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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 CO₂
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 19th 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 mg kg^{-1}) on the
104 work of Hong et al. (2015), where it was reported that 100 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 mg kg^{-1} all the way to 1943 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⁻¹ 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⁻¹ 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⁻¹ 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⁻¹,
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 μ l total volume containing 1x iTaq universal SYBR®
180 Green reaction mix supplemented with 300 μ M of each primer (Integrated DNA
181 Technologies™) and 5 μ l of DNA template at a concentration around 5-10ng μ L⁻¹ 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 α -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 β -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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