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1 **Beneficial traits of root endophytes and rhizobacteria associated with plants growing in**  
2 **phytomanaged soils with mixed trace metal-polycyclic aromatic hydrocarbon contamination**

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27 **ABSTRACT**

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The diversity of cultivable bacteria associated with plants from phytomanaged soils with mixed trace metal (TM) and polycyclic aromatic hydrocarbon (PAH) contamination in Pierrelaye (France) was evaluated. The emphasis was on the cultivable bacterial community since the overall objective is to obtain bioinoculants to improve the remediation of this type of contaminated site. Root endophytic and rhizosphere soil bacterial counts were determined, and isolates were pooled by amplified rDNA restriction analysis and identified by 16S rDNA sequencing. Isolates were further characterised for the production of plant growth-promoting (PGP) substances, and resistance to TM. The selected strains were evaluated for their ability to degrade PAHs. The potential of cell-free microbial supernatant to increase the mobilisation of PAHs from the polluted soil of Pierrelaye was also evaluated. Proteobacteria and Actinobacteria dominated the collection of isolates, and differences in taxonomic diversity were observed between plant species (*Populus* or *Zea mays*) and depending on the remediation treatment (*Populus* inoculation with mycorrhizae or *Populus* intercropping with *Alnus*). The majority of isolates exhibited at least one of the tested PGP traits, as well as resistance to more than one TM. Several rhizosphere, endophyte and even one bulk soil isolate showed high rates of fluoranthene and pyrene dissipation. The endophyte *Rhizobium* strain MR28 isolated from maize and degrading pyrene produced bioemulsifying molecules capable of improving the availability of PAHs from the soil of Pierrelaye. A selection of the most interesting strains was made for other re-inoculation experiments in order to assess their potential in rhizoremediation processes.

50 **Keywords:** organic pollutants; phytomanagement; plant-growth promoting bacteria; *Populus*;  
51 polycyclic aromatic hydrocarbon; trace metals

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## 75 **1. Introduction**

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77 Phytomanaging contaminated soils can successfully restore biological fertility and diversity.  
78 Phytomanagement of metal-contaminated sites has increasingly been combined with short rotation  
79 coppicing (SRC) for the production of bioenergy (Chalot et al., 2020; Mench et al., 2009;  
80 Vangronsveld et al., 2009; Ziegler et al., 2019) or fiber-based material (Jeannin et al., 2020). SRC  
81 consists of densely planted high yielding poplar and willow species that can be harvested every 2-  
82 5 years to produce woody biomass. Willow and poplar species are recognised for their tolerance to  
83 metals and, in some species or cultivars, for their potential to extract trace metals (TM) such as Cd  
84 and Zn from contaminated soils (Chalot et al., 2020; Kidd et al., 2015). The use of polyclonal  
85 stands of *Salix* and *Populus* clones rather than monocultures has also been recommended in the  
86 phytomanagement of contaminated soils using SRC. They can offer several benefits in terms of  
87 plant productivity and nutrition, soil quality and biodiversity, and pest control (Kidd et al., 2015;  
88 Mench et al., 2010). Alder is an important pioneer species of degraded and metal-contaminated  
89 soils, and intercropping this woody plant with *Populus* or *Salix* has been proposed as a means of  
90 enhancing phytoremediation efficiency (Kidd et al., 2015).

91 Plants have been shown to promote the abundance of beneficial microorganisms in their  
92 environment, and in particular in the endosphere and rhizosphere (Thijs et al., 2017). These  
93 mutualistic plant-microbial symbioses can dramatically improve soil phytoremediation processes  
94 by promoting plant health and growth and by modifying the mobility and availability of  
95 contaminants (Mendes et al., 2013). Plant growth-promoting bacteria (PGPB) can improve plant  
96 nutrition by increasing the availability of essential plant nutrients, such as nitrogen (N<sub>2</sub>-fixing  
97 organisms), phosphorus (by solubilisation or mineralization through the production of organic  
98 acids and/or phosphatases) or iron (by releasing siderophores). They can also directly influence

99 plant growth and physiology through the production of phyto-hormones [e.g. indoleacetic acid  
100 (IAA)].

101 The application of plant-associated bacterial inoculants is gaining increasing interest in sites of  
102 mixed contamination, caused by the presence of organic and inorganic compounds. Polycyclic  
103 aromatic hydrocarbons (PAHs) are some of the most widespread organic pollutants; their fate in  
104 soils is of great environmental concern due to their potential toxicity, mutagenicity and  
105 carcinogenicity (Baoune et al., 2019). Mixed contaminated soils represent a source of potentially  
106 beneficial bacteria associated with plants that could be used as part of microbial-assisted  
107 remediation strategies targeting this type of site (Weyens et al., 2009). Selection of PAH degraders  
108 with metal tolerance and surfactant production abilities, or combining PAH degraders with  
109 biosurfactant producers, is of particular interest since surfactants enhance the contaminant  
110 solubility (Hickey et al., 2007). Endophytic bacteria can be a great option as bioinoculants because  
111 they have the advantage of proliferating in plant tissue, thus facing less competition for nutrients  
112 and being protected from the high stress environments of polluted soils (Pawlik et al., 2020).  
113 However, few studies have identified PAH-degrading or biosurfactant-producing endophytes.

114 The objectives of this study were to: (a) assess the cultivable bacterial diversity associated with  
115 plants under different phytomanagement options based on poplar SRC established in a  
116 contaminated mixed site in Pierrelaye (France), (b) isolate root endophytes and rhizobacteria  
117 associated with plants, (c) characterize the bacterial strains for traits promoting plant growth, (d)  
118 analyse bacterial strains for PAH degradation capacity, biosurfactant production potential and TM  
119 tolerance, and (e) assess the influence of certain biosurfactant producers on the availability of  
120 PAHs. The general hypothesis is that certain cultivable bacteria isolated from contaminated sites  
121 can produce bioinoculants which can greatly improve the remediation of PAH-contaminated soils.

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## 123 **2. Materials and methods**

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## 125 **2.1 Soil and plant material**

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127 Soil and plant samples were collected from the experimental field site of Pierrelaye (northwest of  
128 Paris, France). Long-term (>100 years) irrigation of these agricultural soils with wastewater from  
129 Paris resulted in a mixed contamination of both TM (mostly Zn, Cd, Pb and Cu) and organic  
130 pollutants (including PAHs). A description of the general soil properties on this site is given by  
131 Ciadamidaro et al. (2017), while data of TM concentration in soil were published by Lamy et al.  
132 (2006) and Pottier et al. (2015). Two phytomanagement field trials were established on the  
133 Pierrelaye site, one in 2007 (project PHYTOPOP) based on the SRC using 14 different poplar  
134 cultivars (Foulon et al., 2016a, 2016b; Pottier et al., 2015), the second in 2011 (project  
135 BIOFILTREE) to assess the effect of mycorrhizal inoculation by intercropping *Populus* with  
136 *Alnus glutinosa* (Ciadamidaro et al., 2017).

137 The present work is based on rhizosphere soil, plant samples and bulk soil collected from plots  
138 with the clone *Populus trichocarpa* × *Populus maximowiczii* Skado [non-inoculated (NI) or  
139 inoculated (INOC) with mycorrhizal fungi], from plots with the non-inoculated poplar Skado  
140 intercropped with *Alnus* (referred to as intercropped *Populus* plots in the whole manuscript), from  
141 plots adjacent to the BIOFILTREE trial planted with maize and from non-planted soil (bulk soil)  
142 from the BIOFILTREE field trial. In addition, the rhizosphere soils from *Populus* and bulk soil  
143 from the PRPHYTOPOP field trial located also in Pierrelaye were studied. Sampling of soils and  
144 plant roots was carried out according to Foulon et al. (2016a, 2016b). Five replicate of each  
145 sample were studied.

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## 147 **2.2 Isolation of rhizobacterial and root endophytic bacterial strains**

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149 Five grams of bulk soil or fresh rhizosphere soil of five *Populus* and maize plants were suspended  
150 in 45 mL sterile sodium hexametaphosphate solution (1%) and shaken for 30 min in an end-over-  
151 end shaker. Soil suspensions were diluted in 10-fold series and plated in duplicate onto 284 agar  
152 medium (Schlegel et al., 1991) supplemented with 100 µg/mL of cycloheximide. The 284 medium  
153 contains (g/L): 6.06 Tris-HCl, 4.68 NaCl, 1.49 KCl, 1.07 NH<sub>4</sub>Cl, 0.43 Na<sub>2</sub>SO<sub>4</sub>, 0.2 MgCl<sub>2</sub>·6H<sub>2</sub>O,  
154 0.03 CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.04 Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O, 10 mL Fe(III)NH<sub>4</sub> citrate solution (containing 48  
155 mg/100mL) plus oligoelements (1.5 mg FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.3 mg H<sub>3</sub>BO<sub>4</sub>, 0.19 mg CoCl<sub>2</sub>·H<sub>2</sub>O, 0.1 mg  
156 MnCl<sub>2</sub>·4H<sub>2</sub>O, 0.08 mg ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.02 mg CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.036 mg Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O) adjusted  
157 to pH 7. The medium was supplemented with a mixture of different carbon sources (g/L): 0.7  
158 lactate, 0.5 glucose, 0.7 gluconate, 0.5 fructose, and 0.8 succinate. After seven days of incubation  
159 at 28 °C, colony forming units (CFUs) were counted and calculated per gram dry weight (DW)  
160 soil. Distinct colony morphotypes associated with each sample group were sub-cultured at least  
161 three times to ensure purity and cryopreserved.

162 To isolate the root endophytic bacteria of NI *Populus*, root samples (0.2-0.3 g) were surface-  
163 sterilised for 10 min in 2% NaClO solution supplemented with one droplet Tween 80 per 100 ml  
164 solution, and were subsequently rinsed three times for 1 min in sterile deionised water. For maize,  
165 root samples (0.2-0.3 g) were surface-sterilised by sequential immersion in 5% NaClO solution  
166 supplemented with one droplet Tween 80 per 100 ml solution for 10 min and in 75% (v/v) ethanol  
167 for 1 min. The surface-sterilisation process was checked by plating aliquots of the third rinsing  
168 solution on 869 medium [(g/L): 1.0 tryptone, 0.5 yeast extract, 0.5 NaCl, 0.1 glucose, and 0.035  
169 CaCl<sub>2</sub>·2H<sub>2</sub>O]. No growth on these control plates after seven days indicated successful sterilisation.  
170 The concentrations of NaClO and the sterilisation times were empirically determined in previous  
171 studies. After surface sterilisation, the root tissue was macerated in 10 mL of 10 mM MgSO<sub>4</sub> using  
172 a Polytron PT1200E homogeniser (Kinematica AG). Ten-fold serial dilutions were plated in  
173 duplicate on 1/10 strength 869 agar medium, CFUs were determined as above and calculated per



174 gram fresh root weight. Distinct colony morphotypes were sub-cultured at least three times to  
175 ensure purity and cryopreserved.

176

### 177 **2.3 Phenotypic characterisation of bacterial isolates**

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179 The tolerance to TM was tested growing the strains at 28 °C for seven days in 284 agar medium  
180 (see above) supplemented with Cd, 0.1 mM; Cu, 1.0 mM; Pb, 1.0 mM or Zn 2.5 mM. The ability  
181 to solubilize inorganic phosphate was assessed in NBRIP agar medium adjusted to pH 7.0,  
182 modified from Nautiyal (1999) supplied with 5 g/L of hydroxyapatite and incubated at 28 °C for 5  
183 days. Yeast extract was added since some strains were unable to grow in yeast-free NBRIP  
184 medium. Siderophore production was detected in a modified 284 liquid medium (without Fe)  
185 using the Chrome Azurol S (CAS) method described by Schwyn and Neilands (1987). The ability  
186 of isolated strains to produce IAA was evaluated in liquid medium, modified from Sheng et al.  
187 (2008) supplemented with 0.5 mg/mL of tryptophan. After five days of incubation at 28 °C,  
188 cultures were centrifuged and the presence of IAA was evaluated spectrophotometrically (535 nm)  
189 after incubating the supernatant with Salkowski reagent for 25 min. Strains were screened for  
190 potential biosurfactant production using the qualitative method of Chen et al. (2007).

191

### 192 **2.4 Genotypic characterisation of bacterial isolates**

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194 For DNA extraction, purified strains were grown in 1/10 strength 869 liquid medium; and  
195 genomic DNA was extracted from bacterial cell pellets according to Mergeay et al. (1985).  
196 Polymerase chain reaction (PCR) amplification of 16S rRNA genes was carried out in mixtures  
197 containing 100 ng/μl DNA, 1× High Fidelity PCR buffer (Invitrogen, Carlsbad, CA, USA), 0.2  
198 mM of each of the four deoxynucleoside triphosphates, 2 mM MgCl<sub>2</sub>, 0.2 μM each of the forward

199 and reverse primers, and 1 U of High Fidelity Platinum Taq DNA polymerase (Invitrogen,  
200 Carlsbad, CA, USA) per 50 µl. The universal 1392R (5'-ACGGGCGGTGTGTRC-3') and the  
201 bacteria-specific 26F (5'-AGAGTTTGATCCTGGCTCAG-3') primers were used for prokaryotic  
202 16S rRNA gene amplification. Cycling conditions consisted of: one denaturation cycle at 94 °C  
203 for 5 min, followed by 30 cycles at 94 °C for 1 min, 45 °C for 45 s, and 72 °C for 1.5 min, and  
204 completed with an extension cycle of 10 min at 72 °C. PCR products were purified using the  
205 QIAquick PCR purification columns (Qiagen, Valencia, CA, USA) and quantified  
206 spectrophotometrically, using the Nanodrop spectrophotometer (ND-1000, Isogen Life Science).  
207 The PCR products were subjected to 16S rDNA restriction analysis (ARDRA), used for screening  
208 the collection of isolates.

209 For ARDRA, aliquots of the PCR products were digested overnight at 37 °C with 1 U of the  
210 four-base-specific restriction endonuclease HpyCH4 IV in 1×NEB buffer 1 (New England  
211 Biolabs, Beverly, MA, USA). The digestion products obtained were separated by electrophoresis  
212 in a 1.5 % agarose gel, and visualized by red safe staining and UV illumination. Bacterial strains  
213 with the same ARDRA patterns were grouped using the Pearson correlation coefficient and  
214 UPGMA clustering algorithm of the Gel Compar Bionumerics programme (Bionumerics Version  
215 5.1, Applied Maths, Belgium). One representative strain of each group was selected for  
216 sequencing. Sequence data were checked using the Chromas v.1.45 software (Technelysium Pty.  
217 Ltd., Australia) and assessed for similarity with sequences of the Ribosomal Database Project  
218 (Cole et al., 2009).

219

## 220 **2.5 PAH degradation capacity**

221

222 Based on their plant growth promoting characteristics, a selected number of isolates were  
223 cultivated in minimal salt medium (MSM) with a mixture of phenanthrene and fluoranthene and

224 pyrene (Sigma-Aldrich). The PAHs were chosen on the basis of the Pierrelaye soil analyses  
225 (Supplementary Table 1). The composition of the MSM was as follows: 100 mL phosphate buffer  
226 [ $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  (140 g/L),  $\text{KH}_2\text{PO}_4$  (20 g/L)], 10 mL salt solution [ $(\text{NH}_4)_2\text{SO}_4$  (100 g/L),  
227  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (20 g/L)], 1 mL  $\text{Ca}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$  (4.62 g/L), 28 mL  $\text{FeNH}_4$  enriched with 18% Fe  
228 (48 mg/100mL), 1 mL oligoelements solution (1L: HCl 37% 5.2 mL,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  1.5 g,  
229  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  0.08 g,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  0.1 g,  $\text{H}_3\text{BO}_3$  0.3 g,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  0.19 g,  $\text{CuSO}_4$  0.004 g,  
230  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  0.024 g,  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  0.036 g), 860 water. A stock solution of the three PAHs  
231 dissolved in acetone (120  $\mu\text{g}/\text{mL}$ ) was added to test tubes containing 4 mL of MSM to reach a  
232 final concentration of 2  $\mu\text{g}$  of PAHs per ml medium. The test tubes were left uncapped for 30 min  
233 to allow evaporation of the acetone prior to the microbial inoculation inside a laminar sterile hood.

234 To prepare the bacterial inoculants, strains were cultivated in 1/10 strength 869 liquid medium  
235 for two days, harvested by centrifugation (4000 x g, 15 min, 4 °C), washed two times and re  
236 suspended in 10 mM  $\text{MgSO}_4$  to an optical density of 1.0 at 600 nm (about  $10^7$ - $10^8$  cells per mL).  
237 Tubes inoculated with 100  $\mu\text{L}$  of each bacterial strain suspension were incubated at 28 °C and 150  
238 rpm for two, four and seven days. Non-inoculated tubes with MSM were used as a control and  
239 four replicates of each treatment were prepared.

240 PAHs were extracted with 10 mL of hexane added to each test tube in an end-over-end shaker  
241 for 30 min. Extracts were dried with anhydrous  $\text{Na}_2\text{SO}_4$  and then stored at -20 °C for posterior  
242 chromatographic analysis.

243 The concentration of PAHs was determined using a gas chromatograph (Varian 450-GC) and a  
244 mass spectrometer with ion trap (Varian 220-MS) equipped with an automatic sampler (CP-8400,  
245 Varian Inc., Palo Alto, CA, USA). The split/splitless injector worked at 300 °C and in splitless  
246 mode. Sample injection volume was 1  $\mu\text{L}$ . The chromatographic column was a Thermo Trace  
247 Gold TG-5SILMS (30 m x 0.25 mm x 0.25  $\mu\text{m}$ ) of 5% phenyl methyl polysiloxane. The column  
248 oven temperature was varied as follows: 60 °C held for 5 min, followed by an increase of 8 °C

249  $\text{min}^{-1}$  up to 300 °C which was held for 4 min. The carrier gas was helium at constant flow of 1.5  
250  $\text{mL min}^{-1}$ .

251 The mass spectrometer operated in MS/MS mode, ionization of the molecules was carried out  
252 by electronic impact (EI) and the transfer line temperature and the ion trap temperature was fixed  
253 at 280 °C and at 220 °C, respectively.

254

## 255 **2.6 Influence of a biosurfactant on PAH availability**

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257 The potential effect of a microbial biosurfactant on the soil PAHs availability was tested using a  
258 cell free culture supernatant as soil extractant. The MR28 strain was cultured in 1/10 strength 869  
259 liquid medium (30 °C, 150 rpm) for two days, and then centrifuged at  $9,000 \times g$  for 10 min. The  
260 supernatant was separated and filtered at 0.22  $\mu\text{m}$  to remove bacterial cells.

261 Soil from the Pierrelaye site was air-dried and sieved to <2 mm. Four g of soil were placed in  
262 separate glass test tubes sterilized (Alvarez et al., 2015) and extracted with 10 mL of the filtered  
263 culture supernatant. The extraction was performed by shaking the tubes at 150 rpm, 30 °C for five  
264 days. Control tubes were prepared in parallel with soil samples treated with 869 liquid medium  
265 (1/10 strength) filtered at 0.22  $\mu\text{m}$  instead of culture supernatant. Four replicates of each treatment  
266 were performed. The soil extracts were separated from soil particles by centrifugation (9,000  $\times g$ ,  
267 10 min, 4 °C) and the PAHs in the extracts were obtained with 10 ml of hexane by 60 min of  
268 agitation in an end-over end shaker. PAHs in hexane were determined as explained above.

269

## 270 **3. Results**

271

### 272 **3.1 Abundance, identification and phylogenetic analysis of cultivable rhizosphere and root** 273 **endophytic bacteria**

274  
275 The densities of cultivable bacteria in soil (bulk and rhizosphere) and root endophytes (poplar vs.  
276 maize) are presented in Table 1. Rhizobacterial densities did not differ significantly between plant  
277 species but were up to one logarithmic unit higher than those found in bulk soils. On the other  
278 hand, densities endophytes in the root of poplar or maize were not significantly different, but were  
279 between two to three logarithmic units lower than rhizobacterial densities (Table 1).

280 A total of 644 different colony morphotypes was isolated, including 327 rhizobacterial strains,  
281 161 strains from bulk soil and 156 root endophytic strains (108 from NI *Populus* and 48 from *Zea*  
282 *mays*). The diversity of cultivable bacteria was estimated using ARDRA and 16S rDNA  
283 sequencing. At a similarity of >90% ARDRA profiles of isolates from PHYTOPOP were allocated  
284 into 64 and 28 ARDRA groups for rhizobacteria associated with *Populus* and for bacteria from the  
285 bulk soil, respectively. In the case of the ARDRA profiles of the bacteria isolated from the  
286 rhizosphere of *Populus* of the different BIOFILTREE plots, were allocated in the following  
287 groups: 32 groups for INOC *Populus*, 33 groups for NI *Populus*, 28 groups for intercropped  
288 *Populus* and 29 ARDRA groups for bulk soil. Rhizobacterial isolates associated with *Zea mays*  
289 adjacent to the BIOFILTREE plots were classified in 19 ARDRA groups which reflects a lower  
290 variety of taxa compared to rhizobacterial isolated from *Populus*.

291 The ARDRA profiles of root endophyte isolates from BIOFILTREE plots were divided into 31  
292 and 14 groups for *Populus* (NI) and *Zea mays*, respectively. Thus, the cultivable root endophytic  
293 community of *Populus* showed a greater variety of taxa than that of *Zea mays*.

294 The 16S rDNA of one representative isolate per ARDRA group was partially sequenced.  
295 Gram-positive and Gram-negative bacteria were adequately represented in samples from both  
296 PHYTOPOP and BIOFILTREE plots (Table 2). In the PHYTOPOP field trial, isolates belonging  
297 to the phylum Actinobacteria dominated the collections obtained from both the *Populus*  
298 rhizosphere and the bulk soil. Within this phylum, most of the isolates were classified as

299 *Streptomyces*, although the genus *Rhodococcus* was also well represented in the rhizosphere of  
300 *Populus*. Firmicutes was the second most dominant phylum in the PHYTOPOP samples. In both  
301 the *Populus* rhizosphere and the bulk soil, most of the isolated Firmicutes belonged to the genera  
302 *Bacillus* or *Paenibacillus*. Proteobacterial strains were also found: 19% of the rhizobacterial  
303 isolates associated with *Populus* and <10% of strains from the bulk soil. The variety of  
304 Proteobacteria was higher in the rhizobacterial community of *Populus* in which isolates belonging  
305 to the Alpha, Beta- and Gamma-proteobacteria were found. Gamma-proteobacteria was not  
306 represented in the collection of the bulk soil.

307 The collections of isolates obtained from the rhizosphere of *Populus* growing in the  
308 BIOFILTREE plots were also dominated by members of the phylum Actinobacteria.  
309 Actinobacteria isolated from the rhizosphere of NI *Populus* grown in monocultures mainly  
310 belonged to the genera *Streptomyces*, *Rhodococcus* and *Arthrobacter*. On the other hand, in the  
311 collections of INOC or intercropped *Populus*, these genera, in particular *Streptomyces*, were also  
312 represented but the variety of Actinobacteria was higher: members of the genera *Microthricus*,  
313 *Nocardioides*, *Micrococcus*, and *Microbacterium* were identified in the rhizosphere of INOC  
314 *Populus* whereas *Microbacterium*, *Kribbella*, *Knoella* and *Agromyces* were found in the  
315 rhizobacterial community of intercropped *Populus*. By comparing the *Populus* plots and  
316 intercropped *Populus* plots, the genera *Kribbella* and *Agromyces* were only found in the later. In the  
317 collections of NI and INOC *Populus* grown in monoculture, Firmicutes were mainly represented  
318 by *Bacillus* strains, while in the samples collected in intercropped *Populus* plots, the three genera  
319 *Paenibacillus*, *Bacillus* and *Lysinibacillus* were similarly represented.

320 Cultivated Proteobacteria from the rhizosphere of *Populus* included members of the Alpha-,  
321 Beta- and Gamma- classes and the variety of taxa was higher in NI than in INOC *Populus*. In fact,  
322 strains of the *Rhizobiales*, *Ensifer*, *Pseudochrobactrum* and of *Lysobacter* were isolated only from  
323 NI samples. The collection obtained from the rhizosphere of intercropped *Populus* plots included

324 Alpha and Betaproteobacteria; however, most of the isolates were affiliated to genera different  
325 than those found in monoculture plots. In the intercropped *Populus* plots, strains of the  
326 *Rhizobiales*, *Devosia*, *Mesorhizobium* and *Rhizobium* represented about 1/3 of the cultivated  
327 Proteobacteria. Regarding the phylum Bacteroidetes, strains of *Flavobacterium* and *Chitinophaga*  
328 were cultivated from NI *Populus* rhizosphere and isolates of *Pedobacter* were obtained from  
329 INOC plots. Bacteroidetes were absent in the collection of intercropped *Populus* (Table 2).

330 Unlike the rhizobacterial communities of *Populus*, in the rhizosphere of maize grown on the  
331 BIOFILTREE plots, Proteobacteria predominated, while a lower percentage of Actinobacteria was  
332 found (Table 2). The percentage of isolates affiliated to both Firmicutes and Bacteroidetes was  
333 12.5%. The variety of Proteobacteria and Actinobacteria was lower in maize rhizosphere than in  
334 *Populus* rhizosphere. Proteobacteria of the Alpha-, Beta- and Gamma classes were isolated and the  
335 strains were classified in five different genera: *Rhizobium*, *Variovorax*, *Duganella*, *Pseudomonas*  
336 and *Lysobacter*. The isolated Actinobacteria were classified as *Streptomyces*, *Rhodococcus* and  
337 *Arthrobacter*. The Bacteroidetes and Firmicutes were only represented by members of the genera  
338 *Flavobacterium* and *Bacillus*, respectively.

339 The taxonomic breakdown of the root endophytic communities of NI *Populus* and *Zea* mays  
340 from BIOFILTREE plots was in line with that observed in corresponding rhizosphere soils: 45%  
341 of the root endophytes of *Populus* were classified as Proteobacteria, 32.3% as Actinobacteria and  
342 23% as Firmicutes, while the maize root endophytes were clearly dominated by members of the  
343 Proteobacteria (75%). In maize root the proportion of isolates classified as Actinobacteria and  
344 Firmicutes were 7% and 8%, respectively (Table 2).

345

### 346 **3.2 Plant growth-promoting characteristics of rhizosphere and root endophytic bacteria**

347

348 The majority of the isolates obtained from *Populus* and maize plants had at least one of the  
349 PGP traits tested (Supp. Fig. 1A). Soil rhizosphere bacteria were generally able to produce IAA  
350 (>75% of all isolates) and the higher IAA-producers were mostly associated with *Populus* rather  
351 than *Zea mays*. However, the majority of these bacteria produced low levels of IAA (<5 µg/mL),  
352 while the higher IAA-producers were mainly associated with *Populus* rather than *Zea mays*. In  
353 addition, greatest number of higher IAA-producers was observed in the intercropped *Populus* plots  
354 (8 strains produced 5-20 µg/mL IAA and these were identified as *Bacillus* sp., *Rhizobium* sp. and  
355 *Streptomyces* sp.). In contrast, maize rhizobacteria harboured a greater number of bacterial strains  
356 producing siderophore (approx. 75% of isolates). Only 30% of the isolates obtained from  
357 intercropped *Populus* plots showed this trait. P-solubilizers were predominantly associated with  
358 INOC *Populus* (no P-solubilizers were found in NI *Populus*) or with maize. The ability to produce  
359 biosurfactants was less common among isolate collections: between 5 to 20% of rhizosphere soil  
360 isolates exhibited this trait, and the greatest number of biosurfactant-producing strains were found  
361 in rhizosphere soil of INOC *Populus* or maize. In general, between 30 and 60% of isolates were  
362 able to grow in the presence of Cd, Cu, Pb or Zn (Supp. Fig. 1B).

363 In the case of root endophytes, 20-25% of root isolates were strains producing siderophore  
364 (Supp. Fig. 1A). P-solubilisers were again more associated with roots of *Zea mays* than *Populus*,  
365 and up to 40% of maize root endophytes were able to solubilise mineral phosphate. Producers of  
366 biosurfactants were also less frequent and were mainly associated with *Populus* and identified as  
367 *Bacillus*. Only two strains isolated from maize roots were capable of producing biosurfactants and  
368 were identified as *Rhizobium* and *Pseudomonas*.

369 The most frequently observed PGP trait was the ability to produce IAA (although the majority  
370 of the strains produced <5 µg/mL IAA), and a higher number of these IAA-producing strains were  
371 associated with *Populus* rather than maize. With the exception of Cu, metal-resistant strains were



372 more frequent in root endophytes from *Populus* than maize (50-60% of *Populus* isolates could  
373 grow in the presence of Cd, Pb and Zn compared to 15-20% of maize isolates) (Supp. Fig. 1B).

374

### 375 **3.3 PAHs degradation**

376

377 Bacterial strains belonging to different collection of isolates were evaluated for their ability to  
378 degrade phenanthrene, fluoranthene and pyrene as the only C source. Isolates showing at least two  
379 PGP traits (including biosurfactant production) as well as at least one metal tolerance were  
380 selected for this purpose (Table 3).

381 Thus, eleven bacterial isolates including endophyte, rhizospheric and bulk soil strains were  
382 assessed for their ability to degrade PAHs. Mean concentrations of phenanthrene, fluoranthene and  
383 pyrene in the non-inoculated medium were  $1248.6 \pm 53.1$   $\mu\text{g/L}$ ,  $1531.5 \pm 119.6$   $\mu\text{g/L}$  and  $1726.9 \pm$   
384  $131.4$   $\mu\text{g/L}$ , respectively (Figure 1). The mean phenanthrene concentration decreased after seven  
385 days of testing with most of the strains. The final concentrations were between  $815.3 \pm 182.6$   $\mu\text{g/L}$   
386 and  $1236.2 \pm 88.6$   $\mu\text{g/L}$ ; however, the differences were no statistically significant compared to the  
387 non-inoculated medium ( $p > 0.05$ ) (Figure 1A). A notable exception was recorded by the strain  
388 *Bacillus* sp. B32.23, which by the fourth day of cultivation exhibited a significant decrease in  
389 phenanthrene concentration ( $758.0 \pm 111.7$   $\mu\text{g/L}$  vs  $1248.6 \pm 53.1$   $\mu\text{g/L}$ , in B32.23 tubes and in the  
390 non-inoculated control, respectively) ( $p < 0.05$ ). On the fourth day of the experiment, the observed  
391 decrease in fluoranthene concentration was even more pronounced especially in the case of the  
392 strain *Bacillus* sp. B32.23 ( $773.6 \pm 136.9$   $\mu\text{g/L}$  residual fluoranthene,  $p < 0.05$ ). At the end of the  
393 incubation, the strains *Streptomyces* sp. Sk.2012, *Bacillus* sp. Sk1.23, *Rhodococcus* sp. Sk12.6,  
394 *Microbacterium* sp. Sk11.4 and *Bacillus* sp. Sk2.3 also reduced the fluoranthene concentration in  
395 the culture media ( $p < 0.05$ ). In the case of these isolates pronounced fluoranthene dissipation took  
396 place between day four and day seven of incubation (Figure 1B). Again, at the end of the

397 experiment, the lowest fluoranthene concentration ( $734.8 \pm 347.0 \mu\text{g/L}$ ) was found in tubes  
398 inoculated with *Bacillus* sp. B32.23, although similar concentration of residual fluoranthene was  
399 detected in tubes inoculated with the strains *Streptomyces* sp. Sk.20.12, *Bacillus* sp. Sk1.23,  
400 *Rhodococcus* sp. Sk12.6, *Microbacterium* sp. Sk11.4 and *Bacillus* sp. Sk2.3 strains ( $p>0.05$ ).

401 A significant decrease in the pyrene concentration was observed in the presence of the strain  
402 *Bacillus* sp. B32.23 on the second day of the incubation (Fig. 1C) ( $p<0.05$ ). On the fourth day, the  
403 pyrene concentration was also significantly reduced by *Pseudomonas* sp. MR47 and  
404 *Sphingomonas* sp. SR62. In addition to *Sphingomonas* sp. SR62 and *Bacillus* sp. B32.23 strains, a  
405 pronounced decrease in residual pyrene was recorded towards the end of the experiment in tubes  
406 inoculated with strains *Rhizobium* sp. MR28, *Microbacterium* sp. Sk11.4, *Rhodococcus* sp.  
407 Sk12.6, *Bacillus* sp. Sk1.23, *Streptomyces* sp. Sk20.12 and *Bacillus* sp. Sk2.3. As in the case of  
408 fluoranthene, the dissipation of pyrene by these isolates was only detected at the end of the  
409 incubation. For instance, in the case of strain Sk11.4, pyrene concentration dropped sharply from  
410  $1723.0 \pm 49.5 \mu\text{g/L}$  at day four, to  $968.0 \pm 191.6 \mu\text{g/L}$  at day seven. The lowest residual pyrene  
411 concentration was detected in the presence of *Bacillus* sp. B32.23 strain ( $843.1 \pm 407.3 \mu\text{g/L}$ ),  
412 although this value was not significantly different than those observed for isolates MR28, Sk11.4,  
413 Sk12.6, Sk1.23 and Sk20.12 ( $p>0.05$ ).

414

### 415 **3.4 Influence of a biosurfactant on PAH availability**

416

417 The maize-endophyte strain *Rhizobium* sp. MR28 was selected for its high capacity to produce  
418 bioemulsifiers. Thus, the cell-free culture supernatant of *Rhizobium* sp. MR28 was evaluated as a  
419 soil extractant to test if bioemulsifiers potentially released to the growth medium could increase  
420 the bioavailability of PAHs in Pierrelaye's soil. Nine different PAHs were detected in the soil  
421 extracts, and the concentration of four of them, namely naphthalene, fluorine, anthracene and

422 phenanthrene was significantly higher in extracts obtained from MR28 cell-free growth medium  
423 than in those obtained with sterile culture medium (control) ( $p<0.05$ ) (Fig. 2).

424

#### 425 **4. Discussion**

426

427 The use of microorganisms for treating soils contaminated with TM or organic compounds is  
428 an environmentally safe technology which can be improved by plants via the stimulation of  
429 microbial growth (Baoune et al., 2019). In the present study, the cultivable bacterial diversity  
430 associated with plants established in a mixed contaminated site in Pierrelaye (France) under  
431 different SRC-based phytomanagement options was evaluated.

432 Rhizobacterial densities did not differ significantly between plant species (poplar vs maize)  
433 but were up to one logarithmic unit higher than those found in bulk soils. This is a common  
434 observation, which can be related, among many other factors with a greater availability of  
435 carbon in the rhizosphere (Kidd et al., 2008). The main driver for the rhizosphere effect is the  
436 massive root supply of organic substrates to the soil, which can increase the bioavailability of  
437 PAHs but also induce a selection of rhizospheric microbial inhabitants (Arslan et al., 2014;  
438 Kidd et al., 2009). Root exudates and mucilage-derived nutrients attract large numbers of  
439 organisms to the rhizosphere environment. However, the endophytic competence/colonization  
440 is limited to specific bacterial species (Durand et al., 2017). Densities of endophytes in the  
441 root of poplar or maize were not significantly different, but were between two to three  
442 logarithmic units lower than in the rhizosphere. The lower diversity of the endophytic  
443 communities compared to soil communities can be explained by the oligotrophic environment  
444 present inside plants, leading to a restriction of bacteria capable of using the available  
445 resources (Baoune et al., 2018).

446 According to the partially sequenced 16S rDNA, both Gram-positive and Gram-negative  
447 bacteria were widely represented in the samples from the PHYTOPOP and BIOFILTREE trials  
448 suggesting that the variety of different taxa may reflect the adaptation of the community to the  
449 contaminated soil of Pierrelaye over several decades (Bourceret et al., 2016). The dataset obtained  
450 for the roots and soil samples in this work corroborates other previous studies carried out at the  
451 same site (Pierrelaye) on native tree species (poplar and willow), revealing the dominance of  
452 Proteobacteria and Actinobacteria (Durand et al., 2017; Foulon et al., 2016).

453 The collection of isolates obtained from the rhizosphere of *Populus* was dominated by  
454 members of the phylum Actinobacteria. Gram-positive bacteria, and in particular the  
455 Actinobacteria, have frequently been found to dominate cultivable bacterial communities of TM  
456 contaminated soils and natural soils rich in TM, such as serpentine soils (Álvarez-López et al.,  
457 2016; Becerra-Castro et al., 2012). Several actinobacterial inoculants, selected for their PGP traits,  
458 have been used successfully in bioaugmentation assays to enhance TM extraction (phytoextraction  
459 and phytomining) (Becerra-Castro et al., 2013; Cabello-Conejo et al., 2014). Actinobacteria are  
460 also known for their ability to remove xenobiotics, such as pesticides, from aquatic and terrestrial  
461 environments (Álvarez et al., 2017).

462 Significant reductions in CaCl<sub>2</sub>-extractable TM concentrations under poplar-planted soils at the  
463 Pierrelaye site were observed compared to unplanted soils or to baseline values prior to the  
464 implementation of the phytomanagement trials (Foulon et al., 2016a; Ciadamidaro et al., 2017).  
465 Mycorrhizal inoculation also significantly increased the biomass yield of the *Populus* Skado clone  
466 at the Pierrelaye site (Ciadamidaro et al., 2017). Foulon et al. (2016a) reported that higher  
467 bacterial diversity and richness indices were observed in poplar soils compared to unplanted soils,  
468 which is in agreement with the results of the present study. In the present study, the bacterial  
469 communities associating with the INOC *Populus* were found to be significantly different from the  
470 NI *Populus* in the BIOFILTREE plots. At the phyla level, the dominant bacterial groups remained

471 the same (Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria) but, surprisingly, the  
472 variety of cultivable bacteria was lower when the trees had been previously inoculated (INOC) by  
473 a mycorrhizal consortium. Inoculation led to a decrease in the relative abundance of members of  
474 the Bacteroidetes and Proteobacteria, and to an increase in those belonging to Firmicutes.

475 A clear enrichment in the Proteobacteria phylum in the endospheric compartment has been  
476 recorded in previous publications (Afzal et al., 2014). Such as in the case of the rhizosphere, a  
477 higher variety of isolates was observed in the root endophytic community of *Populus* compared to  
478 that of maize. Blain et al. (2017) characterized the endophytic root bacteria associated with the  
479 natural vegetation growing on a hydrocarbon-contaminated site. In agreement with our results, the  
480 authors found that the bacterial orders more represented within these communities were  
481 Rhizobiales, Pseudomonadales, Burkholderiales, Sphingomonadales and Actinomycetales.  
482 Members of these orders seem to be well adapted to PAH contaminated soils and useful for  
483 remediation strategies (Blain et al., 2017).

484 Bioaugmentation with PGP and PAH-degrading bacterial strains has been shown to improve  
485 dissipation of PAHs in contaminated soils. Recent studies have identified rhizosphere and  
486 endophytic bacteria which have the potential to degrade PAH and are widely distributed in the  
487 organs and rhizosphere of plants growing in PAH-polluted sites (Baoune et al., 2018; Pawlik et al.,  
488 2020). Nevertheless, studies on endophytes or rhizosphere bacteria with PAH degradation capacity  
489 are still rare. In the present work, several bacterial strains were evaluated for their ability to  
490 degrade phenanthrene, fluoanthene and pyrene. As was previously mentioned, all the strains  
491 selected to evaluate their capacity to degrade PAHs showed at least two PGP traits and were  
492 tolerant to at least one TM. Among the fluoanthene degrading bacteria, the actinobacterium  
493 *Streptomyces* sp. Sk2012 was found to be able to produce the highest IAA amount among all the  
494 isolates assessed. The IAA is the most important phyto-hormone responsible for root development  
495 stimulating plant cell proliferation and plant cell elongation (Glick, 2014). Another fluoanthene

496 degrading actinobacterium, *Rhodococcus* Sk12.6, was the only soil strain capable of solubilizing  
497 phosphate, a rare trait for members of the phylum as they are rarely described for their ability to  
498 produce the organic acid responsible for the solubilization of phosphate (Jog et al., 2014). It is  
499 important to note that two endophytes (*Sphingomonas* SR62 isolated from *Populus* and *Rhizobium*  
500 sp. MR28 isolated from maize) were among the best pyrene degrading strains. According to Afzal  
501 et al. (2014), endophytic bacteria with PGP and pollutant degradation traits performed better than  
502 bacteria with only one of these characteristics. In addition, the PAH-degrading activity could be  
503 considered itself as a PGP characteristic, because contaminants, have a negatively effect on plant  
504 development.

505 A wide range of bacteria are able to degrade low molecular weight PAHs, while relatively few  
506 microorganisms are able to degrade high molecular weight recalcitrant PAHs (Ghosal et al., 2016).  
507 Interestingly, although fluoranthene and pyrene have been used as a model of high molecular  
508 weight PAHs, several isolates studied here were able to dissipate these compounds. On the other  
509 hand, one of the most frequently cited and generally accepted constraints for PAHs bioremediation  
510 is the low water solubility of these compounds which results in low bioavailability for pollutant-  
511 degrading organisms (Makkar and Rockne 2003). It has been hypothesized that the synthesis and  
512 release of bioemulsifying compounds is one of the numerous microbial adaptations to access  
513 poorly soluble substrates such as hydrocarbons (Uzoigwe et al., 2015). In the present work, the  
514 cell-free growth medium of the pyrene-degrading endophyte *Rhizobium* sp. MR28 showed higher  
515 capacity for solubilising PAHs from contaminated Pierrelaye soil than sterile culture medium used  
516 as control. Hickey et al. (2007) found that biosurfactants considerably increased the desorption of  
517 PAHs from soil and improved their biodegradation by *Pseudomonas alcaligenes* PA-10.  
518 Kuppusamy et al. (2017) isolated two strains belonging to the genera *Bacillus* and *Pseudomonas*  
519 from animal faeces which completely degraded phenanthrene, pyrene and benzo-a-pyrene after 6,

520 7 and 40–50 days, respectively. These isolates were found to produce a biosurfactant of  
521 rhamnolipid nature upon PAH degradation.

522 In a phytoremediation scenario, endophytic bacteria with specific metabolic capabilities might  
523 be able to degrade organic pollutants and reduce both phytotoxicity and evapotranspiration of  
524 volatile compounds. For instance, Sheng et al. (2008) found that the endophytic *Enterobacter* sp.  
525 12J1 could efficiently remove pyrene and improve maize and wheat growth in pyrene  
526 contaminated soils. Recently, Pawlik et al., (2020) demonstrated that in an aged petroleum  
527 hydrocarbon polluted soil, the method of inoculation of endophytic strains determines the  
528 efficiency of the phytoremediation process.

529 The inoculation of plants grown in mixed contaminated sites such as Pierrelaye with the group  
530 of bacterial strains studied could be a successful phytotechnology to improve SRC-based  
531 phytomanagement options.

532

## 533 **5. Conclusion**

534

535 The cultivable bacterial diversity associated with trees under different SRC-based  
536 phytomanagement options in a PAH and TM contaminated site in Pierrelaye (France) was  
537 assessed.

538 The collections of isolates were dominated by Proteobacteria and Actinobacteria. Differences  
539 in taxonomic composition were observed between plant species and depending on the  
540 phytomanagement option. A greater variety of taxa was found in the endophytic community of  
541 *Populus* than inside the root of *Zea mays*.

542 On the basis of the PGP characteristics analysed, the tolerance to TM and the ability for  
543 increasing the dissipation of different PAHs, the following strains could be selected as potential  
544 interesting candidates for further re-inoculation experiments: *Bacillus* sp. B32.23, *Streptomyces*

545 sp. Sk.2012, *Rhodococcus* sp. Sk12.6, *Microbacterium* sp. Sk11.4, *Bacillus* sp. Sk1.23, *Bacillus*  
546 sp. Sk2.3, *Sphingomonas* sp. SR62, *Pseudomonas* sp. MR47 and *Rhizobium* sp. MR28.

547

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552 This article is dedicated to the memory of our dearly missed colleague and friend Petra Kidd  
553 (1972-2020).

554

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#### 747 **Author contributions**

748 Petra S. Kidd: investigation, conceptualization, visualization, writing (original draft), funding  
749 acquisition

750 Analía Alvarez: investigation, writing, review and editing, supervision.

751 Vanessa Álvarez-López: investigation, review and editing.

752 Andrea Cerdeira-Pérez: investigation.

753 Beatriz Rodriguez-Garrido: investigation.

754 Ángeles Prieto-Fernández: review and editing, supervision.

755 Michel Chalot: investigation, review and editing, supervision, funding acquisition.

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757 Figure 1. Mean concentrations of PAHs detected in the culture medium after growing a  
 758 selected group of strains from bulk soil, rhizosphere and roots of poplar and maize plants  
 759 from Pierrelaye. (A) phenanthrene; (B) fluoranthene; (C) pyrene. The mean concentrations of  
 760 non-inoculated samples (NI) are indicated by a continuous line. Bars with an asterisk indicate  
 761 that they were statistically different from the NI control ( $p < 0.05$ , Tukey's post-test). MR and  
 762 SR: endophyte strains from maize and poplar Skado (respectively) from the BIOFILTREE  
 763 plots; Sk: rhizosphere strains from poplar Skado from PHYTOPOP plots; B: strain from bulk  
 764 soil (PHYTOPOP plots).

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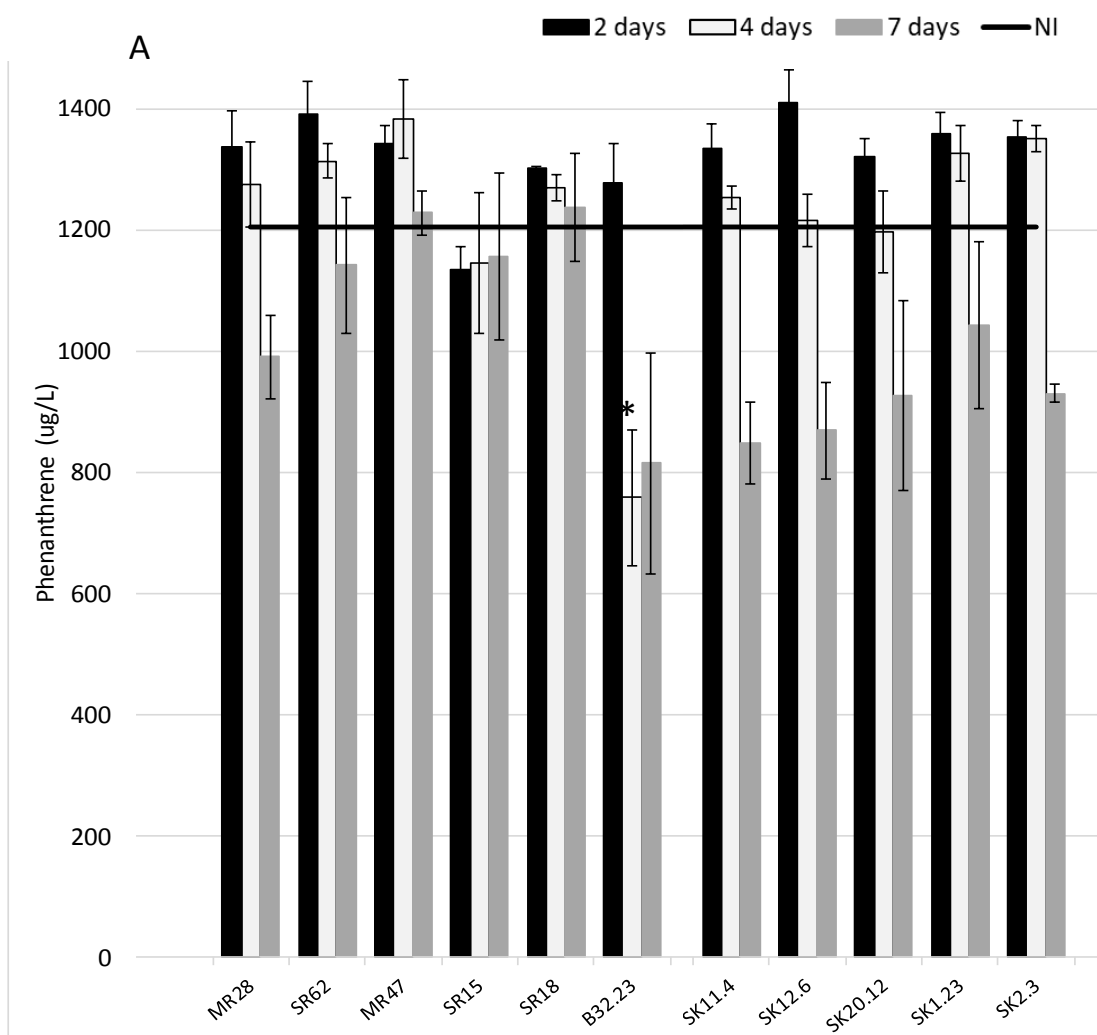
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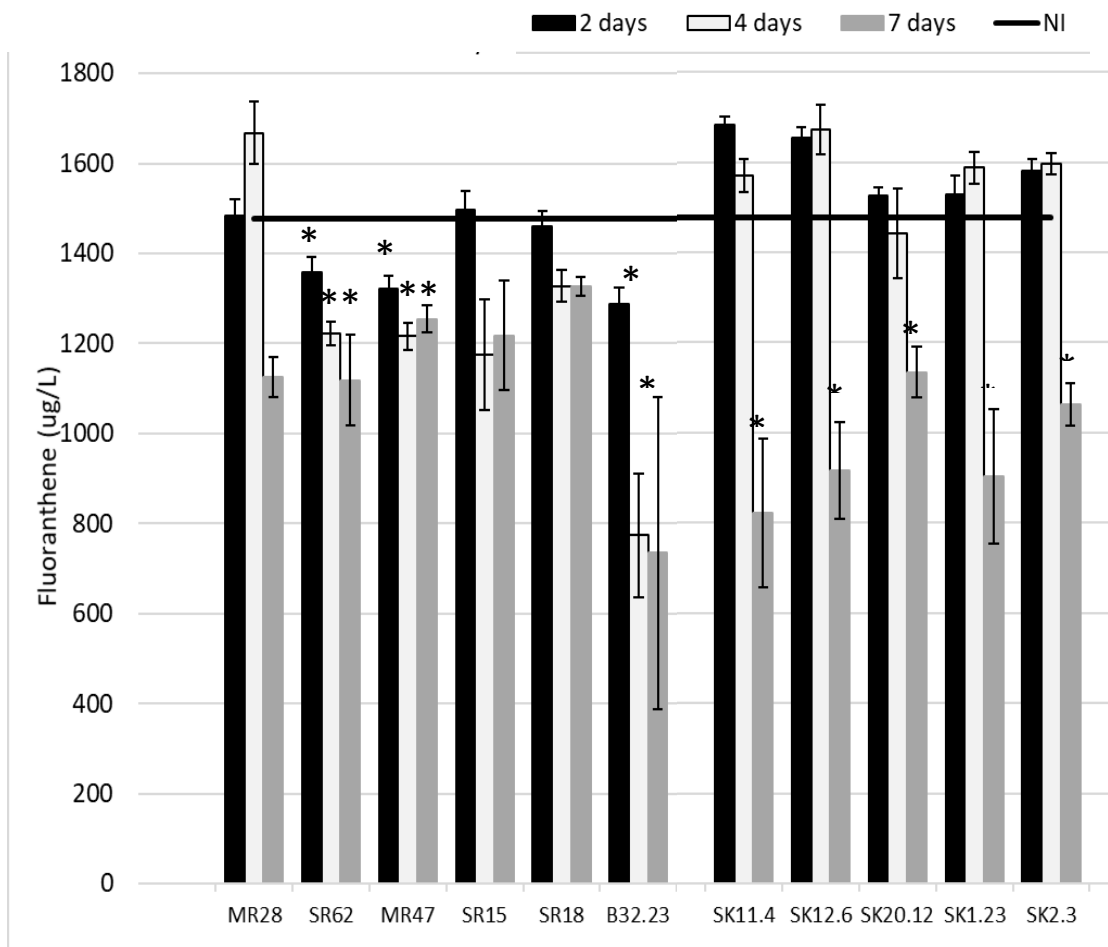
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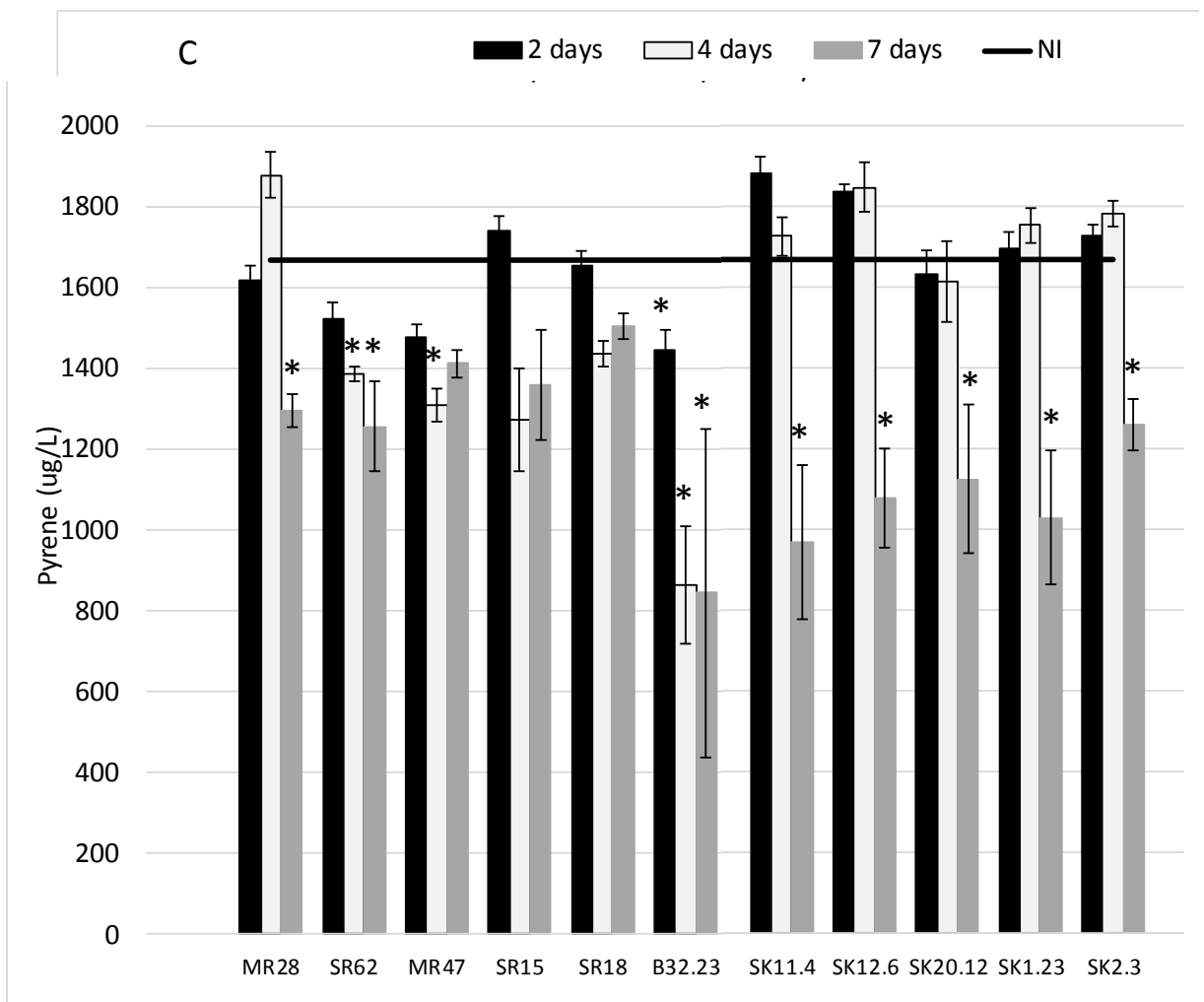
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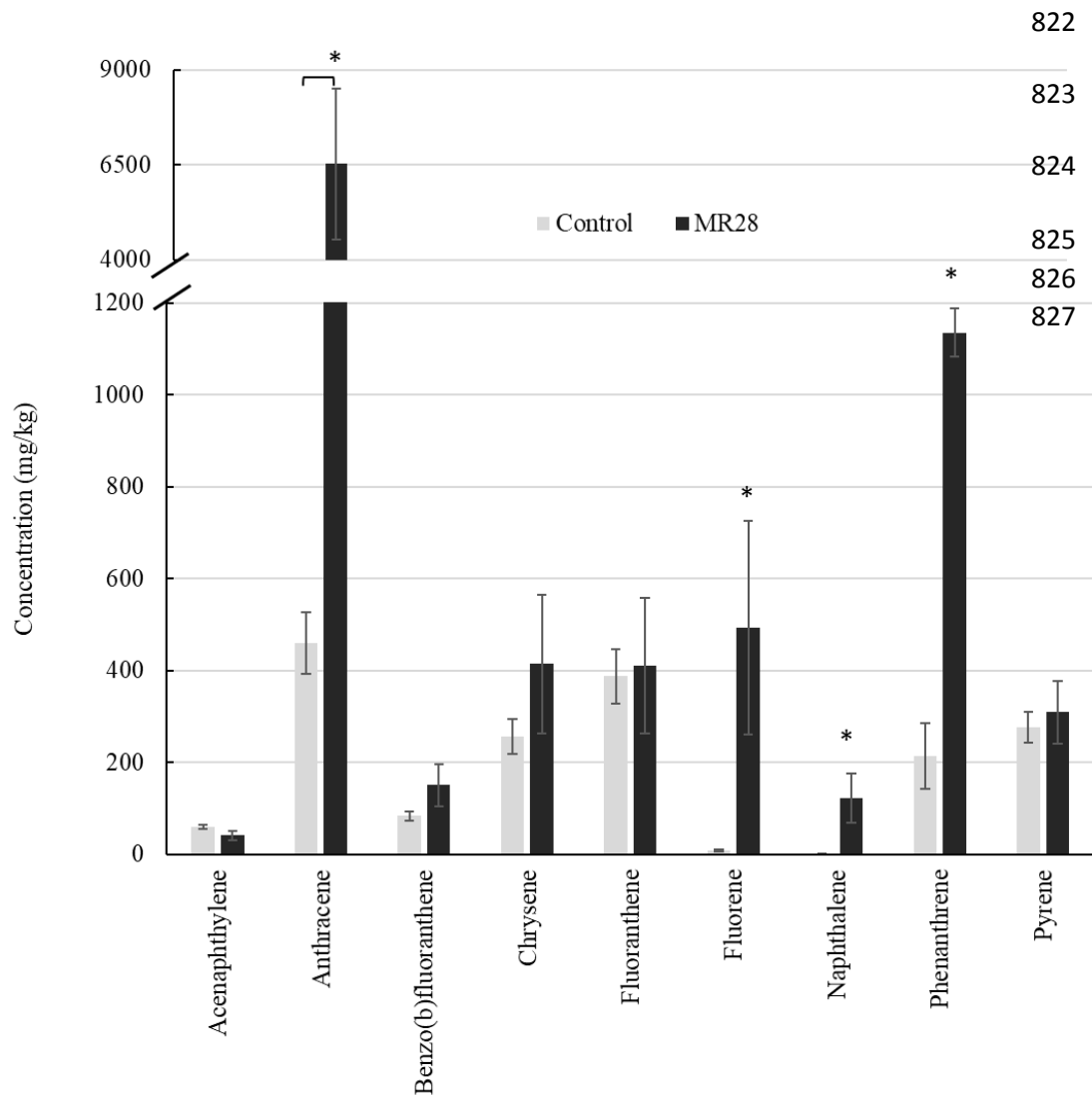
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819 Figure 2. PAHs detected before use the cell-free culture supernatant of *Rhizobium* sp. MR28  
 820 as soil extractant, isolated from the Pierrelaye site. Asterisk indicates significant differences  
 821 among treatments ( $p < 0.05$  Tukey's post-test)



828 Table 1. Description of samples from Pierrelaye soil which were used for the isolation of  
 829 bacterial strains.

Plot and sample name	Description*
<i>Phytopop</i>	
Bulk soil	Isolation of soil bacteria
Poplar Skado	Isolation of rhizobacteria
<i>Biofintree</i>	
Bulk soil	Isolation of soil bacteria
Maize	Isolation of rhizobacteria & root endophytes
Poplar Skado NI**	Isolation of rhizobacteria & root endophytes
Poplar Skado INOC**	Isolation of rhizobacteria
Poplar Skado/ Alder	Isolation of rhizobacteria

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831 \*Five replicates of each sample were studied

832 \*\*NI: non-inoculated plots with mycorrhiza; INOC: inoculated plots with mycorrhiza

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836 Table 2. Taxonomic affiliation of culturable strains isolated from bulk soil, rhizosphere and

Plot name	PHYTOPOP		BIOFILTREE						
Treatment	Bulk soil PHYTOPOP	<i>Populus</i> rhizosphere	Bulk soil BIOFILTREE	<i>Populus</i> rhizosphere			<i>Zea mays</i> rhizosphere	<i>Zea mays</i> root	<i>Populus</i> root
Phylum	Actinobacteria								
Total (%)	47%	51%	15%	48%	47%	54%	31%	17%	32%
Genera (%)	Streptomyces (25%)	Streptomyces (29%)	Streptomyces (8%)	Streptomyces (23%)	Streptomyces (13%)	Streptomyces (25%)	Streptomyces (19%)	Microbacterium (17%)	Streptomyces (13%)
Rhodococcus (4%)	Rhodococcus (8%)	Rhodococcus (8%)	Rhodococcus (10%)	Rhodococcus (10%)	Rhodococcus (7%)	Rhodococcus (4%)	Rhodococcus (6%)		Microbacterium (16%)
Arthrobaacter (2%)	Arthrobaacter (2%)		Arthrobaacter (10%)	Arthrobaacter (10%)	Arthrobaacter (7%)	Arthrobaacter (4%)	Arthrobaacter (6%)		Mycobacterium (3%)
Nocardiooides (4%)	Nocardiooides (6%)		Promicromonospora (3%)	Promicromonospora (7%)	Nocardiooides (7%)	Agromyces (7%)			
Microcococcus (2%)	Promicromonospora (2%)		Lentzea (3%)	Lentzea (3%)	Microcococcus (3%)	Microbacterium (4%)			
Kribbella (2%)	Longispora (2%)				Microbacterium (7%)	Kribbella (7%)			
Longispora (2%)	Okibacterium (2%)				Microlunatus (3%)	Knoellia (4%)			
Mycobacterium (6%)	Williamsia (2%)								
Phylum	Firmicutes								
Total (%)	39%	27%	31%	13%	30%	14%	13%	8%	23%
Genera (%)	Bacillus (33%)	Bacillus (24%)	Bacillus (31%)	Bacillus (13%)	Bacillus (27%)	Bacillus (4%)	Bacillus (12%)	Paenibacillus (8%)	Bacillus (16%)
Paenibacillus (6%)	Paenibacillus (3%)				Paenibacillus (3%)	Paenibacillus (7%)	Lysinibacillus (4%)		Paenibacillus (6%)

837 roots of *Populus* and maize growing in the PHYTOPOP and BIOFILTREE field trials of

838 Pierrelaye (France).

839 \*NI: non-inoculated plots with mycorrhiza; INOC: inoculated plots with mycorrhiza

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Plot name	Phytopop		Biofintree						
	Bulk soil Phytopop	<i>Populus</i> rhizosphere	Bulk soil Biofintree	<i>Populus</i> rhizosphere			<i>Zea mays</i> rhizosphere	<i>Zea mays</i> root	<i>Populus</i> root
NI*				INOC*	Intercropped <i>Alnus</i>				
Treatment									

841 Table 2 (continuation)

Phylum	Proteobacteria								
Total (%)	10%	19%	31%	29%	20%	32%	44%	75%	45%
Class	$\alpha$ -Proteobacteria								
Genera (%)	Methylobacterium (2%)	Methylobacterium (2%)	Ensifer (15%)	Pseudochrobactrum (3%)	Sphingopyxis (3%)	Rhizobium (4%)	Rhizobium (6%)	Rhizobium (17%)	Rhizobium (3%)
	Microvirga (2%)	Microvirga (2%)		Ensifer (3%)		Devosia (4%)			Devosia (3%)
	Ensifer (2%)	Bosea (2%)		Sphingomonas (3%)		Mesorhizobium (4%)			Bosea (6%)
		Sphingomonas (2%)							Sphingomonas (3%)
		Roseomonas (2%)							Tardiphaga (10%)
		Phylobacterium (2%)							
Class	$\beta$ -Proteobacteria								
Genera (%)	Variovorax (2%)	Cupriavidus (3%)	Variovorax (8%)	Variovorax (6%)	Variovorax (3%)	Variovorax (11%)	Variovorax (19%)	Achromobacter (8%)	
	Duganella (2%)	Massilia (2%)		Massilia (3%)		Achromobacter (4%)	Duganella (6%)		
Class	$\gamma$ -Proteobacteria								
Genera (%)		Pseudomonas (3%)	Lyso bacter (8%)	Pseudomonas (3%)	Pseudomonas (3%)	Unclassified Oxal* (4%)	Pseudomonas (6%)	Pseudomonas (17%)	Pseudomonas (16%)
		Pseudoxanthomonas (2%)		Pseudoxanthomonas (6%)	Pseudoxanthomonas (7%)	Shinella (4%)	Lysobacter (6%)		Steroidobacter (3%)
				Lysobacter (3%)					Stenotrophomonas (33%)

842 \*NI: non-inoculated plots with mycorrhiza; INOC: inoculated plots with mycorrhiza

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Plot name	Phytopop		Biofintree			
Treatment	Bulk soil Phytopop	<i>Populus</i> rhizosphere	Bulk soil Biofintree	<i>Populus rhizosphere</i>		
Phylum	Bacteroidetes					
Total (%)	4%	3%	23%	10%	3%	
Genera (%)	Chryseobacterium (2%) Terrimonas (2%)	Chryseobacterium (3%)	Chryseobacterium (15%)	Pedobacter (8%) Chitinophaga (3%)	Flavobacterium (6%)	Pedobacter (3%)

845 Table 2 (continuation)

846 \*NI: non-inoculated plots with mycorrhiza; INOC: inoculated plots with mycorrhiza

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850 Table 3. Bacterial strains isolated from the Pierrelaye field trials and selected for PAHs  
 851 degradation tests, based on their plant growth promoting traits, surfactant production and  
 852 metal resistance ability. They were isolated from bulk soil, from the rhizosphere of poplar  
 853 or maize, or from the endosphere of poplar.

Isolate	Identification (16S rDNA) and ID*	Origin	Sider.	Surf.	SoluP	Cd 0.1 mM	Cu 1mM	Pb 1 mM	Zn 2.5 mM	IAA ( $\mu\text{g/mL}$ )
SR15	<i>Bacillus</i> MW036883	Endophyte poplar	+	+	-	-	-	-	+	6.58
SR18	<i>Bacillus</i> MW036882	Endophyte poplar	-	+	-	+	-	+	+	2.52
SR62	<i>Sphingomonas</i> MW036888	Endophyte poplar	-	-	+	+	-	+	+	10.8
MR28	<i>Rhizobium</i> MW036864	Endophyte maize	+	+	+	+	+	+	-	1.38
MR47	<i>Pseudomonas</i> MW036871	Endophyte maize	-	+	+	+	-	-	-	2.89
Sk2.3	<i>Bacillus</i> MW037076	Rhizosphere poplar	-	+	-	-	-	+	-	2.07
Sk12.6	<i>Rhodococcus</i> MW037075	Rhizosphere poplar	-	+	+	+	+	+	+	5.35
Sk20.12	<i>Streptomyces</i> MW037077	Rhizosphere Poplar	+	-	-	+	-	-	+	12.13
Sk1.23	<i>Bacillus</i> MW036928	Rhizosphere Poplar	+	+	-	+	-	-	-	5.14
Sk11.4	<i>Microbacterium</i> MW037063	Rhizosphere poplar	+	+	-	+	-	-	-	0.81
B32.24	<i>Bacillus</i> MW037184	Bulk soil	+	+	-	+	-	+	-	8.08

854 Sider: siderophore-producers; Surf: biosurfactant-producers; PSolub: inorganic P solubilizers;

855 IAA: indoleacetic acid-producers

856 (\*) ID: GenBank Accession number

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