</td>
<td>Tabatabai and Bremmer (1970)</td>
</tr>
<tr>
<td>Cellulase</td>
<td>Carboxymethylcellulose</td>
<td>Cellulose hydrolysis</td>
<td>Deng and Tabatabai (1994)</td>
</tr>
<tr>
<td>Fluorescein diacetate hydrolase (FDase)</td>
<td>Fluorescein diacetate</td>
<td>Ester hydrolisis</td>
<td>Green et al. (2006), Alarcon et al. (2008)</td>
</tr>
<tr>
<td>Lipase</td>
<td>p-nitrophenyl palmitate</td>
<td>Lipide hydrolisis</td>
<td>Gupta et al. (2002)</td>
</tr>
<tr>
<td>Alkaline and acid phosphomonoesterases</td>
<td>p-nitrophenyl phosphate</td>
<td>Phosphomonoester hydrolisis</td>
<td>Tabatabai and Bremmer (1969)</td>
</tr>
<tr>
<td>Urease</td>
<td>Urea</td>
<td>Urea hydrolisis</td>
<td>Kandeler and Gerber (1988)</td>
</tr>
</tbody>
</table>

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the Shannon diversity index for bacteria and fungi); a second analysis was performed on fauna data (protozoa, nematodes, microarthropods, and Collembola identified at the species level); and a third analysis assessed all information collected, but with Collembola grouped into three ecomorphological groups (see 2.3.2.). For each PRC, diagrams representing deviations in time of treatments used BS as the reference treatment.

3. Results

3.1. Bioindicator levels

The abundance and diversity of bioindicators and activity levels are presented in Table 3. Among microorganisms, bacteria were more abundant than fungi. Microorganism abundance was significantly ($p < 0.001$) higher in September 2009 than for the other sampling dates, and was higher in Msm-TD soil compared to other treatments. Abundance and diversity of fungi was significantly ($P < 0.001$) lower in bare soils (BS and BSt) compared to treatments with growing vegetation. Most microbial activity levels were significantly higher in planted soils (SV, Ms and Msm) compared to Msm-TD and bare soils (BS, BSt). Over time, activity levels were variable, with some increasing with time (FDase, arylamidase, urease) and some higher in September than in April (respiration, cellulase, acid and alkaline phosphomonoesterase). There was no significant difference with time and treatment for microfauna indicators except for amoeba, the most abundant protozoan group. Amoeba abundance was lower in Msm-TD soil, while the abundance of nematodes was higher in this treatment compared to other treatments. Mesofauna indicators were more abundant in April 2009. Microarthropods were significantly more abundant in SV and Msm-TD soils, while bare soil (BS and BSt) had a significant negative effect on their density.

3.2. Multivariate analyses

PRC calculated for microbiological data is presented in Fig. 1. The difference among sampling dates accounted for 41.1% of total variance; the treatment effect accounted for 36.3%. The first and second axes (Fig. 1A and B) explained 39.9% and 20.9% of variance captured by the treatment effect, respectively. The first axis distinguished planted contaminated soil treatments (SV, Ms and Msm) from bare soils (BS and BSt). At all sampling dates, microbial indicators of tilled bare soil treatment (BS) were similar to control treatment (BS). Planted contaminated soil treatments (Ms, Msm and SV except for April 2009) were associated with high enzymatic activities (bioindicator weight of arylsulfatase: 1.89; acid phosphatase: 1.70; lipase: 1.49; alkaline phosphatase: 1.21; and arylamidase: 1.02) and a high abundance and Shannon diversity index for fungi (bioindicator weight of 0.52 and 0.73, respectively). The Msm-TD soil showed greater variability through time than other treatments. In April 2008, September 2008 and September 2009, Msm-TD soil was similar to bare soils (BS and BSt), whereas in April 2009, it resembled planted contaminated soil treatments (SV, Ms and Msm). The second canonical axis of the PRC (Fig. 1B) for all sampling dates distinguished contaminated NM soil treatments from thermally desorbed soil treatment (Msm-TD). The Msm-TD treatment was associated with high microbial abundance and diversity (bioindicator weight of fungi abundance: 1.48, PAH-degrading Gram-positive bacteria: 1.40; bacteria abundance: 1.33; Shannon diversity index for fungi: 1.24) and low lipase activity (bioindicator weight of −2.04). This axis also highlighted a time effect in that all NM treatments tended, with time, to resemble the control BS. For all canonical axes, the results of Monte Carlo’s test of significance showed a significant treatment effect ($p < 0.01$). Comparisons between treatments throughout the study are presented in Table 4. Significant differences ($p < 0.05$) were found between bare soils (BS and BSt) and M. sativa-planted contaminated soil treatments (Ms and Msm). Msm-TD was significantly different from all other treatments ($p < 0.05$).

PRC calculated for the fauna data set is presented in Fig. 2. The difference between sampling dates accounted for 21.7% of total variance; the treatment effect accounted for 31.3%. The first and second axes (Fig. 2A and B) explained 35.1% and 15.6% of the variance captured by the treatment effect, respectively. The first axis showed three groups of treatment: M. sativa-planted contaminated soil treatments (Ms and Msm), bare soils (BS and BSt) and two other treatments (SV and Msm-TD). SV and Msm-TD were associated with a high abundance of fauna (bioindicator weight of nematodes: 2.34, Gamasidae: 1.92, “other microarthropods”: 1.55, Orbitidae: 1.30 and several Collembola species, including Parisotoma notabilis, Pseudosinella sexoculata, Lepidocyrtus cyaneus, Mesaphorura sp. and Protaphorura gr. armata with bioindicator weights from: 1.16 to 1.65). The second canonical axis of the PRC (Fig. 2B) showed fluctuation between sampling dates, but identified differences between Msm-TD and SV soil treatments. Msm-TD was associated with some microarthropods (bioindicator weight of P. armata: −2.12, Sminturinus elegans: −1.31, and Acaridae: −1.62) for April 2009. SV was associated with other microarthropods (bioindicator weight of Lepidocyrtus lignorum: 2.67; Isotoma sp.: 2.33; Lepidocyrtus sp.: 1.38; and Orbitidae: 1.08) for September 2008. The results of Monte Carlo’s test of significance performed on all canonical axes showed a significant treatment effect ($p < 0.01$). Comparisons between treatments throughout the study are presented in Table 4. All treatments were significantly different ($p < 0.05$) except for M. sativa-planted contaminated soil treatments (Ms and Msm) and Msm compared to BS.

PRC calculated for the global data set is presented in Fig. 3. The difference between sampling dates accounted for 35.5% of total variance; the treatment effect accounted for 37.1%. The first and second axes (Fig. 3A and B) explained 33.9% and 23.2% of variance captured by the treatment effect, respectively. The first axis distinguished vegetation treatments (SV, Ms, Msm, Msm-TD) from bare soils (BS, BSt). Vegetation treatments were associated with high fauna abundance

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to vary much over time even when vegetation fluctuated and the PAH concentration decreased from April 2008 to September 2009 (Table 1). In the current study, plant influence was assessed by comparison between bare soil (BS) and planted contaminated soils (SV, Ms, and Msm), while the effect of tillage was assessed by comparison between BS and BSt and that of thermal desorption treatment was assessed by comparison between Msm and Msm-TD.

Vegetation appeared to be the major factor in discriminating between soil treatments. Indeed, vegetation consistently appeared on the first axis of the PRCs. Plants had an influence on all organisms examined in our study at all trophic levels. Plant organic matter has been shown to increase the abundance of fungi and enzymatic activities (Smith et al., 2008; Palmroth et al., 2007). As the resources for saprotrophic fauna increased, a higher abundance of nematodes, Oribatidae and Collembola was observed. Finally, predatory mites or Gamasidae were consistently increased. Our results confirmed models proposed concerning trophic patterns in the soil (Moore et al., 2005). Arylsulfatases, acid and alkaline phosphatases and lipases, with their higher bioindicator weight values, indicated that the most microbiologically active soils were Ms, Msm and SV. Regulation of enzyme production by microorganisms is strongly related to the richness, availability and quality of organic matter (OM) as well as mineral nutrient (N, P, K and S) content of soil (Alarcón-Gutiérrez et al., 2009; Criquet and Braud, 2008). Moreover, soils with vegetation were more abundantly enriched with easily degradable OMs originating from plant exudates, litter and root decay, explaining the greater abundance of fungi and enzyme

(bioindicator weight of nematodes: −2.17; Gamasidae: −1.70; “other microarthropods”: −1.48; Oribatidae: −1.40; hemi-edaphic Collembola: −1.04), microbial enzymatic activities (bioindicator weight of arylsulfatase: −1.48; alkaline and acid phosphatase: −1.28; arylamidase: −1.21) and fungi abundance (bioindicator weight of −1.13). The second canonical axis of the PRC (Fig. 3B), for all sampling dates, distinguished contaminated NM soil treatments from thermally desorbed soil treatment (Msm-TD). Msm-TD treatment was associated with high fauna abundance (bioindicator weight of Acarida: 1.62, nematodes: 1.10, and eu-edaphic Collembola: 0.94). In contrast, vegetation treatments were associated with high enzymatic activities (bioindicator weight of lipase: −2.70; acid phosphatase: −1.74; arylsulfatase: −1.71; urease: −1.11; and cellulase: −1.01). The results of Monte Carlo’s test of significance performed on all canonical axes showed a significant treatment effect \((p < 0.01)\). Comparisons between treatments throughout the study are presented in Table 4. All treatments were significantly different \((p < 0.05)\) except for M. sativa-planted contaminated soil treatments (Ms and Msm).

### 4. Discussion

Our study highlights the value of using a range of complementary bioindicators (assessing diversity and functions) to evaluate the influence of vegetation and tillage and to distinguished polluted from thermal-desorption-treated soils. The fluctuation in the abundance of organisms (Table 3) did not drastically influence the treatment effect; indeed, using PRC analyses, differences between treatments did not appear

### Table 3

Bioindicator levels for the 6 treatments studied and the 4 sampling dates.

| BS | BS | BS | BS | BS | BS | BS | BS/
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Micro-organisms</strong></td>
<td><strong>Abundance</strong></td>
<td><strong>Bacteria</strong></td>
<td>(16S rRNA gene copies ( \times 10^7 ) g soil)</td>
<td>157</td>
<td>136</td>
<td>886</td>
<td>2184</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td>(18S rRNA gene copies ( \times 10^7 ) g soil)</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>22</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Shannon diversity index</strong></td>
<td><strong>Activities</strong></td>
<td><strong>Bacteria</strong></td>
<td>Respiration</td>
<td>(mgO(_2)/g soil)</td>
<td>2.96</td>
<td>3.01</td>
<td>2.77</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td>Lipase</td>
<td>(mgU/g soil)</td>
<td>2.11</td>
<td>1.76</td>
<td>1.65</td>
<td>1.77</td>
<td>1.80</td>
</tr>
<tr>
<td><strong>Acmidase</strong></td>
<td>(mgU/g soil)</td>
<td>0.73</td>
<td>0.65</td>
<td>0.71</td>
<td>2.92</td>
<td>0.64</td>
<td>0.76</td>
</tr>
<tr>
<td><strong>Arylsulfatase</strong></td>
<td>(mgU/g soil)</td>
<td>0.26</td>
<td>0.08</td>
<td>0.15</td>
<td>0.06</td>
<td>0.02</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Cellulose</strong></td>
<td>(mgU/g soil)</td>
<td>2.0</td>
<td>3.8</td>
<td>0.7</td>
<td>5.2</td>
<td>2.6</td>
<td>3.0</td>
</tr>
<tr>
<td><strong>Lipase</strong></td>
<td>(mgU/g soil)</td>
<td>3.9</td>
<td>4.5</td>
<td>2.1</td>
<td>2.9</td>
<td>4.0</td>
<td>2.1</td>
</tr>
<tr>
<td><strong>Acid phosphomonoesterase</strong></td>
<td>(mgU/g soil)</td>
<td>2.2</td>
<td>4.9</td>
<td>3.8</td>
<td>5.2</td>
<td>2.5</td>
<td>4.2</td>
</tr>
<tr>
<td><strong>Alkaline phosphomonoesterase</strong></td>
<td>(mgU/g soil)</td>
<td>3.4</td>
<td>7.3</td>
<td>5.4</td>
<td>6.0</td>
<td>3.2</td>
<td>5.6</td>
</tr>
<tr>
<td><strong>Urease</strong></td>
<td>(mgU/g soil)</td>
<td>10.8</td>
<td>15.5</td>
<td>18.2</td>
<td>15.9</td>
<td>9.9</td>
<td>12.9</td>
</tr>
<tr>
<td><strong>Protozoa</strong></td>
<td><strong>Flagellates</strong></td>
<td>(ng/g soil)</td>
<td>32</td>
<td>9</td>
<td>44</td>
<td>34</td>
<td>32</td>
</tr>
<tr>
<td>** amoeba**</td>
<td>(ng/g soil)</td>
<td>10433</td>
<td>1631</td>
<td>1048</td>
<td>471</td>
<td>2790</td>
<td>1425</td>
</tr>
<tr>
<td><strong>Ciliates</strong></td>
<td>(ng/g soil)</td>
<td>32</td>
<td>9</td>
<td>44</td>
<td>34</td>
<td>32</td>
<td>29</td>
</tr>
<tr>
<td><strong>Nematodes</strong></td>
<td>(Abundance/mg soil)</td>
<td>0.35</td>
<td>0.27</td>
<td>0.24</td>
<td>0.50</td>
<td>1.79</td>
<td>1.86</td>
</tr>
<tr>
<td><strong>Meso-fauna</strong></td>
<td><strong>Acarida</strong></td>
<td>(Abundance/mg soil)</td>
<td>8519</td>
<td>3921</td>
<td>35462</td>
<td>4923</td>
<td>1710</td>
</tr>
<tr>
<td><strong>Collembola</strong></td>
<td>Epi-edaphic</td>
<td>(Abundance/mg soil)</td>
<td>2152</td>
<td>0</td>
<td>1091</td>
<td>0</td>
<td>295</td>
</tr>
<tr>
<td><strong>Hemi-edaphic</strong></td>
<td>(Abundance/mg soil)</td>
<td>5188</td>
<td>560</td>
<td>4510</td>
<td>1444</td>
<td>2093</td>
<td>619</td>
</tr>
<tr>
<td><strong>Eu-edaphic</strong></td>
<td>(Abundance/mg soil)</td>
<td>3095</td>
<td>2565</td>
<td>2299</td>
<td>737</td>
<td>2004</td>
<td>1268</td>
</tr>
<tr>
<td><strong>Total collembola</strong></td>
<td>(Abundance/mg soil)</td>
<td>10435</td>
<td>3215</td>
<td>7900</td>
<td>2181</td>
<td>4392</td>
<td>1975</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>(Abundance/mg soil)</td>
<td>19396</td>
<td>8342</td>
<td>4356</td>
<td>4923</td>
<td>6367</td>
<td>2771</td>
</tr>
</tbody>
</table>

\(^{a}\) groups from two-way analysis of variance ANOVA(\(p < 0.001\)) and pair-wise multiple comparison test (Newman-Keuls).
activities (de Boer et al., 2005). Concerning Collembola, the increase was mainly observed in epi- and hemi-edaphic species. This is not surprising, as these Collembola benefit from the presence of litter (Gisin, 1943). It is interesting to note that spontaneous vegetation (SV) differed from M. sativa-planted soils (Ms and Msm) by harboring more microarthropods, especially epi-edaphic species like Isotoma sp. In contrast to spontaneous vegetation, M. sativa was harvested each year, leading to less organic matter in the soil and disruption of vegetation cover, which has been shown to decrease epi-edaphic Collembola (Gillet and Ponge, 2003). Moreover, PRCs showing fauna data identified differences between BSt and BS; fewer Gamasidae and nematodes were observed after soil tillage. These results confirm the notion that fauna is sensitive to physical disturbances in soil, such as during agricultural practices (Cortet et al., 2002, 2007). No difference between Ms and Msm was observed. We previously showed that mycorrhizal colonization of M. sativa roots with the two inoculated Glomus strains was very low (Ouvard et al., in press), which could be due to soil toxicity with the combination of PAH and heavy metals (Leyval and Binet, 1998; Leyval et al., 1997) and possibly to high sulfate concentrations.

TD treatment appeared to be the second major factor in identifying differences in soil treatment on the second axis of the PRCs. Although this technique is among the most efficient methods for removing organic pollutants and is commonly used for PAH remediation, the long-term evolution of TD-treated soils has never been examined, especially regarding biological function. Little information on restoration of biological diversity and function in this type of soil, considered to be an extreme environment, is available. In a previous study, we observed that during the first two years after TD treatment, the bacterial community of the TD soil re-colonized and diversified and the main bacterial representatives closely resembled bacteria detected in extreme environments (i.e. characterized by high pollution, low pH, high salinity, low nutrient availability; Cébron et al., 2009). Similarly, our results show that despite strong physical and chemical modifications (increased pH level, decreased nitrogen content and higher C/N ratio), biological communities (various trophic levels) re-colonized TD soil after more than two years on the site. Indeed, increases in bacteria, Gram-positive PAH-degrader abundance, fungi abundance and diversity were observed in the TD soil. Lower PAH concentrations might favor development of fungi (Blakely et al., 2002). Moreover, thermally desorbed soils as well as post-fire soils are known to be enriched in condensed and recalcitrant organic matter (González-Pérez et al., 2004; Biache et al., 2008). Changes in composition of organic matter and nitrogen limitation could favor fungal growth and colonization (Strickland and Rousk, 2010; Hong et al., 2010). Moreover, fungi and Actinobacteria (e.g., Gram-positive PAH degraders) are favored by vegetation (Smith et al., 2008; Cébron et al., 2009) and are commonly found in extreme environments, such as dry volcanic soils or deserts (Costello et al., 2009; Connon et al., 2007). Extreme soil environments and hostile conditions select for organisms with ecological strategies for survival under harsh conditions, such as desiccation, freeze-thaw, UV resistance, and resting spore formation (Ensing,
1978; Hong et al., 2010), and molecular techniques cannot distinguish spores from active cells. In our study, most of the enzymatic activities were decreased in Msm-TD compared to the contaminated soil, Msm. As soil enzyme activities are a direct response by the soil community to metabolic requirements and environmental stress (Caldwell, 2005), these data highlight the poor nutrient availability, physical disturbance and the particular organic matter composition and reactivity of TD soil. Lipase activity was especially low in TD soil. Such C-acquiring enzymes are known to be stimulated by the availability of their own substrates (e.g. aliphatic carbon; Alarcón-Gutiérrez et al. 2009). On this point, Biache et al. (2008) demonstrated that the TD process strongly reduces the content of aliphatic C of soil, a decrease which may explain the lower lipase activity measured in TD soil. Concerning fauna, TD led to an increase in eu-edaphic Collembola species known to be sensitive to the presence of organic pollutants but tolerant to the presence of heavy metals, as previously shown for the species *P. armata* (Gillet and Ponge, 2003). Furthermore, the TD process induced an increase in pH.

Fig. 1. Principal response curve (PRC) for the microbial data set (activities, abundance and diversity index). The first (A) and second (B) axes indicate differences between plot treatments. The lines represent the course of treatment over time. Values deviating from the reference value of 0 (BS control treatment) indicate treatment effects. The bioindicator weight can be interpreted as the affinity of the taxon with the principal response curves. Only species with a weight of 0.5 or higher and −0.5 or lower are considered significant indicators (bold type).
levels that may have modified the communities (Garnier and Ponge, 2004), and lower PAH content could explain increases in Acarididae and nematode abundance.

We used a number of bioindicator tests, including abundance, diversity and functional parameters. The principal response curve (PRC) technique had the advantage of multivariate analysis constrained by the evolution of treatments compared to a control over time. This statistical treatment is powerful and well-suited to compare bioindicator data. This type of analysis demonstrated that, depending on the observed treatment effects (vegetation or TD), the response of each bioindicator could differ. When considering the effect of vegetation, all trophic levels responded in a similar manner; abundance of organisms and activities increased with the presence of vegetation, indicating redundancy when using all bioindicators together. For micro- and mesofauna development, the most important parameter is the food resource. Thus, abundance of fauna mainly depends on fungal and bacterial biomass and not on microbial diversity or community structure. This basic knowledge about the food web explains the redundancy among bioindicators referring to organism abundances. In contrast, when studying the effects of TD, various bioindicators appeared to be complementary because of contradictory responses (e.g. the high abundance and diversity of microorganisms was not linked to their low activity levels). A more in-depth characterization of phylogenetic diversity of...
microorganisms (Buèe et al., 2009) and their metabolic profiles (i.e. BIOLOG; Garland and Mills, 1991) would probably help to understand the functions and physiology of the microbial community. Activity levels directly depend on the functional diversity of microorganisms, but not necessarily on the phylogenetic diversity assessed here through molecular fingerprinting. Microorganisms were present but not active, indicating a narrow range of functions represented in the community (high functional redundancy or high specificity).

This could be due to specific and highly selective environmental conditions that differ from natural soil and, as a consequence, traditionally studied functions are not the best indicators for highly anthropogenic soils. Due to the special status of the soil after the TD remediation process, lipase activity could be an interesting bioindicator complementary to microorganism abundance, as explained above. It is thus important in this type of study to consider both diversity and functional bioindicators, since the ecology of anthropized soils

Fig. 3. PRC for all indicators studied (microbial data, except for the Shannon diversity index, and micro- and mesofauna data with Collembola grouped into three ecomorphological groups). The first (A) and second (B) axes indicate differences between plot treatments. The three ecomorphological groups of Collembola are the following: 1) epi-edaphic (Entomobrya sp., Bourletiella hortensis, Isotoma sp., Isotoma viridis), 2) hemi-edaphic (Anurida tullbergi, Cryptopygus thermoophilus, Folsomia sp., Heteromurus major, Hypogastrura manubrialis, Lepidocyrtus cyaneus, Lepidocyrtus lanuginosus, Lepidocytus lignorum, Lepidocytus curvicolis, Lepidocytus sp., Macrosomia sp., Proisotoma minuta, Pseudosinella secucdata, Pseudosinella carca, Smiathirtus elegans) and 3) eu-edaphic (Mesaphorura sp., Protaphorura armata group, Stenaphorura denisi, Stenaphorura quadrispina). The lines represent the course of treatment over time. Values deviating from the reference value of 0 (BS control plots) indicate treatment effects. The bioindicator weight can be interpreted as the affinity of the taxon with the principal response curves. Only species with a weight of 0.5 or higher and−0.5 or lower are considered significant indicators (bold type).
Table 4

Results of the Monte Carlo test of significance for all canonical axes.

<table>
<thead>
<tr>
<th>Microbiological data</th>
<th>BS</th>
<th>BSt</th>
<th>SV</th>
<th>Ms</th>
<th>Msm</th>
<th>Msm-TD</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>a</td>
<td>ab</td>
<td>bc</td>
<td>c</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td>Micro- and meso-fauna data</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>d</td>
<td>ad</td>
<td>e</td>
</tr>
<tr>
<td>Global data</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>d</td>
<td>d</td>
<td>e</td>
</tr>
</tbody>
</table>

Significant treatment effects ($p < 0.01$) are shown and comparisons between treatments through the study were performed separately for the three PRC data sets (microbiological, micro- and mesofauna, and overall). Different letters between treatments indicate significant differences ($p < 0.05$).

is not well understood and the relationship between diversity and function is not as clear as in natural soils (Naeem et al., 2002; O’Donnell et al. 2001). Alternatively, we observed that the response of some parameters (e.g., protozoa, respiration and FDase activities) were less discriminative compared to others (e.g., nematodes, enzymatic activities, fungi and microarthropods). Furthermore, our study pointed out that the ecomorphological classification of Collembola could give patterns similar to those of classical Linnean species identification, suggesting that the life-trait-based classification of microarthropods is a very promising method for exploring data. Finally, it is essential to carry out a number of analyses, including organisms at several trophic levels, and structural and functional parameters.

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References


