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Differential composition of bacterial communities as influenced by phenanthrene and dibenzo[a,h]anthracene in the rhizosphere of ryegrass (*Lolium perenne* L.)

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Abstract

Bioremediation technologies of Polycyclic Aromatic Hydrocarbons (PAH) are often limited by the recalcitrance to biodegradation of high molecular weight (HMW) PAH. Rhizosphere is known to increase the biodegradation of PAH but little is known about the biodegradability of these HMW compounds by rhizosphere bacteria. This study compared the effects of a 3 and a 5-ring PAH, phenanthrene (PHE) and dibenzo[a,h]anthracene (dBA) respectively, on the composition of bacterial community, the bacterial density and the biodegradation activity. Compartmentalized devices were designed to harvest three consecutive sections of the rhizosphere. Rhizosphere and non-rhizosphere compartments were filled with PHE or dBA spiked or unspiked sand and inoculated with a soil bacterial inoculum. Different bacterial communities and degradation values were found 5 weeks after spiking with PHE (41–76% biodegradation) and dBA (12–51% biodegradation). In sections closer to the root surface, bacterial populations differed as a function of the distance to roots and the PAH added, whereas in further rhizosphere sections, communities were closer to those of the non-planted treatments. Biodegradation of PHE was also a function of the distance to roots, and decreased from 76 to 42% within 9 mm from the roots. However, biodegradation of dBA was significantly higher in the middle section (3–6 mm from roots) than the others. Rhizosphere degradation of PAH varies with the nature of the PAH, and C fluxes from roots could limit the degradation of dBA.

Introduction

Polycyclic Aromatic Hydrocarbons (PAH) are produced by incomplete combustion of organic matter, and share a similar molecular skeleton of at least two fused aromatic rings. These compounds are usually classified as low molecular weight (LMW) PAH for the molecules from two to three rings and as high molecular weight (HMW) PAH for the molecules with more than three rings. It is assumed that the molecular weight and the angularity of the molecule determine the physical and chemical properties of PAH (Wickle 2000). The hydrophobicity and the molecular stability of PAH increase with respect to the number of rings (Marschner 1999). For example, water solubility is 1.6 mg/l for phenanthrene (PHE), 0.132 mg/l for pyrene and 0.0038 mg/l for benzo[a]pyrene, which are respectively 3, 4 and 5-ring PAH (Dabestani & Ivanov 1999). These chemical properties determine the fate of PAH in the environment and their biological degradation processes. The half-life of

PAH in soil depends on their molecular weight and varies from 16 to 126 days for PHE, and up to 4 years for benzo[a]pyrene (Dabestani & Ivanov 1999; Wickle 2000). The persistence of HMW PAH in soil results from sorption onto the soil organic matter (Means 1980; Weissenfels et al. 1992) but also from their lower biodegradability (Juhasz & Naidu 2000). Compared to LMW compounds, HMW PAH are more stable and recalcitrant to biodegradation.

Numerous bacterial species have been identified and isolated for their catabolic capabilities on LMW PAH. However, a limited number of studies report bacterial species which can partially oxidize HMW PAH. Juhasz and Naidu (2000) investigated the biodegradation of HMW PAH by a pure culture of *Stenotrophomonas maltophila*. The findings suggest biodegradation for benzo[a]pyrene and dibenzo[a,h]anthracene (dBA) to be 22 and 55% for coronene. Boochan et al. (2000) observed an increased biodegradation in fungal–bacterial cocultures of *Penicillium janthinellum* and *S. maltophila* with 53% of benzo[a]pyrene mineralization in 100 days. *Bjerkandera*, a white rot fungus, was described to rapidly biotransform benzo[a]pyrene into polar metabolites which could be metabolized by indigenous microflora from PAH contaminated sediments (Kotterman et al. 1998).

Chemical and physical treatments, such as solvent flushing or thermodesorption, are the most efficient technologies to remove PAH from soils. However, these treatments remain expensive and are non-specific as the entire soil organic matter is destroyed. Bioremediation technologies are usually cheaper and environmentally friendly and are based on the capabilities of endogenous or inoculated microorganisms to specifically degrade organics such as PAH. Therefore, enhancing biodegradation of HMW PAH is the main challenge for future improvements of bioremediation technologies. Planting, using high density covering grasses for example, has the advantage to limit the spreading of PAH contaminated dusts that represent the main risk of exposure to HMW PAH. The efficiency of plants to increase biodegradation of LMW PAH has been amply demonstrated (Anderson & Coats 1994; Günther et al. 1996; Höflich & Günther 2000). The effect of rhizosphere on PAH biodegradation hypothesizes the beneficial effect of root exudates that increase density and biodegradation activities (direct catabolism, cometabolism) of rhizosphere-selected bacteria (Yoshitomi & Shann 2001; Siciliano et al. 2003). However, few studies report the biodegradation of HMW PAH in the rhizosphere (Liste & Alexander 2000; Yoshitomi & Shann 2001) while none describes the effect of HMW PAH on rhizosphere communities.

In pot experiments, Binet et al. (2000a, b) reported the dissipation of eight PAH, including HMW PAH, in the rhizosphere of ryegrass. Biodegradation (or biotransformation) of PAH in soils is difficult to identify among other mechanisms such as photooxidation, leaching, and sequestration that are involved in the dissipation of PAH in the environment. With similar experimental set-ups, Joner & Leyval (2003) also observed spatial gradients of HMW PAH dissipation in the rhizosphere using PAH contaminated industrial soils, with a highest dissipation in the volume of soil strongly adhering to roots. The authors concluded that HMW molecules were less affected by rhizosphere than LMW PAH. It has been previously shown that the biodegradation of PHE in the rhizosphere is a function of proximity to roots (Corgié et al. 2003) and is therefore associated with the composition of different microbial populations (Corgié et al. 2004). In such a complex environment as rhizosphere, the nature of the PAH itself is then a crucial parameter that could dramatically affect the microbial communities and the resulting biodegradation activities. This study aims to: (i) compare the effect of a LMW PAH (PHE) and a HMW PAH (dBA) on the composition of bacterial communities, (ii) study the spatial effect of rhizosphere on the composition of these communities in the presence of two different PAH, and (iii) follow the bacterial biodegradation of PHE and dBA in this 2-D defined rhizosphere. The compartmentalized pots were spiked with either a 3-ring PAH (PHE), or a 5-ring PAH (dBA). The biodegradation of PAH, the structure of bacterial communities and the densities of culturable bacteria were followed as a function of the distance to roots.

Material and methods

Experimental design

Compartmentalized devices, as previously described by Corgié et al. (2003), were made with a T-shape PVC tube forming a vertical root compartment (3.5 cm diam, 250 cm^3) and 2 horizontal root-free compartments (3.5 cm diam, 30 cm^3) inserted at the bottom of the vertical compartment. In order to restrict root entry the root-free compartments were separated from the root compartment by a 37 μm nylon mesh. Ryegrass seeds (*Lolium perenne* L. cv. Barclay) were individually pre-grown in vermiculite for 2 weeks. One plant was transferred in each vertical root compartment filled with 60% Terragreen (Oil Dry, Lobbe), 30% sand (0.125–2 mm), and 10% vermiculite. Transferred plants were grown for another 2 weeks to ensure that the roots fully colonized the central compartment. Water and mineral nutrients were provided by a cotton wick connecting the root compartment to a 250 ml reservoir of sterile nutrient solution (1 mM NH_4NO_3, 1 mM $Ca(NO_3)_2$, 1 mM $Na_2HPO_4{\cdot}2H_2O$, 1 mM K_2SO_4, 0.75 mM $MgSO_4{\cdot}7H_2O$, 12.5 μM H_3BO_3, 2.5 μM $MnSO_4{\cdot}H_2O$, 0.3 μM $CuSO_4{\cdot}5H_2O$, 1 μM $ZnSO_4{\cdot}7H_2O$, 0.05 μM $Na_2MoO_4{\cdot}2H_2O$, 0.2 μM $CoSO_4{\cdot}7H_2O$, 20 μM Fe-EDTA; pH 6). The sterile nutrient solution was refilled on a weekly basis.

Washed quartz sand (0.125–2 mm) with low organic carbon content was used as mineral matrix in the lateral compartments. The sand was spiked with PHE (500 mg kg^{-1} of sand, Fischer Scientific, 99% purity) or dBA (50 mg kg^{-1} of sand, Fischer Scientific, 99% purity) dissolved in chloroform. A bacterial inoculum was isolated from an industrial PAH-contaminated soil from the North of France (Joner et al. 2002) and isolated as described by Corgié et al. (2003). Briefly, 80 g of soil was agitated with glass beads (1 h, 500 ml of 0.8% NaCl) and the solution was filtered at 10, 5 and 2 μm to remove soil particles, fungi and fungal spores. The solution was finally filtered at 0.2 μm and bacteria were collected from the surface of the 0.2 μm filter and suspended in 100 ml of 0.8% NaCl. Bacteria cells were counted using a Thoma cell. The microbial suspension was diluted and added to the PAH-spiked and PAH-free sands for a final concentration of approx. 10^5 cells g^{-1} sand. Compartments were then filled with the sands (same inoculum, same initial bacterial density) and fixed on the existing central compartments containing the pre-grown ryegrass.

Five treatments with four replicates were performed: planted pots without PAH in the lateral compartments (P), planted pots with a phenanthrene-contaminated compartment (P + PHE) on one side and a dibenzo[a,h]anthracene-contaminated compartment (P + dBA) on the other side, and non-planted pots where a lateral compartment contained phenanthrene (NP + PHE) and the other one dibenzo[a,h]anthracene (NP + dBA). The pots were incubated in a growth chamber (24/20 °C day/night, 16 h light period, 60% RH, 300–350 μmol m^{-2} s^{-1} PAR) and harvested 5 weeks after lateral compartment addition. Three consecutive sections were sampled as the sand from the lateral compartments was sequentially removed at defined distances from the root mat: 0–3mm (S1), 3–6 mm (S2) and 6–9 mm (S3). One gram of sand from each layer was immediately used for microbial enumerations and quantification of soluble organic carbon, 1 gram was frozen at −80 °C for molecular analysis and the remaining sand air-dried overnight and stored at 4 °C prior to PAH analysis.d

Analysis of organic carbon and PAH quantification

Sand sample (1 g) was agitated for 30 min in 10 ml NaCl (0.8%) and filtrated at 0.2 μm. Organic carbon was quantified with a Total Organic Carbon Analyser (TOC, Dhorman) for two replicates of the non-planted treatment and PAH-free treatment, and for one device of the planted and polluted treatment. Soxhlet extractions with chloroform (Leyval & Binet 1998) were performed on 2 g of air-dried samples (four replicates of each treatment). The extracts were analyzed for PHE and dBA by GC–MS according to Binet et al. (2000a, b). This analysis was carried out with a 3400 CX Varian gas chromatograph coupled to a mass spectrometer (ION TRAP Saturn III, Varian) at the SARM (CNRS, Vandoeuvre-lès-Nancy, France). Compound separation was performed on a DB5 MS column (30 m, 0.25 mm internal diameter, 0.25 μm coating). The temperature of the column was programmed as follows: 70–150 °C at 10 °C min^{-1}, 150–300 °C at 6 °C min^{-1} and then an isotherm level of 10 min at 300 °C. The ion trap

temperature was set to 220 °C and the mass spectrometer was operated at 70 eV in an electron impact mode. Identification and quantification of PHE and dBA were performed by single ion monitoring. PHE and dBA standard solutions were injected every 20 samples. Quantification performed on non-inoculated sands spiked with PHE and dBA indicated a percentage of recovery of 99% (+/−0.8%) compared to initial added concentrations.

Microbial enumerations

Culturable heterotrophic bacteria, PHE and dBA degraders were counted on Petri dishes. Culturable heterotrophs were counted on Nutrient Broth (1 g l^{-1}, DIFCO) with 1.5% bacteriological agar (Sigma). PHE or dBA degrading bacteria were cultivated using either PHE or dBA as a sole source of carbon. Plates were spiked with PHE (0.5 mg per plate) or dBA (0.25 mg per plate) dissolved in chloroform. PAH solutions (20 μl) were homogenously spread onto the plate surface (Bushell Hass medium, 3.27 g l^{-1}, DIFCO, and bacteriological agar, 1.5%, Sigma) and chloroform was left to evaporate under the fumehood for 30 min each. Serial dilutions (10^{-2} to 10^{-5}) of suspended sand (1 g sand in 10 ml of 0.8%NaCl) were carefully plated with sterile glass beads (2 mm diam.) to avoid disturbing the thin PAH crust onto agar surface. Plates were then incubated at 28 °C. CFU were counted for heterotrophs after 5 days and for PHE and dBA degraders after 10 and 15 days respectively. The number of bacteria per gram of sand was calculated using the numbers of CFU for the two highest positive dilutions.

Molecular analysis

Total DNA isolation from the samples was carried out based on a protocol from Corgié et al. (2004). It was performed with a bead beating procedure in an extraction buffer (100 mM Tris, 100 mM EDTA, 100 mM NaCl, 1% PVPP wt/vol, 2% SDS wt/vol, pH 8) and a phenol/chloroform purification. DNA pellet was then dissolved in 100 μl of ultrapure water and stored at −80 °C for further analysis. A 475 bp sequence of the Bacteria 16S rDNA (V6–V8 region) was amplified by PCR with the primers GC-968f and 1401r (Heuer et al. 1999) from 1 μl of extracted DNA according to Corgié et al. (2004). Temporal temperature gradient electrophoresis (TTGE) was performed with a BioRad electrophoresis (Dcode, Universel Mutation Detection System, Biorad) in polyacrylamide gels (6% (wt/vol) acrylamide, 0.21% (wt/vol) bisacrylamide, 8 M urea, 1.25× TAE and 0.2% (vol/vol) glycerol). PCR products (5 μl) were electrophoresed in 1.25× TAE at a constant of 100 V, with a temperature gradient from 57 to 67 °C (temperature increment of 0.7 °C per hour). Two reference samples were loaded in each gel. Gels were stained with ethidium bromide, and digitalized under UV light.

Statistical analysis

Band mobility and band intensity were quantified in each profile using the Kodak 1D 3.5.2. software. Profiles obtained from different gels were aligned using the profile of the reference samples. Principal Component Analysis was performed on the relative intensity of bands in each profile (Marschner & Baumann 2003) using the ADE-4 software. Kruskal–Wallis tests (Statview software) were used to compare mean values of remaining PHE and dBA in each section. Fisher Least Significant Difference (LSD) tests were performed on PAH biodegradation values, bacterial numbers and bacterial community from the PCA analysis.

Results

The number of culturable heterotrophs was the highest in both planted and PAH-spiked treatments (Figure 1). In these treatments, heterotroph density was higher in the S1 sections, than in the following sections S2 and S3 where density was 4-fold lower. In the unplanted treatments, no significant difference between the sections was observed in the heterotroph density. The number of PHE degrader was significantly higher in PAH-spiked than in PAH-free treatments, and in the S1 section of the P + PHE treatment than in the following sections S2 and S3. The density of PHE degraders in sections S2 and S3 of P + PHE was not different from the three sections of the P + dBA treatment. The number of PHE degraders was significantly lower in the NP + PHE treatment, with similar density in S1, S2 and S3, compared to the other PAH-spiked treatments. The

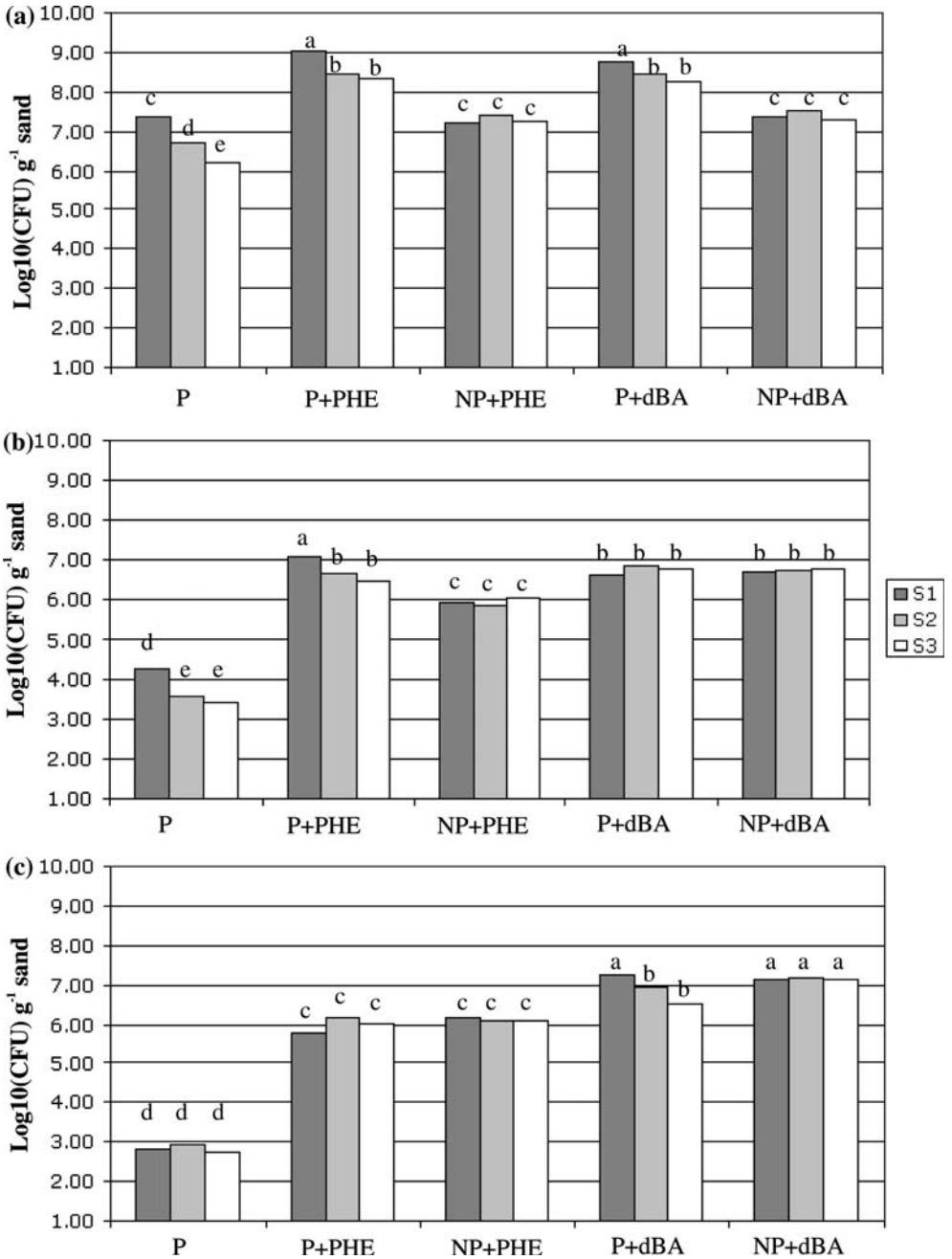

Figure 1. Number of culturable bacteria (log10 CFU per g sand) for the planted (P) and non-planted (NP) treatments after 5 weeks of incubation in function of distance from roots (S1 = 0–3 mm, S2 = 3–6 mm and S3 = 6–9 mm) growing on Nutrient Broth (a), growing on phenanthrene (b) and growing on dibenzo[a,h]anthracene (c). Level of significance (Fisher LSD) for each medium is indicated by lower-case letters, the same letters indicate no significant difference ($n = 4$, LSD (A) = 0.41, LSD (B) = 0.19, LSD (C) = 0.28).

highest number of dBA degraders was found in S1 of P + dBA and in the three sections of NP + dBA. Their density was lower in the sections S2 and S3 of P + dBA treatment than in S1. In PAH-free treatments, PHE and dBA degrader density was lower than in PAH-spiked pots; the number of PHE degraders was higher in S1 than in S2 and S3 section, whereas dBA degraders reached only

250–800 bacteria per g sand, without any significant difference between the 3 sections.

The variations in community structure arose from both the presence of plant and the nature of the PAH. Variability in community structure was distributed for 47% on the C1 axis, discriminating communities as a function of distance to roots, and for 36% on the C2 axis, which discriminated the presence of PAH (Figure 2). Communities from NP plots were coordinated in the top part of the PCA and no significant difference was observed in the structure of community between the sections S1, S2 and S3 for NP + PHE treatment and NP + dBA treatment (data not shown). Without plant, the communities from PHE contaminated sand were significantly different from those developed on dBA as primary source of C. Community of the S3 layer of the P + PHE treatment had the same structure as the one of the NP + PHE treatment. Communities from the S2 and S3 layers of the P + dBA treatment were close to the one of the NP + dBA treatment. On the opposite, the PAH-free treatment profiles grouped in the bottom part of the PCA plot, as well as the S1 sections of the planted and PAH-spiked treatments. The sections S1 of the planted treatments were not significantly different on the C1 axis but were significantly discriminated by the C2 axis. The bacterial communities of these sections were always significantly different from those of the following sections S2 and S3 of their corresponding treatment.

PHE biodegradation was significantly higher in the S1 section of the planted treatment than in the following layers, but not significantly different from the values in the non-planted treatment (Table 1). Biodegradation of dBA was higher in the sections of the non-planted treatment reaching about 50%. In the presence of plant, dBA biodegradation was lower (12–22%) with the highest percentage of degradation in the section S2. The initial content of soluble organic carbon was 10, 23 and 14 μg C g^{-1} respectively for unspiked, PHE and dBA spiked sands. After 5 weeks, the quantity of soluble organic carbon had increased in all the sections of the planted treatments, and decreased from S1 to S2 and S3 sections (Table 2). Organic carbon concentration was similar in the three sections of the non-planted treatments.

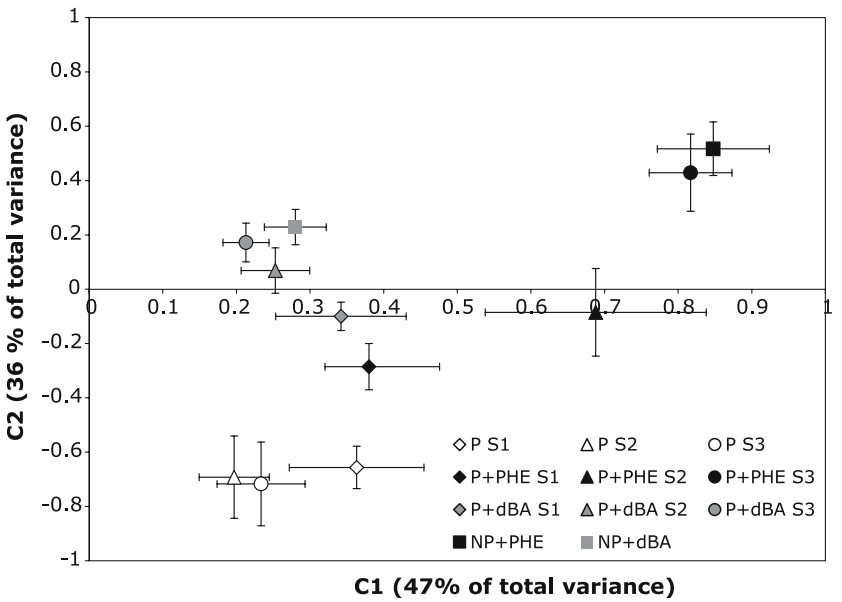

Figure 2. Microbial community structure based on PCA analysis from relative abundance of detected species by TTGE analysis for the planted (P) and non-planted (NP) treatments after 5 weeks of incubation. Data points represent mean coordinate values ($n = 4$ for planted treatments and $n = 12$ for non-planted treatments, within each treatment. Bars are SEM, Level of significance (Fisher LSD) for the X and Y axis (LSD (X) = 0.12, LSD (Y) = 0.07).

Table 1. Phenanthrene or dibenzoanthracene (expressed in μg of C per g of sand) for the planted (P) and non-planted (NP) treatments after 5 weeks of incubation: remaining PAH (standard deviation in brackets) and percentage of biodegradation (within a column, the same letter indicate no significant difference; $p > 0.05$; $n = 4$)

	Remaining	% Biodegradation	Remaining	% Biodegradation
	P + PHE		P + dBA	
S1	107 (20)	76 (a)	41 (3)	12.5 (c)
S2	240 (36)	46 (b)	37 (2)	22 (b)
S3	260 (17)	42 (b)	40 (4)	15 (c)
	NP + PHE		NP + dBA	
S1	114 (22)	74 (a)	22 (6)	51 (a)
S2	123 (32)	72 (a)	21 (5)	52 (a)
S3	126 (28)	72 (a)	22 (6)	49 (a)

Table 2. Total soluble organic carbon (μg C per g sand) in sections S1, S2 and S3 for the planted (P) and non-planted (NP) treatments after 5 weeks of incubation

	P	P + PHE	NP + PHE	P + dBA	NP + dBA
S1	158	137	44	113	32
S2	47	58	46	37	30
S3	33	41	45	38	31

Discussion

In the specific environmental conditions of our experimental design, we observed a double effect of plants in function of distance to the roots. The first one was a selection of bacterial species in the rhizosphere sections that extended up to 6 mm in the presence of PHE and 3 mm in the presence of dBA. In absence of PAH, the plant also modified the composition of bacterial communities up to 3 mm. The second effect was the inhibition of biodegradation of both PAH in the furthest sections and no stimulation by the roots although the bacterial communities were similar to those in the non-polluted soil. Using similar compartmentalized devices, it was demonstrated that the density, the structure of bacterial community, and the degradation of PHE were function of the distance from the roots (Corgié et al. 2003).

Furthermore this study demonstrates that two PAH of different molecular weight and biodegradability induce a different composition of bacterial communities in function of the distance to root surface. In the sections closest to the roots, bacterial species were specific for the rhizosphere and clearly differed from the communities from the unplanted treatments (Figure 2). The density of total heterotrophic bacteria was the highest in these sections in the presence of either PHE or dBA (Figure 1). In the second section (3–6 mm to the roots), bacterial community structure was intermediary to the S1 and the S3 communities when polluted with PHE. This result indicates that the plant still has a selection effect at similar distance from the roots in the presence of PHE. However, it was not the case with dBA treatment where the communities were similar to the ones observed in the non-planted treatment (Figure 2). In the third section (6–9 mm from the roots), with PHE as well as dBA, roots had no selection effect on bacterial communities. However, the number of heterotrophic bacteria was higher in these rhizosphere sections.

In the previously stated device, sorption of PAH to the substrate was expected to be extremely low due to the low reactivity and low hydrophobicity of the sand (the percentages of recovery for both PAH from sand were 99% +/− 0.8%). Photooxidation was not likely to occur in the devices as lateral compartments were kept away from light. Moreover, preliminary analyses showed that PAH were not transferred in the central compartments (data not shown). Binet et al. (2000a, b) have shown that the transfer of PAH to the plants was negligible. Therefore, in the current experimental set-up, the quantification of

the remaining PAH was directly linked to the catalysis of the parent molecules. In unplanted treatments, PHE or dBA were the only source of C available for bacterial growth. The catabolic activities were seemingly high for both compounds with biodegradation reaching 50 and 70% for dBA and PHE respectively (Table 1). PHE and dBA community structures were distinctly different in the non-planted treatments (Figure 2). In the dBA treatment, the number of dBA-degrading bacteria was similar to the number of total heterotrophs and PHE-degraders (Figure 1). This result indicates that dBA-selected communities had members that could use dBA or PHE, or both, as a primary source of carbon. However, it was not the case for communities selected by PHE, where the number of total heterotrophs was higher than PHE degraders and only few members of the PHE-selected community could use dBA as sole source of carbon. A special effort should be undertaken to characterize the bacterial consortium growing on dBA by identifying the species and their abilities to degrade other HMW PAH.

In this study, three different rhizosphere sections differing according the bacterial density, community structure and biodegradation efficiency were observed. The goal of these compartmentalized devices was to simplify the rhizosphere ecosystem to provide more insights about the spatial distribution of bacterial communities and biodegradation activities under two different PAH pollutions. Using these compartmentalized devices, one could gather details on the bacterial composition and biodegradation activities, but the main drawback of these devices is that the results could not be directly extrapolated to more complex ecosystems, such as polluted soils. Compartmentalized and model devices are usually well-suited to understand biological interaction and regulation mechanisms such as spatial rhizosphere gradients. The results of this study are not in direct contradiction with the previously established efficiency of phytoremediation in the field or in soils (Anderson & Coats 1994; Liste & Alexander 2000, Macek et al. 2000; Maier & Soberon-Chavez 2000), they describe some of the microbe-related biodegradation mechanisms that are likely to occur in these far more complex ecosystems. Indeed, most of technology-assessment studies about phytoremediation were performed at different scale and time of sampling with real soils differing in microbial and organic carbon content and diversity. For example, Joner et al. (2002) observed that both biodegradation and extractability of 3, 4 and 5-ring PAH was improved by addition of artificial root exudates (ARE), when supplemented with mineral nutrients. HMW PAH extractability however increased after incubation with ARE whereas biodegradation was not necessarily improved. These results demonstrated the combined effects of sequestration and biodegradation that control the biodegradation of HMW PAH (Tang et al. 1998). Similarly, the bioavailability of PAH is known to decrease via humification processes that could stabilize PAH in the rhizosphere (Walton et al. 1994; Eschenbach & Mahro 2000). On the other hand, increased quantity of organic compounds as well as the production of biosurfactants by plant and bacteria could enhance solubility of PAH (Déziel et al. 1996; Löser et al. 2000). Binet et al. (2000a, b) showed that dissipation of HMW PAH, including dBA, was enhanced in the rhizosphere of ryegrass. This effect was reduced after an ageing period of the artificially contaminated soil. Joner et al. (2001) showed an enhanced dissipation of the 4 and 5 ring PAH, chrysene and dBA, in the rhizosphere of clover. In their experiment, the authors found that 98% of the LMW PAHs were already degraded in unplanted treatments after 8 weeks. After 8 weeks, the dissipation was significantly higher compared to unplanted controls, whereas the dissipation was similar after 16 weeks.

Moreover, Joner and Leyval (2003) observed a rhizosphere zone in contaminated soils, from 0.2 to 0.4 mm to the roots, where the dissipation of PAH was lower compared to the non-planted control. It has been previously reported that there is an inhibition of PHE biodegradation in sections from 6 to 9 mm from the roots (Corgié et al. 2003). In the present study, biodegradation of PHE was lower in the 3–6 and 6–9 mm sections for planted treatments compared to the non-planted treatment (Table 1). PHE biodegradation in the closest section from the roots (0–3 mm) was similar to the non-planted treatment. Biodegradation of dBA was always lower in the presence of the plant. In the conditions under this study, plants clearly inhibited dBA biodegradation (Table 1). However, no significant correlation was observed between biodegradation and the variations in the structure of bacterial communities, or the densities

of bacteria. Culture-based and molecular screening methods usually allow quantifying the most abundant species within a complex bacterial community. For example, most sensitive staining methods (silver nitrate or fluorescent staining) could improve the profiling of these communities especially by revealing minority, but potentially highly active, species during biodegradation processes.

In the presence of dBA, the structures of communities were similar for both planted and unplanted treatments for the sections S2 and S3. Similar results were observed in the S3 sections in the presence of PHE. These results indicate that the inhibition of PHE and dBA biodegradation in the furthest rhizosphere sections is not due to a selection of the microbial species, but rather due to an alteration of their catabolic activities. Analysis of organic carbon in solution confirmed that plants created a gradient of organic carbon from S1 to S3 (Table 2). The growth of PAH-degrading CFU using soluble organic carbon flowing from roots rather than on PAH could however partly explain the decrease of biodegradation activities. Plant $^{14}CO_2$ continuous or flush labeling could be performed to track the precise flow of root organic carbon (Warembourg & Estelrich 2000) and to understand its use by rhizosphere bacteria in the presence of PAH. Indeed, the inhibition of PHE and dBA biodegradation could be a transitory effect. As root exudates have a short life span, and may be mineralized or assimilated in microbial biomass, organic carbon analyze do not reflect the total C exuded by plants during the 5 weeks of incubation. The variations in root exudation and accumulation of organic carbon in the lateral compartments could greatly modify the dynamics of the microbial component compared to the non-planted treatment, and inhibition of bacterial biodegradation processes could be a transitory rhizosphere effect. Therefore, time course experiments should be undertaken to follow the extension of the rhizosphere and its related effects on rhizosphere-selected species, dynamics of bacterial communities and biodegradation activities.

It has been shown that the biodegradation of HMW PAH is enhanced by the unspecific attack of aromatic rings by fungal enzymes such as peroxidases, laccases, and phenol-oxidases (Bogan et al. 1996; Pickard et al. 1999). Catabolism of HMW PAH is enhanced by co-culture of fungi and bacterial consortia (Boochan et al. 2000). In this study, fungi were excluded of our initial consortium. Bacterial catabolism of HMW PAH is supposed to share similar enzymatic reactions with LMW PAH catabolism. Indeed, dihydrodiol metabolites of benzo(a)pyrene were found in *Mycobacterium* cultures (Schneider et al. 1996) suggesting early dioxygenation steps. Degradation of HMW PAH could involve cometabolic processes in monoxenic bacteria cultures. *Burkholderia cepacia* cometabolizes dBA and BaP in the presence of PHE (Juhasz et al. 1997). Kanaly and Bartha (2000) demonstrated that a specific addition of fuel or oil fraction in soil could improve the degradation of BaP by cometabolism whereas pure compounds, such as aliphatic hydrocarbons, alicyclic and aromatic hydrocarbons, have no effect. In the rhizosphere, the diversity and quantity of organic carbon is increased (Rovira 1969) and this organic carbon was expected to favor cometabolism of dBA. However, this study did not find cometabolism to be occurring for dBA since biodegradation was considerably lower in the planted treatments.

Conclusions

The results of this study confirmed that, in function of the distance to roots, the mechanisms of selection for bacterial communities in the rhizosphere (selection of bacterial species and selection of degradation activities) are the result of root exudation gradients as well as the PAH. The distributions of microbial communities and the biodegradation activities should be furthest studied to better understand plant-based technologies for the remediation of PAH contaminated soil. Considering the complexity of the microbial ecology in the rhizosphere and the lack of data on the biodegradation mechanisms of HMW PAH in soil, the use of compartmentalized devices of increasing complexity would provide a better understanding of plant, microbe and pollutant interactions.

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