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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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Key words: historically contaminated soil, alfalfa, rhizosphere, RNA, bacterial diversity, PAH-degraders, functional community

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 favoured the

activity of many *Gamma-Proteobacteria* (*Pseudomonadaceae*, *Stenotrophomonas* and *Acinetobacter*) while in unplanted soil *Alpha-Proteobacteria* (*Sphingomonadaceae*, *Sphingobium* and *Magnetospirillum*) and *Actinobacteria* (*Iamia*, *Geodermatophilaceae*, and *Solirubrobacterales*) were more active.

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 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 is 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 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 three 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 seeds 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-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 x 29.8 cm long x 2.3 cm wide) without bottom, that fits into the second part: a rhizotron (30 cm high x 30 cm long x 2.5 cm wide) with a removable front face allowing spatially localized depth sampling as described by Bourceret et al. (2015). First, 8 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 4 of these devices by seeding 2.4 g of seeds (c.a. 26 seeds per cm^2) and the other 4 devices were left unplanted. After germination, the 8 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 hours photoperiod) and were maintained at 80% of the WHC by weighing the devices every two 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

130 rhizotrons were filled with spiked NM soil (1418 ± 193 mg of Σ13 PAHs per kg of dw soil),
131 bolting cloths of the mini-rhizotrons were removed and devices were fitted in their
132 corresponding rhizotron to allow root penetration and growth in spiked soil. This time
133 corresponded to T0. Initial spiked NM soil was sampled: six aliquots from each of the eight
134 rhizotrons corresponding to 48 T0 samples were stored at -80°C for further analyses. Then the
135 4 planted and 4 unplanted devices were incubated in plant growth chamber in conditions
136 described above. After 6 (T1), 12 (T2), 16 (T3) and 22 (T4) days of plant growth, two
137 rhizotrons (one planted and one unplanted) were harvested and opened to allow core sampling
138 using a stainless steel cylinder equipped with a PTFE piston (1.6 cm diameter, 2.5 cm long,
139 thus enabling to collect soil cores of around 5 cm^3). At 3 cm from the interface of the two
140 compartments, 6 samples with 3 cm between the core centers were collected per device **(Fig.**
141 **1)**. Soil samples were prepared as previously described by Bourceret et al. (2015). Briefly,
142 after 3 rinsing by sterile water, roots were spread on a black paper and photographed to
143 estimate the size of each root fragment using ImageJ software (http://rsbweb.nih.gov/ij/).
144 Roots were then air-dried at 60°C and weighed to quantify dry root biomass. Rhizosphere
145 soil, collected from root rinsing water by centrifugation at 4400 rpm for 10 minutes, and bulk
146 soil were weighed to calculate the ratio between the two fractions and were mixed together to
147 obtain a unique soil sample stored at -80°C before analyses: soil characteristics (pH, DOC,
148 organic acids, and sugar concentrations), PAH and pPAC concentrations, and DNA/RNA
149 extractions. Soil humidity was measured by collecting 3 other samples on the side of the
150 rhizotrons and by weighing the soil before and after drying at 60°C for 48 hours.

151 **Soil characteristics**

152 Soil solutions were prepared with 1 g of soil in distilled water (1:5 w/v ratio), and pH was
153 measured using a pH-meter electrode (BioBlock scientific, pHM210 Radiometer analytical).
154 After centrifugation (10 min, 4000 rpm, 20°C) supernatant was filtered (0.45 µm) and diluted
155 20 times to quantify dissolved organic carbon (DOC) with a total organic carbon analyzer
156 (TOC-V CSH, Shimadzu). Sugars (inositol, trehalose, sucrose, glucose, fructose) and organic
157 acids (formate, butyrate, oxalate) were quantified from non-diluted extract with an ion-
158 exchange chromatograph ICS 5000 (CarboPac SA10 column, 40°C) and ICS 3000 (AS 11-
159 HC column, 35°C), respectively.

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

161 An aqueous solution of hydroxypropyl-β-cyclodextrin (20 ml, 50 mM) (Acros organics, New
162 Jersey, USA) was used to extract available PAH fraction from 1 g of fresh soil from 5 NM

163 soil samples before spiking, 6 T0 samples, and 48 rhizotron samples (6 samples from both
164 planted and bare rhizotrons at each sampling time) in Oak Ridge Centrifuge tubes (FEP,
165 Nalgene, USA) (Reid et al. 2000). After stirring (100 rpm) for 16 hours at 24 °C and 2
166 centrifugation steps (10000 rpm, 20 min), dichloromethane was added (24 ml) to the aqueous
167 supernatant (c.a. 30 ml) to extract available PAHs. An Accelerated Solvent Extractor
168 (ASE350 Dionex ®) was used to extract total PAHs and pPACs with dichloromethane
169 (130°C, 100 bars) from 0.25 g of soil (stored at -80°C) from 12 NM soil samples before
170 spiking, 48 T0 samples, and 48 rhizotron samples previously lyophilized and crushed (<500
171 µm). Dichloromethane extracts were split in two fractions to quantify PAHs using HPLC/UV-
172 Fluo (High Pressure Liquid Chromatography) and pPACs using GC-MS (gas chromatography
173 coupled to mass spectrometry). Firstly, the two dichloromethane extracts containing available
174 and total PAHs were evaporated under nitrogen flow and diluted in 5 ml and 20 ml of
175 acetonitrile, respectively. HPLC (Dionex Ultimate 3000) system, with a 100-mm long and
176 4.6-mm internal diameter separation column (SupelcoAscendis Express C10), 2.6-mm
177 granulometry allowed quantifying PAHs from 10 µl of extract. From total and available
178 extracts, 13 PAHs (16 US-EPA PAHs excluding the three more volatile molecules:
179 naphtalene, acenaphtylene and acenaphtene) were quantified by using a UV (254 nm) and a
180 fluorescence detector, respectively. Secondly, from dichloromethane extracts pPACs were
181 quantified on a GC-6890 (Agilent Technologies) equipped with a DB5- MS column (60 ×
182 0.25 mm id × 0.25µm film thickness) coupled to a MS-5973 Inert (Agilent Technologies)
183 operating SIM mode (Single Ion Monitoring). The oven temperature program was as follows:
184 2 minutes at 70 °C, then from 70 °C to 130 °C at 15 °C min^{-1}, then from 130 °C to 315 °C at 4
185 °C min^{-1} and then a 25 min hold at 315 °C. The carrier gas was helium at 1.4 ml min^{-1}
186 constant flow. Fifteen pPACs including 11 oxygenated pPACs (O-PACs) and 4 nitrogenated
187 pPACs (heterocyclic PACs containing nitrogen N-PACs) were quantified by an internal
188 calibration using deuterated internal standards (the list of the 15 quantified polar PAC is given
189 in Hanser et al. (2015). During a sequence, calibration controls were injected every 12
190 samples to check the overall calibration.

191 **DNA/RNA extraction**

192 Nucleic acids were extracted from 0.5 g of soil using a FastDNA SPIN Kit for soil (MP
193 Biomedicals, France). DNA and RNA were co-extracted from the same aliquots of samples
194 stored at -80°C. DNA was resuspended in 100 µl of DES (Dnase-free water) and quantified at
195 480 nm (Xenius, SAFAS) by using Quant-iTPicoGreenasDNA Assay kit (Invitrogen), and

196 then extracts were diluted to 2 ng/µl before amplification. RNA was collected from the
197 filtrates at the DNA binding to the column step. After sodium acetate (0.1Vol, 3M, pH 5.2)
198 and isopropanol precipitation for one night at 4°C and centrifugation, RNA was resuspended
199 in 45 µl of Rnase-free water. Two steps of DNase (Thermo scientific) treatment (addition of
200 10 µl of DNase buffer (10X), 1 µl of DNase (1u/µl) and incubated 1 hour at 37°C) and
201 purification using RNeasyMinElute Cleanup kit (Qiagen) allowed obtaining 30 µl of pure
202 extract (presence of DNA contamination was PCR checked). After quantification at 480 nm
203 (Xenius, SAFAS) by Quant-iTRiboGreen RNA Assay kit (Invitrogen), extracts were
204 converted in cDNA using SuperScript III First-Strand kit (Invitrogen). Reverse transcription
205 reaction (20 µl) was performed using 1 µl of random hexamer (50 ng/µl), 1 µl of hybridization
206 buffer and 22.27 ng of RNA (5 min at 65°C and 1 min on ice), then 10 µl of reaction buffer
207 (2X) and 2 µl of enzyme mix were added and incubated 10 min at 25°C, 50 min at 50°C and 5
208 min at 85°C.

209 **Real-time PCR quantification**

210 The primer sets Fung5F/FF390R (Lueders et al. 2004; Thion et al. 2013), 968F/1401R (Felske
211 et al. 1998; Cébron et al. 2008) and PAH-RHD$_{\alpha}$GN F/R, PAH-RHD$_{\alpha}$ GP F/R (Cébron et al.
212 2008), targeting the fungal 18SrRNA genes, the bacterial 16S rRNA genes, and the PAH-ring
213 hydroxylating dioxygenase genes from Gram-negative and Gram-positive bacteria,
214 respectively, were used to quantify by real-time PCR the abundance of active and total fungal,
215 bacterial and PAH-degrading bacterial populations. Amplification reactions were performed
216 on 20 µl of reaction mix containing 10 µl of iQ SYBR green SuperMix (Bio-Rad), 0.8 µl of
217 each primer (10 µM), 0.4 µl of 3% BSA (bovine serum albumin) solution, 0.2 µl of DMSO
218 (dimethyl sulfoxide), 0.08 µl of T4 bacteriophage gene 32 product (MP Biomedicals, France)
219 and 1 µl of diluted gDNA or pure cDNA. Amplification and quantification were achieved
220 using a CFX96 Real-Time PCR detection system (Bio-Rad) according to standard plasmid
221 dilution series and program described by Cébron et al. (2008) and Thion et al. (2012). After
222 soil humidity correction, data were expressed in number of gene copies per gram of dry
223 weight soil, in percentage of transcripts relative to gene copies, and in percentages of 18S
224 rRNAor PAH-dioxygenase relative to 16S rRNA gene or transcript copies.

225 **Pyrosequencing and sequence analysis**

226 The primer set 515F ((5'-GTG CCA GCM GCC GCG GTA A-3') / 907R (5'-CCG TCA ATT
227 CMT TTR AGT TT-3')) (Turner et al. 1999) was used to amplify the V4-V5 region of
228 bacterial 16S rDNA from gDNA and cDNA. Samples were identified with a MID (Multiplex

229 IDentifier, Roche). PCR reactions were achieved as described in Bourceret et al. (2016).
230 Beckman Coulter Genomics (Danvers, MA, USA) sequenced libraries using a 454/Roche GS-
231 FLX Titanium system. Data were deposited in the NCBI Sequence Read Archive (SRA)
232 under the BioProject ID: PRJNA324193. Denoised 16S rDNA sequenced were analysed in
233 QIIME 1.8.0 pipeline (Caporaso et al. 2010) as previously described by Bourceret et al.
234 (2017). Briefly, after filtering and reverse complement non-sense sequences, data from gDNA
235 and cDNA were gathered and clustered in Operational Taxonomic Units (OTUs) at 97% of
236 similarity and their representative sequences were affiliated at different taxonomic levels
237 (from phyla to genus) using RDP classifier (Wang et al. 2007) and the Greengenes 13_5
238 database (McDonald et al. 2012). Then subsampling was performed to the lowest number of
239 sequences counted in one of the samples (4,739 sequences per sample) and alpha-diversity
240 indexes (richness and diversity estimates) were calculated using QIIME 1.8.0 pipeline.

241 **Statistical analyses**

242 Statistical analyses were performed using XLStat 2013 software (Addinsoft). One-way
243 analysis of variance (ANOVA, with $p<0.05$) followed by a Newman-Keuls multiple
244 comparison test was performed to measure the time effect in each condition (planted and
245 unplanted) between 0 and 22 days. Two-way analysis of variance (ANOVA, with $p<0.05$)
246 followed by a Newman-Keuls multiple comparison tests were performed to measure the time
247 and plant effect between T1 and T4. Box plots were drawn using PAH data to compare their
248 distribution and variability. Significant differences in relative abundance of dominant OTUs
249 (≥1% in at least one sample) over time, were identified in cDNA samples with STAMP
250 version 2.0.8 (Parks and Beiko 2010) by a Tukey Kramer ANOVA, with a Benjamini
251 Hochberg correction of p value ($q<0.05$ and Effect size (ES) >0.26). Significant differences in
252 the ratio cDNA/gDNA OTU abundance, according to the presence of plant was studied by
253 Student-t test with a Benjamini Hochberg correction of p value with the differential
254 expression function in XLStatOmics package. Canonical correspondence analysis (CCA)
255 followed by a Monte Carlo permutation test (1000 permutations) was performed by using
256 XLStat-ADA 2015 software (Addinsoft) to study, considering both conditions (alfalfa and
257 unplanted soil) and 4 sampling times (0, 6, 12, 16 and 22 days), relationship between the
258 relative abundance of the 41 most abundant OTUs (≥1% in at least one sample) and
259 environmental variables (root biomass and length, pH, percentage of Gram-negative and
260 Gram-positive PAH dioxygenase transcripts and genes, sugars, organic acids, and PAH
261 content).

262 # Results

263 ## Rhizosphere parameters

264 Humidity stayed constant (around 80% of the WRC) over time in both planted and unplanted
265 soil rhizotrons but was globally significantly higher in unplanted soil (78.36 ± 20.91 and
266 70.85 ± 9.98 %, in unplanted and alfalfa planted soil, respectively). In alfalfa rhizotron, root
267 biomass was significantly ($p<0.05$) higher after 22 days of plant development than after 6
268 days, whereas root length significantly increased over time and was significantly different
269 between 6 and 12 days and then between 16 and 22 days (**Fig. 2A)**. Related to the plant
270 growth, several parameters, highlighting the effect of root exudation on soil properties,
271 increased over time. Globally sugar concentration and DOC increased significantly over time
272 in the planted compared to unplanted soil. Indeed sugar concentration was significantly higher
273 after 12 days in planted than in unplanted soil where no sugar was detected **(Fig. 2B)** and
274 DOC stayed constant over time in both rhizotrons until the 16th day, and then increased
275 significantly in planted soil on the 22nd day **(Fig. 2C).** At this time point, organic acids were
276 detected in alfalfa planted rhizotron (0.21 ± 0.13 mg.kg^{-1}) but none in unplanted soil.
277 Surprisingly soil pH increased significantly over time in both conditions and was stable after
278 12 days. However it increased more rapidly in the planted than in the unplanted soil rhizotron
279 with a significant difference at 6 days **(Fig. 2D)**.

280 ## PAHs and pPACs contents

281 The total aged PAH concentration (Σ13 PAHs) of the NM soil (1033 ± 148 mg.kg^{-1}dw soil)
282 was significantly increased ($p<0.05$), i.e 27 %, after soil spiking with the organic pollutant
283 extract and reached 1418 ± 193 mg.kg.dw soil^{-1} at the beginning (T0) of the experiment (**Fig.**
284 **3)**. The bioavailable PAH fraction was also increased by 41 % and reached 63.7 ± 18.0 mg.kg^{-}
285 ^{1}dw soil, while it represented 37.3 ± 15.6 mg.kg^{-1}dw soil before spiking (data not shown).
286 Spiking increased the concentration in total and bioavailable fractions of most of the 13 PAH
287 compounds (**Fig. S1**). The total PAH concentration decreased significantly between 0 and 6
288 days and stayed constant until the 22nd day in both planted and unplanted rhizotrons **(Fig. 3)**.
289 From the 6th days, the total PAH concentration dropped down to 1200 ± 210 and 1024 ± 190
290 mg.kg.dw soil^{-1} in unplanted and planted soil, respectively, corresponding to a decrease in 19
291 and 24% relative to the initial concentration, and to 70 and 100% of the spiked PAH fraction,
292 respectively. The concentration in total PAHs was significantly lower in alfalfa than in
293 unplanted soil when considering all times together (from 6 to 22 days, two-way ANOVA) and
294 was mostly explained by a significant difference after 12 days (Student t-test) of incubation

295 **(Fig. 3)**. The concentrations of several compounds decreased significantly from the 6th day
296 (fluorene, phenanthrene, fluoranthene, pyrene, benzo(k)fluoranthene, benzo(a)pyrene and
297 benzo(g,h,i)perylene only in planted condition)whereas the concentration of other PAHs
298 remained constant over the time course (anthracene, benzo(a)anthracene, chrysene,
299 benzo(b)fluoranthene, dibenzo(a,h)anthracene, indeno(1,2,3-cd)pyrene)) or even increased
300 significantly (benzo(g,h,i)perylene) from the 22nd day, in presence of plant (**Fig. S2**).
301 Bioavailable PAH fraction decreased also significantly between T0 and the 6th day in both
302 conditions, reaching 0.05 ± 0.01 and 0.08 ± 0.03 mg.kg^{-1}dw soil, corresponding to ca.100 %
303 decreases, for both planted and unplanted soil rhizotron. After one week, the bioavailable
304 PAH fraction was significantly lower than in the soil before spiking. Ten oxygenated and one
305 nitrogenous pPAC compounds were detected in initial soil (T0) and in both conditions after 6
306 days **(Table 1)**. After 6 days the concentration of 4 oxygenated pPAC (methylanthracene-
307 9,10-dione, benzoanthracenedione, napthacene-5,12-dione) was significantly higher than at
308 T0, without significant differences between planted and unplanted soils. Benzo(cd)pyrenone
309 was only significantly higher at 6 days than at T0 in the planted rhizotron. The concentration
310 of the other pPAC stayed constant over the first week for both planted and unplanted soils.
311
312 **Microbial communities**
313 *Abundance of active and total microbial communities*
314 Bacterial abundance was higher than fungal, and bacteria were also more active over time in
315 both planted and unplanted soil (mean value of 7.84 x 10^{8} ± 6.64 x 10^{8} 16S rRNA gene copies
316 and 1.12 x 10^{10} ± 1.25 x 10^{10} transcript copies g dw soil^{-1}) while fungi were 100 times less
317 represented (mean value of 9.94 x 10^{6} ± 7.04 x 10^{6}18S rRNA gene copies and 2.05 x 10^{8} ±
318 1.48 x 10^{8} transcript copies g dw soil^{-1}) **(Fig. 4A, B)**. Abundance of 16S rRNA genes tended
319 to increase over time in alfalfa and stayed constant in unplanted rhizotron whereas number of
320 16S rRNA transcripts increased significantly between T0 and the 6thday for both rhizotrons
321 and then stayed constant. Abundance of 16S rRNA genes and transcripts was significantly
322 higher in planted than unplanted rhizotron and plant effect was mainly detected at 12 (T2) and
323 22 days (T4). Abundance of 18S rRNA genes and transcripts tended to be constant in both
324 unplanted and planted rhizotrons and no plant effect was observed.
325 Abundance of Gram-negative PAH-dioxygenase genes and transcripts increased significantly
326 between T0 and the 6th day in both rhizotron types, and were higher in planted than in
327 unplanted soils, with a significant plant effect after 12 days **(Fig. 4A and B)**. Abundance of
328 Gram-positive PAH-dioxygenase genes slightly increased over time whereas transcripts

329 largely increased between T0 and 6 days in both soil and further increased in planted soil till
330 12 days, resulting in a higher content in planted than in unplanted rhizotron, significant at
331 each sampling time. The level of PAH-dioxygenase gene transcription was expressed by the
332 ratio of the number of transcripts relative to corresponding gene copies **(Fig. 4C)**. The level of
333 PAH-dioxygenase gene transcription was significantly higher in alfalfa than in unplanted soil
334 specifically after 12 days, and then decreased (for Gram-negative bacteria) and stayed
335 constant (for Gram-positive bacteria) over time without significant differences with unplanted
336 soil **(Fig. 4C)**.
337
338 *Richness, evenness and diversity of the active community*
339 Globally, bacterial richness (Chao1) and diversity (Inverse of Simpson's) indices were
340 significantly higher in unplanted than in alfalfa planted rhizotron (**Table 2**). This difference
341 was significant after 16 days for richness and 12 days for diversity index **(Table 2)**. No
342 significant impact of plant was detected on the bacterial community evenness. For both
343 studied conditions, bacterial richness, diversity and evenness indices decreased significantly
344 during the 6 first days and after this time point increased significantly over time.
345 To explore the potential bacterial population involved into the rapid PAH degradation
346 appearing at the beginning of the incubation period, we looked for bacterial community
347 structure modification over time **(Fig. 5)**. Among the active community, 12 phyla were
348 identified and their proportion changed over time. In both rhizotrons, *Proteobacteria* was the
349 more active phylum all over the incubation to the detriment of *Chloroflexi*, *Actinobacteria*
350 and *Nitrospirae* that represented a lower proportion of the active community along the time
351 course compared to the T0. Activity of *Beta-* and *Gamma-Proteobacteria* increased between 0
352 and 6 days and stayed constant or decreased until the end of incubation while the proportion
353 of active *Alpha*-subdivision members further increased. An opposite trend was observed for
354 the activity of *Planctomycetes*, *Acidobacteria* and *Delta-Proteobacteria* that was first
355 repressed between 0 and 12 days and then favored at the end of the incubation **(Fig. 5)**. The
356 relative abundance of some active phyla evolved also differently over time according to the
357 presence of alfalfa. In alfalfa planted rhizotron, *Bacteroidetes* and *TM6* were more and less
358 active over time, respectively, while the activity of *Unclassified Proteobacteria* was
359 transiently high and then low over the 22 days. In planted soil, 3 OTUs affiliated to *Beta-*
360 *Proteobacteria* (*Thiobacillus*, and Un.*Oxalobacteraceae*), 1 OTU affiliated to *Alpha-*
361 *Proteobacteria* (*Mycoplana*) and 1 OTU affiliated to *Firmicutes* (*Bacillus selenatarsenatis*)
362 were significantly more abundant among the active community after 6 days of incubation than

363 at all other times, contrary to the unplanted rhizotron where no OTU was favored after 6 days
364 **(Fig. S3)**. After 12 days of incubation, three other OTUs belonging to *Alpha-* (*Sphingobium*),
365 *Beta-* (*Janthinobacterium*) and *Gamma-* (PYR10d3) *Proteobacteria* were favored in planted
366 soil. In unplanted rhizotron, 5 OTUs affiliated to *Alpha-* (*Mycoplana*, *Phenylobacterium*) and
367 *Beta-* (*Thiobacillus*, *unclassified. Comamonadaceae*) *Proteobacteria* were significantly more
368 abundant among the active community after 12 days of incubation.
369 Significant positive or negative impact of plants on shaping active bacterial community was
370 observed on 7 and 18 OTUs after 6 and 12 days of incubation, respectively **(Fig. 6)**. After 6
371 days, the activity ratio between 16S rRNA and rDNA for 3 OTUs belonging to *Gamma-*
372 (*Pseudomonadaceae*), *Beta-*(*Achromobacter*) *Proteobacteria* and *Firmicutes* (*Paenibacillus*)
373 was significantly higher in alfalfa planted than in unplanted soil. Four other OTUs, affiliated
374 to *Alpha-* (*Un. Sphingomonadaceae*), *Beta-* (*Un. Burkolderiales*) *Protebacteria* and
375 *Actinobacteria* (*Un. Micrococcaceae*) were more actives in unplanted soil. After 12 days,
376 plant growth favored significantly the activity of 11 OTUs belonging to *Alpha-* (*Devosia*),
377 *Beta-* (*Methylophilaceae*), *Gamma-* (*Pseudomonadaceae*, *Stenotrophomonas*, *Acinetobacter*)
378 *Proteobacteria*, *Bacteroidetes* (*Dyadobacter*) and *Actinobacteria* (*Un. Micrococcaceae*). In
379 unplanted soil the activity of 6 OTUs affiliated to *Alpha-Proteobacteria* (*Un.*
380 *Sphingomonadaceae*, *Sphingobium*, *Magnetospirillum*) and *Actinobacteria* (*Un.*
381 *Solirubrobacterales*, *Un. Geodermatophilaceae*) was significantly higher than in alfalfa
382 planted soil. After 16 and 22 days of incubation, 56% and 76% of the OTUs whose activity is
383 significantly impacted by the presence of plant, were more active in alfalfa planted rhizotron
384 **(Fig. S4)**.
385
386 **Multivariate analysis**
387 The two first axes of the canonical correspondence analysis (CCA) explained 62.5 % of
388 bacterial diversity variability among samples **(Fig. 7A and B)**. The F1 axis (explaining 41.8%
389 of the variability) separates samples from the T0 (located on the right) to all other sampling
390 times. The F2 axis (explaining 20.8% of the variability) separates samples according to the
391 presence of plant and time, with 6to 22 day samples from the bottom to the top. Alfalfa
392 planted samples were separated from unplanted soil samples according to sugar and DOC
393 contents, root development (biomass and length), abundance of Gram-negative PAH-
394 dioxygenase genes and transcripts, and abundance of Gram-positive PAH-dioxygenase
395 transcripts **(Fig. 7A)**. These parameters are correlated to the higher activity of 11 bacterial
396 OTUs, most belonging to *Proteobacteria* except one *Bacteriodetes* (*Dyalobacter*), in the

397 alfalfa planted rhizotron **(Fig. 7B)**. These OTUs gathered bacteria affiliated to
398 *Pseudomonadales* (*Acinetobacter* and *Pseudomonas*), *Sphingomonadales* (*Sphingobium*),
399 *Burkolderiales* (*Janthinobacterium* and *Achromobacter*) and *Rhizobiales* (*Un.*
400 *Methylobacteriaceae*) orders. Unplanted soil samples were mostly discriminated according to
401 the higher PAH content (∑13 PAHs and 2-3, 4 and 5-6 cycles). According to the Pearson
402 correlation matrix **(Table S2)**, pH and the root biomass and length were positively and
403 significantly correlated to the percentage of Gram-positive PAH-dioxygenase transcripts
404 contrary to the abundance of 18S transcripts, showing a negative correlation. Moreover
405 concentrations of 2-3, 4- rings and ∑13PAHs were significantly and negatively correlated to
406 the percentage of Gram-negative PAH-dioxygenase transcripts, shown as the most active
407 degrading community at the beginning of the monitoring.
408

409 ## Discussion

410 **Rapid PAH dissipation in a spiked aged-contaminated soil**

411 After 6 days of incubation 100 and 70% of the spiked PAH fraction was dissipated in the
412 planted and unplanted rhizotrons, respectively. A similar rapid dissipation, of bioavailable
413 PAHs after a few days was previously observed in simplified sand or soil microcosms spiked
414 with phenanthrene (Louvel et al. 2011; Cébron et al. 2011), but never observed with such a
415 complex PAH contaminant mixture. We evidenced the degradation of almost all spiked PAHs
416 including fluorene, phenanthrene, fluoranthene, pyrene, benzo(k)fluoranthene and
417 benzo(a)pyrene (which have from 2 to 5 aromatic cycles) in both conditions. In the same soil
418 (NM) incubated in bioslurry, 30 to 55% of the aged PAH compounds (fluorene, phenanthrene,
419 fluoranthene, pyrene, benzo(a)anthracene and chrysene) were degraded after 1.5 months of
420 incubation (Biache et al. 2017), indicating the presence of a very effective microbial
421 community for the degradation of a wide range of PAH compounds. Degradation of PAHs by
422 microbial consortia and co-metabolism are often described as playing crucial roles the in
423 degradation of PAH mixtures in the environment (Ghosal et al. 2016) because the presence of
424 low-molecular-weight PAHs could have a synergistic effect on the degradation of more
425 recalcitrant compounds (Van Herwijnen R et al. 2003; Roy et al. 2013). During the first 6
426 days of the experiment, spiked PAHs were probably the main available carbon sources in both
427 the planted and unplanted rhizotrons, as no increase in DOC or sugar content was observed in
428 either condition. As the microbial community of the aged-contaminated NM coking plant soil
429 was well adapted to PAH pollutants (Cébron et al. 2009; Bourceret et al. 2016), the bacterial

430 community was rapidly activated, and the spiked PAH fraction was in turn rapidly
431 biodegraded. The main parameter that limited the biodegradation of aged PAHs in this soil
432 was their low bioavailability (Cébron et al. 2013) andspiking transiently led to more easily
433 biodegradable PAHs. In our experiment, before being irreversibly adsorbed to soil mineral or
434 organic materials, such as clay, black carbon or coal-tar substances (Ghosal et al. 2016) ,
435 PAHs were rapidly degraded, proving that their availability is crucial for biodegradation.
436 Besides PAH degradation, oxygenated polar compounds (pPAC) were produced during the
437 first week of the experiment. This kind of bioavailable compounds, previously described in
438 water from industrial wasteland (Rollin et al. 2005; Lundstedt et al. 2006) and in coal tar
439 (Northcott and Jones 2001; Benhabib et al. 2010), could result from biotic PAH degradation.
440 Lundstedt et al. (2007) indeed described the production of oxygenated compounds during
441 microbial biodegradation, with apparent concentrations resulting from the balance between
442 pPAC degradation and pPAC production during PAH biodegradation (Biache et al. 2017).
443 The production of oxygenated pPAC possibly more available and more toxic than parent
444 molecules could contribute to shape the bacterial community structure.
445 Together with PAH biodegradation, we observed that bacterial community abundance and
446 activity rapidly increased during the first 6 days of incubation. Moreover the community
447 composition was drastically modified in both planted and unplanted conditions. Most of
448 bacterial OTUs with increasing activity were affiliated to *Beta-* and *Gamma-Proteobacteria,*
449 at the expense of *Actinobacteria* whose proportions fell sharply. PAH amendment may have
450 caused a shift of bacterial communities from oligotroph, K-strategist, Gram-positive bacteria
451 (such as *Actinobacteria*) to copiotroph, r-strategist, Gram-negative bacteria (such as
452 *Proteobacteria*) (Bevivino and Dalmastri 2017). These *Proteobacteria* are potentially Gram-
453 negative PAH-degraders. We did find a predominance of bacteria known to be involved in
454 PAH degradation, such as OTUs affiliated to the *Achromobacter, Janthinobacterium,*
455 Pyr10d3, *Sphingobium*, and *Thiobacillus* genera and *Sphingomonadaceae*, *Microccocaceae,*
456 and *Comamonadaceae* families (Fernández-Luqueño et al. 2011; Kunihiro et al. 2013). The
457 abundance and transcription level of PAH-dioxygenase genes increased significantly during
458 the first 6 days in both conditions, confirming the higher potential of PAH bacterial
459 degradation. Greater activity of Gram-negative than Gram-positive (*Actinobacteria*) PAH
460 degraders was evidenced during these early days, indicating that *Proteobacteria* were most
461 probably the main phylum involved in the biodegradation of the spiked PAHs. This finding
462 was also supported by a higher activity of *Proteobacteria* as compared to other taxa in both
463 planted and unplanted soils, and by a negative correlation between the activity of Gram-

464 negative PAH-degrading bacteria and PAH contents. Several studies indicated that PAH
465 contamination induces a selection of and an enrichment in PAH degraders (Cébron et al.
466 2008; Zhang et al. 2013; Sawulski et al. 2014), and also up-regulates the transcription of the
467 PAH-dioxygenase gene in pure culture (Kim et al. 2006) or complex communities (Louvel et
468 al. 2011). A negative correlation between the transcription of PAH-dioxygenase genes of
469 Gram-negative bacteria and PAH contents would confirm the major involvement of this
470 population in early pollutant degradation, although we cannot rule out the involvement of
471 Gram-positive *Actinobacteria* that could be the main active PAH-degraders after PAH spiking
472 (de Menezes et al. 2012). Furthermore, fungi could also be involved in PAH degradation
473 (Cerniglia and Sutherland 2010), but we did not detect any increase of fungal 18S rRNA gene
474 or transcript copies during incubation in either condition, suggesting a fairly limited
475 involvement of these microorganisms in our soil.
476
477 **Plant effect**
478 The presence of plants induced a shift in the active bacterial community structure and the
479 PAH-degrading population. The timescale of the experiment (22 days) allowed us to monitor
480 the root development of alfalfa, and compare processes between planted and unplanted soils.
481 In addition to increasing the PAH biodegradation capacity, alfalfa plants globally increased
482 the abundance and activity of bacteria, the abundance of Gram-negative PAH-degrading
483 bacteria and the expression of both Gram-negative and Gram-positive PAH-dioxygenase
484 genes. However, the presence of plants led to a decrease of the bacterial richness and diversity
485 indexes. This finding contrasts with previous observation from the same soil (NM) spiked
486 with phenanthrene and planted with ray-grass. The authors found that plants indeed induced
487 an increase of bacterial species richness and slightly inhibited phenanthrene degradation, even
488 if the abundance of PAH-dioxygenase genes increased, yet without modification of the
489 transcription level as compared to unplanted soil (Thomas and Cébron 2016). These
490 contrasting results can be explained by two different types of contamination, i.e. a PAH
491 mixture *vs*. phenanthrene, and two different plant species with contrasted root systems and
492 root exudation (Bourceret et al. 2015). Root exudates probably partly inhibit the degradation
493 of one sole PAH compound, while they might help in co-metabolism processes when PAH
494 contamination is complex and contains high-molecular-weight compounds (Rentz et al. 2005)
495 as shown for PCB degradation (Vrchotová et al. 2013).
496 After 6 days, even if root biomass and length were low, the presence of alfalfa had already
497 modified a few parameters. The pH, the abundance and proportion of active Gram-negative

498 PAH-degrading bacteria were significantly higher in the planted rhizotron than in the
499 unplanted one. Similar findings about PAH-degrading bacterial activity were reported in
500 batch experiments with sand spiked with phenanthrene (Louvel et al. 2011). After 6 days,
501 plants had already shaped the bacterial community structure: several OTUs belonging to *Beta-*
502 and *Gamma-Proteobacteria* were significantly more active in the planted rhizotron, while
503 other OTUs belonging to *Alpha-*, *Beta-Proteobacteria* and *Actinobacteria* were favoured in
504 the unplanted rhizotron. In our experiment, after 12 days, root length and sugar content were
505 significantly higher than after 6 days, and root biomass and DOC tended to increase,
506 suggesting a stronger rhizosphere effect. At this sampling time, significantly higher PAH-
507 degradation was observed in the planted rhizotron than in the unplanted one. This could be
508 linked to an increasing level of PAH-dioxygenase transcription in both Gram-negative and
509 Gram-positive PAH-degrading bacterial populations that was significantly higher in the
510 planted soil than in the unplanted one. Our results clearly show a positive and rapid impact of
511 the rhizosphere on the PAH degradation potential *via* the increased activity of a wider
512 diversity (both GN and GP) of PAH-degrading bacteria. Even if *Actinobacteria* were enriched
513 in the unplanted soil, the activity of the PAH degraders belonging to this phylum was favored
514 in the rhizosphere. The higher activity of Gram-positive PAH degraders could help in the
515 degradation of more recalcitrant PAH compounds (Kanaly and Harayama 2000). We
516 highlighted a time-dependent effect in the course of the process dynamics, which could
517 explain why some authors did not observe an increase in the activity of PAH-degrading
518 populations in certain rhizospheres (Kawasaki et al. 2015). The addition of artificial root
519 exudates can increase the density of soil PAH degraders, with an increase (Joner et al. 2002)
520 or no difference (Cébron et al. 2011) in the degradation of spiked PAHs. We showed the
521 activity of growing roots in a more natural rhizosphere system. A rhizotron device is probably
522 the most adapted laboratory system to study the dynamics of rhizosphere processes because it
523 makes it possible to perform analyses on precisely located samples with specific
524 characteristics (depth, root density, roots age…) instead of mean rhizosphere samples usually
525 obtained from pot experiments. Rhizotrons can allow studying the root turnover (Leigh et al.
526 2002), the isothiocyanate degradation rate, microbial communities (Rumberger and
527 Marschner 2003) or spatial heterogeneity of PAH degradation and microbial communities
528 (Bourceret et al. 2015).
529 In our study, the carbon released by actively growing roots and also leached from the top part
530 of the device presenting a higher root density may have activated PAH degradation through
531 co-metabolism (Ambrosoli et al. 2005). This phenomenon could also be attributed to a

532 priming effect, whereby the release of labile carbon stimulates microbial density and activity
533 (Haichar et al. 2014), promoting PAH degradation or biotransformation (Rentz et al. 2005).
534 As shown by the canonical analysis the higher differentiation between the planted and
535 unplanted conditions was observed after 6 and 12 days of incubation. Root growth seemed to
536 activate bacterial population mostly belonging to *Proteobacteria*, such as OTUs affiliated to
537 the *Gamma-* (*Pseudomonadaceae*, *Stenotrophomonas* and *Acinetobacter*), *Alpha-*
538 (*Sphingobium*, *Devosia*), and *Beta*-(*Methylophilaceae, janthinobacterium* and
539 *Achromobacter*) subdivisions. In addition to their ability to degrade PAHs (Fernández-
540 Luqueño et al. 2011; Tauler et al. 2015; Gupta et al. 2015), OTUs affiliated to *Proteobacteria*
541 can be favoured in the presence of plants as compared to unplanted soil (Thomas and Cébron
542 2016; Thomas et al. 2016). On the contrary, in unplanted soil other members of the *Alpha*
543 (*Sphingomonadaceae*, *Sphingobium, Sphingomonas, Mycoplana, Magnetospirillum*) and Beta
544 (*Ralstonia*, *Oxalobacteraceae*, *Comamonadaceae*, *Thiobacillus*) subdivisions were favoured,
545 as well as *Actinobacteria* (*Iamia*, *Geodermatophilaceae, Georgenia, and Micrococcaceae*).
546 After 16 and 22 days of root development, when no further PAH dissipation was observed,
547 the transcriptional level of the PAH-dioxygenase gene from Gram-negative and Gram-
548 positive bacteria decreased or remained constant over time in both planted and unplanted
549 soils. At these time points, root development seemed to mainly influence the structure of the
550 bacterial community. The proportion of many OTUs indeed differed between the planted and
551 unplanted soils, but organic pollutant degradation no longer did.
552

553 ## Conclusion

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

565 in planted soil while *Alpha-Proteobacteria* and *Actinobacteria* were more active in unplanted
566 soil.
567

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575
576

577 ## Figure captions

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

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

589 **Figure 1.** Experimental devices and sampling points. (A) The device was composed of a
590 mini-rhizotron (in orange) fitting in a corresponding rhizotron (in black). According to the
591 sampling map (in white) at the depth 3 cm, six samples (in green) were collected, 3 samples
592 (in gray) were used for soil humidity measurements, and 4 supplemental samples collected
593 between green locations allowed quantification of dissolved organic carbon (DOC). (B)
594 Pictures of alfalfa rhizotron devices after 6 , 12 , 16 and 22 days of plant growth.
595

596 **Figure 2.** Soil and root colonization parameters over time. (A) Dry root biomass and length,
597 (B) sugar content, (C) dissolved organic carbon (DOC)and (D) pH after 6, 12 , 16 and 22
598 days in alfalfa planted (green) and unplanted (brown) soils. Means (n=6 for root biomass and
599 length, and n=4 for pH and DOC) and standard errors. Letters indicate significant ($p<0.05$)
600 combined effect of time and presence of plant evaluated by using two-way ANOVA followed
601 by Neuman-Keuls multiple comparison test.
602
603 **Figure 3.** PAH concentration (sum of 13 PAH, i.e. the 16 US-EPA PAHs, except
604 naphthalene, acenaphthylene and acenaphthene) after 6, 12, 16 and 22 days in planted (green)
605 and unplanted (brown) soils. Box plots show data variability and means (black cross), n=24
606 (for T0 samples) and n=6 (from 6 to 22 day samples). Dashed line indicate mean of PAH
607 concentration in the NM soil before spiking. Black asterisks indicate significant differences
608 between planted and unplanted soil (Student t test, $p<0.05$) and letters indicate significant
609 differences depending on time (one-way ANOVA).
610
611 **Figure 4**. Abundance values of genes (A) and transcripts (B) and ratio of transcripts relative
612 to their corresponding genes (C) for fungal 18S rRNA, bacterial 16S rRNA and PAH-
613 degrading bacterial communities after 6, 12, 16 and 22 days in alfalfa planted (green) and
614 unplanted (brown) soils. Values are means and standard deviations of 3 replicates (T0) and 6
615 replicates for 6 to 22 days. The effect of time was evaluated for each condition (planted,
616 unplanted) by using one-way ANOVA ($p<0.05$) and presence of plant was evaluated between
617 6 and 22 days by using two-way ANOVA ($p<0.05$) followed by Neuman Keuls multiple
618 comparison test. In A and B, different letters indicate significant changes in microbial
619 community abundance depending on time for each condition, black asterisks indicate plant
620 effect. In C, letters indicate cross effect of plant and time on the ratio of transcript relative to
621 gene copies.
622
623 **Figure 5.** Relative proportion (mean, n=4) of dominant active bacterial phyla (≥1% in at least
624 one sample) in alfalfa planted (Alf) and unplanted soil (BS) at 0, 6, 12, 16 and 22 days of
625 incubation. The twelve significant phyla were identified by a Tukey Kramer ANOVA with a
626 Benjamini Hochberg correction of p-value ($q<0.05$, and effect size >0.26).

627 **Figure 6.** Ratio of the 16S rRNA relative to 16S rDNA for OTU (≥10 sequences in one
628 sample) significantly discriminating plant effect, after 6 and 12 days of incubation. Both

629 conditions were compared using Student t-tests with Benjamini-Hochberg correction of p-
630 value. Asterisks denote degree of significance (green and brown for higher ratio with alfalfa
631 and unplanted soils, respectively; * q<0.05; ** q<0.01; *** q<0.001). OTUs were affiliated at
632 97% of similarities to the deeper taxonomic level, leading to affiliations at different levels.

633 **Figure 7.** Canonical correspondence analysis (CCA) ordination including abiotique (A) and
634 (B) biotique variables. (A) environmental variables(in red) (DOC, sugar and organic acid
635 contents, pH, percentage of 18S rRNA, PAH-RHD$_{\alpha}$ GN and GPgenes and transcripts relative
636 to 16S rRNA), root biomass (RootB) and length (RootL), sum of 13 PAH concentrations, 2-3-
637 ring PAHs (fluorene, phenanthrene, anthracene), 4-ring PAHs (fluoranthene, pyrene,
638 benzo(a)anthracene, chryzene), 5-6-ring PAHs (benzo(b)fluoranthene, benzo(k)fluoranthene,
639 benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene, indeno(1,2,3-cd)pyrene), and
640 (B) the relative abundance of dominant active bacterial OTUs (≥1% in at least one sample,
641 taxonomic affiliation at 97% of similarities to the deeper taxonomic level, leading to
642 affiliations at different levels) (in black) were used to discriminate the 24 samples (4
643 replicates for each sampling time corresponding to 0, 6, 12, 16 and 22 days) collected for
644 alfalfa planted (green) and unplanted (brown) rhizotrons. A Monte Carlo permutation test
645 (1000 permutations) was performed and showed a significant (p<0.0001) effect of
646 environmental variables on the relative abundance of dominant OTUs. Un. Coma.: Un.
647 Comamonadaceae ; Un. Pseudo.: Un. Pseudomonadaceae ; Un.Sphingo.: Un.
648 Sphingomonadaceae ; Un. Geo.: Un. Geodermatophilaceae ; Un. Meth.:Un.
649 Methylobacteriaceae ; Phen.: Phenylobacterium ;Phaeos. Fulvum: PhaeospirillumFulvum ;
650 Un. Xantho.: Un. Xanthomonadaceae ; Un. Oxalo.: Un. Oxalobacteraceae.
651

652 ## Supplemental data

653 **Table S1.** PAH concentration (mg.L^{-1}) in the organic extract used to spike NM soil. Values
654 are means (n=3) and standard errors.
655 **Table S2.** Pearson's correlation matrix between soil properties and the percentage of PAH-
656 dioxygenase transcripts and genes. Values in bold correspond to significant correlation
657 between variables (p<0.05).
658

659 **Figure S1.** Bioavailable (A) and total (B) PAH concentration in NM soil before (grey) and
660 after (blue) spiking. Black asteriks indicates significant differences between both conditions

661 (Student t test, p<0,05). Values are means and standard deviation of 12 and 48 samples (total
662 fraction) and 8 and 6 samples (bioavailable fraction), before and after spiking respectively.

663 **Figure S2.** Total PAH concentration of 13 individual compounds (the 16 US-EPA PAHs
664 except naphthalene, acenaphthylene and acenaphthene) after 6, 12, 16 and 22 days in alfalfa
665 planted (green) and unplanted (brown) soils. Values are means and standard deviations of 24
666 replicates (0 day) and 6 replicates from 6 to 22 days. The effect of time was evaluated for
667 each condition (planted, unplanted) independently by using one-way ANOVA (p<0.05) and
668 effect of presence of plant was evaluated between T1 and T4 by using two-way ANOVA
669 (p<0.05) followed by Neuman Keuls multiple comparison test. Different letters indicate
670 significant differences in PAH concentration depending on time for each condition, black
671 asterisks indicate plant effect.

672 **Figure S3.** Evolution over time of the relative proportion of dominant OTUs (≥1% in at least
673 one sample), in alfalfa planted and unplanted soil, reported to the transcript 16S rRNA copy
674 number based on cDNA analysis. Forty-seven significant OTUs were identified by a Tukey
675 Kramer ANOVA with a Benjamini Hochberg correction of p-value (q<0.05, and effect size
676 >0.26) and non-significant OTUs correspond to population presenting constant relative
677 proportion over time. Asterisks denote OTUs with significant differences in relative
678 abundance over time specifically in planted (green) or in unplanted soil (brown). OTUs were
679 affiliated at the most precise level.

680 **Figure S4.** Ratio of the 16S rRNA relative to 16S rDNA for OTU (with at least 10 sequences
681 in one sample) significantly discriminating plant effect, after 16 and 22 days of incubation.
682 Both conditions were compared using Student t-tests with Benjamini-Hochberg correction of
683 p-value. Asterisks denote degree of significance (green and brown for higher ratio with alfalfa
684 planted and unplanted soils, respectively; * q<0.05; ** q<0.01; *** q<0.001).
685

686

687 ## References

688 Allard A-S, Remberger M, Neilson AH (2000) The negative impact of aging on the loss of
689 PAH components in a creosote-contaminated soil. Int Biodeterior Biodegradation 46:43–
690 49 . doi: 10.1016/S0964-8305(00)00050-0

Ambrosoli R, Petruzzelli L, Minati JL, Marsan FA (2005) Anaerobic PAH degradation in soil by a mixed bacterial consortium under denitrifying conditions. Chemosphere 60:1231–1236 . doi: 10.1016/j.chemosphere.2005.02.030

Baudoin E, Benizri E, Guckert A (2002) Impact of growth stage on the bacterial community structure along maize roots, as determined by metabolic and genetic fingerprinting. Appl Soil Ecol 19:135–145 . doi: 10.1016/S0929-1393(01)00185-8

Bell TH, El-Din Hassan S, Lauron-Moreau A, et al (2014) Linkage between bacterial and fungal rhizosphere communities in hydrocarbon-contaminated soils is related to plant phylogeny. Isme J 8:331–343 . doi: 10.1038/ismej.2013.149

Benhabib K, Faure P, Sardin M, Simonnot MO (2010) Characteristics of a solid coal tar sampled from a contaminated soil and of the organics transferred into water. Fuel 89:352–359 . doi: 10.1016/j.fuel.2009.06.009

Bevivino A, Dalmastri C (2017) Composition and Function of Microbial Communities in Soil Ecosystems. In: Soil Biological Communities and Ecosystem Resilience. pp 79–81

Biache C, Ouali S, Cébron A, et al (2017) Bioremediation of PAH-contamined soils: Consequences on formation and degradation of polar-polycyclic aromatic compounds and microbial community abundance. J Hazard Mater 329:1–10 . doi: 10.1016/j.jhazmat.2017.01.026

Bogan BW, Sullivan WR (2003) Physicochemical soil parameters affecting sequestration and mycobacterial biodegradation of polycyclic aromatic hydrocarbons in soil. Chemosphere 52:1717–1726 . doi: 10.1016/S0045-6535(03)00455-7

Bourceret A, Cébron A, Tisserant E, et al (2016) The Bacterial and Fungal Diversity of an Aged PAH- and Heavy Metal-Contaminated Soil is Affected by Plant Cover and Edaphic Parameters. Microb Ecol 1–14 . doi: 10.1007/s00248-015-0682-8

Bourceret A, Leyval C, De Fouquet C, Cébron A (2015) Mapping the centimeter-scale spatial variability of PAHs and microbial populations in the rhizosphere of two plants. PLoS One 10:

Bourceret A, Leyval C, Thomas F, Cébron A (2017) Rhizosphere effect is stronger than PAH concentration on shaping spatial bacterial assemblages along centimetre-scale depth

720 gradients. Can J Microbiol 63:881–893 . doi: 10.1139/cjm-2017-0124

721 Caporaso JG, Kuczynski J, Stombaugh J, et al (2010) QIIME allows analysis of high-
722 throughput community sequencing data. Nat Methods 7:335–336 . doi:
723 10.1038/nmeth.f.303

724 Castro-Silva C, Ruíz-Valdiviezo VM, Valenzuela-Encinas C, et al (2013) The bacterial
725 community structure in an alkaline saline soil spiked with anthracene. Electron J
726 Biotechnol 16:1–20 . doi: 10.2225/vol16-issue5-fulltext-14

727 Cébron A, Beguiristain T, Faure P, et al (2009) Influence of vegetation on the in situ bacterial
728 community and polycyclic aromatic hydrocarbon (PAH) degraders in aged PAH-
729 contaminated or thermal-desorption-treated soil. Appl Environ Microbiol 75:6322–6330
730 . doi: 10.1128/AEM.02862-08

731 Cébron A, Cortet J, Criquet S, et al (2011) Biological functioning of PAH-polluted and
732 thermal desorption-treated soils assessed by fauna and microbial bioindicators. Res
733 Microbiol 162:897–907 . doi: 10.1016/j.resmic.2011.02.011

734 Cébron A, Faure P, Lorgeoux C, et al (2013) Experimental increase in availability of a PAH
735 complex organic contamination from an aged contaminated soil: Consequences on
736 biodegradation. Environ Pollut 177:98–105 . doi: 10.1016/j.envpol.2013.01.043

737 Cébron A, Norini MP, Beguiristain T, Leyval C (2008) Real-Time PCR quantification of
738 PAH-ring hydroxylating dioxygenase (PAH-RHDα) genes from Gram positive and
739 Gram negative bacteria in soil and sediment samples. J Microbiol Methods 73:148–159 .
740 doi: 10.1016/j.mimet.2008.01.009

741 Cerniglia CE, Sutherland JB (2010) Degradation of polycyclic aromatic hydrocarbons by
742 fungi. In: (Berlin: Springer;) (ed) Handbook of Hydrocarbon and Lipid Microbiology,
743 Timmis K. pp 2080–2110

744 D'Orazio V, Ghanem A, Senesi N (2013) Phytoremediation of pyrene contaminated soils by
745 different plant species. Clean - Soil, Air, Water 41:377–382 . doi:
746 10.1002/clen.201100653

747 de Menezes A, Clipson N, Doyle E (2012) Comparative metatranscriptomics reveals
748 widespread community responses during phenanthrene degradation in soil. Environ

749 Microbiol 14:2577–2588 . doi: 10.1111/j.1462-2920.2012.02781.x

750 Felske A, Akkermans ADL, De Vos WM (1998) Quantification of 16S rRNAs in complex
751 bacterial communities by multiple competitive reverse transcription-PCR in temperature
752 gradient gel electrophoresis fingerprints. Appl Environ Microbiol 64:4581–4587

753 Fernández-Luqueño F, Valenzuela-Encinas C, Marsch R, et al (2011) Microbial communities
754 to mitigate contamination of PAHs in soil—possibilities and challenges: a review.
755 Environ Sci Pollut Res 18:12–30

756 Gartler J, Wimmer B, Soja G, Reichenauer TG (2014) Effects of Rapeseed Oil on the
757 Rhizodegradation of Polyaromatic Hydrocarbons in Contaminated Soil. Int J
758 Phytoremediation 16:671–683 . doi: 10.1080/15226514.2013.856841

759 Ghosal D, Ghosh S, Dutta TK, Ahn Y (2016) Current state of knowledge in microbial
760 degradation of polycyclic aromatic hydrocarbons (PAHs): A review. Front Microbiol 7: .
761 doi: 10.3389/fmicb.2016.01369

762 Gupta S, Pathak B, Fulekar MH (2015) Molecular approaches for biodegradation of
763 polycyclic aromatic hydrocarbon compounds: a review. Rev Environ Sci Biotechnol
764 14:241–269 . doi: 10.1007/s11157-014-9353-3

765 Haichar F el Z, Santaella C, Heulin T, Achouak W (2014) Root exudates mediated
766 interactions belowground. Soil Biol Biochem 77:69–80 . doi:
767 10.1016/j.soilbio.2014.06.017

768 Haichar F el Z, Marol C, Berge O, et al (2008) Plant host habitat and root exudates shape soil
769 bacterial community structure. ISME J 2:1221–1230 . doi: 10.1038/ismej.2008.80

770 Hannula SE, de Boer W, van Veen JA (2010) In situ dynamics of soil fungal communities
771 under different genotypes of potato, including a genetically modified cultivar. Soil Biol
772 Biochem 42:2211–2223 . doi: 10.1016/j.soilbio.2010.08.020

773 Hanser O, Biache C, Boulangé M, et al (2015) Evolution of dissolved organic matter during
774 abiotic oxidation of coal tar—comparison with contaminated soils under natural
775 attenuation. Environ Sci Pollut Res 22:1431–1443

776 Haritash AK, Kaushik CP (2009) Biodegradation aspects of Polycyclic Aromatic
777 Hydrocarbons (PAHs): A review. J Hazard Mater 169:1–15 . doi:

10.1016/j.jhazmat.2009.03.137

Hinsinger P, Gobran GR, Gregory PJ, Wenzel WW (2005) Rhizosphere geometry and heterogeneity arising from root-mediated physical and chemical processes. New Phytol 168:293–303 . doi: 10.1111/j.1469-8137.2005.01512.x

Joner EJ, Corgié SC, Amellal N, Leyval C (2002) Nutritional constraints to degradation of polycyclic aromatic hydrocarbons in a simulated rhizosphere. Soil Biol Biochem 34:859–864 . doi: 10.1016/S0038-0717(02)00018-4

Kanaly R, Harayama S (2000) Biodegradation of High-Molecular-Weight Polycyclic Aromatic Hydrocarbons by Bacteria MINIREVIEW Biodegradation of High-Molecular-Weight Polycyclic Aromatic Hydrocarbons by Bacteria. J Bacteriol 182:2059–2067 . doi: 10.1128/JB.182.8.2059-2067.2000.Updated

Kawasaki A, Warren CR, Kertesz MA (2015) Specific influence of white clover on the rhizosphere microbial community in response to polycyclic aromatic hydrocarbon (PAH) contamination. Plant Soil 1–15

Kawasaki A, Watson E, Kertesz M (2012) Indirect effects of polycyclic aromatic hydrocarbon contamination on microbial communities in legume and grass rhizospheres. Plant Soil 358:169–182 . doi: 10.1007/s11104-011-1089-z

Kim S-J, Kweon O, Freeman JP, et al (2006) Molecular Cloning and Expression of Genes Encoding a Novel Dioxygenase Involved in Low- and High-Molecular-Weight Polycyclic Aromatic Hydrocarbon Degradation in Mycobacterium vanbaalenii PYR-1. Appl Environ Microbiol 72:1045–1054

Kirk JL, Klironomos JN, Lee H, Trevors JT (2005) The effects of perennial ryegrass and alfalfa on microbial abundance and diversity in petroleum contaminated soil. Environ Pollut 133:455–465 . doi: 10.1016/j.envpol.2004.06.002

Kunihiro M, Ozeki Y, Nogi Y, et al (2013) Benz[a]anthracene biotransformation and production of ring fission products by Sphingobium sp. strain KK22. Appl Environ Microbiol 79:4410–4420 . doi: 10.1128/AEM.01129-13

Leigh MB, Fletcher JS, Fu X, Schmitz FJ (2002) Root turnover: An important source of microbial substrates in rhizosphere remediation of recalcitrant contaminants. Environ Sci

807 Technol 36:1579–1583 . doi: 10.1021/es015702i

808 Liste H-H, Alexander M (2000) Plant-promoted pyrene degradation in soil. Chemosphere
809 40:7–10 . doi: 10.1016/S0045-6535(99)00216-7

810 Louvel B, Cébron A, Leyval C (2011) Root exudates affect phenanthrene biodegradation,
811 bacterial community and functional gene expression in sand microcosms. Int Biodeterior
812 Biodegrad 65:947–953 . doi: 10.1016/j.ibiod.2011.07.003

813 Lueders T, Wagner B, Claus P, Friedrich MW (2004) Stable isotope probing of rRNA and
814 DNA reveals a dynamic methylotroph community and trophic interactions with fungi
815 and protozoa in oxic rice field soil. Environ Microbiol 6:60–72 . doi: 10.1046/j.1462-
816 2920.2003.00535.x

817 Lundstedt S, Persson Y, Öberg L (2006) Transformation of PAHs during ethanol-Fenton
818 treatment of an aged gasworks' soil. Chemosphere 65:1288–1294

819 Lundstedt S, White PA, Lemieux CL, et al (2007) Sources, fate, and toxic hazards of
820 oxygenated polycyclic aromatic hydrocarbons (PAHs) at PAH-contaminated sites.
821 AMBIO A J Hum Environ 36:475–485

822 McDonald D, Price MN, Goodrich J, et al (2012) An improved Greengenes taxonomy with
823 explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME J
824 6:610–8 . doi: 10.1038/ismej.2011.139

825 Muratova AY, Turkovskaya O V., Hübner T, Kuschk P (2003) Studies of the Efficacy of
826 Alfalfa and Reed in the Phytoremediation of Hydrocarbon-Polluted Soil. Appl Biochem
827 Microbiol 39:599–605 . doi: 10.1023/A:1026238720268

828 Northcott GL, Jones KC (2001) Partitioning, extractability, and formation of nonextractable
829 PAH residues in soil. 2. Effects on compound dissolution behavior. Environ Sci Technol
830 35:1111–1117

831 Parks DH, Beiko RG (2010) Identifying biologically relevant differences between
832 metagenomic communities. Bioinformatics 26:715–721

833 Peng A, Liu J, Ling W, et al (2015) Diversity and distribution of 16S rRNA and phenol
834 monooxygenase genes in the rhizosphere and endophytic bacteria isolated from PAH-
835 contaminated sites. Sci Rep 5:12173 . doi: 10.1038/srep12173

836 Reid BJ, Stokes JD, Jones KC, Semple KT (2000) Nonexhaustive cyclodextrin-based
837 extraction technique for the evaluation of PAH bioavailability. Environ Sci Technol
838 34:3174–3179 . doi: 10.1021/es990946c

839 Rentz JA, Alvarez PJJ, Schnoor JL (2005) Benzo[a]pyrene co-metabolism in the presence of
840 plant root extracts and exudates: Implications for phytoremediation. Environ Pollut
841 136:477–484 . doi: 10.1016/j.envpol.2004.12.034

842 Rollin C, Quiot F, DENYS S, BUREAU J (2005) Hydrocarbures Aromatiques Polycycliques.
843 Guide méthodologique. Acquisition des données d'entrée des modèles analytiques ou
844 numériques de transferts dans les sols et les eaux souterraines. INERIS–DRC–
845 66244/DESP–R01

846 Roy M, Khara P, Basu S, Dutta TK (2013) Catabolic Versatility of Sphingobium sp. Strain
847 PNB Capable of Degrading Structurally Diverse Aromatic Compounds. J
848 Bioremediation Biodegrad 4:1–6 . doi: 10.4172/2155-6199.1000173

849 Rumberger A, Marschner P (2003) 2-Phenylethylisothiocyanate concentration and microbial
850 community composition in the rhizosphere of canola. Soil Biol Biochem 35:445–452 .
851 doi: 10.1016/S0038-0717(02)00296-1

852 Sawulski P, Clipson N, Doyle E (2014) Effects of polycyclic aromatic hydrocarbons on
853 microbial community structure and PAH ring hydroxylating dioxygenase gene
854 abundance in soil. Biodegradation 25:835–847 . doi: 10.1007/s10532-014-9703-4

855 Siciliano SD, Germida JJ, Banks K, Greer CW (2003) Changes in Microbial Community
856 Composition and Function during a Polyaromatic Hydrocarbon Phytoremediation Field
857 Trial. Appl Environ Microbiol 69:483–489

858 Storey S, Ashaari MM, Mccabe G, et al (2014) Microbial community structure during
859 fluoranthene degradation in the presence of plants. J Appl Microbiol 117:74–84 . doi:
860 10.1111/jam.12518

861 Tauler M, Vila J, Nieto JM, Grifoll M (2015) Key high molecular weight PAH-degrading
862 bacteria in a soil consortium enriched using a sand-in-liquid microcosm system. Appl
863 Microbiol Biotechnol 1–16

864 Tejeda-Agredano MC, Gallego S, Vila J, et al (2013) Influence of the sunflower rhizosphere

on the biodegradation of PAHs in soil. Soil Biol Biochem 57:830–840 . doi: 10.1016/j.soilbio.2012.08.008

Thion C, Cébron A, Beguiristain T, Leyval C (2013) Inoculation of PAH-degrading strains of Fusarium solani and Arthrobacter oxydans in rhizospheric sand and soil microcosms: microbial interactions and PAH dissipation. Biodegradation 24:569–581 . doi: 10.1007/s10532-013-9628-3

Thion C, Cébron A, Beguiristain T, Leyval C (2012) Long-term in situ dynamics of the fungal communities in a multi-contaminated soil are mainly driven by plants. FEMS Microbiol Ecol 82:169–181 . doi: 10.1111/j.1574-6941.2012.01414.x

Thomas F, Cébron A (2016) Short-term rhizosphere effect on available carbon sources, phenanthrene degradation, and active microbiome in an aged-contaminated industrial soil. Front Microbiol 7:1–15 . doi: 10.3389/fmicb.2016.00092

Thomas F, Lorgeoux C, Faure P, et al (2016) Isolation and substrate screening of polycyclic aromatic hydrocarbon degrading bacteria from soil with long history of contamination. Int Biodeterior Biodegradation 107:1–9

Trapp S, Karlson U (2001) Aspects of phytoremediation of organic pollutants. J Soils Sediments 1:37–43 . doi: 10.1065/jss2001.04.007

Turner S, Pryer KM, Miao VP, Palmer JD (1999) Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis1. J Eukaryot Microbiol 46:327–338

van Herwijnen R, Wattiau P, Bastiaens L, et al. (2003) Elucidationn of the metabolic pathway of fluorene and cometabolicc pathways of phenanthrene , fluoranthene , anthracenee and dibenzothiophene by Sphingomonas sp . Res Microbiol 154:199–206

Vrchotová B, Lovecká P, Dražková M, et al (2013) Influence of root exudates on the bacterial degradation of chlorobenzoic acids. Sci World J 2013: . doi: 10.1155/2013/872026

Walker TS, Bais HP, Grotewold E, Vivanco JM (2003) Root exudation and rhizosphere biology. Plant Physiol 132:44–51

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. Appl Environ

894 Microbiol 73:5261–5267

895 Yang C-H, Crowley DE (2000) Rhizosphere Microbial Community Structure in Relation to
896 Root Location and Plant Iron Nutritional Status. Appl Environ Microbiol 66:345–351 .
897 doi: 10.1128/AEM.66.1.345-351.2000

898 Yergeau E, Sanschagrin S, Maynard C, et al (2014) Microbial expression profiles in the
899 rhizosphere of willows depend on soil contamination. ISME J 8:344–58 . doi:
900 10.1038/ismej.2013.163

901 Zhang Z, Zhao X, Liang Y, et al (2013) Microbial functional genes reveal selection of
902 microbial community by PAHs in polluted soils. Environ Chem Lett 11:11–17 . doi:
903 10.1007/s10311-012-0370-6

904 Zhou W, Yang J, Lou L, Zhu L (2011) Solubilization properties of polycyclic aromatic
905 hydrocarbons by saponin, a plant-derived biosurfactant. Environ Pollut 159:1198–1204

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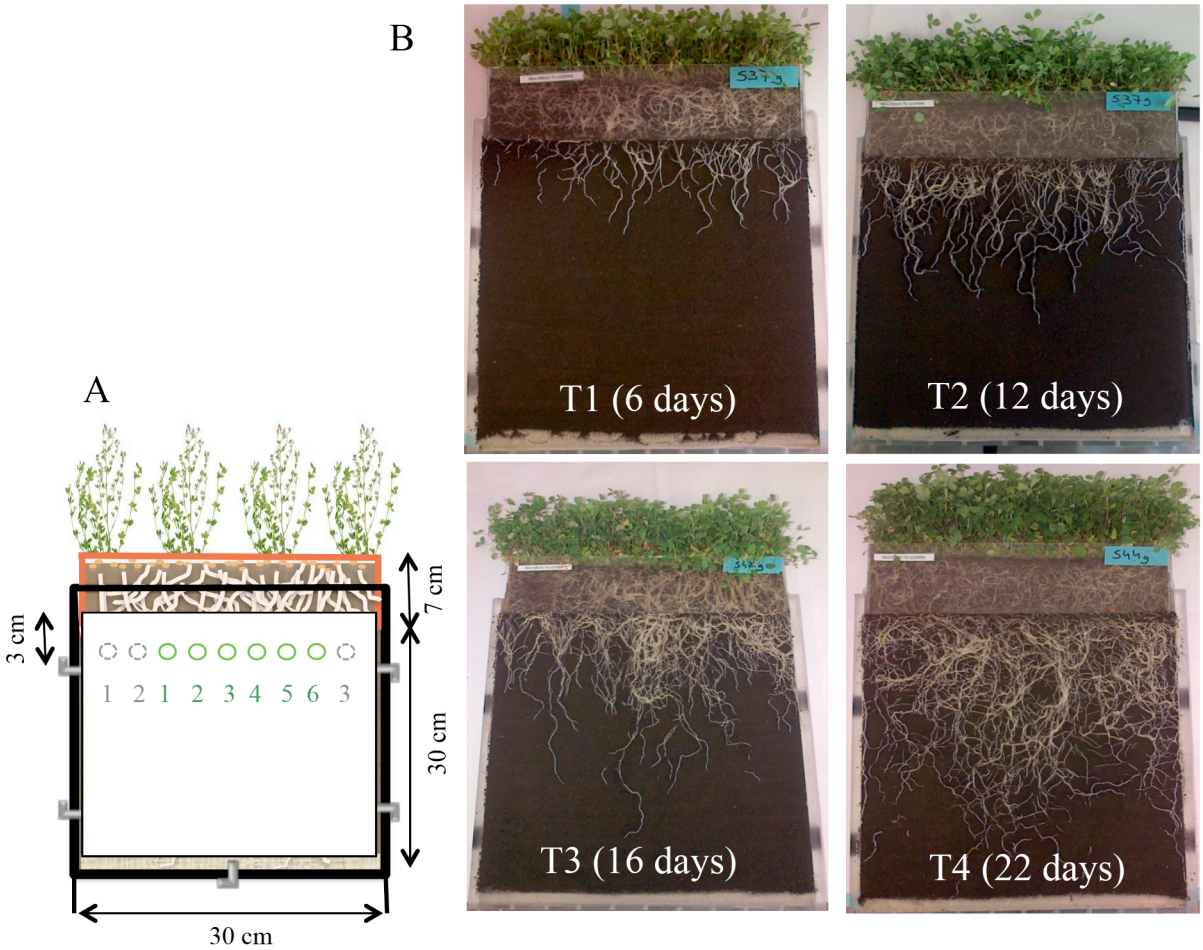
Figure 1.


A
B
3 cm
7 cm
30 cm
30 cm
1 2 1 2 3 4 5 6 3
T1 (6 days)
T2 (12 days)
T3 (16 days)
T4 (22 days)

911 Figure 2.

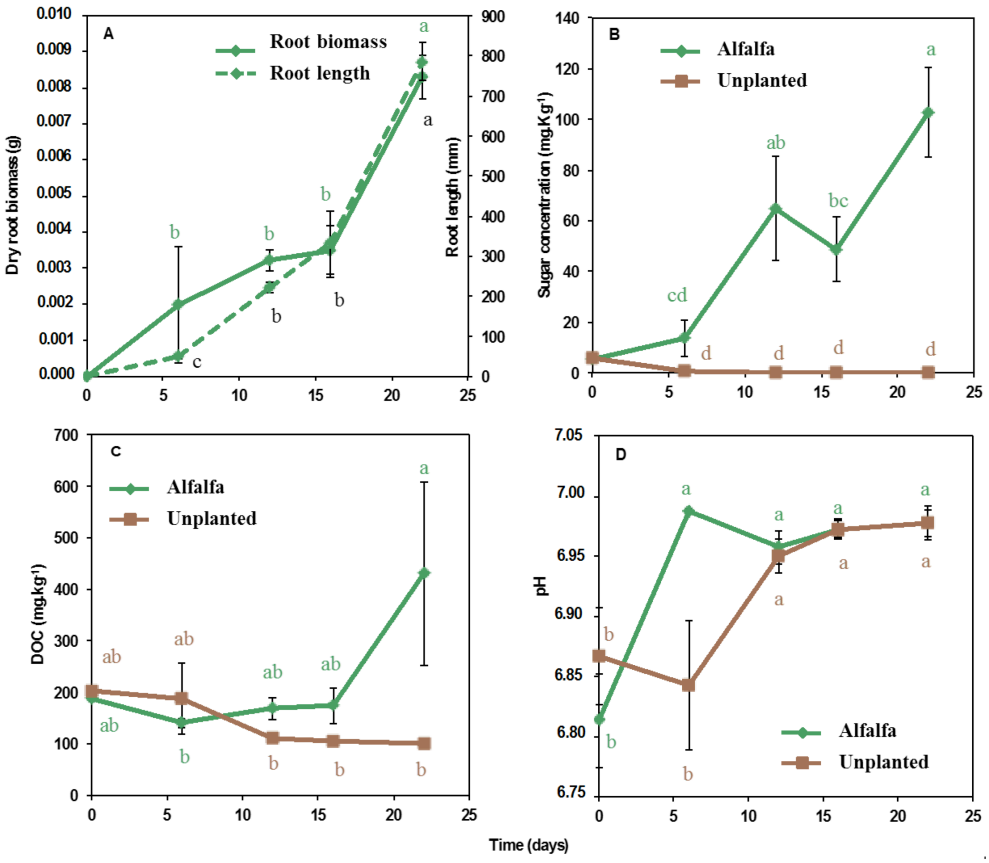


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Figure 3.

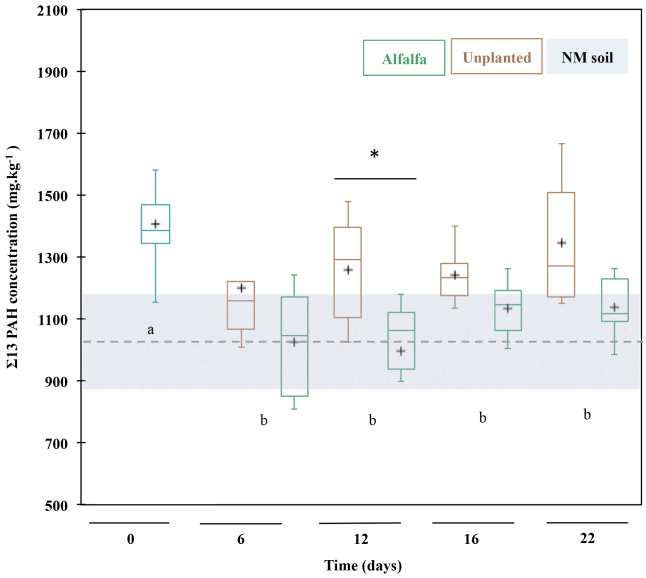
Alfalfa
Unplanted
NM soil
Σ13 PAH concentration (mg.kg-1)
2100
1900
1700
1500
1300
1100
900
700
500
*
a
b
b
b
b
0
6
12
16
22
Time (days)

Figure 4.

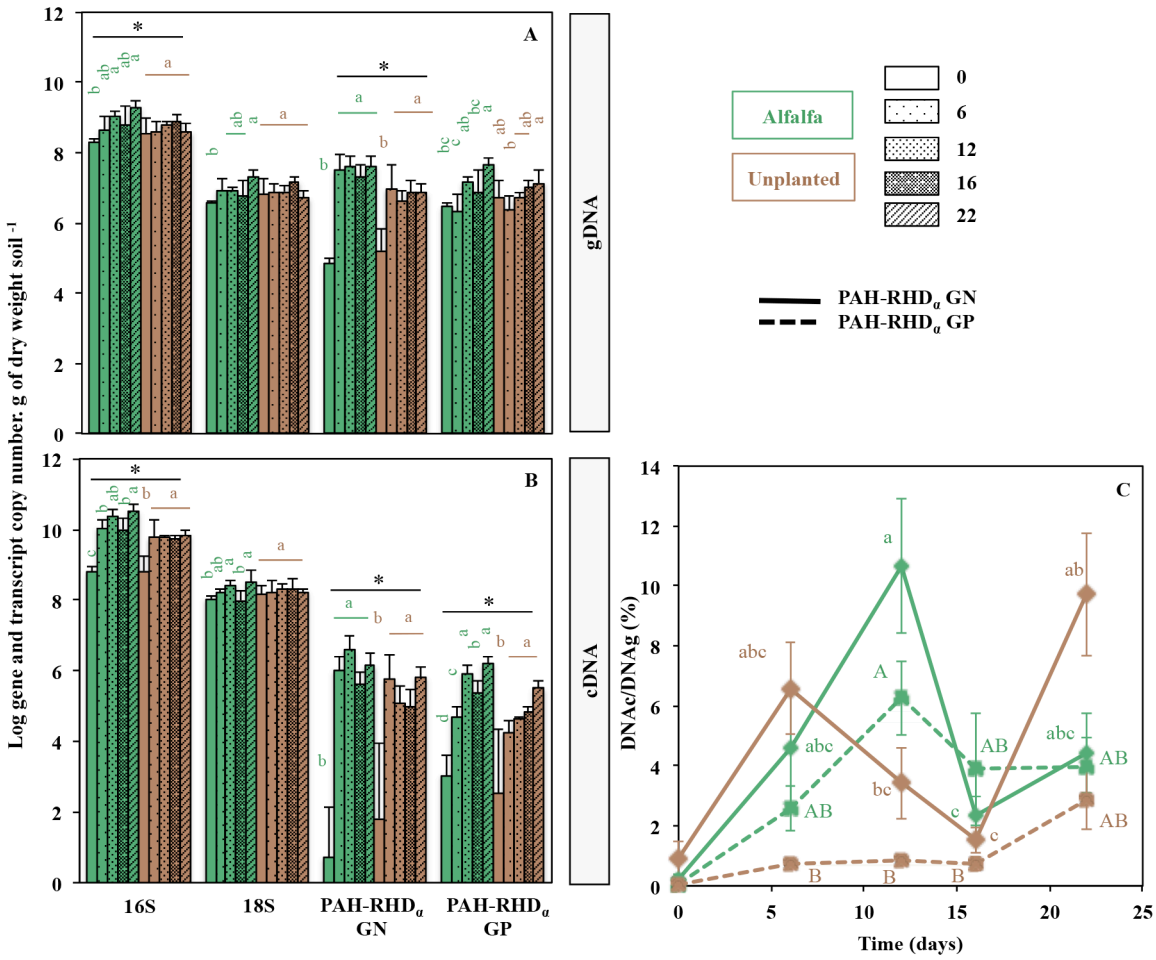

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Figure 5.

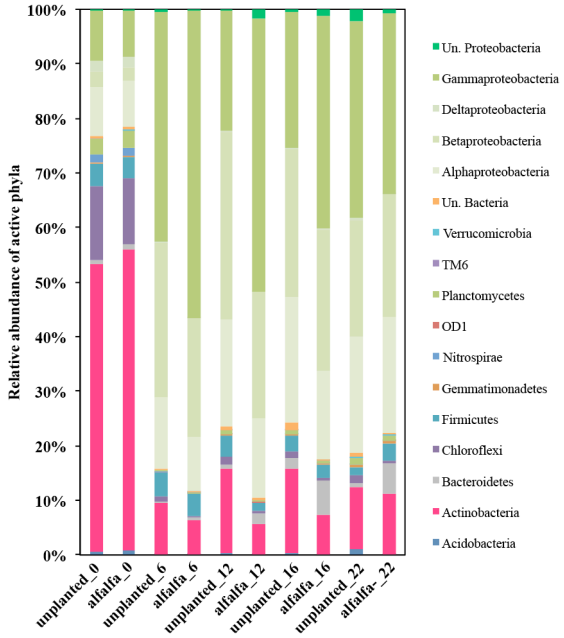

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Relative abundance of active phyla
unplanted_0
alfalfa_0
unplanted_6
alfalfa_6
unplanted_12
alfalfa_12
unplanted_16
alfalfa_16
unplanted_22
alfalfa- _22
Un. Proteobacteria
Gammaproteobacteria
Deltaproteobacteria
Betaproteobacteria
Alphaproteobacteria
Un. Bacteria
Verrucomicrobia
TM6
Planctomycetes
OD1
Nitrospirae
Gemmatimonadetes
Firmicutes
Chloroflexi
Bacteroidetes
Actinobacteria
Acidobacteria

Figure 6.

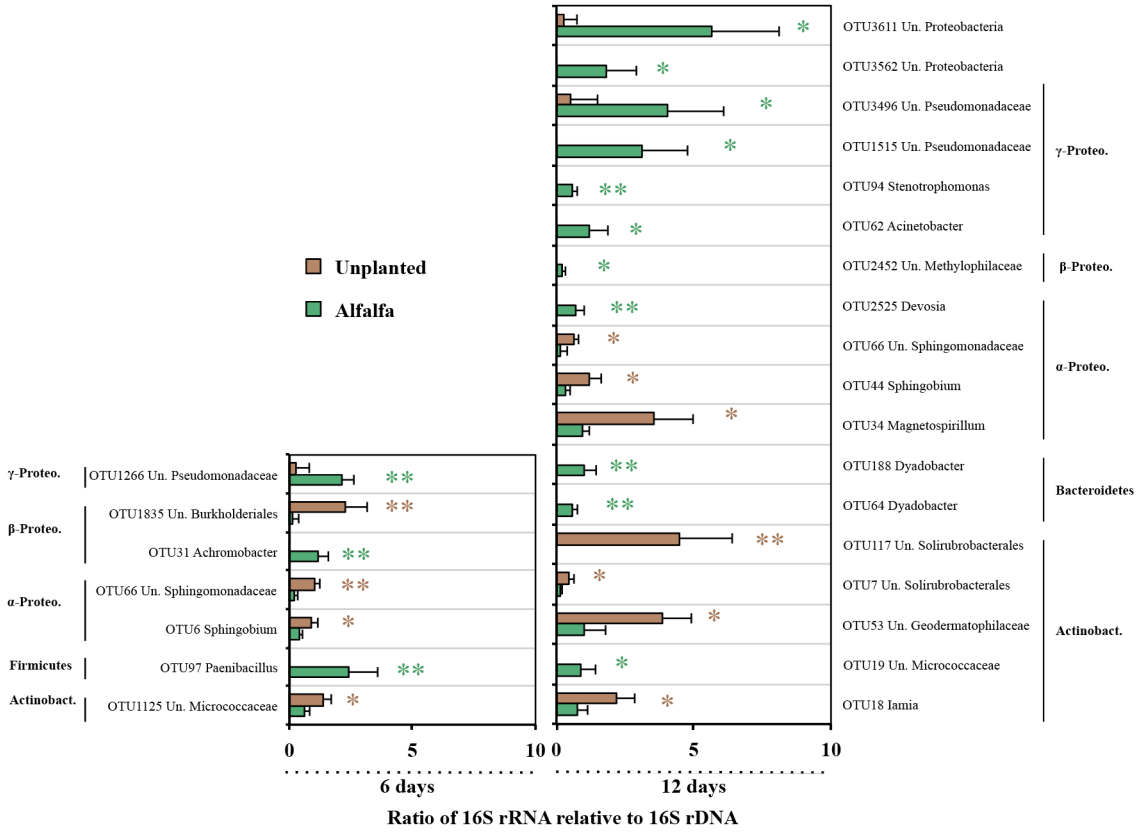
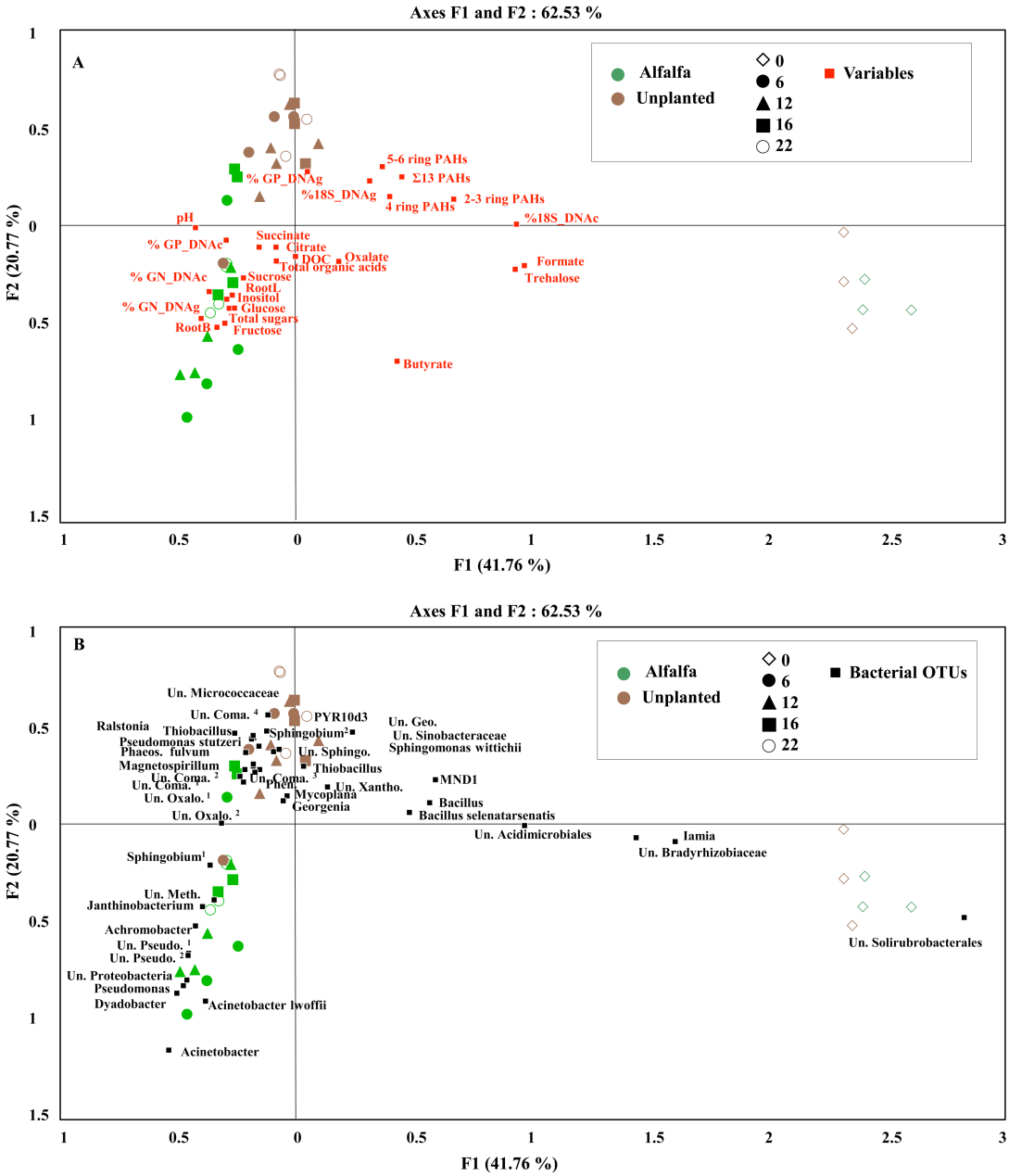

Unplanted
Alfalfa
OTU3611 Un. Proteobacteria
OTU3562 Un. Proteobacteria
OTU3496 Un. Pseudomonadaceae
OTU1515 Un. Pseudomonadaceae
OTU94 Stenotrophomonas
OTU62 Acinetobacter
γ-Proteo.
OTU2452 Un. Methylophilaceae
β-Proteo.
OTU2525 Devosia
OTU66 Un. Sphingomonadaceae
OTU44 Sphingobium
OTU34 Magnetospirillum
α-Proteo.
OTU188 Dyadobacter
OTU64 Dyadobacter
Bacteroidetes
OTU117 Un. Solirubrobacterales
OTU7 Un. Solirubrobacterales
OTU53 Un. Geodermatophilaceae
OTU19 Un. Micrococcaceae
OTU18 Iamia
Actinobact.
γ-Proteo.
OTU1266 Un. Pseudomonadaceae
β-Proteo.
OTU1835 Un. Burkholderiales
OTU31 Achromobacter
α-Proteo.
OTU66 Un. Sphingomonadaceae
OTU6 Sphingobium
Firmicutes
OTU97 Paenibacillus
Actinobact.
OTU1125 Un. Micrococcaceae
0
5
10
6 days
12 days
Ratio of 16S rRNA relative to 16S rDNA

Figure 7.


Axes F1 and F2 : 62.53 %
A
Alfalfa
Unplanted
0
6
12
16
22
Variables
5-6 ring PAHs
Σ13 PAHs
% GP_DNAg
%18S_DNAg
4 ring PAHs
2-3 ring PAHs
%18S_DNAc
pH
Succinate
Citrate
% GP_DNAc
DOC
Oxalate
Total organic acids
Formate
Trehalose
% GN_DNAc
Sucrose
RootL
Inositol
% GN_DNAg
Glucose
Total sugars
RootB
Fructose
Butyrate
F2 (20.77 %)
F1 (41.76 %)
Axes F1 and F2 : 62.53 %
B
Alfalfa
Unplanted
0
6
12
16
22
Bacterial OTUs
Un. Micrococcaceae
Un. Coma. 4
PYR10d3
Un. Geo.
Ralstonia
Thiobacillus
Sphingobium2
Un. Sinobacteraceae
Pseudomonas stutzeri
Un. Sphingo.
Sphingomonas wittichii
Phaeos. fulvum
Thiobacillus
Magnetospirillum
Un. Coma. 2
Un. Coma. 3
MND1
Un. Coma. 1
Phen.
Un. Xantho.
Un. Oxalo. 1
Mycoplana
Bacillus
Georgenia
Un. Oxalo. 2
Bacillus selenatarsenatis
Un. Acidimicrobiales
Iamia
Un. Bradyrhizobiaceae
Sphingobium1
Un. Meth.
Janthinobacterium
Achromobacter
Un. Pseudo. 1
Un. Pseudo. 2
Un. Solirubrobacterales
Un. Proteobacteria
Pseudomonas
Dyadobacter
Acinetobacter lwoffii
Acinetobacter
F2 (20.77 %)
F1 (41.76 %)

Supplmentary material

Figure S1.

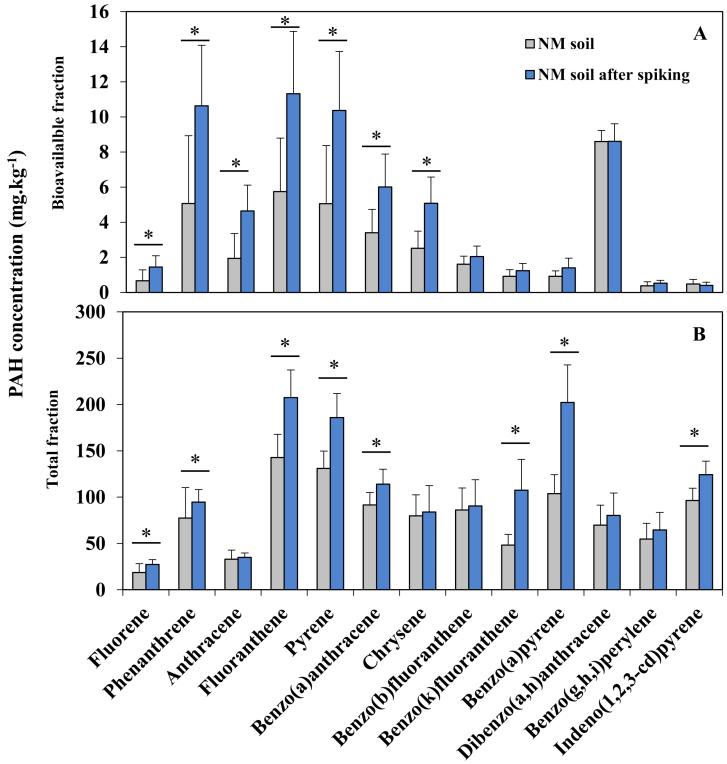


Figure S2.

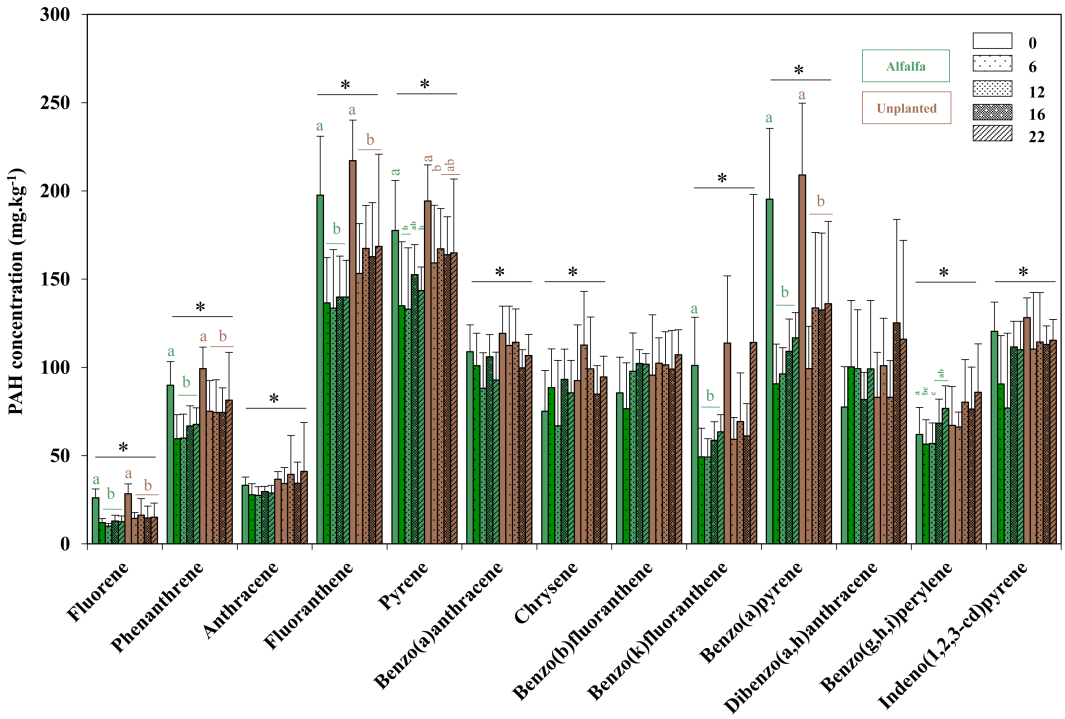


Figure S3.

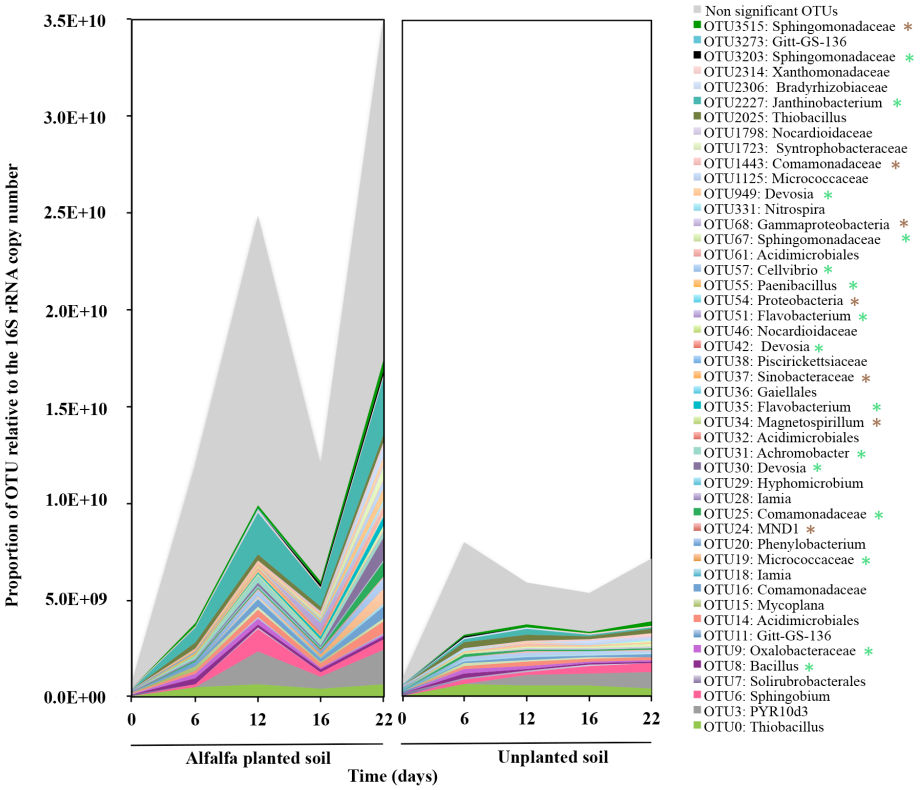
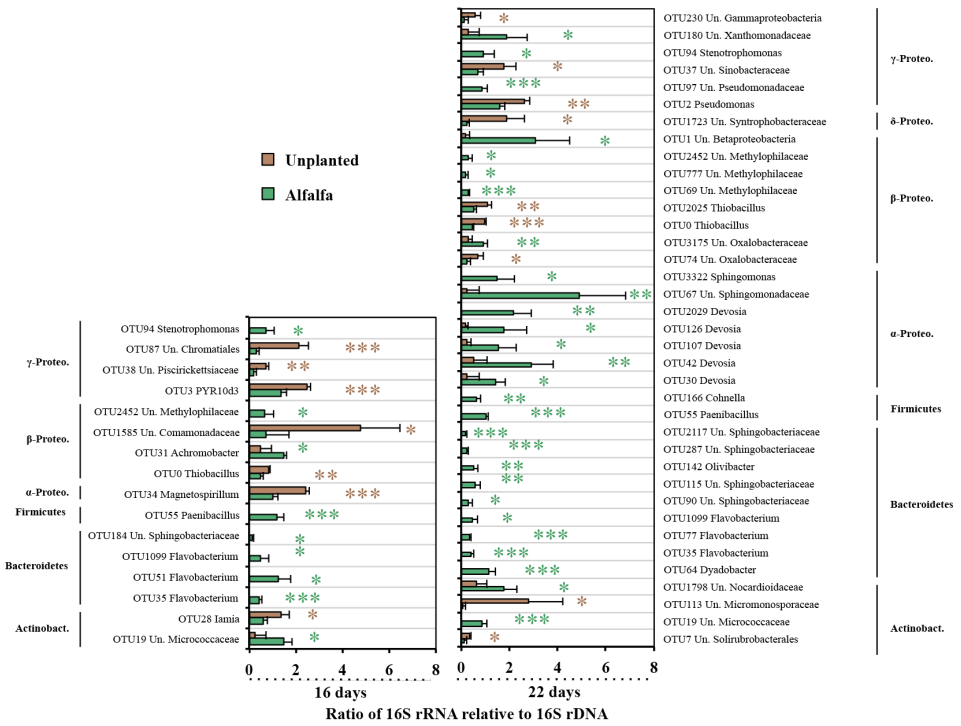


Figure S4



938

939 **Table S2.** PAH concentration (mg.L^{-1}) in the organic extract used to spike NM soil. Values are means (n=3) and standard errors.

940

941

942

PAHs concentration (mg/L)												
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**
24.8 ± 2.3	98.7 ± 4.2	35.9 ± 1.6	221.8 ± 16.7	200.9 ± 11.7	126.3 ± 12.2	101.1 ± 6.8	115.7 ± 10.8	70.7 ± 10.3	28.2 ± 7.6	70.3 ± 7.0	77.2 ± 7.8	101.7 ± 10.3

943

Variables	Time	2-3 ring PAH	4 ring PAH	5-6 ring PAH	Σ13 PAHs	pH	DOC	RootB	RootL
%18S_DNAc	**-0.646**	**0.686**	**0.451**	**0.361**	**0.474**	**-0.603**	-0.028	**-0.360**	**-0.338**
% PAH-RHDα GN_DNAc	0.106	**-0.434**	**-0.391**	-0.220	**-0.339**	0.059	-0.094	0.062	0.010
% PAH-RHDα GP_DNAc	**0.733**	-0.204	-0.253	0.137	-0.053	**0.409**	0.154	**0.452**	**0.538**
%18S_DNAg	**-0.502**	0.283	0.199	0.064	0.154	**-0.386**	-0.151	**-0.363**	**-0.485**
% PAH-RHDα GN_DNAg	-0.032	**-0.512**	**-0.448**	**-0.530**	**-0.547**	0.213	-0.029	**0.349**	0.052
% PAH-RHDα GP_DNAg	**0.497**	0.163	0.094	**0.388**	0.277	0.170	0.092	0.049	0.120

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949 Table S1. Pearson's correlation matrix between soil properties and the percentage of PAH-dioxygenase transcript and genes. Values in bold correspond to
950 significant correlation between variables ($p<0.05$).

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