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RESEARCH ARTICLE

High PAH degradation and activity of degrading bacteria during alfalfa growth where a contrasted active community developed in comparison to unplanted soil

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Abstract

PAH biodegradation in plant rhizosphere has been investigated in many studies, but the timescale of degradation and degrading bacteria activity was rarely considered. We explored the impact of plants on the temporal variability of PAH degradation, microbial abundance, activity, and bacterial community structure in a rhizotron experiment. A historically contaminated soil was spiked with PAHs, planted or not with alfalfa, over 22 days with sampling once a week. In both conditions, most of the spiked PAHs were dissipated during the first week, conducting to polar polycyclic aromatic compound production and to decreased richness and diversity of bacterial communities. We showed a rapid impact of the rhizosphere on PAH degradation via the increased activity of PAH-degrading bacteria. After 12 days, PAH degradation was significantly higher in the planted (100% degradation) than in unplanted (70%) soil. Gram-negative (*Proteobacteria*) PAH-dioxygenase genes and transcripts were higher in planted than unplanted soil and were correlated to the spiked PAH degradation. Conversely, Gram-positive (*Actinobacteria*) PAH-dioxygenase gene transcription was constant over time in both conditions. At 12 days, plant growth favored the activity of many *Gammaproteobacteria* (*Pseudomonadaceae*, *Stenotrophomonas*, and *Acinetobacter*) while in unplanted soil *Alphaproteobacteria* (*Sphingomonadaceae*, *Sphingobium*, and *Magnetospirillum*) and *Actinobacteria* (*Iamia*, *Geodermatophilaceae*, and *Solirubrobacterales*) were more active.

Keywords Historically contaminated soil · Alfalfa · Rhizosphere · RNA · Bacterial diversity · PAH-degraders · Functional community

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic pollutants resulting from coal-related activities (coking plants, gas plants) and derived activities (iron- and steel-production plants). These toxic compounds are persistent in the environment. Their high hydrophobicity, which leads to adsorption to the soil matrix, reduces their bioavailability to microorganisms (Bogan and Sullivan 2003). This aging process decreases PAH bioavailability in soils over time, and slows down the rate of PAH biodegradation (Allard et al. 2000). Microbial diversity also evolves over time, suggesting an adaptation to contamination (Bourceret et al. 2016). Thus, it is essential to better understand the temporal variations in microbial activity, to monitor it, and to relate it to the dynamics of PAHs in soils. To address this, many studies have used soils spiked with

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model PAH compounds (Allard et al. 2000; Thomas and Cébron 2016), but this makes them more bioavailable and therefore more degradable than aged PAHs in historically contaminated soils. Therefore, it appeared to us more realistic to study the biodegradation of a complex mixture of PAHs so as to investigate the activity of autochthonous microbial populations from aged-contaminated soils and involved in biodegradation (Cébron et al. 2013).

Rhizodegradation is a cost-effective and environment-friendly process using microorganisms associated to roots to dissipate soil organic pollutants, such as PAHs (Haritash and Kaushik 2009). Plants release root exudates composed of sugars, amino, and organic acids, among other compounds (Walker et al. 2003; Gao et al. 2015). They provide carbon growth sources for microorganisms and biosurfactant compounds that increase PAH bioavailability and dissipation (Joner et al. 2002; Zhou et al. 2011). In PAH-contaminated soils, root exudates can increase microbial abundance (Kirk et al. 2005) and activity (Cébron et al. 2011). Several studies showed that plants enhanced PAH dissipation by modifying microbial diversity (Kawasaki et al. 2012) and activity (Yergeau et al. 2014; Peng et al. 2015), and selecting PAH degraders (Storey et al. 2014; Siciliano et al. 2003) in the rhizosphere. Consequently, most studies indicated that the combination of all these processes increased PAH degradation in the presence of plants (Liste and Alexander 2000; Tejeda-Agredano et al. 2013) and more precisely in the rhizosphere. However, other experiments also evidenced no effect (Gartler et al. 2014), or slower PAH degradation in planted soil as compared to unplanted soil (Thomas and Cébron 2016). These contrasting results could be explained by spatio-temporal variations that occur within the rhizosphere and activate various processes during plant development. This hypothesis justifies further research on the time course of PAH-degradation processes and on the activity of the functional microbial community in soil-plant systems.

The temporal dynamics of rhizospheric processes could explain the variable efficiency of PAH degradation observed with plants. In uncontaminated soil, it has been extensively shown that the composition and quantity of root exudates can vary depending on the plant developmental stage and on root location (Hinsinger et al. 2005; Walker et al. 2003), and can thus modify the microbial community (Yang and Crowley 2000; Hannula et al. 2010; Baudoin et al. 2002), functional diversity (Haichar F et al. 2008), and microbial activity (Baudoin et al. 2002). On the other hand, these rhizosphere processes have received little attention in contaminated environments where pollution is an additional factor impacting microorganism activity. It is therefore essential to assess the impact of both PAH pollution and root development on overall microbial activity and on the PAH-biodegrading function to know which factors are the most determining ones in a rhizoremediation context.

Among plant species tested for PAH rhizodegradation, the efficiency of legumes e.g., alfalfa is relevant, because they have extensive root systems that allow soil colonization and nutrient uptake (Muratova et al. 2003; D'Orazio et al. 2013). Alfalfa is a plant commonly used in situ during land-farming treatment of organic pollutants (Trapp and Karlson 2001). It is also commonly used in microcosm studies to evaluate rhizodegradation efficiency, and more precisely study the processes involved (Bourceret et al. 2015). A legume such as alfalfa is thus a good and realistic model plant to evaluate the time course of PAH rhizodegradation in aged-contaminated soil.

This study aimed to evaluate (1) the dynamics of PAH dissipation in alfalfa rhizosphere as compared to unplanted soil and (2) the variability of microbial community activity and PAH-degrading bacterial abundance and activity over 3 weeks of plant growth. We developed a specific 2-compartment rhizotron device to rapidly establish an active root system and put it in contact with PAH-spiked soil so as to avoid the time gap between root growth from seed germination and the degradation of bioavailable pollutants. Rhizotrons were filled with an aged PAH-contaminated soil, initially spiked with a complex mixture of PAHs (i.e., a soil organic extract). Rhizotrons were sampled once a week to monitor concentrations of PAHs and potential metabolites (polar polycyclic aromatic compounds, pPAC). Dissolved organic carbon (DOC), pH, and humidity were measured to characterize the rhizosphere and soil conditions. Working on extracted DNA and RNA fractions, we also assessed total bacterial and fungal abundance, PAH-degrading bacterial abundance and transcriptional activity levels. Finally, we assessed active bacterial community structures by pyrosequencing of 16S rRNA transcripts.

Materials and methods

Experimental device

We used an aged PAH-contaminated soil, from a former coking plant (Neuves-Maisons (NM), Lorraine, France) to perform rhizotron experiments. Soil characteristics and spiking procedure were described previously by Bourceret et al. (2015). Briefly, the NM soil (1033 ± 148 mg per kg of dw soil of Σ13 PAHs (fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene, indeno(1,2,3-cd)pyrene) was air-dried, sieved at 2 mm and spiked with a complex organic pollutant extract (Table S1), rich in PAHs and obtained from the same soil using chloroform extraction (Cébron et al. 2013). After solvent evaporation, the spiked soil was mixed with NM soil not exposed to chloroform, in a one-

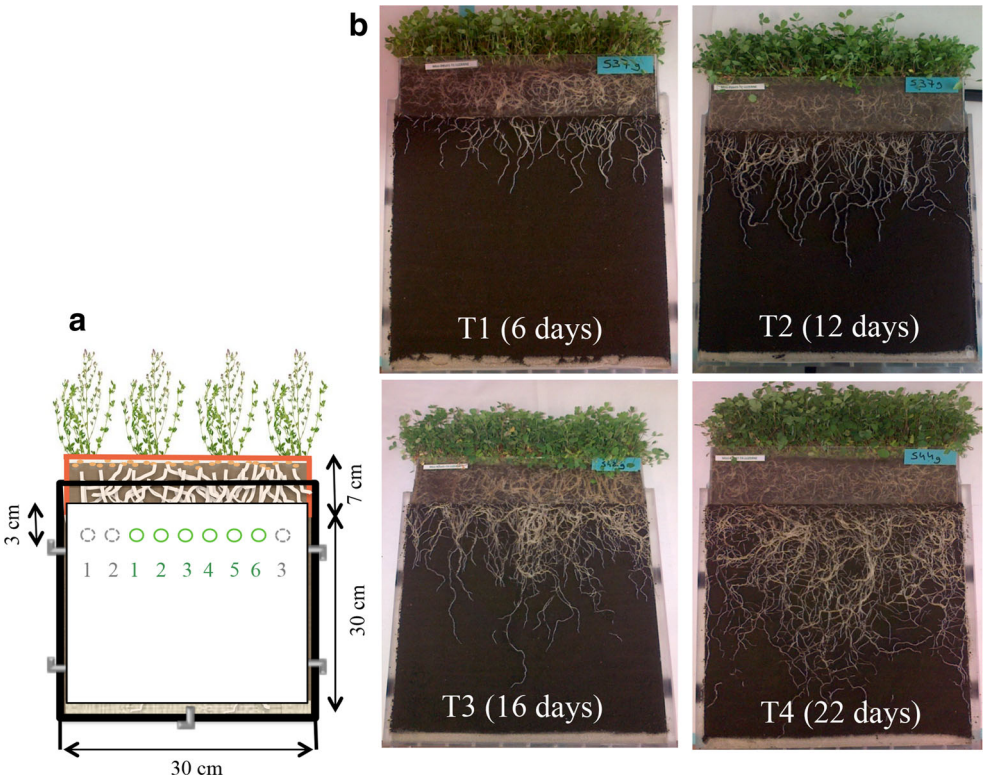

Fig. 1 Experimental devices and sampling points. **a** The device was composed of a mini-rhizotron (in orange) fitting in a corresponding rhizotron (in black). According to the sampling map (in white) at the depth 3 cm, six samples (in green) were collected, three samples (in gray) were used for soil humidity measurements, and four supplemental samples collected between green locations allowed quantification of dissolved organic carbon (DOC). **b** Pictures of alfalfa rhizotron devices after 6, 12, 16, and 22 days of plant growth

tenth ratio. Experimental device was divided in two parts (Fig. 1): the top part (mini-rhizotron) was a poly-methyl methacrylate box (7 cm high × 29.8 cm long × 2.3 cm wide) without bottom, that fits into the second part: a rhizotron (30 cm high × 30 cm long × 2.5 cm wide) with a removable front face allowing spatially localized depth sampling as described by Bourceret et al. (2015). First, eight mini-rhizotrons were filled with NM soil, closed at the bottom by bolting cloth (5 μm mesh), and adjusted to 80% of the soil water holding capacity (WHC) (corresponding to 203 ml deionized water per kg of dw soil). Alfalfa (*Medicago sativa* var. Europe) was planted on four of these devices by seeding 2.4 g of seeds (c.a. 26 seeds per cm^2) and the other four devices were left unplanted. After germination, the eight devices were placed in a plant growth chamber under controlled conditions (22 °C/18 °C day/night, 80% relative humidity, c.a. 250 μmol photons m^{-2} s^{-1}, 16 h photoperiod) and were maintained at 80% of the WHC by weighing the devices every 2 days. The previous growth of plant in NM soil allowed to avoid the delay between time needed for root development and the PAH degradation, that was already shown to be fast in spiked soil with a previous study (Bourceret et al. 2015). After 15 days, when roots reached the bottom of the mini-rhizotron devices, eight rhizotrons were filled with spiked NM soil (1418 ± 193 mg of Σ13 PAHs per kg of dw soil), bolting cloths of the mini-rhizotrons were removed, and devices were fitted in their corresponding rhizotron to allow root penetration and growth in spiked soil. This time corresponded to T0. Initial spiked NM soil was sampled: six aliquots from each of the eight rhizotrons corresponding to 48 T0 samples were stored at − 80 °C for further analyses. Then the four planted and four unplanted devices were incubated in plant growth chamber in conditions described above. After 6 (T1), 12 (T2), 16 (T3), and 22 (T4) days of plant growth, two rhizotrons (one planted and one unplanted) were harvested and opened to allow core sampling using a stainless steel cylinder equipped with a PTFE piston (1.6 cm diameter, 2.5 cm long, thus enabling to collect soil cores of around 5 cm^3). At 3 cm from the interface of the two compartments, six samples with 3 cm between the core centers were collected per device (Fig. 1). Soil samples were prepared as previously described by Bourceret et al. (2015). Briefly, after three rinsing by sterile water, roots were spread on a black paper and photographed to estimate the size of each root fragment using the ImageJ software (http://rsbweb.nih.gov/ij/). Roots were then air-dried at 60 °C and weighed to quantify dry root biomass. Rhizosphere soil, collected from root rinsing water by centrifugation at 4400 rpm for 10 min, and bulk soil were weighed to calculate the ratio between the two fractions and were mixed together to obtain a unique soil sample stored at − 80 °C before analyses: soil characteristics (pH, DOC, organic acids, and sugar concentrations), PAH and pPAC concentrations, and DNA/RNA extractions. Soil humidity was measured by collecting three other samples on the side of the rhizotrons and by weighing the soil before and after drying at 60 °C for 48 h.

Soil characteristics

Soil solutions were prepared with 1 g of soil in distilled water (1:5 *w/v* ratio), and pH was measured using a pH-meter electrode (BioBlock scientific, pHM210 Radiometer analytical). After centrifugation (10 min, 4000 rpm, 20 °C) supernatant was filtered (0.45 μm) and diluted 20 times to quantify dissolved organic carbon (DOC) with a total organic carbon analyzer (TOC-V CSH, Shimadzu). Sugars (inositol, trehalose, sucrose, glucose, fructose) and organic acids (formate, butyrate, oxalate) were quantified from non-diluted extract with an ion-exchange chromatograph ICS 5000 (CarboPac SA10 column, 40 °C) and ICS 3000 (AS 11-HC column, 35 °C), respectively.

Extraction and quantification of total PAHs, pPACs, and bioavailable PAHs

An aqueous solution of hydroxypropyl-β-cyclodextrin (20 ml, 50 mM) (Acros organics, NJ, USA) was used to extract available PAH fraction from 1 g of fresh soil from 5 NM soil samples before spiking, 6 T0 samples, and 48 rhizotron samples (6 samples from both planted and bare rhizotrons at each sampling time) in Oak Ridge centrifuge tubes (FEP, Nalgene, USA) (Reid et al. 2000). After stirring (100 rpm) for 16 h at 24 °C and two centrifugation steps (10,000 rpm, 20 min), dichloromethane was added (24 ml) to the aqueous supernatant (c.a. 30 ml) to extract available PAHs. An Accelerated solvent extractor (ASE350 Dionex ®) was used to extract total PAHs and pPACs with dichloromethane (130 °C, 100 bars) from 0.25 g of soil (stored at − 80 °C) from 12 NM soil samples before spiking, 48 T0 samples, and 48 rhizotron samples previously lyophilized and crushed (< 500 μm). Dichloromethane extracts were split in two fractions to quantify PAHs using HPLC/UV-Fluo (High Pressure Liquid Chromatography) and pPACs using GC-MS (gas chromatography coupled to mass spectrometry). Firstly, the two dichloromethane extracts containing available and total PAHs were evaporated under nitrogen flow and diluted in 5 and 20 ml of acetonitrile, respectively. HPLC (Dionex Ultimate 3000) system, with a 100-mm long and 4.6-mm internal diameter separation column (SupelcoAscendis Express C10), 2.6-mm granulometry allowed quantifying PAHs from 10 μl of extract. From total and available extracts, 13 PAHs (16 US-EPA PAHs excluding the three more volatile molecules: naphthalene, acenaphthylene, and acenaphthene) were quantified by using a UV (254 nm) and a fluorescence detector, respectively. Secondly, from dichloromethane extracts, pPACs were quantified on a GC-6890 (Agilent Technologies) equipped with a DB5-MS column (60 × 0.25 mm id × 0.25-μm film thickness) coupled to a MS-5973 Inert (Agilent Technologies) operating SIM mode (single ion monitoring). The oven temperature program was as follows: 2 min at 70 °C, then from 70 to 130 °C at 15 °C min^{-1}, then from 130 to 315 °C at 4 °C min^{-1} and then a 25-min hold at 315 °C. The carrier gas was helium at 1.4 ml min^{-1} constant flow. Fifteen pPACs including 11 oxygenated pPACs (O-PACs) and 4 nitrogenated pPACs (heterocyclic PACs containing nitrogen N-PACs) were quantified by an internal calibration using deuterated internal standards (the list of the 15 quantified polar PAC is given in Hanser et al. (2015). During a sequence, calibration controls were injected every 12 samples to check the overall calibration.

DNA/RNA extraction

Nucleic acids were extracted from 0.5 g of soil using a FastDNA SPIN Kit for soil (MP Biomedicals, France). DNA and RNA were co-extracted from the same aliquots of samples stored at − 80 °C. DNA was resuspended in 100 μl of DES (Dnase-free water) and quantified at 480 nm (Xenius, SAFAS) by using Quant-iTPicoGreen dsDNA Assay kit (Invitrogen), and then extracts were diluted to 2 ng/μl before amplification. RNA was collected from the filtrates at the DNA binding to the column step. After sodium acetate (0.1Vol, 3 M, pH 5.2) and isopropanol precipitation for one night at 4 °C and centrifugation, RNA was resuspended in 45 μl of Rnase-free water. Two steps of DNase (Thermo scientific) treatment (addition of 10 μl of DNase buffer (10 ×), 1 μl of DNase (1 u/μl) and incubated 1 h at 37 °C) and purification using RNeasyMinElute Cleanup kit (Qiagen) allowed obtaining 30 μl of pure extract (presence of DNA contamination was PCR checked). After quantification at 480 nm (Xenius, SAFAS) by Quant-iTRiboGreen RNA Assay kit (Invitrogen), extracts were converted in cDNA using SuperScript III First-Strand kit (Invitrogen). Reverse transcription reaction (20 μl) was performed using 1 μl of random hexamer (50 ng/μl), 1 μl of hybridization buffer, and 22.27 ng of RNA (5 min at 65 °C and 1 min on ice), then 10 μl of reaction buffer (2 ×) and 2 μl of enzyme mix were added and incubated 10 min at 25 °C, 50 min at 50 °C, and 5 min at 85 °C.

Real-time PCR quantification

The primer sets Fung5F/FF390R (Lueders et al. 2004; Thion et al. 2013), 968F/1401R (Felske et al. 1998; Cébron et al. 2008), and PAH-RHD$_\alpha$GN F/R, PAH-RHD$_\alpha$ GP F/R (Cébron et al. 2008), targeting the fungal 18SrRNA genes, the bacterial 16S rRNA genes, and the PAH-ring hydroxylating dioxygenase genes from Gram-negative and Gram-positive bacteria, respectively, were used to quantify by real-time PCR the abundance of active and total fungal, bacterial, and PAH-degrading bacterial populations. Amplification reactions were performed on 20 μl of reaction mix containing 10 μl of iQ SYBR green SuperMix (Bio-Rad), 0.8 μl of each primer

(10 μM), 0.4 μl of 3% BSA (bovine serum albumin) solution, 0.2 μl of DMSO (dimethyl sulfoxide), 0.08 μl of T4 bacteriophage gene 32 product (MP Biomedicals, France), and 1 μl of diluted gDNA or pure cDNA. Amplification and quantification were achieved using a CFX96 real-time PCR detection system (Bio-Rad) according to standard plasmid dilution series and program described by Cébron et al. (2008) and Thion et al. (2012). After soil humidity correction, data were expressed in number of gene copies per gram of dry weight soil, in percentage of transcripts relative to gene copies, and in percentages of 18S rRNA or PAH-dioxygenase relative to 16S rRNA gene or transcript copies.

Pyrosequencing and sequence analysis

The primer set 515F ((5′-GTG CCA GCM GCC GCG GTA A-3′)/907R (5'-CCG TCA ATT CMT TTR AGT TT-3′)) (Turner et al. 1999) was used to amplify the V4-V5 region of bacterial 16S rDNA from gDNA and cDNA. Samples were identified with a MID (multiplex identifier, Roche). PCR reactions were achieved as described in Bourceret et al. (2016). Beckman Coulter Genomics (Danvers, MA, USA) sequenced libraries using a 454/Roche GS-FLX Titanium system. Data were deposited in the NCBI Sequence Read Archive (SRA) under the BioProject ID: PRJNA324193. Denoised 16S rDNA sequenced were analyzed in QIIME 1.8.0 pipeline (Caporaso et al. 2010) as previously described by Bourceret et al. (2017). Briefly, after filtering and reverse complement non-sense sequences, data from gDNA and cDNA were gathered and clustered in operational taxonomic units (OTUs) at 97% of similarity and their representative sequences were affiliated at different taxonomic levels (from phyla to genus) using RDP classifier (Wang et al. 2007) and the Greengenes 13_5 database (McDonald et al. 2012). Then subsampling was performed to the lowest number of sequences counted in one of the samples (4739 sequences per sample) and alpha-diversity indexes (richness and diversity estimates) were calculated using QIIME 1.8.0 pipeline.

Statistical analyses

Statistical analyses were performed using the XLStat 2013 software (Addinsoft). One-way analysis of variance (ANOVA, with $p < 0.05$) followed by a Newman-Keuls multiple comparison test was performed to measure the time effect in each condition (planted and unplanted) between 0 and 22 days. Two-way analysis of variance (ANOVA, with $p < 0.05$) followed by a Newman-Keuls multiple comparison test were performed to measure the time and plant effect between T1 and T4. Box plots were drawn using PAH data to compare their distribution and variability. Significant differences in relative abundance of dominant OTUs (≥ 1% in at least one sample) over time, were identified in cDNA samples with STAMP version 2.0.8 (Parks and Beiko 2010) by a Tukey Kramer ANOVA, with a Benjamini Hochberg correction of p value ($p < 0.05$ and effect size (ES) > 0.26). Significant differences in the ratio cDNA/gDNA OTU abundance, according to the presence of plant was studied by the Student t test with a Benjamini Hochberg correction of p value with the differential expression function in XLStatOmics package. Canonical correspondence analysis (CCA) followed by a Monte Carlo permutation test (1000 permutations) was performed by using the XLStat-ADA 2015 software (Addinsoft) to study, considering both conditions (alfalfa and unplanted soil) and 4 sampling times (0, 6, 12, 16,s and 22 days), relationship between the relative abundance of the 41 most abundant OTUs (≥ 1% in at least one sample) and environmental variables (root biomass and length, pH, percentage of Gram-negative and Gram-positive PAH dioxygenase transcripts and genes, sugars, organic acids, and PAH content).

Results

Rhizosphere parameters

Humidity stayed constant (around 80% of the WRC) over time in both planted and unplanted soil rhizotrons but was globally significantly higher in unplanted soil (78.36 ± 20.91 and 70.85 ± 9.98%, in unplanted and alfalfa planted soil, respectively). In alfalfa rhizotron, root biomass was significantly ($p < 0.05$) higher after 22 days of plant development than after 6 days, whereas root length significantly increased over time and was significantly different between 6 and 12 days and then between 16 and 22 days (Fig. 2a). Related to the plant growth, several parameters, highlighting the effect of root exudation on soil properties, increased over time. Globally, sugar concentration and DOC increased significantly over time in the planted compared to unplanted soil. Indeed, sugar concentration was significantly higher after 12 days in planted than in unplanted soil where no sugar was detected (Fig. 2b) and DOC stayed constant over time in both rhizotrons until the 16th day, and then increased significantly in planted soil on the 22nd day (Fig. 2c). At this time point, organic acids were detected in alfalfa planted rhizotron (0.21 ± 0.13 mg kg^{-1}) but none in unplanted soil. Surprisingly, soil pH increased significantly over time in both conditions and was stable after 12 days. However, it increased more rapidly in the planted than in the unplanted soil rhizotron with a significant difference at 6 days (Fig. 2d).

PAH and pPAC contents

The total-aged PAH concentration (Σ13 PAHs) of the NM soil (1033 ± 148 mg kg^{-1} dw soil) was significantly increased ($p < 0.05$), i.e., 27%, after soil spiking with the organic pollutant

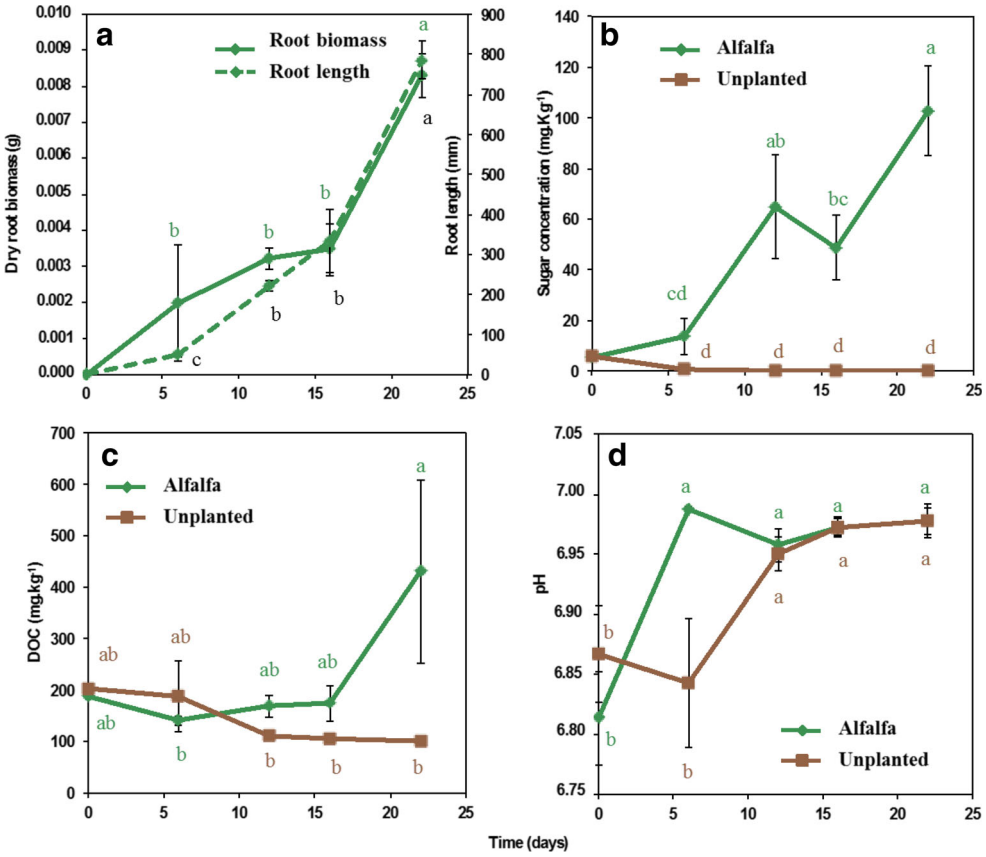

Fig. 2 Soil and root colonization parameters over time. **a** Dry root biomass and length. **b** Sugar content. **c** Dissolved organic carbon (DOC). **d** pH after 6, 12, 16, and 22 days in alfalfa planted (green) and unplanted (brown) soils. Means ($n = 6$ for root biomass and length, and $n = 4$ for pH and DOC) and standard errors. Letters indicate significant ($p < 0.05$) combined effect of time and presence of plant evaluated by using two-way ANOVA followed by Neuman-Keuls multiple comparison test

extract and reached 1418 ± 193 mg kg dw soil^{-1} at the beginning (T0) of the experiment (Fig. 3). The bioavailable PAH fraction was also increased by 41% and reached 63.7 ± 18.0 mg kg^{-1} dw soil, while it represented 37.3 ± 15.6 mg kg^{-1} dw soil before spiking (data not shown). Spiking increased the concentration in total and bioavailable fractions of most of the 13 PAH compounds (Fig. S1). The total PAH concentration decreased significantly between 0 and 6 days and stayed constant until the 22nd day in both planted and unplanted rhizotrons (Fig. 3). From the 6th day, the total PAH concentration dropped down to 1200 ± 210 and 1024 ± 190 mg kg dw soil^{-1} in unplanted and planted soil, respectively, corresponding to a decrease in 19 and 24% relative to the initial concentration, and to 70 and 100% of the spiked PAH fraction, respectively. The concentration in total PAHs was significantly lower in alfalfa than in unplanted soil when considering all times together (from 6 to 22 days, two-way ANOVA) and was mostly explained by a significant difference after 12 days (Student's t test) of incubation (Fig. 3). The concentrations of several compounds decreased significantly from the 6th day (fluorene, phenanthrene, fluoranthene, pyrene, benzo(k)fluoranthene, benzo(a)pyrene, and benzo(g,h,i)perylene only in planted condition)whereas the concentration of other PAHs remained constant over the time course (anthracene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, dibenzo(a,h)anthracene, indeno(1,2,3-cd)pyrene)) or even increased significantly (benzo(g,h,i)perylene) from the 22nd day, in presence of plant (Fig. S2). Bioavailable PAH fraction decreased also significantly between T0 and the 6th day in both conditions, reaching 0.05 ± 0.01 and 0.08 ± 0.03 mg kg^{-1} dw soil, corresponding to ca.100% decreases, for both planted and unplanted soil rhizotron. After 1 week, the bioavailable PAH fraction was significantly lower than in the soil before spiking. Ten oxygenated and one nitrogenous pPAC compounds were detected in initial soil (T0) and in both conditions after 6 days (Table 1). After 6 days, the concentration of four oxygenated pPAC (methylanthracene-9,10-dione, benzoanthracenedione, napthacene-5,12-dione) was significantly higher than at T0, without significant differences between planted and unplanted soils. Benzo(cd)pyrenone was only significantly higher at 6 days than at T0 in the planted rhizotron. The concentration of the other pPAC stayed constant over the first week for both planted and unplanted soils.

Microbial communities

Abundance of active and total microbial communities

Bacterial abundance was higher than fungal, and bacteria were also more active over time in both planted and unplanted soil (mean value of $7.84 \times 10^8 \pm 6.64 \times 10^8$ 16S rRNA gene copies

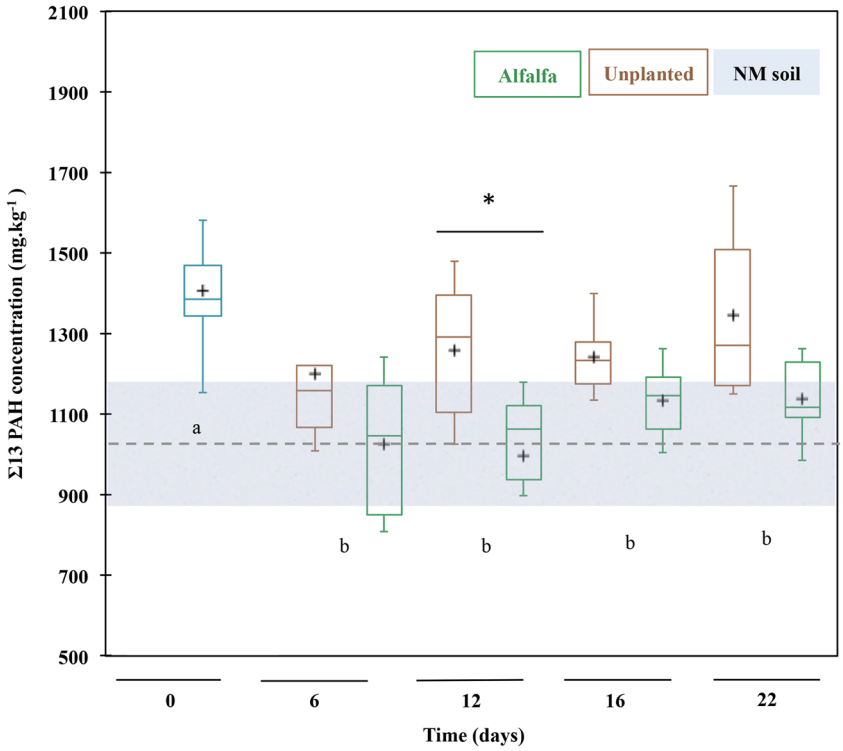

Fig. 3 PAH concentration (sum of 13 PAH, i.e., the 16 US-EPA PAHs, except naphthalene, acenaphthylene, and acenaphthene) after 6, 12, 16, and 22 days in planted (green) and unplanted (brown) soils. Box plots show data variability and means (black cross), $n = 24$ (for T0 samples) and $n = 6$ (from 6 to 22 day samples). Dashed line indicate mean of PAH concentration in the NM soil before spiking. Black asterisks indicate significant differences between planted and unplanted soil (Student's t test, $p < 0.05$) and letters indicate significant differences depending on time (one-way ANOVA)

and $1.12 \times 10^{10} \pm 1.25 \times 10^{10}$ transcript copies g dw soil^{-1}) while fungi were 100 times less represented (mean value of $9.94 \times 10^{6} \pm 7.04 \times 10^{6}$18S rRNA gene copies and $2.05 \times 10^{8} \pm 1.48 \times 10^{8}$ transcript copies g dw soil^{-1}) (Fig. 4a, b). Abundance of 16S rRNA genes tended to increase over time in alfalfa and stayed constant in unplanted rhizotron whereas number of 16S rRNA transcripts increased significantly between T0 and the 6th day for both rhizotrons and then stayed constant. Abundance of 16S rRNA genes and transcripts was significantly higher in planted than unplanted rhizotron and plant effect was mainly detected at 12 (T2) and 22 days (T4). Abundance of 18S rRNA genes and transcripts tended to be constant in both unplanted and planted rhizotrons and no plant effect was observed.

Abundance of Gram-negative PAH-dioxygenase genes and transcripts increased significantly between T0 and the 6th day in both rhizotron types, and were higher in planted than in unplanted soils, with a significant plant effect after 12 days (Fig. 4a, b). Abundance of Gram-positive PAH-dioxygenase genes slightly increased over time whereas transcripts largely increased between T0 and 6 days in both soil and further increased in planted soil till 12 days, resulting in a higher content in planted than in unplanted rhizotron, significant at each sampling time. The level of PAH-dioxygenase gene transcription was expressed by the ratio of the number of transcripts relative to corresponding gene copies (Fig. 4c). The level of PAH-dioxygenase gene transcription was significantly higher in alfalfa than in unplanted soil specifically after 12 days, and then decreased (for Gram-negative bacteria) and stayed constant (for Gram-positive bacteria) over time without significant differences with unplanted soil (Fig. 4c).

Richness, evenness, and diversity of the active community

Globally, bacterial richness (Chao1) and diversity (Inverse of Simpson's) indices were significantly higher in unplanted than in alfalfa planted rhizotron (Table 2). This difference was significant after 16 days for richness and 12 days for diversity index (Table 2). No significant impact of plant was detected on the bacterial community evenness. For both studied conditions, bacterial richness, diversity, and evenness indices decreased significantly during the six first days and after this time point increased significantly over time.

To explore the potential bacterial population involved into the rapid PAH degradation appearing at the beginning of the incubation period, we looked for bacterial community structure modification over time (Fig. 5). Among the active community, 12 phyla were identified and their proportion changed over time. In both rhizotrons, *Proteobacteria* was the more active phylum all over the incubation to the detriment of *Chloroflexi*, *Actinobacteria*, and *Nitrospirae* that represented a lower proportion of the active community along the time course compared to the T0. Activity of *Beta-* and *Gammaproteobacteria* increased between 0 and 6 days and stayed constant or decreased until the end of incubation while the proportion of active *Alpha*-subdivision members further increased. An opposite trend was observed for the activity of *Planctomycetes*, *Acidobacteria*, and *Deltaproteobacteria* that

Table 1 Nitrogenous and oxygenated pPAC concentration (mg kg^{-1}) over time (at T0 and T1) in planted (Alf) and unplanted (BS) soils(Means ± SD, $n = 6$). Letters indicate significant differences in pPAC concentrations between T0 and 6 days, and depending on presence of plant (one-way ANOVA)

Oxygenated pPAC						
Conditions	Dibenzofuran	9H-fluorenone	Anthraquinone	Cyclopenta(def)phenanthrone	Methylanthracene-9.10-dione	Benzo(a) fluorenone
T0	26.6 ± 2.9	26.9 ± 1.4	12.9 ± 0.9	16.1 ± 0.5	2.5 ± 1.6[b]	16.9 ± 1.6
BS_T1	21.9 ± 2.7	23.4 ± 3.1	12.8 ± 1.1	15.8 ± 0.8	6.6 ± 0.1[a]	20.5 ± 1.1
Alf_T1	18.5 ± 1.3	19.8 ± 1.1	12.0 ± 0.4	15.8 ± 0.5	6.7 ± 0.1[a]	21.0 ± 1.0

Oxygenated pPAC					Nitrogenous pPAC
Conditions	Benzanthrone	Benzo anthracenedione	Naphtacene-5.12-dione	Benzo(cd)pyrenone	Carbazole
T0	14.5 ± 2.3	3.4 ± 2.1[b]	4.4 ± 2.8[b]	19.9 ± 2.4[b]	6.4 ± 1.4
BS_T1	16.9 ± 1.1	8.8 ± 0.4[a]	11.2 ± 0.4[a]	23.6 ± 0.6[ab]	6.9 ± 1.5
Alf_T1	18.3 ± 0.7	9.1 ± 0.4[a]	12.0 ± 0.4[a]	25.8 ± 0.9[a]	7.0 ± 0.3

was first repressed between 0 and 12 days and then favored at the end of the incubation (Fig. 5). The relative abundance of some active phyla evolved also differently over time according to the presence of alfalfa. In alfalfa planted rhizotron, *Bacteroidetes* and *TM6* were more and less active over time, respectively, while the activity of *unclassified Proteobacteria* was transiently high and then low over the 22 days. In planted soil, three OTUs affiliated to *Betaproteobacteria* (*Thiobacillus* and Un. *Oxalobacteraceae*), one OTU affiliated to *Alphaproteobacteria* (*Mycoplana*), and one OTU affiliated to *Firmicutes* (*Bacillus selenatarsenatis*) were significantly more abundant among the active community after 6 days of incubation than at all other times, contrary to the unplanted rhizotron where no OTU was favored after 6 days **(**Fig. S3**)**. After 12 days of incubation, three other OTUs belonging to *Alpha-* (*Sphingobium*), *Beta-* (*Janthinobacterium*), and *Gamma-* (PYR10d3) *Proteobacteria* were favored in planted soil. In unplanted rhizotron, five OTUs affiliated to *Alpha-* (*Mycoplana*, *Phenylobacterium*) and *Beta-* (*Thiobacillus*, *unclassified Comamonadaceae*) *Proteobacteria* were significantly more abundant among the active community after 12 days of incubation.

Significant positive or negative impact of plants on shaping active bacterial community was observed on 7 and 18 OTUs after 6 and 12 days of incubation, respectively **(**Fig. 6**)**. After 6 days, the activity ratio between 16S rRNA and rDNA for three OTUs belonging to *Gamma-* (*Pseudomonadaceae*), *Beta-*(*Achromobacter*) *Proteobacteria* and *Firmicutes* (*Paenibacillus*) was significantly higher in alfalfa planted than in unplanted soil. Four other OTUs, affiliated to *Alpha-* (*Un. Sphingomonadaceae*), *Beta-* (*Un. Burkolderiales*) *Protebacteria*, and *Actinobacteria* (*Un. Micrococcaceae*) were more active in unplanted soil. After 12 days, plant growth favored significantly the activity of 11 OTUs belonging to *Alpha-* (*Devosia*), *Beta-* (*Methylophilaceae*), *Gamma-* (*Pseudomonadaceae*, *Stenotrophomonas*, *Acinetobacter*) *Proteobacteria*, *Bacteroidetes* (*Dyadobacter*), and *Actinobacteria* (*Un. Micrococcaceae*). In unplanted soil, the activity of six OTUs affiliated to *Alphaproteobacteria* (*Un. Sphingomonadaceae*, *Sphingobium*, *Magnetospirillum*) and *Actinobacteria* (*Un. Solirubrobacterales*, *Un. Geodermatophilaceae*) was significantly higher than in alfalfa planted soil. After 16 and 22 days of incubation, 56 and 76% of the OTUs whose activity is significantly impacted by the presence of plant were more active in alfalfa planted rhizotron **(**Fig. S4**)**.

Multivariate analysis

The two first axes of the canonical correspondence analysis (CCA) explained 62.5% of bacterial diversity variability among samples **(**Fig. 7a, b**)**. The F1 axis (explaining 41.8% of the variability) separates samples from the T0 (located on the right) to all other sampling times. The F2 axis (explaining 20.8% of the variability) separates samples according to the presence of plant and time, with 6- to 22-day samples from the bottom to the top. Alfalfa-planted samples were separated from unplanted soil samples according to sugar and DOC contents, root development (biomass and length), abundance of Gram-negative PAH-dioxygenase genes and transcripts, and abundance of Gram-positive PAH-dioxygenase transcripts **(**Fig. 7a**)**. These parameters are correlated to the higher activity of 11 bacterial OTUs, most belonging to *Proteobacteria* except one *Bacteriodetes* (*Dyalobacter*), in the alfalfa planted rhizotron **(**Fig. 7b**)**. These OTUs gathered bacteria affiliated to *Pseudomonadales* (*Acinetobacter* and

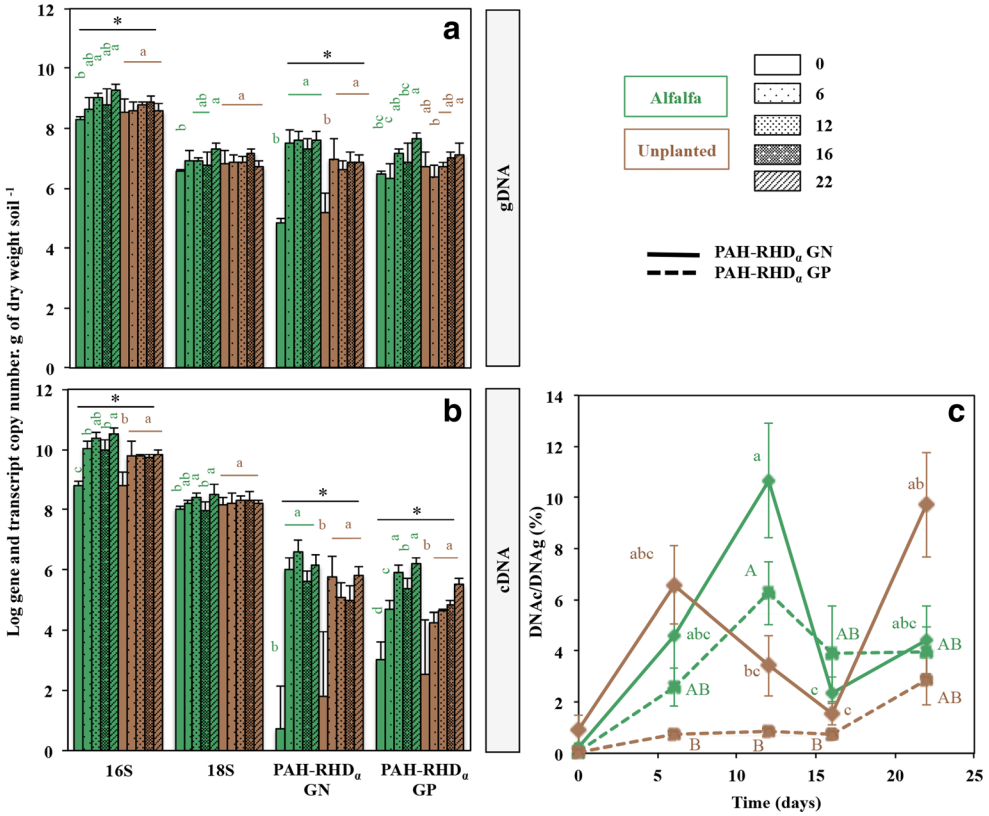

Fig. 4 Abundance values of genes (**a**) and transcripts (**b**) and ratio of transcripts relative to their corresponding genes (**c**) for fungal 18S rRNA, bacterial 16S rRNA, and PAH-degrading bacterial communities after 6, 12, 16, and 22 days in alfalfa planted (green) and unplanted (brown) soils. Values are means and standard deviations of three replicates (T0) and six replicates for 6 to 22 days. The effect of time was evaluated for each condition (planted, unplanted) by using one-way ANOVA ($p < 0.05$) and presence of plant was evaluated between 6 and 22 days by using two-way ANOVA ($p < 0.05$) followed by Neuman-Keuls multiple comparison test. In **a** and **b**, different letters indicate significant changes in microbial community abundance depending on time for each condition, black asterisks indicate plant effect. In **c**, letters indicate cross effect of plant and time on the ratio of transcript relative to gene copies

Table 2 Bacterial richness (Chao1) and diversity estimators (Inverse of Simpson's index and Evenness) in unplanted soil (BS) and soil planted with alfalfa (Alf) at the beginning (T0) and after 6 , 12 , 16 and 22 days of incubation from cDNA data (Means ± SD, $n = 4$). The plant effect was tested (from 6 to 22 days) using a two-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (letters represents groups with significant different values ($p < 0.05$)). One-way ANOVA was used to test time effect (from T0 to 22 days), separately for each condition

Samples	Conditions	Time (days)	Chao1	Inverse of Simpson's index (1/day)	Evenness (E)
BS_T0	Bare soil	0	597 ± 39	27.71 ± 4.70	0.731 ± 0.016
BS_T1	Bare soil	6	425 ± 56^{cd}	11.49 ± 8.90^{bc}	0.593 ± 0.124^{b}
BS_T2	Bare soil	12	467 ± 28^{abc}	28.10 ± 4.55^{a}	0.724 ± 0.021^{a}
BS_T3	Bare soil	16	529 ± 28^{ab}	24.90 ± 4.04^{a}	0.717 ± 0.016^{a}
BS_T4	Bare soil	22	541 ± 30^{a}	21.26 ± 2.24^{ab}	0.707 ± 0.010^{a}
Alf_T0	Alfalfa	0	637 ± 29	26.81 ± 2.52	0.729 ± 0.005
Alf_T1	Alfalfa	6	353 ± 39^{d}	7.80 ± 2.89^{c}	0.585 ± 0.043^{b}
Alf_T2	Alfalfa	12	448 ± 41^{bc}	12.98 ± 5.83^{bc}	0.643 ± 0.048^{ab}
Alf_T3	Alfalfa	16	427 ± 67^{cd}	19.69 ± 8.16^{ab}	0.693 ± 0.045^{a}
Alf_T4	Alfalfa	22	485 ± 40^{abc}	25.62 ± 3.16^{a}	0.734 ± 0.009^{a}
ANOVA 2F	Plant effect (T1-T4) letters		BS > Alf	BS > Alf	n.s.
ANOVA 1F	Time effect (T0-T4) BS		T1, T2 < others	T1 < others	T1 <others
ANOVA 1F	Time effect (T0-T4) Alf		T0 > others T1 < others	T1 < others	T1 < others

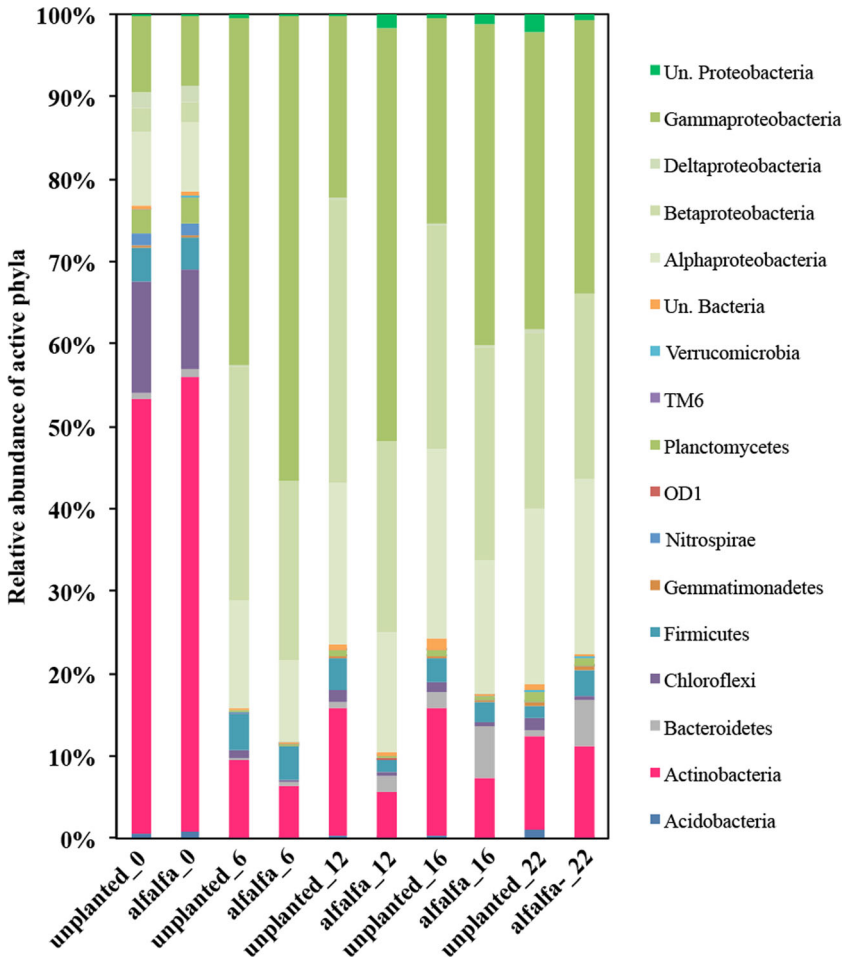

Fig. 5 Relative proportion (mean, $n = 4$) of dominant active bacterial phyla (≥ 1% in at least one sample) in alfalfa planted (Alf) and unplanted soil (BS) at 0, 6, 12, 16, and 22 days of incubation. The 12 significant phyla were identified by a Tukey Kramer ANOVA with a Benjamini Hochberg correction of p value ($q < 0.05$, and effect size > 0.26)

Pseudomonas), *Sphingomonadales* (*Sphingobium*), *Burkolderiales* (*Janthinobacterium* and *Achromobacter*), and *Rhizobiales* (*Un. Methylobacteriaceae*) orders. Unplanted soil samples were mostly discriminated according to the higher PAH content (∑13 PAHs and 2–3, 4, and 5–6 cycles). According to the Pearson correlation matrix (Table S2), pH and the root biomass and length were positively and significantly correlated to the percentage of Gram-positive PAH-dioxygenase transcripts contrary to the abundance of 18S transcripts, showing a negative correlation. Moreover, concentrations of 2–3, 4- rings and ∑13PAHs were significantly and negatively correlated to the percentage of Gram-negative PAH-dioxygenase transcripts, shown as the most active degrading community at the beginning of the monitoring.

Discussion

Rapid PAH dissipation in a spiked aged-contaminated soil

After 6 days of incubation, 100 and 70% of the spiked PAH fraction was dissipated in the planted and unplanted rhizotrons, respectively. A similar rapid dissipation of bioavailable PAHs after a few days was previously observed in simplified sand or soil microcosms spiked with phenanthrene (Louvel et al. 2011; Cébron et al. 2011), but never observed with such a complex PAH contaminant mixture. We evidenced the degradation of almost all spiked PAHs including fluorene, phenanthrene, fluoranthene, pyrene, benzo(k)fluoranthene, and benzo(a)pyrene (which have from two to five aromatic cycles) in both conditions. In the same soil (NM) incubated in bioslurry, 30 to 55% of the aged PAH compounds (fluorene, phenanthrene, fluoranthene, pyrene, benzo(a)anthracene, and chrysene) were degraded after 1.5 months of incubation (Biache et al. 2017), indicating the presence of a very effective microbial community for the degradation of a wide range of PAH compounds. Degradation of PAHs by microbial consortia and co-metabolism are often described as playing crucial roles in the degradation of PAH mixtures in the environment (Ghosal et al. 2016) because the presence of low-molecular-weight PAHs could have a synergistic effect on the degradation of more recalcitrant compounds (van Herwijnen et al. 2003; Roy et al. 2013). During the first 6 days of the experiment, spiked PAHs were probably the main available carbon sources in both the planted and

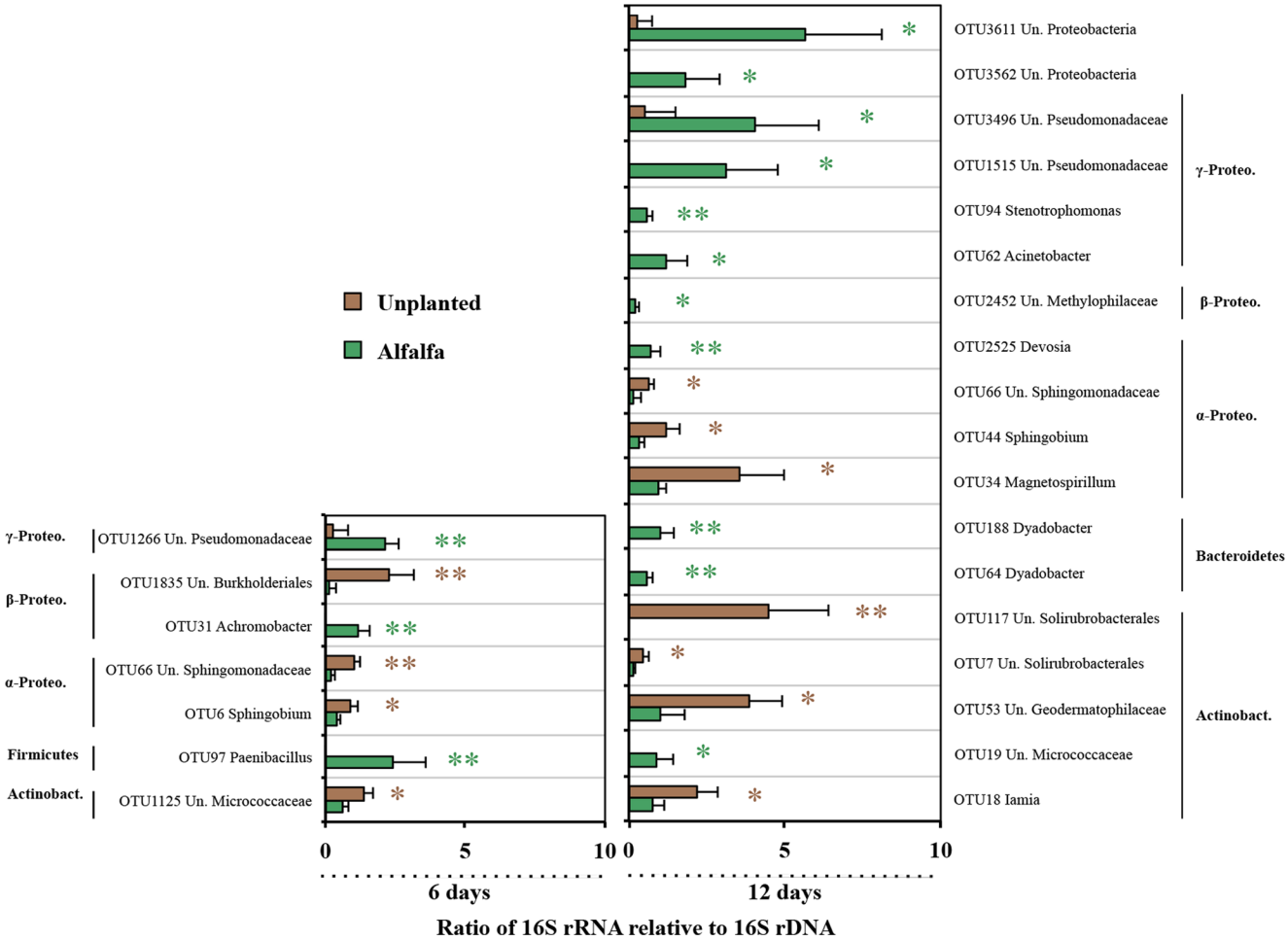

Fig. 6 Ratio of the 16S rRNA relative to 16S rDNA for OTU (≥ 10 sequences in one sample) significantly discriminating plant effect, after 6 and 12 days of incubation. Both conditions were compared using Student's *t* tests with Benjamini-Hochberg correction of *p* value. Asterisks denote degree of significance (green and brown for higher ratio with alfalfa and unplanted soils, respectively; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$). OTUs were affiliated at 97% of similarities to the deeper taxonomic level, leading to affiliations at different levels

unplanted rhizotrons, as no increase in DOC or sugar content was observed in either condition. As the microbial community of the aged-contaminated NM coking plant soil was well adapted to PAH pollutants (Cébron et al. 2009; Bourceret et al. 2016), the bacterial community was rapidly activated, and the spiked PAH fraction was in turn rapidly biodegraded. The main parameter that limited the biodegradation of aged PAHs in this soil was their low bioavailability (Cébron et al. 2013) and spiking transiently led to more easily biodegradable PAHs. In our experiment, before being irreversibly adsorbed to soil mineral or organic materials, such as clay, black carbon, or coal-tar substances (Ghosal et al. 2016), PAHs were rapidly degraded, proving that their availability is crucial for biodegradation. Besides PAH degradation, oxygenated polar compounds (pPAC) were produced during the first week of the experiment. This kind of bioavailable compounds, previously described in water from industrial wasteland (Rollin et al. 2005; Lundstedt et al. 2006) and in coal tar (Northcott and Jones 2001; Benhabib et al. 2010), could result from biotic PAH degradation. Lundstedt et al. (2007) indeed described the production of oxygenated compounds during microbial biodegradation, with apparent concentrations resulting from the balance between pPAC degradation and pPAC production during PAH biodegradation (Biache et al. 2017). The production of oxygenated pPAC that is possibly more available and more toxic than parent molecules could contribute to shape the bacterial community structure.

Together with PAH biodegradation, we observed that bacterial community abundance and activity rapidly increased during the first 6 days of incubation. Moreover, the community composition was drastically modified in both planted and unplanted conditions. Most of bacterial OTUs with increasing activity were affiliated to *Beta-* and *Gammaproteobacteria*, at the expense of *Actinobacteria* whose proportions fell sharply. PAH amendment may have caused a shift of bacterial communities from oligotroph, K-strategist, and Gram-positive bacteria (such as *Actinobacteria*) to copiotroph, r-strategist, and Gram-negative bacteria (such as *Proteobacteria*)

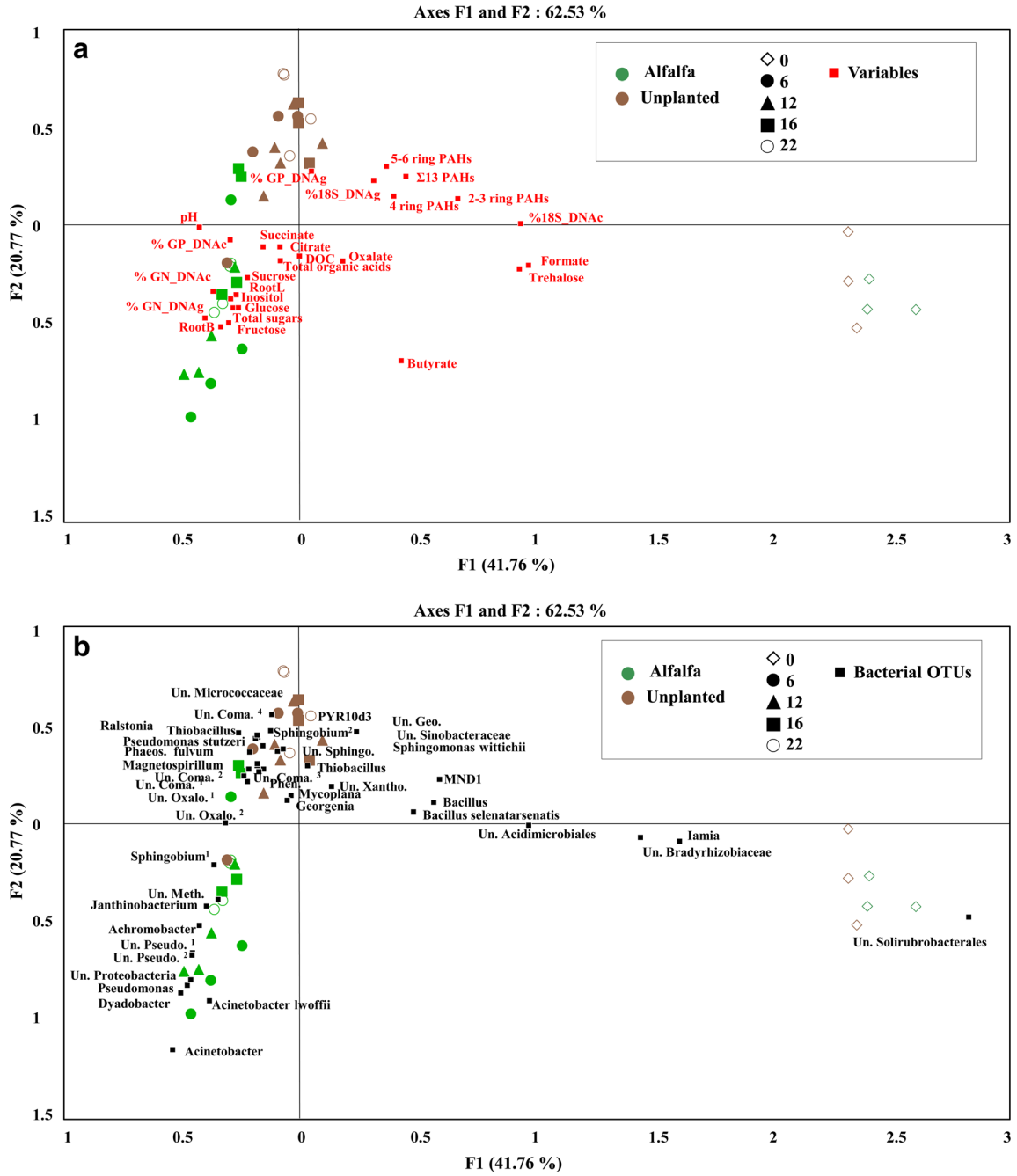

Fig. 7 Canonical correspondence analysis (CCA) ordination including abiotique (**a**) and biotique (**b**) variables. **a** Environmental variables (in red) (DOC, sugar and organic acid contents, pH, percentage of 18S rRNA, PAH-RHD$_{\alpha}$ GN and GPgenes, and transcripts relative to 16S rRNA), root biomass (RootB) and length (RootL), sum of 13 PAH concentrations, 2–3-ring PAHs (fluorene, phenanthrene, anthracene), 4-ring PAHs (fluoranthene, pyrene, benzo(a)anthracene, chryzene), 5–6-ring PAHs (benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene, indeno(1,2,3-cd)pyrene), and **b** the relative abundance of dominant active bacterial OTUs (≥ 1% in at least one sample, taxonomic affiliation at 97% of similarities to the deeper taxonomic level, leading to affiliations at different levels) (in black) were used to discriminate the 24 samples (4 replicates for each sampling time corresponding to 0, 6, 12, 16, and 22 days) collected for alfalfa planted (green) and unplanted (brown) rhizotrons. A Monte Carlo permutation test (1000 permutations) was performed and showed a significant ($p < 0.0001$) effect of environmental variables on the relative abundance of dominant OTUs. Un. Coma., Un. Comamonadaceae; Un. Pseudo., Un. Pseudomonadaceae; Un.Sphingo., Un. Sphingomonadaceae; Un. Geo., Un. Geodermatophilaceae; Un. Meth., Un. Methylobacteriaceae; Phen., Phenylobacterium; Phaeos. Fulvum, PhaeospirillumFulvum; Un. Xantho., Un. Xanthomonadaceae; Un. Oxalo., Un. Oxalobacteraceae

(Bevivino and Dalmastri 2017). These *Proteobacteria* are potentially Gram-negative PAH-degraders. We did find a predominance of bacteria known to be involved in PAH degradation, such as OTUs affiliated to the *Achromobacter*,

Janthinobacterium, Pyr10d3, *Sphingobium*, and *Thiobacillus* genera and *Sphingomonadaceae*, *Microccocaceae*, and *Comamonadaceae* families (Fernández-Luqueño et al. 2011; Kunihiro et al. 2013). The abundance and transcription level of PAH-dioxygenase genes increased significantly during the first 6 days in both conditions, confirming the higher potential of PAH bacterial degradation. Greater activity of Gram-negative than Gram-positive (*Actinobacteria*) PAH degraders was evidenced during these early days, indicating that *Proteobacteria* were most probably the main phylum involved in the biodegradation of the spiked PAHs. This finding was also supported by a higher activity of *Proteobacteria* as compared to other taxa in both planted and unplanted soils, and by a negative correlation between the activities of Gram-negative PAH-degrading bacteria and PAH contents. Several studies indicated that PAH contamination induces a selection of and an enrichment in PAH degraders (Cébron et al. 2008; Zhang et al. 2013; Sawulski et al. 2014), and also up-regulates the transcription of the PAH-dioxygenase gene in pure culture (Kim et al. 2006) or complex communities (Louvel et al. 2011). A negative correlation between the transcription of PAH-dioxygenase genes of Gram-negative bacteria and PAH contents would confirm the major involvement of this population in early pollutant degradation, although we cannot rule out the involvement of Gram-positive *Actinobacteria* that could be the main active PAH-degraders after PAH spiking (de Menezes et al. 2012). Furthermore, fungi could also be involved in PAH degradation (Cerniglia and Sutherland 2010), but we did not detect any increase of fungal 18S rRNA gene or transcript copies during incubation in either condition, suggesting a fairly limited involvement of these microorganisms in our soil.

Plant effect

The presence of plants induced a shift in the active bacterial community structure and the PAH-degrading population. The timescale of the experiment (22 days) allowed us to monitor the root development of alfalfa, and compare processes between planted and unplanted soils. In addition to increasing the PAH biodegradation capacity, alfalfa plants globally increased the abundance and activity of bacteria, the abundance of Gram-negative PAH-degrading bacteria and the expression of both Gram-negative and Gram-positive PAH-dioxygenase genes. However, the presence of plants led to a decrease of the bacterial richness and diversity indexes. This finding contrasts with previous observation from the same soil (NM) spiked with phenanthrene and planted with ray-grass. The authors found that plants indeed induced an increase of bacterial species richness and slightly inhibited phenanthrene degradation, even if the abundance of PAH-dioxygenase genes increased, yet without modification of the transcription level as compared to unplanted soil (Thomas and Cébron 2016). These contrasting results can be explained by two different types of contamination, i.e., a PAH mixture vs. phenanthrene, and two different plant species with contrasted root systems and root exudation (Bourceret et al. 2015). Root exudates probably partly inhibit the degradation of one sole PAH compound, while they might help in co-metabolism processes when PAH contamination is complex and contains high-molecular-weight compounds (Rentz et al. 2005) as shown for PCB degradation (Vrchotová et al. 2013).

After 6 days, even if root biomass and length were low, the presence of alfalfa had already modified a few parameters. The pH, the abundance, and proportion of active Gram-negative PAH-degrading bacteria were significantly higher in the planted rhizotron than in the unplanted one. Similar findings about PAH-degrading bacterial activity were reported in batch experiments with sand spiked with phenanthrene (Louvel et al. 2011). After 6 days, plants had already shaped the bacterial community structure: several OTUs belonging to *Beta-* and *Gammaproteobacteria* were significantly more active in the planted rhizotron, while other OTUs belonging to *Alpha-*, *Betaproteobacteria*, and *Actinobacteria* were favored in the unplanted rhizotron. In our experiment, after 12 days, root length and sugar content were significantly higher than those after 6 days, and root biomass and DOC tended to increase, suggesting a stronger rhizosphere effect. At this sampling time, significantly higher PAH-degradation was observed in the planted rhizotron than in the unplanted one. This could be linked to an increasing level of PAH-dioxygenase transcription in both Gram-negative and Gram-positive PAH-degrading bacterial populations that was significantly higher in the planted soil than in the unplanted one. Our results clearly show a positive and rapid impact of the rhizosphere on the PAH degradation potential via the increased activity of a wider diversity (both GN and GP) of PAH-degrading bacteria. Even if *Actinobacteria* were enriched in the unplanted soil, the activity of the PAH degraders belonging to this phylum was favored in the rhizosphere. The higher activity of Gram-positive PAH degraders could help in the degradation of more recalcitrant PAH compounds (Kanaly and Harayama 2000). We highlighted a time-dependent effect in the course of the process dynamics, which could explain why some authors did not observe an increase in the activity of PAH-degrading populations in certain rhizospheres (Kawasaki et al. 2015). The addition of artificial root exudates can increase the density of soil PAH degraders, with an increase (Joner et al. 2002) or no difference (Cébron et al. 2011) in the degradation of spiked PAHs. We showed the activity of growing roots in a more natural rhizosphere system. A rhizotron device is probably the most adapted laboratory system to study the dynamics of rhizosphere processes because it makes it possible to perform analyses on precisely located samples with specific characteristics (depth, root density, roots age…) instead of mean rhizosphere samples usually

obtained from pot experiments. Rhizotrons can allow studying the root turnover (Leigh et al. 2002), the isothiocyanate degradation rate, microbial communities (Rumberger and Marschner 2003), or spatial heterogeneity of PAH degradation and microbial communities (Bourceret et al. 2015).

In our study, the carbon released by actively growing roots and also leached from the top part of the device presenting a higher root density may have activated PAH degradation through co-metabolism (Ambrosoli et al. 2005). This phenomenon could also be attributed to a priming effect, whereby the release of labile carbon stimulates microbial density and activity (Haichar F et al. 2014), promoting PAH degradation or biotransformation (Rentz et al. 2005). As shown by the canonical analysis the higher differentiation between the planted and unplanted conditions was observed after 6 and 12 days of incubation. Root growth seemed to activate bacterial population mostly belonging to *Proteobacteria*, such as OTUs affiliated to the *Gamma-* (*Pseudomonadaceae*, *Stenotrophomonas*, and *Acinetobacter*), *Alpha-* (*Sphingobium*, *Devosia*), and *Beta-*(*Methylophilaceae*, *janthinobacterium*, and *Achromobacter*) subdivisions. In addition to their ability to degrade PAHs (Fernández-Luqueño et al. 2011; Tauler et al. 2015; Gupta et al. 2015), OTUs affiliated to *Proteobacteria* can be favored in the presence of plants as compared to unplanted soil (Thomas and Cébron 2016; Thomas et al. 2016). On the contrary, in unplanted soil, other members of the *Alpha* (*Sphingomonadaceae*, *Sphingobium*, *Sphingomonas*, *Mycoplana*, *Magnetospirillum*) and Beta (*Ralstonia*, *Oxalobacteraceae*, *Comamonadaceae*, *Thiobacillus*) subdivisions were favored, as well as *Actinobacteria* (*Iamia*, *Geodermatophilaceae*, *Georgenia*, *and Micrococcaceae*).

After 16 and 22 days of root development, when no further PAH dissipation was observed, the transcriptional level of the PAH-dioxygenase gene from Gram-negative and Gram-positive bacteria decreased or remained constant over time in both planted and unplanted soils. At these time points, root development seemed to mainly influence the structure of the bacterial community. The proportion of many OTUs indeed differed between the planted and unplanted soils, but organic pollutant degradation no longer did.

Conclusion

Our results suggest that the dissipation of spiked PAHs is very fast in the soil when these pollutants represent the main available carbon sources for microbial communities and PAH-degrading bacteria are present. The presence of plants, which release root exudates, could favor microbial degradation by co-metabolism and increase the PAH degradation yield. The transcription of the PAH-dioxygenase gene was higher in the presence of plants than in unplanted soil, and transcription from Gram-negative bacteria (*Proteobacteria*) was high during the first 12 days when most of the PAHs were degraded. In the planted condition, Gram-positive PAH-degrading bacteria (*Actinobacteria*) were also highly active, and probably contributed to the higher level of PAH degradation in the rhizosphere. The characterization of the bacterial community structure and activity over time indicated different dynamics between the two conditions, with *Beta-* and *Gammaproteobacteri*a mostly active in planted soil while *Alphaproteobacteria* and *Actinobacteria* were more active in unplanted soil.

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