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▶ **To cite this version:**

Armand Cavé-Radet, Sara Correa-Garcia, Cécile Monard, Abdelhak El Amrani, Armel Salmon, et al.. Phenanthrene contamination and ploidy level affect the rhizosphere bacterial communities of Spartina spp. FEMS Microbiology Ecology, 2020, 96 (10), pp.AA156. 10.1093/femsec/fiaa156 . hal-02929499v2

HAL Id: hal-02929499
https://hal.science/hal-02929499v2

Submitted on 3 Mar 2021

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

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Keywords: Phenanthrene, rhizosphere, rhizoremediation, Spartina, polyploidy, bacterial communities

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Abstract

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

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Introduction

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

However, one of the most interesting form of phytoremediation is rhizodegradation, where plants stimulate a wide variety of root-associated bacteria and fungi (Correa-García *et al.* 2018; Ghosal *et al.* 2016) to degrade organic contaminants, often all the way to CO_2 (mineralization). Close interactions between plants and soil microorganisms were reported to play a major role in detoxification and metabolization of xenobiotics (El Amrani *et al.* 2015; Oliveira *et al.* 2014), and recent advances in 'omics' approaches now allow to address the plant-microbe complex in more details (Bell *et al.* 2014b). Root exudates were demonstrated to have a central role, modifying the microbial PAH degraders community structure (Cebron *et al.* 2011; Rohrbacher and St-Arnaud 2016) and significantly increasing microbial biomass (Esperschutz *et al.* 2009). Rhizodegradation of PAH was suggested to be a model for understanding and manipulating plant-microbe interactions (Correa-García *et al.* 2018).

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

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

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

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Material and methods

Plant and soil material

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

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

Experimental design

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

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

Bacterial 16S rRNA gene amplification

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

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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 20µl total volume containing 1x iTaq universal SYBR®
180 Green reaction mix supplemented with 300µM of each primer (Integrated DNA
181 Technologies™) and 5µl of DNA template at a concentration around 5-10ng μL^{-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

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

Statistical analyses

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

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Results

Bacterial alpha diversity and community structure

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

Bacterial community composition

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

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

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

Abundance of PAH ring-hydroxylating dioxygenases

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

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Discussion

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

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

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

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

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

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

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

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

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

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

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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

381 **Acknowledgements**

382 A.C.-R. was supported by an Erasmus+ mobility scholarship from the European Commission.
383 This work was supported by the Ministère de l'Enseignement Supérieur et de la Recherche,
384 by the CNRS, the Observatoire des Sciences et de l'Univers de Rennes (OSUR) and by a
385 NSERC Discovery Grant (2014-05274 to EY). This research was enabled in part by support
386 provided by Calcul Québec (www.calculquebec.ca) and Compute Canada
387 (www.computecanada.ca).

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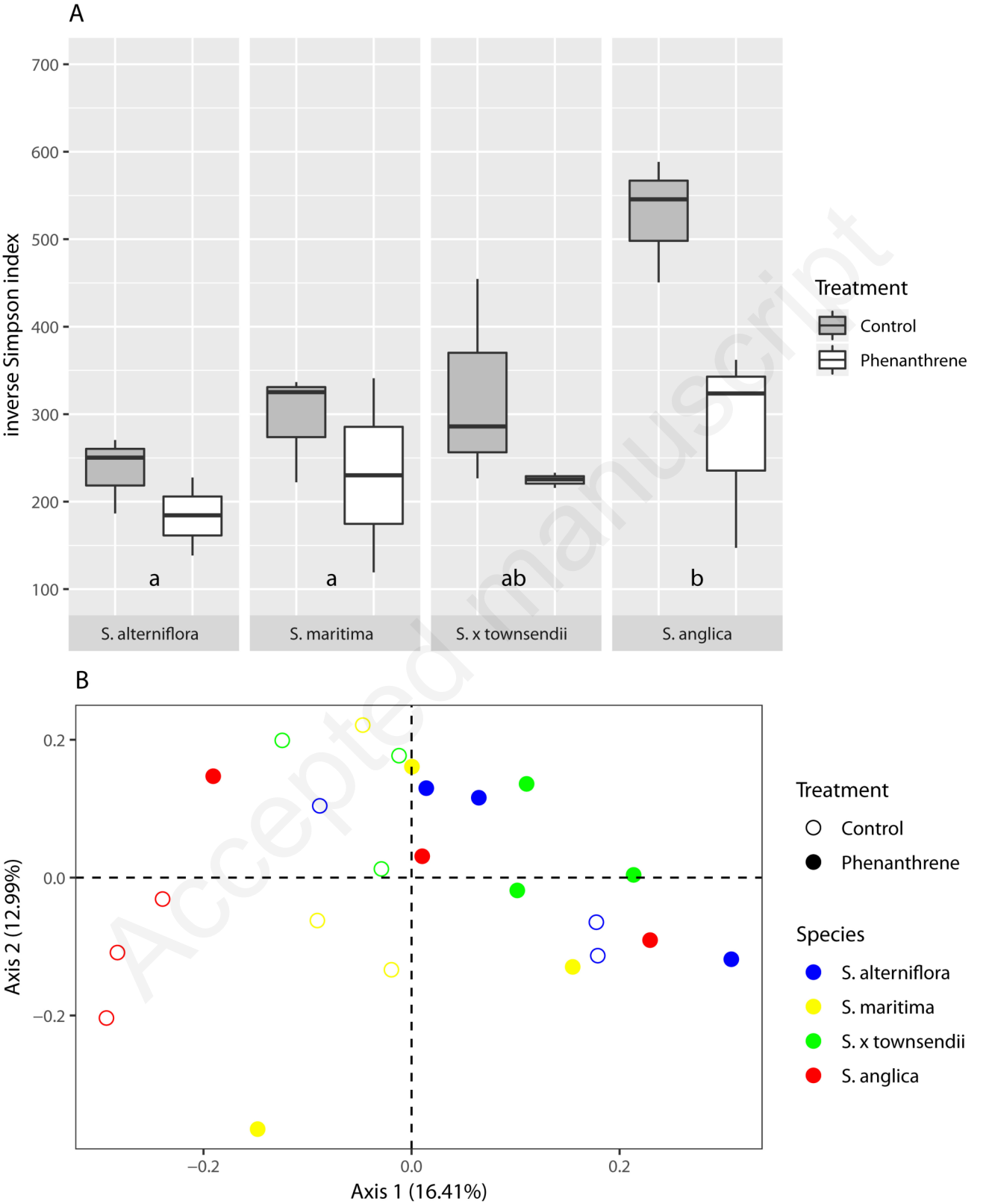
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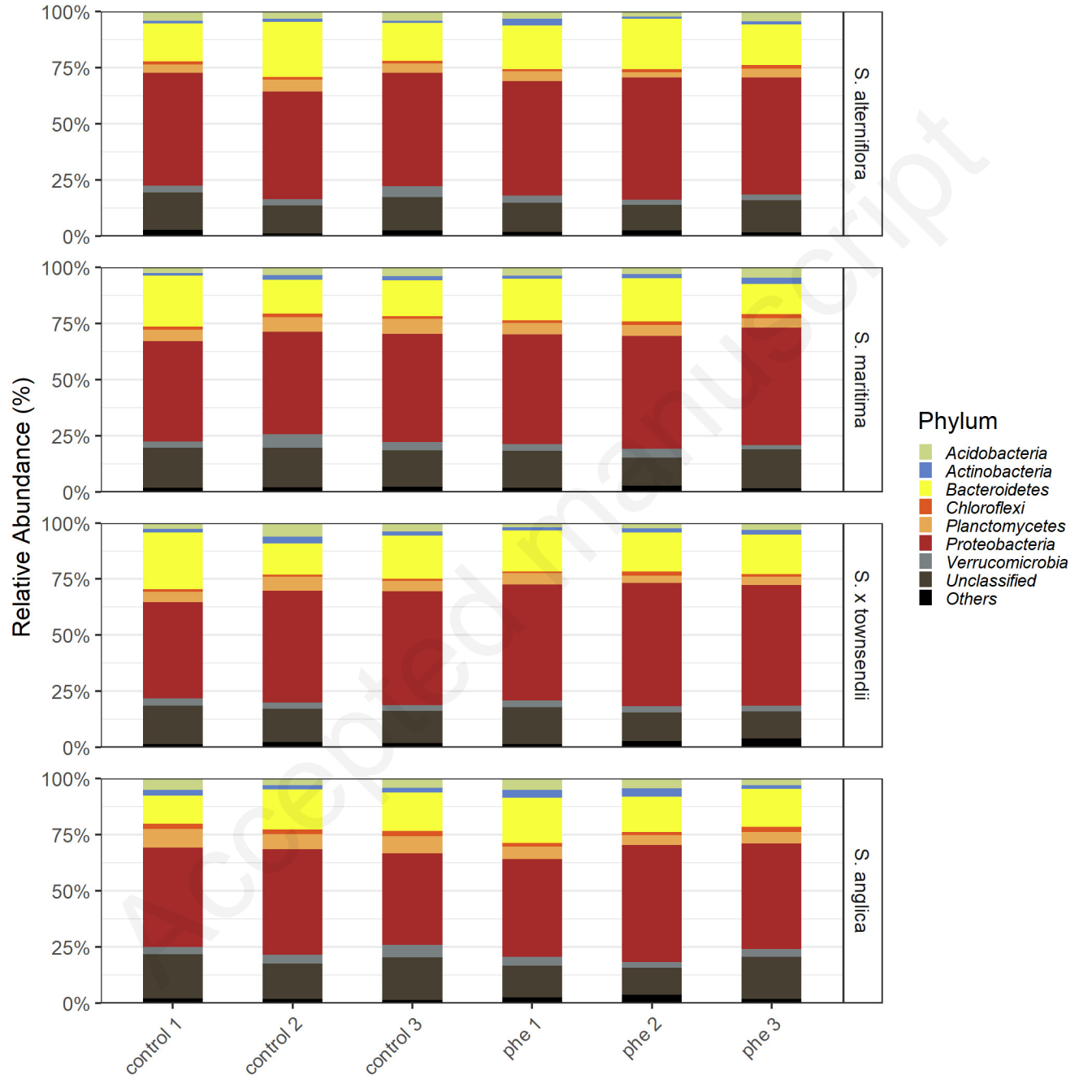
Table 1. Top 10 strongest indicator bacterial OTUs for the phenanthrene-contaminated *Spartina* rhizospheres.

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

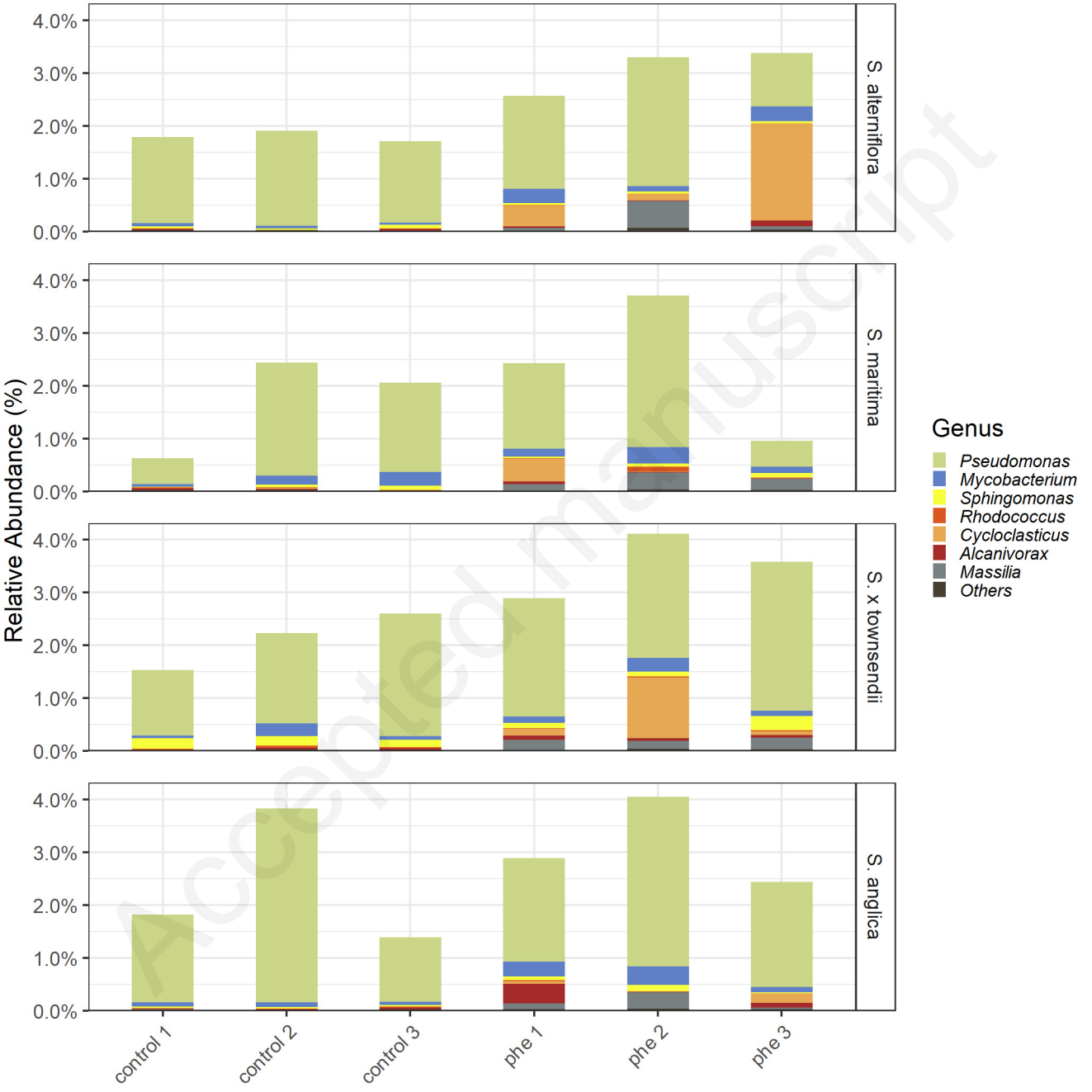
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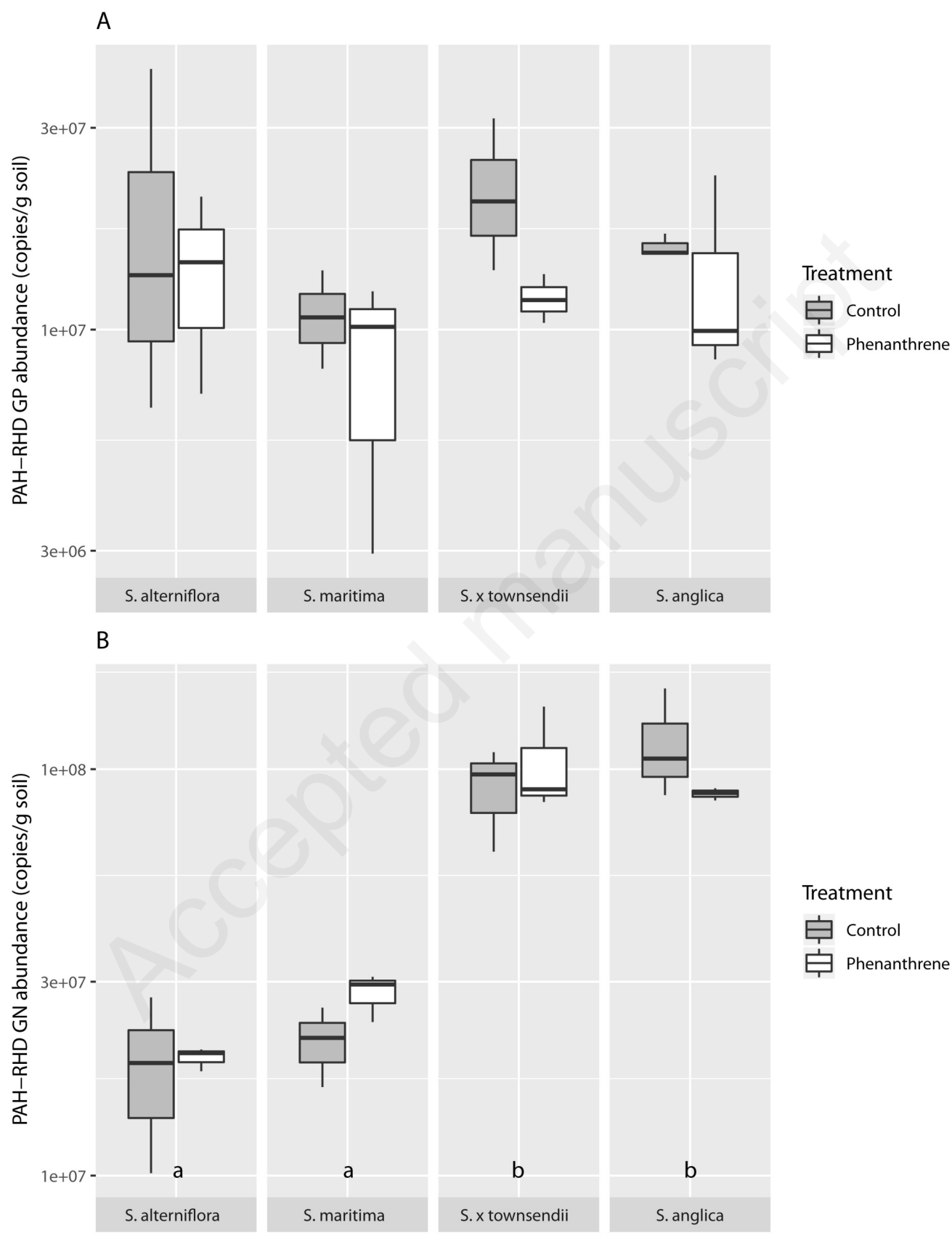
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