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1 **Phenanthrene contamination and ploidy level affect the rhizosphere**  
2 **bacterial communities of *Spartina* spp.**

3

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14

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16 communities

17 **Abstract**

18 *Spartina* spp. are widely distributed salt marsh plants that have a recent history of  
19 hybridization and polyploidization. These events have resulted in a heightened tolerance to  
20 hydrocarbon contaminants, but the effects of this phenomenon on the rhizosphere microbial  
21 communities is unknown. Here, we grew two parental *Spartina* species, their hybrid and the  
22 resulting allopolyploid in salt marsh sediments that were contaminated or not with  
23 phenanthrene. The DNA from the rhizosphere soil was extracted and the bacterial 16S rRNA  
24 gene was amplified and sequenced, whereas the abundances of the genes encoding for the  
25 PAH ring-hydroxylating dioxygenase (RHD) of gram-negative and gram-positive bacteria  
26 were quantified by real-time PCR. Both the contamination and the plant genotype  
27 significantly affected the bacterial communities. In particular, the allopolyploid *S. anglica*,  
28 harbored a more diverse bacterial community in its rhizosphere. The interspecific hybrid and  
29 the allopolyploid also harbored significantly more copies of the PAH-RHD gene of gram-  
30 negative bacteria in their rhizosphere than the parental species, irrespective of the  
31 contamination treatments. Overall, our results are showing that the recent polyploidization  
32 events in the *Spartina* affected its rhizosphere bacterial communities, both under normal and  
33 contaminated conditions, possibly increasing its phytoremediation potential.

## 34 **Introduction**

35 Polycyclic aromatic hydrocarbons (PAHs) are a group of ubiquitous organic pollutants, that  
36 are highly concerning in view of their potentially severe impact on natural ecosystems and  
37 public health. The remediation of sites contaminated by these compounds due to human  
38 activity, such as oil spill, is mainly carried out through excavation, soil leaching or various  
39 techniques based on microbial degradation (Samanta, Singh and Jain 2002; Wilson and Jones  
40 1993). Phytoremediation is an alternative low-cost and environmentally friendly technology  
41 that uses plants and their associated microorganisms to remove pollutants from the  
42 environment. There are various types of phytoremediation, depending on the contaminant  
43 targeted and its fate (Pilon-Smits 2005). For instance, phytoextraction mainly concerns the  
44 extraction of inorganic pollutant from the soil and its translocation and accumulation in plant  
45 tissues. Volatile compounds can also be extracted from the soil by plants, and after  
46 translocation be released into the atmosphere during phytovolatilization.

47 However, one of the most interesting form of phytoremediation is rhizodegradation,  
48 where plants stimulate a wide variety of root-associated bacteria and fungi (Correa-García *et*  
49 *al.* 2018; Ghosal *et al.* 2016) to degrade organic contaminants, often all the way to CO<sub>2</sub>  
50 (mineralization). Close interactions between plants and soil microorganisms were reported to  
51 play a major role in detoxification and metabolization of xenobiotics (El Amrani *et al.* 2015;  
52 Oliveira *et al.* 2014), and recent advances in ‘omics’ approaches now allow to address the  
53 plant-microbe complex in more details (Bell *et al.* 2014b). Root exudates were demonstrated  
54 to have a central role, modifying the microbial PAH degraders community structure (Cebon  
55 *et al.* 2011; Rohrbacher and St-Arnaud 2016) and significantly increasing microbial biomass  
56 (Esperschütz *et al.* 2009). Rhizodegradation of PAH was suggested to be a model for  
57 understanding and manipulating plant-microbe interactions (Correa-García *et al.* 2018).

58 One area of interest that is understudied is the use of phytoremediation for the cleanup  
59 of coastal salt marshes contaminated by hydrocarbons coming from oil spills at sea. In that  
60 context, *Spartina* is a particularly interesting genus, colonizing salt marshes all around the  
61 world and providing key ecosystem services, while being particularly tolerant to the effects of  
62 oil spills (Alvarez *et al.* 2018; Lin and Mendelssohn 2012; Lin *et al.* 2016; RamanaRao *et al.*  
63 2012; Silliman *et al.* 2012). This genus is also characterized by numerous hybridization and  
64 genome doubling events (polyploidy) (Aïnouche *et al.* 2009), which are major evolutionary  
65 mechanisms for eukaryotes, and most specially for plants (Soltis *et al.* 2009; Wendel 2000).  
66 Recent *in vitro* work from our group has shown that genome doubling increased tolerance to  
67 phenanthrene in *S. anglica* as compared to its single-genome parents (Cavé-Radet *et al.*  
68 2019). Indeed, the genomic shock induced by the merging of divergent genomes results in  
69 massive shifts in genetic and epigenetic pathways, that may lead to the emergence of new  
70 adaptive phenotypes to environmental constraints (Comai 2005). Although previous studies  
71 have shown a link between plant genotype and the rhizosphere microbial community structure  
72 and gene expression during phytoremediation (Bell *et al.* 2014a; Yergeau *et al.* 2018), nothing  
73 is known about the effects of genome doubling events on the root-associated microorganisms  
74 and their response to PAH contamination.

75 Here, we hypothesized that the heightened tolerance of polyploid *Spartina* species to  
76 PAH contaminants observed *in vitro* also results in an increased abundance of PAH degraders  
77 within the root-associated microorganisms. We selected four *Spartina* species: the parental  
78 species *Spartina alterniflora* and *S. maritima*, their interspecific sterile hybrid *S. x townsendii*  
79 and the allopolyploid derivative *S. anglica*. These different *Spartina* species were grown in  
80 salt marsh sediments contaminated or not with phenanthrene, after which the bacterial 16S  
81 rRNA gene of the rhizosphere soil was amplified and sequenced, and the PAH ring-

82 hydroxylating dioxygenase (PAH-RHD) genes of gram-positive (GP) and gram-negative  
83 (GN) bacteria were quantified by real-time quantitative PCR.

Accepted manuscript

## 84 **Material and methods**

### 85 *Plant and soil material*

86 Plants were collected in 2016 on their natural habitats along coastlines of France and England.  
87 We selected four *Spartina* species: the parental species *Spartina alterniflora* ( $2n=6x=62$ ) and  
88 *S. maritima* ( $2n=6x=60$ ), their interspecific sterile hybrid *S. x townsendii* ( $2n=6x=62$ ) that  
89 appeared and emerged at the end of the 19<sup>th</sup> century and the allopolyploid derivative *S.*  
90 *anglica* that subsequently appeared following genome doubling ( $2n=12x=120, 122, 124$ ). For  
91 the parental species, *S. alterniflora* was sampled at Le Faou (Roadstead of Brest, France),  
92 whereas *S. maritima* was sampled in Brillac-Sarzeau and Le Hezo (Gulf of Morbihan,  
93 France). The homoploid hybrid *S. x townsendii*, which distribution range is not extended to  
94 France was collected in Hythe (Romney Marsh, England), and the allopolyploid *S. anglica*  
95 was sampled at La Guimorais (Saint-Coulomb, France), where bulk sediments (not associated  
96 with plants) were also collected. The plant roots and rhizomes were washed abundantly with  
97 tap water before their introduction to the new sediment, in order to eliminate foreign plant  
98 residues, stones, and most of the rhizosphere sediments. After that, all the plants were  
99 acclimated in the La Guimorais sediments for three weeks before the start of the experiment.  
100 During plant acclimation, the same sediments were used to prepare phenanthrene treated and  
101 untreated sediments for the experiment. For this, wet sediments were rinsed several times with  
102 tap water and sieved through a 5 mm sieve to reduce salt content and to remove plant residues  
103 and stones. We based our phenanthrene sediment contamination level ( $150 \text{ mg kg}^{-1}$ ) on the  
104 work of Hong et al. (2015), where it was reported that  $100 \text{ mg kg}^{-1}$  of phenanthrene was not  
105 toxic to *S. alterniflora* but affected plant-microorganism interactions. Previous studies have  
106 shown that the PAH concentration in the sediments of polluted rivers, estuaries or salt  
107 marshes around the world ranged from  $2 \text{ mg kg}^{-1}$  all the way to  $1943 \text{ mg kg}^{-1}$  (Budzinski *et al.*  
108 1997; Jiang *et al.* 2007; Shi *et al.* 2005; Watts, Ballestero and Gardner 2006). We selected a

109 concentration of 150 mg kg<sup>-1</sup> of phenanthrene, which represents a non-phytotoxic, moderate  
110 exposure in the range of concentrations reported in polluted environments. To contaminate the  
111 soil, 300 g of wet sediments was air-dried and then split in two 150 g subsamples. One 150 g  
112 subsample was spiked with 100 mM of phenanthrene (1.95 g of phenanthrene diluted in 109.4  
113 mL of absolute ethanol), whereas the other 150 g subsample (control) was spiked with 109.4  
114 mL of absolute ethanol. After total evaporation of ethanol for one day, these sediment  
115 samples were vigorously mixed by hand with an additional 12.85 kg of wet and rinsed  
116 sediments to reach a final concentration of 150 mg phenanthrene kg<sup>-1</sup> substrate for the  
117 contaminated treatment. Some contaminated and control soils were sampled at this step and  
118 kept at -20°C to serve as T0 samples.

119

#### 120 *Experimental design*

121 Both polluted and control sediments were supplemented with sterile vermiculite before the  
122 start of the experiment (volume 1/3) for a better substrate breathability. Individual *Spartina*  
123 plants (four species) were then transplanted in pot containing 1 kg of contaminated or control  
124 substrates, and rhizomes were carefully placed into rhizobags of 3 cm of diameter made from  
125 plastic mesh (1-cm mesh size) inspired from the experimental design of Hong *et al.* (2015).  
126 The rhizobags were filled with the exact same substrate as the remainder of the pot.  
127 Rhizobags were used to create a physical separation between the bulk soil and the rhizosphere  
128 because of the difficulty to retrieve an intact root system when uprooting *Spartina* plants from  
129 very wet sediments. The rhizosphere was thus defined as the soil inside the rhizobags, where  
130 roots and rhizomes were also found. The experiment was replicated three times in a  
131 completely randomized design, resulting in a total of 24 pots that were placed in a phytotronic  
132 chamber with a light/dark regime of 16/8h, in an average ambient temperature of 20°C. Pots  
133 were watered with 200 mL of half-strength Hoagland's nutrient solution (Hoagland and

134 Arnon 1950) every five days. After 60 days of growth, plants were uprooted and the  
135 rhizosphere soil (inside the rhizobags) was collected and stored at -20°C until DNA  
136 extraction.

137

### 138 *Bacterial 16S rRNA gene amplification*

139 Genomic DNA from soil samples was extracted using a MoBio PowerSoil DNA extraction  
140 kit. Amplicons were prepared from total DNA by PCR targeting the bacterial 16S rRNA gene  
141 (forward: 515F: GTGCCAGCMGCCGCGGTAA and reverse: 806R:  
142 GGACTACHVGGGTWTCTAAT) (Caporaso *et al.* 2012). PCR amplifications were  
143 performed in 25 µL final reaction volumes containing final concentrations of 1X KAPA HiFi  
144 HotSart ReadyMix, 0.4 mg.ml<sup>-1</sup> bovine serum albumin, 0.6 µM forward and reverse primers,  
145 and 1 µL of DNA. PCR conditions were as follows: 5 min of initial denaturation (95°C), 25  
146 cycles of 30s denaturation (95°C), 30s for primer annealing (55°C), and 45s elongation  
147 (72°C), followed by final extension for 10 min at 72°C. PCR amplicons were then purified  
148 using AMPure XP beads. Amplicon indexing was carried out by a second PCR in 25 µL final  
149 reaction volumes containing 12.5 µL 2X KAPA HiFi HotSart ReadyMix, 2.5 µL of specific  
150 index primers 1 and 2 from the Nextera Index kit, and 5 µL of purified amplicon. The second  
151 step PCR conditions were as follows: 3 min of initial denaturation (95°C), 8 cycles of 30s  
152 denaturation (95°C), 30s for primer annealing (55°C), and 45s elongation (72°C), followed by  
153 final extension for 5 min at 72°C. Index PCR cleanup was performed using AMPure XP  
154 beads. Finally, PCR amplicons were quantified using PicoGreen, normalized at 1 ng µl<sup>-1</sup>,  
155 pooled together and sent for sequencing on an Illumina MiSeq (paired-end 2 × 250bp) at the  
156 McGill University and Genome Quebec Innovation Center (Montréal, Canada). Raw reads  
157 and associated metadata are available through NCBI BioProject accession PRJNA518897  
158 (<http://www.ncbi.nlm.nih.gov/bioproject/518897>).

159

160 *Sequencing data processing*

161 Raw sequencing reads were processed using the mothur Illumina MiSeq Standard Operating  
162 Procedure (SOP) (Kozich *et al.* 2013). Paired-end reads were first assembled and the joined  
163 paired-end sequences with ambiguous bases or smaller than 275 bp were excluded. The  
164 dataset was dereplicated for faster computation, after which chimera were removed. Unique  
165 sequences were aligned using the SILVA nr database (v128) after which the aligned 16S  
166 rRNA gene sequences were clustered at 97% sequence similarity, and singletons were  
167 removed from the analysis. Sequences were classified with the 16S rRNA PDS reference  
168 from Ribosomal Database Project (RDP version 16; 80% cut-off on bootstrap value for  
169 confidence taxonomic assignment) and undesirable sequences (chloroplast, mitochondria,  
170 unknown, Archaea and Eukaryota) were removed. A total of 4,905,456 sequences were  
171 assembled from the bacterial 16S rRNA gene reads, resulting in 3,132,223 clean sequences,  
172 corresponding to 121,804 unique sequences that clustered into 28,092 OTUs. Individual  
173 samples were represented by 14,671 to 604,114 reads, with a median of 85,052.5 reads.

174

175 *Real-time quantitative PCR*

176 The qPCR experiments were conducted on a Stratagene Mx3005P qPCR system (Agilent  
177 Technologies), associated with the corresponding software MxPro Mx3005P (v4.10; Agilent).  
178 The qPCR reactions were performed using the primers designed by Cébron *et al.* (2008). The  
179 qPCR reactions were performed in 20 $\mu$ l total volume containing 1x iTaq universal SYBR®  
180 Green reaction mix supplemented with 300 $\mu$ M of each primer (Integrated DNA  
181 Technologies™) and 5 $\mu$ l of DNA template at a concentration around 5-10ng  $\mu$ L<sup>-1</sup> or water  
182 (negative control). The amplifications were carried out following the protocol provided in  
183 Cébron *et al.* (2008) with some modifications. Briefly, the first step consisted on heating to

184 95°C (5min) followed by 40 cycles of denaturation at 95°C for 30s, annealing at either 57°C  
185 (PAH-RHD GN) or 54°C (PAH-RHD GP) for 35s and elongation at 72°C for 75s, after which  
186 SYBR Green fluorescence was measured. At the end of the run, a melting curve analysis was  
187 performed where fluorescence was measured at 0.5°C temperature increment every 5s from  
188 51 to 95°C. Standard curves for the two genes were made from 10-fold dilutions of linearized  
189 plasmid containing the gene fragment of interest, cloned from amplified soil DNA (Yergeau  
190 *et al.* 2009).

191

### 192 *Statistical analyses*

193 Statistical analyses were performed in R (v 3.5.1). Inversed Simpson index was calculated  
194 after rarefaction through random subsampling based on the size of the smallest library (14,671  
195 sequences). Comparisons between  $\alpha$ -diversity of bacterial communities, the abundance of the  
196 PAH-RHD genes, the relative abundance of phyla and the relative abundance of selected  
197 putative hydrocarbon degraders according to the *Spartina* species and the treatments (polluted  
198 or control substrate) were conducted using ANOVA with post-hoc Tukey HSD tests (aov and  
199 tukeyHSD functions of the *stats* package). Prior to ANOVA the normality, and the  
200 homoscedasticity of the data to be tested were confirmed using the shapiro.test and  
201 bartlett.test of the *stats* package, respectively. Spearman correlations were calculated using  
202 the cor.test of the *stats* package. Principal coordinate analysis (PCoA) to describe  $\beta$ -diversity  
203 was performed on normalized OTU tables using Bray-Curtis dissimilarity index in the *vegan*  
204 (Dixon 2003) and *ape* (Paradis, Claude and Strimmer 2004) packages. Permanova testing the  
205 impact of *Spartina* species, treatments, and their interactions were conducted through 999  
206 permutations. Indicative species among OTUs were investigated using default parameters  
207 provided by the *indicspecies* package (Cáceres and Legendre 2009).

208

## 209 **Results**

### 210 *Bacterial alpha diversity and community structure*

211 The presence of phenanthrene led to a general decrease of bacterial alpha diversity in the  
212 rhizosphere of all the plant species (two-way ANOVA:  $F = 12.41$ ,  $P = 0.002$ ) (Fig. 1a). The  
213 two-way ANOVA also highlighted a significant effect of plant species on the bacterial alpha  
214 diversity ( $F = 6.19$ ,  $P = 0.005$ ), and Tukey HSD post-hoc tests showed that the rhizosphere of  
215 *S. anglica* was significantly more diverse than the rhizosphere of *S. alterniflora* and *S.*  
216 *maritima* (Fig. 1a). The interaction effect contamination  $\times$  species was not significant for the  
217 bacterial alpha diversity in two-way ANOVA tests. PCoA ordinations based on bacterial OTUs  
218 showed some level of clustering based on plant species and contamination (Fig. 1b).  
219 Permanova tests confirmed that *Spartina* species ( $F = 2.09$ ,  $R^2 = 0.22$ ,  $P < 0.001$ ) and  
220 phenanthrene ( $F = 2.48$ ,  $R^2 = 0.09$ ,  $P < 0.001$ ) had highly significant influences on the  
221 bacterial community structure.

222

### 223 *Bacterial community composition*

224 The relative abundance of various dominant bacterial phyla in the rhizosphere of *Spartina*  
225 varied between the different plant species and between the contaminated and non-  
226 contaminated soils (Fig. 2). These shifts were not significant for some phyla, such as the  
227 *Acidobacteria*, the *Actinobacteria*, and the *Bacteroidetes*. In contrast, plant species  
228 significantly influenced the rhizosphere relative abundance of *Chloroflexi* ( $F = 6.21$ ,  $P = 0.05$ ,  
229 significantly higher in the rhizosphere of *S. anglica* as compared to *S. alterniflora* and *S. x*  
230 *townsendii* in Tukey HSD tests), *Planctomycetes* ( $F = 8.86$ ,  $P = 0.00108$ , significantly higher  
231 in the rhizosphere of *S. anglica* as compared to *S. alterniflora* and *S. x townsendii*, and in the  
232 rhizosphere of *S. maritima* vs. *S. alterniflora*) and total *Proteobacteria* ( $F = 4.54$ ,  $P = 0.02$ ,  
233 significantly lower in the rhizosphere of *S. anglica* as compared to *S. alterniflora* and *S. x*

234 *townsendii*). A significant effect of contamination was also observed for the *Chloroflexi* ( $F =$   
235  $20.93$ ,  $P < 0.001$ ), total *Proteobacteria* ( $F = 14.23$ ,  $P = 0.002$ ), *Verrucomicrobia* ( $F = 4.85$ ,  $P$   
236  $= 0.43$ ) and *Gammaproteobacteria* ( $F = 11.39$ ,  $P = 0.004$ ). The genera *Sphingobacterium*,  
237 *Acinetobacter*, *Nocardia*, *Pseudomonas*, *Mycobacterium*, *Burkholderia*, *Bacillus*,  
238 *Sphingomonas*, *Rhodococcus*, *Paenibacillus*, *Massilia*, *Alcanivorax*, *Cycloclasticus* were  
239 singled out as putative hydrocarbon degraders based on a survey of the available literature.  
240 The summed relative abundance of all these genera varied between around 2% to over 3% of  
241 all reads and was significantly higher in the rhizosphere of plants growing in contaminated  
242 soil ( $F = 8.43$ ,  $P = 0.01$ , Fig. 3), but showed no significant differences between plant species.  
243 For the individual genera, the relative abundance of *Paenibacillus*, *Bacillus*, *Burkholderia*,  
244 *Acinetobacter*, *Alcanivorax*, *Rhodococcus* and *Pseudomonas* did not vary significantly  
245 between the plant species and treatments. However, the relative abundances of *Sphingomonas*  
246 ( $F = 9.85$ ,  $P < 0.001$ , significantly higher in the rhizosphere of *S. x townsendii* as compared to  
247 all other species), *Sphingobacterium* ( $F = 5.41$ ,  $P = 0.009$ , significantly higher in the  
248 rhizosphere of *S. maritima* as compared to all other species), *Nocardia* ( $F = 7.56$ ,  $P = 0.002$ ;  
249 significantly higher in the rhizosphere of *S. anglica* as compared to all other species) showed  
250 significant differences between the rhizosphere of the different plant species. The relative  
251 abundances of *Massilia* ( $F = 17.54$ ,  $P < 0.001$ ), *Cycloclasticus* ( $F = 4.96$ ,  $P = 0.04$ ) and  
252 *Mycobacterium* ( $F = 7.49$ ,  $P = 0.01$ ) were significantly affected by the phenanthrene  
253 treatment, with significantly higher relative abundance in the rhizosphere of plants growing in  
254 the contaminated soil (Fig. 3). In addition, the interaction between contamination and plant  
255 species was significant for *Sphingobacterium* ( $F = 4.73$ ,  $P = 0.001$ ). Indicator species analyses  
256 confirmed some of the trends observed in ANOVA tests, as it identified bacterial OTUs related  
257 to *Massilia* and *Cycloclasticus* as the OTUs with the highest indicator power for contaminated  
258 rhizospheres among all OTUs (Table 1 and Supplementary Table S1). The relative abundance

259 of *Massilia* was significantly and negatively correlated to bacterial alpha diversity (inverse  
260 Simpson index) ( $r_s = -0.48$ ,  $P = 0.017$ ). Apart from a positive correlation for *Rhodococcus* ( $r_s$   
261  $= 0.41$ ,  $P = 0.047$ ), the other genera were not significantly correlated to bacterial alpha  
262 diversity.

263

#### 264 *Abundance of PAH ring-hydroxylating dioxygenases*

265 The abundance of the gene encoding for the alpha subunit of the PAH-RHD of gram-positive  
266 bacteria was not significantly different between the rhizosphere of the four *Spartina* species  
267 and was not affected by sediment contamination, nor by their interaction (Fig. 4a). In contrast,  
268 the abundance of the gram-negative version of the same gene showed significant differences  
269 between the four *Spartina* species ( $F = 63.2$ ,  $P = 4.31 \times 10^{-9}$ ), with the allopolyploid *S.*  
270 *anglica* and the hybrid *S. x townsendii* harboring on average 354 % more copies of the gene in  
271 their rhizospheres than the parental species (Fig. 4b). Contamination and the contamination  $\times$   
272 species interaction did not have any significant effect on the abundance of the gram-negative  
273 version of the PAH-RHD.

274

## 275 **Discussion**

276 Recently, we have shown enhanced *in vitro* tolerance to phenanthrene in the allopolyploid in  
277 *S. anglica* as compared to the parental species (Cavé-Radet *et al.* 2019). The hypothesis  
278 behind the present pot study was that this increased tolerance of the allopolyploid *S. anglica*  
279 to hydrocarbon contamination would also result in an increased abundance of PAH degraders  
280 within its root-associated microorganisms. The results presented here are coherent with this  
281 hypothesis, as we found significant differences between the different pot-grown *Spartina*  
282 species in terms of their bacterial community composition, structure and diversity, and, more  
283 importantly, in the abundance of PAH ring-hydroxylating dioxygenase (RHD) genes in their  
284 rhizosphere. The allopolyploid *S. anglica* harbored a more diverse bacterial community,  
285 composed of relatively more *Nocardia*, *Chloroflexi* and *Planctomycetes* and less  
286 *Proteobacteria* in its rhizosphere as compared to its diploid parents and their hybrid. In  
287 addition, the allopolyploid (*S. anglica*) and the hybrid (*S. x townsendii*) had significantly more  
288 PAH-RHD genes related to gram-negative bacteria in their rhizosphere than the parental  
289 species, suggesting a heightened capacity for PAH degradation by the rhizosphere microbial  
290 community. Previous studies from our group using willows have shown that the phylogeny of  
291 the host plant significantly influenced the fungal community composition under high levels of  
292 contaminant (Bell *et al.* 2014a). We also showed that the metatranscriptomic response of the  
293 rhizosphere microbial communities to contamination varied between different willow  
294 genotypes, and that this response was mirrored in the growth of the genotypes in  
295 contaminated soil (Yergeau *et al.* 2018). However, this is the first time, to our knowledge, that  
296 functional and taxonomical differences between the rhizosphere bacterial communities of  
297 recently naturally speciated plants are reported in the context of soil/sediment contamination.

298 The rhizosphere bacterial communities of the four pot-grown *Spartina* species tested  
299 showed significant responses to the presence of the contaminant. In the presence of PAH, salt

300 marsh plants were reported to harbor different microbial communities, favoring hydrocarbon-  
301 degrading microorganisms (Ribeiro *et al.* 2011). Similarly, we found here an increase in the  
302 relative abundance of putative PAH degraders, and more specifically for the genera  
303 *Mycobacterium*, *Cycloclasticus* and *Massilia*. These genera represent large bacterial groups  
304 that are able to metabolize and degrade PAH (Johnsen, Wick and Harms 2005; Kanaly and  
305 Harayama 2000) and are consistent with previous results about the PAH-degrading bacteria  
306 associated to *Spartina* in salt marshes (Daane *et al.* 2001; Launen *et al.* 2008). Interestingly,  
307 *Cycloclasticus* spp. were often reported as one of the predominant PAH degraders in seawater  
308 (Tremblay *et al.* 2017; Yergeau *et al.* 2015b), whereas *Mycobacterium* are typical soil PAH  
309 degraders (Hennessee and Li 2016) and *Massilia* were found to degrade PAHs in soils or  
310 associated to roots (Liu *et al.* 2014; Wang *et al.* 2016). This diversity of putative PAH  
311 degraders associated with the rhizosphere of *Spartina* might be linked to the nature of its  
312 habitat, at the interface of terrestrial, plant and marine ecosystems. These bacterial genera  
313 probably contain candidates of interest for salt marsh remediation, but their potential would  
314 have to be confirmed.

315         However, these taxonomical shifts in the relative abundance of these putative  
316 microbial hydrocarbon degraders were not reflected in the abundance of the PAH-RHD genes.  
317 Indeed, contamination with phenanthrene did not shift the abundance of PAH-RHD genes in  
318 the rhizosphere of the pot-grown *Spartina*, both for the gram-positive and -negative variants  
319 of the gene. Previous studies had alluded to a similar phenomenon, where genes and  
320 microorganisms related to the degradation of hydrocarbon contaminants were already  
321 enriched in the rhizosphere of plants growing in non-contaminated soil (Daane *et al.* 2001).  
322 These hydrocarbon degrading genes were also expressed by microbial isolates in response to  
323 root exudates (Mark *et al.* 2005; Matilla *et al.* 2007; Ramachandran *et al.* 2011), and found in  
324 the rhizosphere metatranscriptome of willows growing in not-contaminated soils (Yergeau *et*

325 *al.* 2014; Yergeau *et al.* 2018). One of the explanations put forward was the presence of  
326 secondary metabolites in the rhizosphere environment that were structurally analog to  
327 hydrocarbon contaminants (Singer, Crowley and Thompson 2003), resulting in an enrichment  
328 and activation of PAH degradation genes even in the absence of exogenous PAH.  
329 Alternatively, the significant enrichment of PAH-RHD GN genes in the rhizospheres of the  
330 hybrid and the allopolyploid could be due to a larger total bacterial community as recently  
331 reported for *Solidago* polyploids (Wu *et al.* 2019). Regardless of the underlying mechanism,  
332 the higher abundance of PAH-RHD genes in the rhizosphere of the hybrid and the  
333 allopolyploid suggest a heightened capacity for contaminant tolerance and degradation. Of  
334 course, this higher abundance of PAH-RHD genes does not mean that they were actively  
335 expressed, and a study based on the quantification of transcripts is needed to complete this  
336 observation.

337         The discrepancy between the qPCR of functional genes and the relative abundance of  
338 putative hydrocarbon degraders could be explained by two reasons: 1) unknown microbial  
339 groups could harbour these genes, since part of soil microbial diversity is still unexplored; and  
340 2) it is possible that not all members of the genera singled out carried out PAH-RHD  
341 degradation genes, as intra-genus and intra-species variability in that trait is expected. Recent  
342 studies showed that bacterial isolates undistinguishable based on their 16S rRNA gene  
343 harbored different gene pools, had different ecological behaviors and functional capacity  
344 (Irshad and Yergeau 2018; Lopes *et al.* 2018). These results are suggesting that one should  
345 exert caution when trying to extrapolate functional information from amplicon sequencing  
346 data.

347         As bacteria are thought to be the major players in organic contaminant degradation  
348 during rhizoremediation (Bell *et al.* 2014a; Bell *et al.* 2014b; Correa- García *et al.* 2018; El  
349 Amrani *et al.* 2015), and that recent plant-microbe metatranscriptomic studies confirmed that

350 the hydrocarbon degradation genes expressed in the root-rhizosphere environment were  
351 mostly linked to bacteria (Gonzalez *et al.* 2018; Yergeau *et al.* 2018), it is interesting to note  
352 the higher bacterial diversity in the rhizosphere of the allopolyploid *S. anglica* as compared to  
353 the parental *Spartina* species. Upon a contamination event, the plant-associated microbial  
354 diversity could have a central importance, as higher diversity generally results in functional  
355 redundancy, which would enable the microbial communities to cope with a wider variety of  
356 environmental conditions while still providing essential services to the plant. Previous studies  
357 from our group have shown that the initial soil diversity explained better the difference in  
358 willow growth under highly contaminated conditions than diversity at the end of the  
359 experiment (Yergeau *et al.* 2015a). It was further suggested in that study that restoring  
360 microbial diversity of degraded environments could be the key for successful  
361 phytoremediation. This potential link between the higher rhizosphere diversity and the  
362 increased resilience of *S. anglica* to contamination is intriguing and warrants further research.

363 Even though we acclimated the plants in the sediments for three weeks, we cannot  
364 exclude the possibility that the differences in the microbial communities in the rhizosphere of  
365 *S. anglica* were because the bulk soil used for the experiment was from a site where *S. anglica*  
366 predominantly grew. This might also have affected indirectly the microbial communities  
367 through shifts in the physiology of the parental plants and of the hybrid that were adapted to  
368 the conditions found in other sediments. However, some commonalities in the responses of  
369 the allopolyploid and of the hybrid suggest that the effect of the soil origin on some of the  
370 results presented here was not necessarily that strong.

371 We described here for the first time the root-associated bacterial community of four  
372 pot-grown *Spartina* species in a context of allopolyploidization and PAH contamination.  
373 Significant differences were observed between the plant species and between contaminated  
374 and control rhizospheres. The most salient differences were the higher abundance of gram-

375 negative PAH-RHD genes and the higher bacterial diversity in the rhizosphere of the  
376 allopolyploid as compared to the parental species, which could be a major factor contributing  
377 to the increased tolerance of *S. anglica* to contaminant stress. The underlying cause for these  
378 shifts could be related to changes in the root exudation patterns following hybridization, but  
379 more work would be necessary to test this hypothesis.

380

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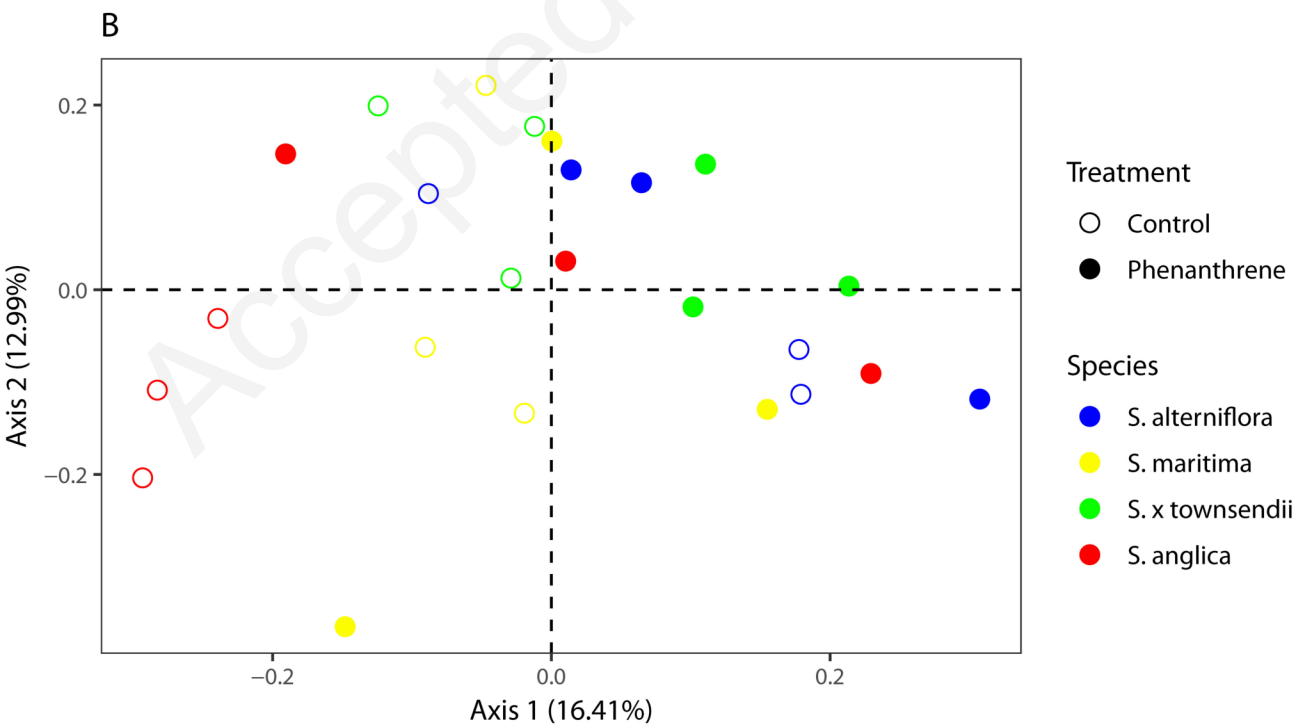
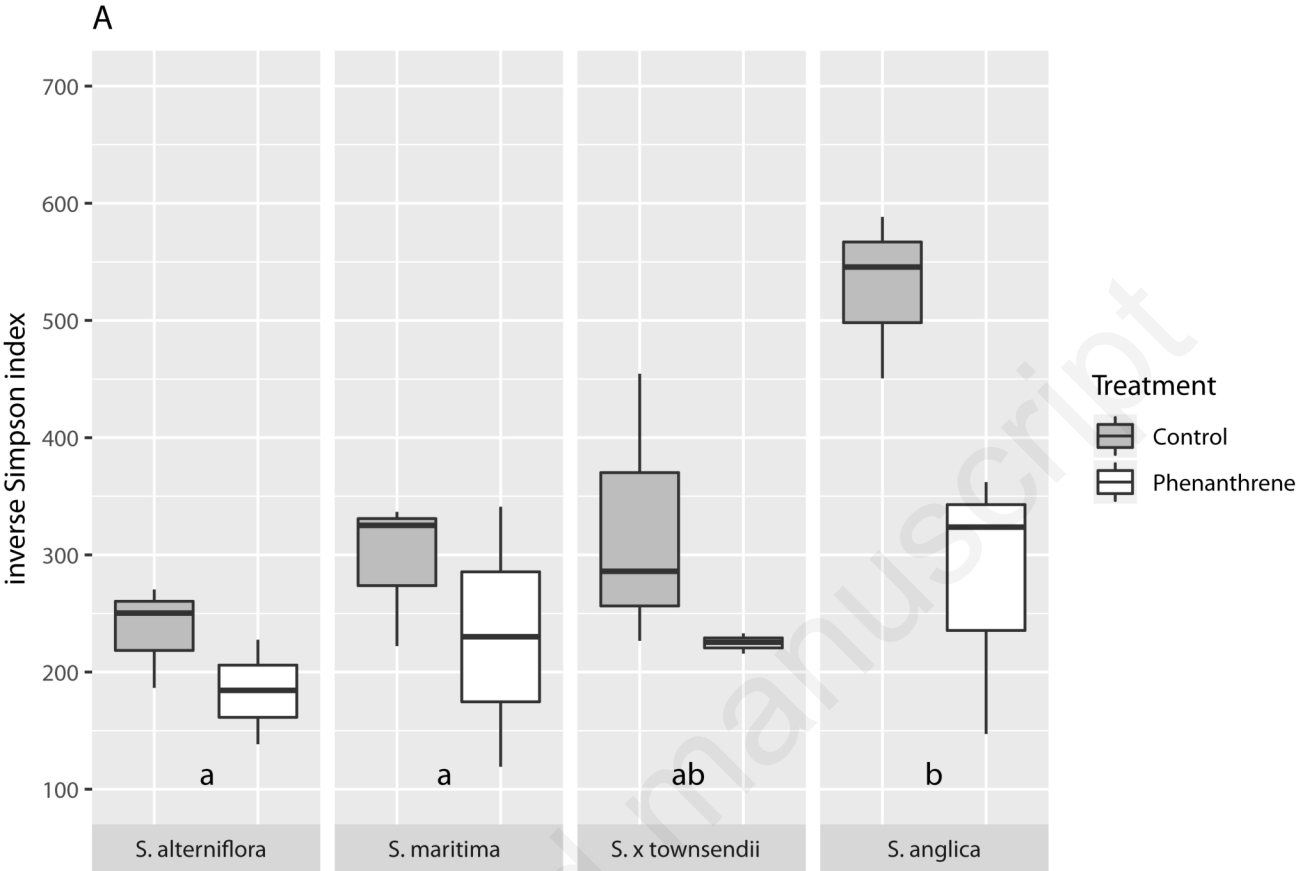
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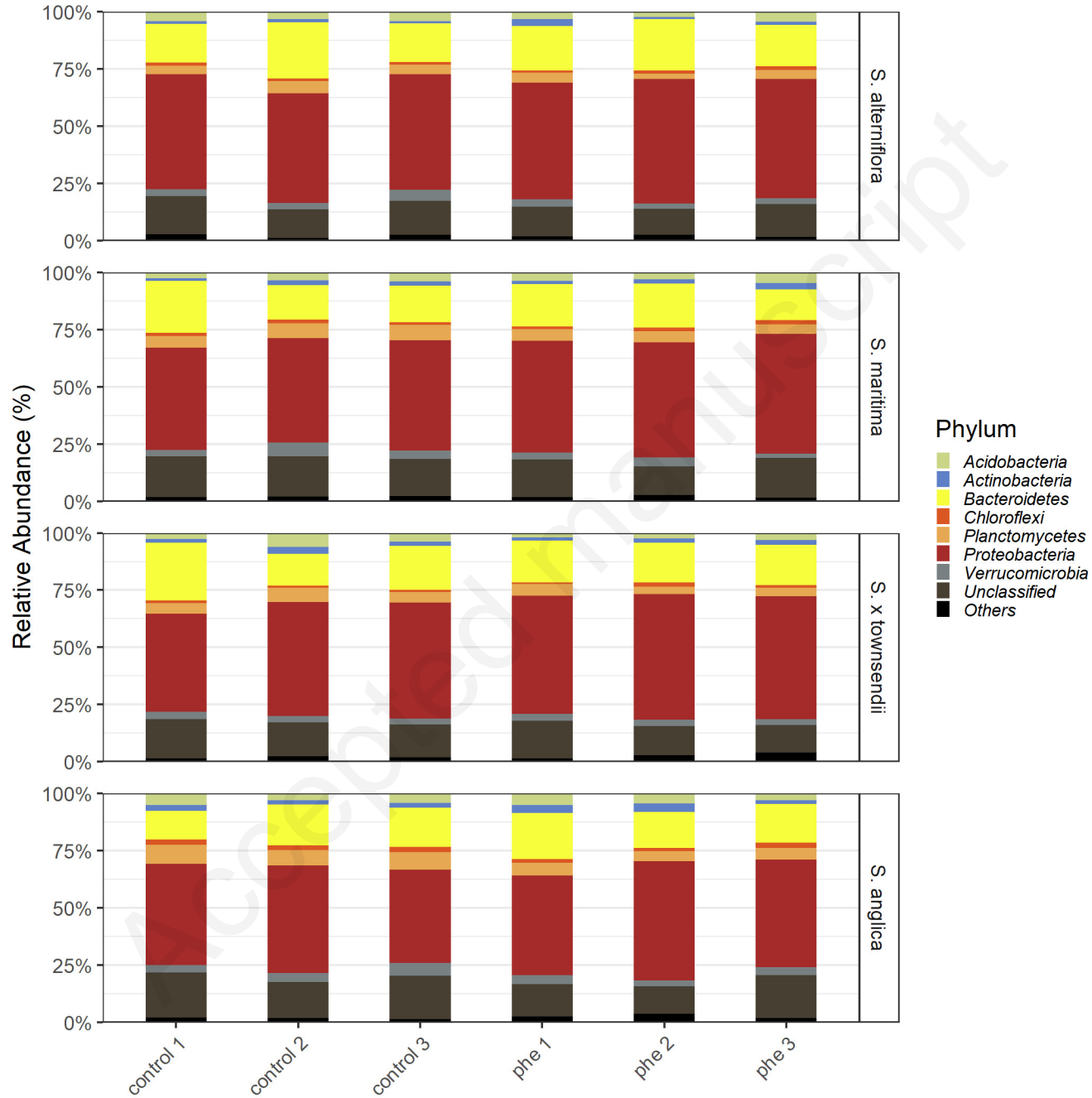
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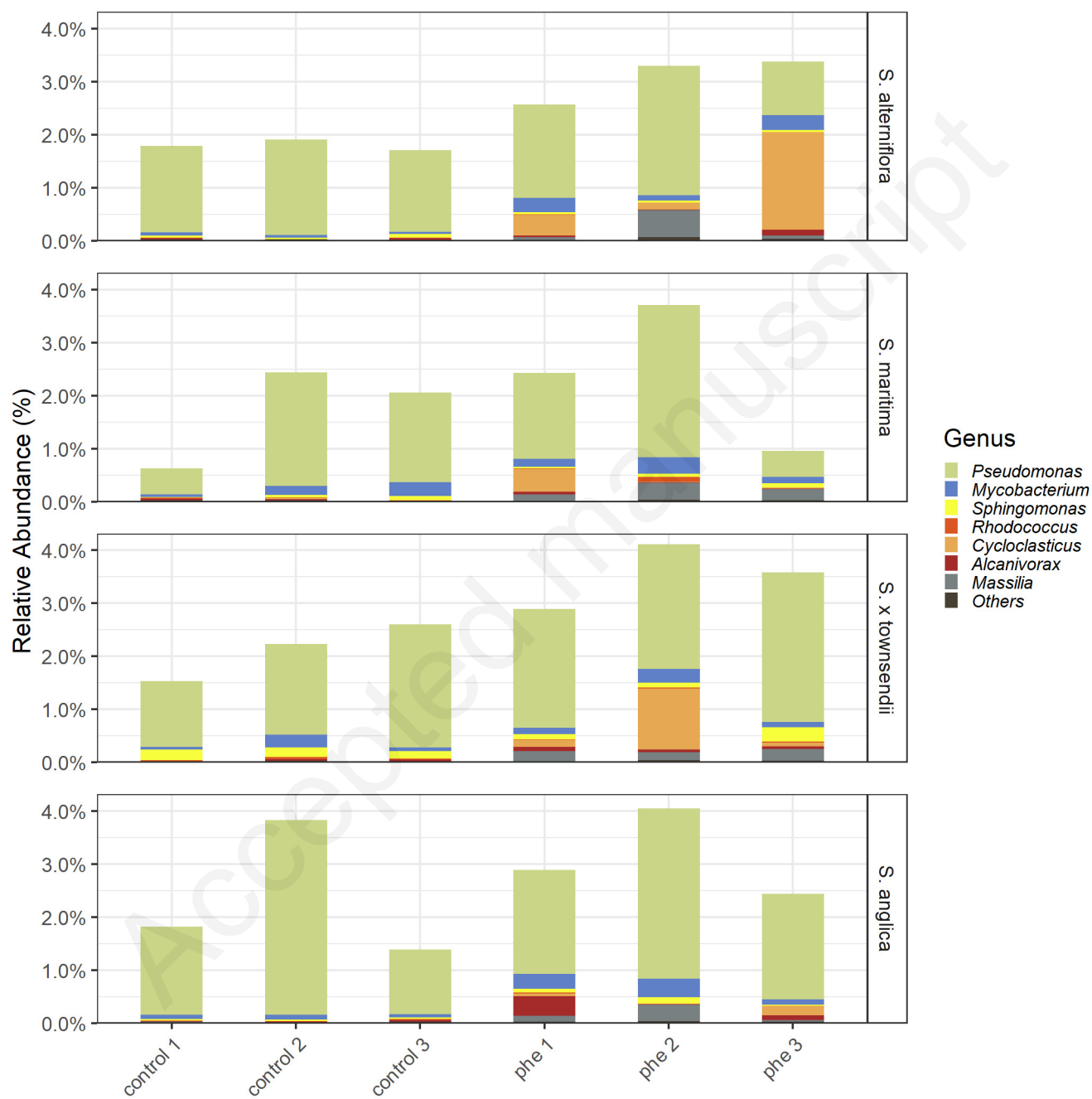
529

**Table 1.** Top 10 strongest indicator bacterial OTUs for the phenanthrene-contaminated *Spartina* rhizospheres.

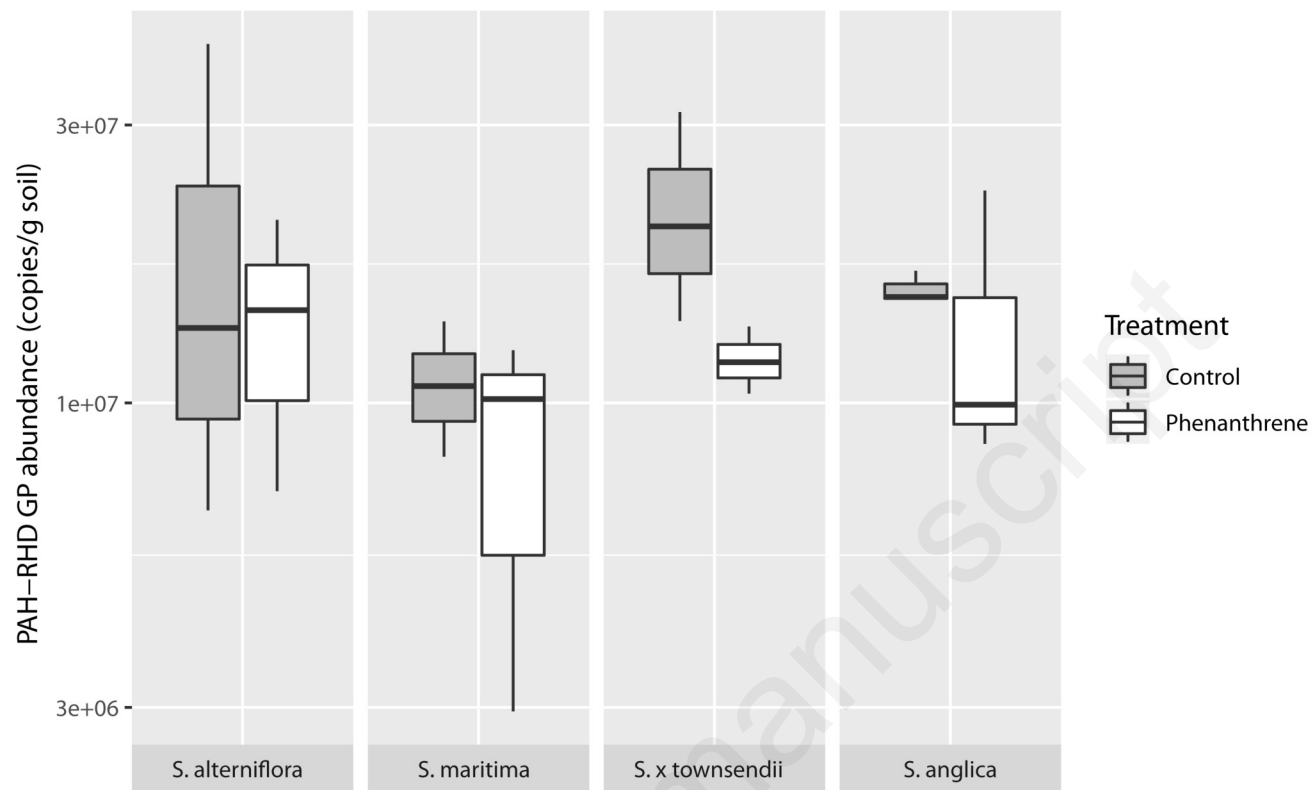
<b>OTU</b>	<b>stat</b>	<b>P-value</b>	<b>Taxonomy</b>
Otu00188	0.998	0.001	<i>Massilia</i>
Otu00140	0.996	0.001	<i>Cycloclasticus</i>
Otu00370	0.991	0.001	<i>Alcaligenaceae</i> unclassified
Otu00552	0.989	0.049	<i>Flavobacteriaceae</i> unclassified
Otu02955	0.960	0.002	<i>Salinirepens</i>
Otu01642	0.954	0.006	<i>Sphingobium</i>
Otu02528	0.954	0.005	<i>Alcaligenaceae</i> unclassified
Otu01089	0.941	0.005	<i>Peredibacter</i>
Otu04988	0.921	0.008	unassigned
Otu03519	0.896	0.006	<i>Gammaproteobacteria</i> unclassified







A



B

