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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

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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 between 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 site and sampling

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 tilled 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.