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Persisting responses of salt marsh fungal communities to the Deepwater Horizon oil spill

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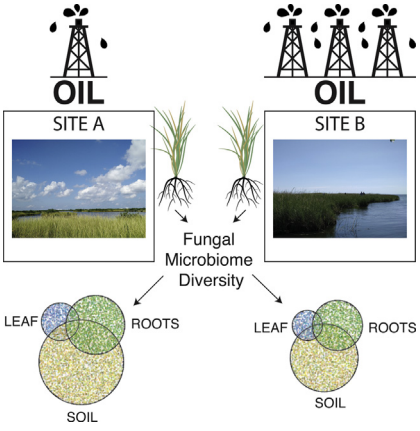
HIGHLIGHTS

- Effects of oil spill on plant-associated microbiomes in salt marshes were investigated.
- Nature and magnitude of oiling effects assessed between sites and across plant host compartments
- Fungal communities inhabiting leaf, root and soil show distinct responses to oiling.
- Magnitude of fungal community responses to oiling constrained by local environment
- Oil pollution effects on plant microbiomes persist six years after initial exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

The plant microbiome, composed of diverse interacting microorganisms, is thought to undergird host integrity and well-being. Though it is well understood that environmental perturbations like oil pollution can alter the diversity and composition of microbiomes, remarkably little is known about how disturbance alters plant-fungal associations. Using Next-Generation sequencing of the 18S rDNA internal transcribed spacer (ITS1) region, we examined outcomes of enduring oil exposure on aboveground leaf and belowground endophytic root and rhizosphere fungal communities of *Spartina alterniflora*, a highly valued ecosystem engineer in southeastern Louisiana marshes affected by the 2010 Deepwater Horizon accident. We found that aboveground foliar fungal communities exhibited site-dependent compositional turnover with consequent loss in diversity according to oiling history. Rhizosphere soil communities also exhibited shifts in community composition associated with oiling history, whereas root endophytic communities did not. Oiling did not increase or decrease similarities among aboveground and belowground communities within an individual host, indicating that host plant characteristics exert stronger control than external factors on fungal community composition. These results show that fungal community responses to oiling vary within tissues of the same host plant, and that differences in the local environment, or alternatively, site-specific differences in residual oil constrain the magnitude of exposure responses. Our study offers novel perspectives on how environmental contaminants and perturbations can influence plant microbiomes, highlighting the importance of assessing long-term ecological outcomes of oil pollution to better

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understand how shifts in microbial communities influence plant performance and ecosystem function. Our findings are relevant to coastal management programs tasked with responding to oil spills and increasing pressures arising from intensifying development and climate change. Understanding how modification of plant-microbiome associations influences plant performance, particularly of ecosystem engineers like *S. alterniflora*, can help guide efforts to protect and restore at-risk coastal ecosystems.

1. Introduction

Plants host diverse complements of microorganisms, with distinct communities inhabiting aboveground and belowground tissues (Mishra et al., 2012; Christian et al., 2015). The diversity and composition of microbes can alter host fitness and behavior (Vandenkoornhuyse et al., 2015), and can have direct and indirect feedbacks on ecosystem processes like carbon and nitrogen cycling (e.g., Saleem et al., 2017). Despite the recent explosion of work on microbial diversity (Vorholt, 2012; Tian et al., 2015), the mechanisms by which environmental perturbations affect plant-microbe interactions are not well understood, particularly for aboveground and belowground fungal communities.

Environmental disturbances can alter plant-microbe interactions by modifying microbial assemblages. Oil pollution, a prevailing environmental concern in industry-laden areas like the Gulf of Mexico, can reduce diversity of plant-associated fungal communities via increased abundance of oil-degrading taxa with consequent loss of diversity, which can persist for years after initial exposure (e.g., Kandalepas et al., 2015; Bourdel et al., 2016). The magnitude of shifts in plant-associated fungal communities, however, can vary across plants (e.g., Bourdel et al., 2016; Rietl et al., 2016) and even among assemblages within plant host tissues. For example, leaf fungal communities can suffer near complete diversity loss compared to more modest responses of root fungal assemblages to oiling (Kandalepas et al., 2015), suggesting that different mechanisms underlie responses to stressor exposure.

Outcomes of perturbations like oiling might depend on several factors, including whether fungal communities are in aboveground and belowground plant tissues (Coince et al., 2014; Tardif et al., 2016) as well as local abiotic and biotic conditions. Fungal symbiont communities occupying different tissues of a host plant (e.g., aboveground vs. belowground, endophytic (inside plant tissues) vs. rhizosphere (surrounding roots)) exhibit distinct ecologies (Wearn et al., 2012; Christian et al., 2015). Each may interact idiosyncratically with different factors that may change according to local environmental conditions. The fungal community residing in host leaves may, for example, have relatively poor and variable nutrient supply (Lindow and Brandl, 2003) while rhizosphere communities draw from a stable and nutrient-rich environment (Badri et al., 2009). Consequently, perturbations can result in varying patterns and degrees of responses between fungal communities. For instance, oil contamination might promote selection of hydrocarbon-degrading microorganisms in the rhizosphere of plants for protection against contaminant toxicity (Siciliano et al., 2001; Bell et al., 2014), altering root endophytic and rhizosphere community composition while exerting little influence on leaf endophytes. It is possible, however, that predominantly belowground perturbations like oiling also alter foliar fungal communities via changes to host tissue chemistry and plant uptake of organic compounds (Desalme et al., 2013; Li et al., 2017).

The nature and magnitude of fungal community response to perturbations may also be due to different processes driving community assembly in different plant tissues. For example, the assembly of aboveground foliar fungal endophytes largely reflects airborne dispersal, while belowground communities are primarily influenced by local environmental filtering (David et al., 2016; Hendershot et al., 2017). Thus, fungal communities within the same plant host can have distinct responses to the same perturbation, especially when local environmental conditions influence stressor exposure. Parallel responses may, however, arise following a disturbance. For example, communities throughout the host plant could be reduced to taxa that are tolerant to a specific disturbance type like oiling. Therefore, microbes generally associated within a particular plant tissue may not persist, where stressor exposure acts as a selective force resulting in greater similarity among communities across a plant host (Herren et al., 2016).

Characterizing exposure responses across tissues within and among plant hosts can offer insight into potential associations between aboveground and belowground processes (Wardle et al., 2004). It would also clarify how plant-microbiota interactions mitigate outcomes of environmental disturbances, including how associations feedback to influence higher-order processes ranging from host demography to ecosystem resilience (e.g., Rudgers and Orr, 2009). We examined the effects of oil exposure on aboveground foliar and belowground endophytic root and rhizosphere fungal communities of the foundational saltmarsh grass, *Spartina alterniflora*, at two sites in southeastern Louisiana six years after the Deepwater Horizon (DWH) oil spill. Through next-generation metabarcoding, we assessed whether (1) aboveground and belowground endophyte and rhizosphere fungal communities of *S. alterniflora* respond idiosyncratically to oiling within and among sites; (2) site differences influence the magnitude of fungal community responses to oiling; and (3) oil exposure alters associations between aboveground and belowground fungal communities. Given that different plant tissues may not be equally susceptible to (direct) oil exposure, we expected to find greater variation in response in aboveground versus belowground communities. Furthermore, we expected that belowground endophytic and rhizosphere communities would exhibit similar response profiles to site-level differences in oiling history given that the two communities are generally tightly linked (i.e. root endophytes are generally derived from the microbial pool inhabiting the rhizosphere zone).

2. Methods

2.1. Site and sample collection

Our two focal sites are located in southeastern Louisiana: Bay Jimmy and Fourchon (Fig. 1). Both sites were affected by the DWH oil spill in 2010, although the Bay Jimmy site experienced greater oil deposition than the Fourchon site (Michel et al., 2013). The Bay Jimmy site, located in Northern Barataria Bay, is salt marsh dominated by *S. alterniflora* with a few *Distichlis spicata* and *Salicornia virginica* occurring infrequently on the shoreline. The marsh is predominantly organic soil with some clay content. The Fourchon site, situated in the adjoining Carminada Bay, consists of organic soil with some sand content. The vegetation is predominantly *S. alterniflora* and *Avicennia germinans*. The two sites are 43 km apart. Our measurement of residual polycyclic aromatic hydrocarbon (PAH) levels in soils directly within the vicinity of our plant samples indicated that Bay Jimmy had significantly higher PAH levels than Fourchon at the time of our study (Fig. S1). Within each site, one transect was placed in an area that received oil (i.e., oiled area) and another transect was placed in a nearby area that did not receive oil (i.e., non-oiled area). The two transects (14 m each) were ca. 600 and 30 m apart in Bay Jimmy and Fourchon, respectively.

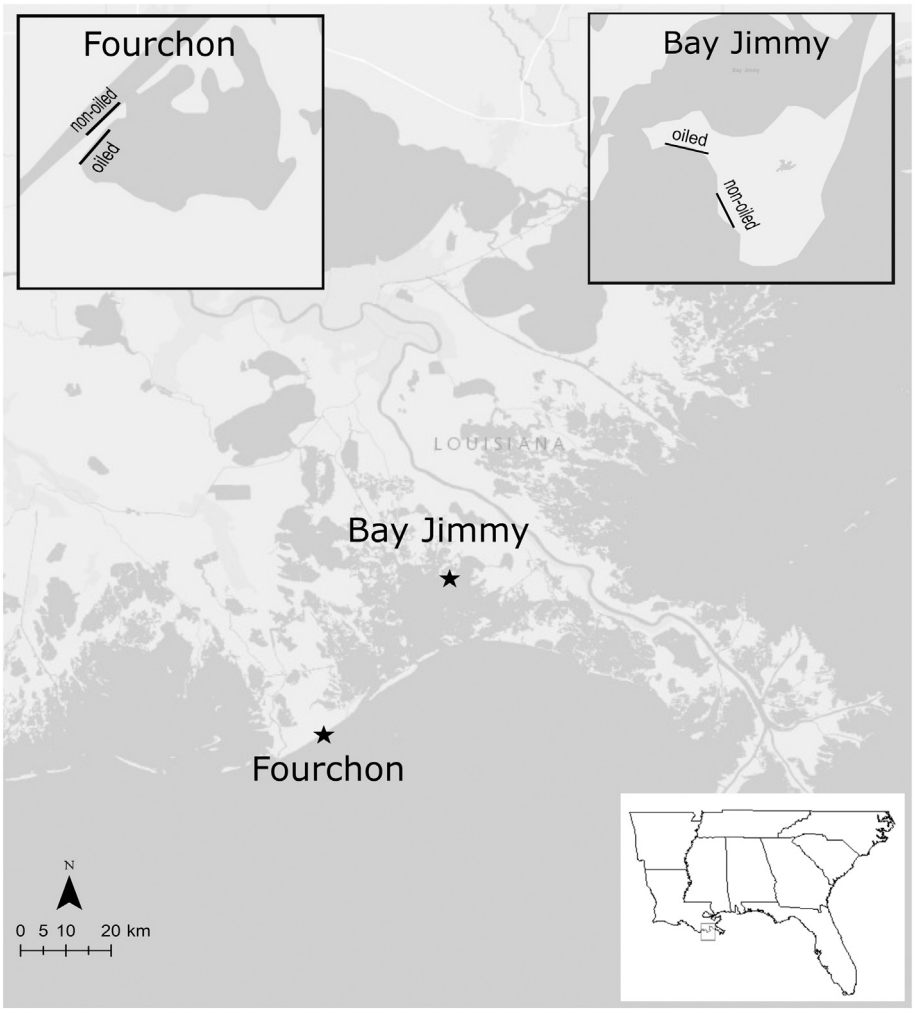

Fig. 1. Map of study sites (stars) on the southeastern coast of Louisiana (square). Top inset maps show the oiled and non-oiled transects in Fourchon (left) and Bay Jimmy (right). Bottom inset is a map of the Gulf Coast. Gray areas in maps denote ocean and white areas are lands.

Spartina alterniflora is a perennial, foundational grass that dominates saltmarshes across the northern Gulf of Mexico and Atlantic coast of North America. The productivity and functional trait variation in *S. alterniflora* govern marsh resilience by influencing platform accretion and shoreline erosion (e.g., Morris et al., 2002). Moreover, marsh resilience is moderated by *S. alterniflora* responses to stressor exposure, including nutrient loading and oiling (Deegan et al., 2012; Bernik, 2015). In September 2016, we collected leaves and roots from 10 *S. alterniflora* plants per transect, separated from each other by 6–10 m and within 1 m from the shoreline. The soil around the root zone of the plant (rhizosphere soil) was also collected for each of the 10 plants. Thus, a total of 120 samples (leaves, roots and soil) were collected from 40 plants.

2.2. Chemical analysis of local environmental conditions

Rhizospheric porewater was sampled using a clean syringe from soil below each plant and analyzed for conductivity, total dissolved solids (parts per thousand) and pH using a multimeter (Myron L, Carlsbad, USA). Approximately 10 g of soil were used for extraction and measurement of crude oil components. Polycyclic aromatic hydrocarbon (PAH) analysis was performed using 6890N gas chromatograph (Agilent Technologies, Santa Clara, USA) equipped with 5973N mass selective detector (see S1).

Weathering ratios (PWR (Eq. (S1.1)) and DWR (Eq. (S1.2)), Supplementary files) were also developed to assess the relative fate of alkylated 3-ring phenanthrenes (PWR) and dibenzothiophenes (DWR) to more recalcitrant 4-ring chrysenes. The selected weathering ratios measure the changes in the most biodegradable PAH groups, alkylated phenanthrenes and dibenzothiophenes, remaining in DWH crude oil after the weathering that occurred at sea, specifically, the loss of 2-ring naphthalenes. Declines in these ratios would be observed in an oil where PAH biodegradation is occurring, especially at the initiation of the process.

2.3. Sample processing and meta-barcoding

All plant materials and soil were immediately stored at 4 °C in the field and brought to lab for processing within 72 h of collection. Leaves were cut into 4–6 mm^2 pieces, while white, healthy primary roots (0.5–2 mm in diameter), including all attached fine roots, were trimmed from the root ball and cut into 4 mm long pieces prior to surface-sterilization. Surface-sterilization of leaves was carried out as follows: sequential washing in 95% ethanol (EtOH) for 10 s, 0.525% sodium hypochlorite bleach for 2 min and 70% EtOH for 2 min. On the other hand, roots were surface-sterilized by sequential immersion in 70% EtOH for 10 s, 2.625% sodium hypochlorite for 2 min, followed by three rinses in sterile distilled water. Samples were frozen at −20 °C until DNA extraction. Soil around the roots was collected and immediately frozen at −20 °C for extraction.

Total genomic DNA was extracted from all plant tissues and soil for use in amplicon sequencing of the 18S internal transcribed spacer (ITS) region of the ribosomal DNA. Leaves and roots were ground in liquid nitrogen, and 0.30 mg of pulverized material was used for extraction

using MoBio PowerPlant Kits (Qiagen, Hilden, Germany). For soil, 0.30 mg was used for extraction using MoBio PowerSoil Kit. To make fungal DNA libraries, genomic DNA was standardized to 20 ng and the ITS1 region was first amplified with standard primers ITS1F and ITS2 modified with Illumina TruSeq adaptor. PCRs were done in triplicate for each sample using Phusion High-Fidelity Master Mix (New England Biolabs, Ipswich, USA), which were then pooled and used as template for indexing PCRs with unique barcode sequences for each sample (further details in S2).

Libraries were purified using Qiaquick PCR Purification Kits (Qiagen, Hilden, Germany), and quantified using the Quant-iT® dsDNA HS Assay kit with Qubit Flourometer (ThermoFisher, Waltham, USA). Equal amounts of purified libraries (25 ng) were pooled and run on paired-end Illumina MiSeq platform at Duke Genome Sequencing and Analysis Core Facility (Durham, USA).

2.4. OTU delineation and taxa identification

MiSeq sequences were filtered for quality and adapters/distal priming sites were removed using cutadapt v.1.7.1 (Martin, 2011) to remove low-quality regions from the ends of reads and keeping a minimum length of 50 base pairs (bp). Forward and reverse sequence reads for each sample were merged using PEAR v.0.9 (Zhang et al., 2014). Further filtering was completed on merged reads by removing homopolymers (maximum homopolymer of 9 bases), short sequences (keeping a minimum length of 125 bp), and ambiguous bases using the screen.seqs in mothur v.1.34.4 (Schloss et al., 2009). Edited sequences were first dereplicated by collapsing identical sequences, and Operational Taxonomic Units (OTUs) were clustered at a 97% threshold, with chimera detection and removal done with the uparse algorithm in USEARCH (Edgar, 2013). It is important to note that setting a 97% threshold for OTU clustering might potentially inflate the number of non-Ascomycota (Nilsson et al., 2008), but most of our sequences were classified as Ascomycota (see Results). Some precautions were taken, however, to reduce the influence of spurious assignments on downstream analyses. For example, all analyses were performed at OTU level, and singleton OTUs (OTUs with sequence count = 1) were removed prior to all analyses. In addition, we discarded OTUs (n = 2) that were assigned to non-fungi (e.g., Protista) at the kingdom level.

Taxonomic assignment of OTUs was done by BLAST alignment against the reference database UNITE v.7. A representative sequence was first picked using pick_rep_set.py command in QIIME (Caporaso et al., 2010), then were assigned taxonomy in BLAST++ v 2.2 (Camacho et al., 2009) with a threshold of 80% hit length alignment and 80% identity. OTUs falling below the threshold were recorded as "unidentified". We also performed an exploratory analysis for assigning guilds to OTUs using the FUNGuild software (Nguyen et al., 2016). Analyses were done separately for leaf, root and soil samples, and by oil spill history within each site to determine if there were changes in guild assignments associated with oiling history. We kept guild assignments of only those OTUs that could be assigned with the confidence ranking of 'probable' and 'highly probable' as recommended by Nguyen et al. (2016) (see Nguyen et al. (2016) for further details of the FUNGuild). The number of OTUs assigned into different guilds were plotted as relative percentages (number of OTUs assigned to a specific guild divided by the total assigned OTUs) in R v.3.4 (R Core Team, 2016).

2.5. Data analyses

We assessed outcomes of oiling in aboveground and belowground fungal endophyte communities by estimating: (1) within-community diversity (alpha diversity); (2) community structure (beta-diversity) and compositional turnover among communities; and (3) associations and patterns of similarities between aboveground and belowground fungal communities within an individual host, between sites and across all plants. All variables were transformed where necessary. Prior to all analyses, community data was rarefied to the lowest total sequence count n = 2000 using the *rrarefy*() function in vegan (Oksanen et al., 2013). All analyses were done at the OTU level (i.e., including those that were not classified below Kingdom level) and conducted separately for leaf, root and soil communities in R.

2.6. Influence of oiling on aboveground and belowground diversity

Because oil contamination can alter patterns of diversity within aboveground and belowground fungal endophyte and rhizosphere communities, we examined within-community diversity based on two metrics: OTU richness (i.e. total number of OTUs), and Shannon diversity (Shannon, 1948), which were calculated using vegan. We first compared richness and diversity between oiled and non-oiled areas in aboveground and belowground fungal communities (1) across our dataset and (2) within each site, with significance determined using *t*-tests.

To identify the factors that exert the greatest influence on site-level fungal diversity, we analyzed the effects of oiling relative to local environmental conditions on diversity using multi-model inference and model averaging (Grueber et al., 2011). To do this, we fit a mixed-effects model constructed with lme4 (Bates et al., 2014) as the base model for the multimodel inference. We used site as a random factor, with oiling history (oiled vs. non-oiled), PAH concentrations, the two weathering ratios (P_{WR} and D_{WR}), conductivity, total dissolved solids (TDS), soil pH, and the interactions of oiling history and PAHs, PAHs and P_{WR}, and of PAH and D_{WR} as fixed factors. The *dredge*() and *model.avg*() functions in the MuMIn library (Barton, 2014) were then used to fit all possible models and identify the best models within four AICc (Akaike Information Criteria) units. Parameter values, errors and AICc-weighted importance values were then averaged for these models. Analysis was conducted for both natural log-transformed (OTU) Shannon diversity and richness. All continuous predictor variables were scaled to standard *z*-scores, with a mean of 0 and standard deviation of 1 in order to compare estimates among variables.

2.7. Influence of oiling on community structure and compositional turnover

Exposure to oil can lead to differences in composition among microbial communities, so we examined dissimilarity and turnover among fungal communities (equivalent to beta-diversity), measured as abundance-weighted Bray-Curtis dissimilarity values. To investigate the best predictor of beta-diversity, and analyze sources of variation among communities, we conducted a Permutational Multivariate Analysis of Variance (PERMANOVA) using the *adonis*() function in vegan at site level. We tested for the main effects of site, oiling, PAHs, P_{WR}, D_{WR}, conductivity, TDS, pH and the interactions of site, oil spill history and PAH.

We visualized patterns of compositional differences between oiled and non-oiled areas underlying the PERMANOVA test by using Bray-Curtis distance matrix described above to conduct (1) nonmetric multidimensional scaling (NMDS) ordination; and (2) transect-level hierarchical community clustering. For hierarchical clustering, we pooled samples by oiling history and site (e.g., all non-oiled communities in the Fourchon group), summed OTU abundances for each group, and calculated abundance-weighted Bray-Curtis dissimilarity index values. We then used the *hclust*() function using ward.D2 agglomeration method (Murtagh and Legendre, 2014), with the resulting clusters visualized as dendogram using dendextend (Galili, 2015).

We also conducted similar PERMANOVA analysis at the family-level delineations to assess compositional differences between individual plants in the abundances of taxa representing different families with respect to oil history and site. OTUs were collapsed into family level, and sequence counts (or abundances) were aggregated to that level. All unclassified OTUs were included in the analyses and were lumped together. Separate analyses were done for leaf, root and soil samples.

We further plotted the top 5 most abundant families within plant tissues with respect to site and oiling history in R.

2.8. Associations between aboveground and belowground fungal communities

In order to test if oiling either increased or decreased similarity among communities, we calculated pairwise tissue comparisons (i.e. leaf-root, leaf-soil and root-soil within each plant) using Bray-Curtis dissimilarity index values. Values of Similarity (1 − Dissimilarity) among each pairwise comparison for individual plants were plotted against a plant's corresponding PAH concentration and then used to perform linear regressions to test for associations with PAH concentrations.

We also examined whether oiling influences associations between aboveground and belowground communities by examining correlations between the diversity and composition of leaf, root and soil communities in oiled areas and compared these to non-oiled areas within each site. Spearman rank correlations were conducted to evaluate associations between the diversity of leaf and root, leaf and soil and root and soil fungal communities within each site. We also investigated the strength of association in community dissimilarity by performing a Mantel Test (Pearson method) on the Bray-Curtis dissimilarity index values for corresponding community pairs (i.e. leaf-root, leaf-soil, and root-soil) within individual plants using vegan. To further assess whether oiling disrupted associations among communities, similar analyses were conducted separately on communities in non-oiled areas within each site. These analyses allowed us to test whether among-community dissimilarity of communities within a host plant tissue (e.g., leaf) was correlated with a similar change in communities in another tissue (e.g., root) in oiled areas, and by further comparing these patterns with non-oiled communities, we could assess the direction and magnitude of potential oiling influence on associations among aboveground and belowground communities.

3. Results

3.1. Fungal communities

We obtained 7.6 million sequences from 120 samples after filtering. We generated 3495 fungal OTUs delineated at 97% sequence identity across all samples. OTUs primarily belonged to the Ascomycota phylum (87% of all OTUs assigned to taxa), followed by Basidiomycota (9.6%) and Glomeromycota (1.8%). All other phyla comprised ~1% of OTUs. Of the 3495 OTUs delineated for all samples, 1382 OTUs (61%) could not be assigned with our relatively stringent BLAST criteria (80% query alignment length, 80% identity match).

We detected differences in fungal communities according to tissue, site and oiling history. Although the top two dominant OTUs were the same in non-oiled and oiled areas of both sites regardless of plant tissue, the rest of the top 10 most abundant OTUs varied between site and oiling history, with some OTUs declining in abundance in oiled areas (Table 1). For instance, the third most abundant OTU (derep_15) found in non-oiled areas in Fourchon (which was not abundant in Bay Jimmy) declined by as much as 64% in oiled areas. Similarly, the third and fourth most abundant OTUs at the non-oiled Bay Jimmy area (derep_4 and derep_3) declined in abundance to negligible numbers in oiled areas (Table 1). On the other hand, some OTUs (e.g., derep_18 in Bay Jimmy) were more abundant in oiled areas and were negligible in non-oiled areas. There were fewer shared OTUs in oiled areas across our dataset (n = 35, comprising 3% of total OTUs in oiled communities) compared to non-oiled communities (n = 73, 6% of all OTUs found in non-oiled areas). Of the shared OTUs among plant tissues, we detected differences in relative abundance and composition of OTUs between non-oiled and oiled areas (Fig. S2).

The relative proportions of class-level taxonomic delineations also varied among aboveground and belowground fungal communities with respect to site and oiling history (Fig. S3). The most abundant classes across our dataset were *Sordariomycetes*, comprising 20% of all total sequence reads, followed by *Dothideomycetes* (7.7%) and *Agaricomycetes* (5.8%). The top five most abundant families also varied among aboveground and belowground communities with respect to site and oil history (Fig. 2). This is supported by the PERMANOVA analysis showing compositional differences at family-level delineations among communities within leaf, root and soil according to site (R^2 leaf = 0.102, p = 0.001; R^2 root = 0. 286, p = 0.001; R^2 soil = 0.111, p = 0.001) and oil history (R^2 leaf = 0.110, p = 0.001; R^2 root = 0. 074, p = 0.003; R^2 soil = 0.037, p = 0.134).

Table 1
Top 10 most dominant OTUs across all plant tissues in non-oiled and oiled areas in Fourchon and Bay Jimmy. Abundance is the raw sequence counts.

Fourchon		Bay Jimmy	
OTU ID	Abundance	OTU ID	Abundance
Non-oiled			
derep_0	35,240	derep_0	20,647
derep_1	7023	derep_1	10,331
derep_15	1385	derep_4	4909
derep_43	1091	derep_3	2790
derep_24	1001	derep_5	1859
derep_60	976	derep_7	1197
derep_25	569	derep_12	1056
derep_108	481	derep_10	1009
derep_64	453	derep_13	949
derep_61	447	derep_17	676
Oiled			
derep_0	30,026	derep_0	29,693
derep_1	9272	derep_1	10,605
derep_43	1055	derep_18	1251
derep_34	622	derep_8	1247
derep_206	569	derep_12	1112
derep_161	568	derep_14	1104
derep_15	489	derep_5	968
derep_5	395	derep_11	823
derep_108	383	derep_76	790
derep_12	336	derep_21	786

Across all OTUs, only 13% could be taxonomically parsed into guilds with the threshold of probable and highly probable confidence rankings (Nguyen et al., 2016). Most of the identifiable OTUs detected in root and soil samples were considered saprotrophs, arbuscular mycorrhizal fungi, and a few pathogens (Figs. S4–S6). This pattern, though, varied between sites and plant tissues. In leaf, for example, majority of OTUs were undefined saprotrophs followed by arbuscular mycorrhizal (AM) regardless of site and oiling history (Fig. S4). In roots, there was an increase in AM in oiled communities (13%) from non-oiled communities (7%) in Fourchon but not in Bay Jimmy (Fig. S5). Regardless of oiling history and site, saprotrophs dominated most of the assigned OTUs. However, for rhizosphere communities in Bay Jimmy, OTUs assigned to AM in non-oiled soil communities comprised 10% of all OTUs, but exhibited a nearly four-fold increase (37%) in oiled communities. An opposite pattern was observed for (undefined) saprotrophs, which were higher in non-oiled (37%) compared to oiled (29%) conditions (Fig. S6). In Fourchon, the relative percentage of OTUs considered as AM remained constant (14%) in both oiled and non-oiled communities, similar to saprotrophs (undefined and soil saprotrophs).

3.2. Influence of oiling history on aboveground endophyte communities

We detected a strong signature of oiling in leaf endophytic community diversity and composition. Communities in oiled areas exhibited significantly lower Shannon diversity (non-oiled = 0.524 ± 0.014, oiled = 0.202 ± 0.095, $p < 0.05$, Fig. 3). Though not statistically significant, richness was also lower (non-oiled = 34.350 ± 156.860, oiled = 21.833 ± 52.083, $p > 0.05$). The effect of oiling varied between our two sites; greater differences were found between oiled and non-oiled

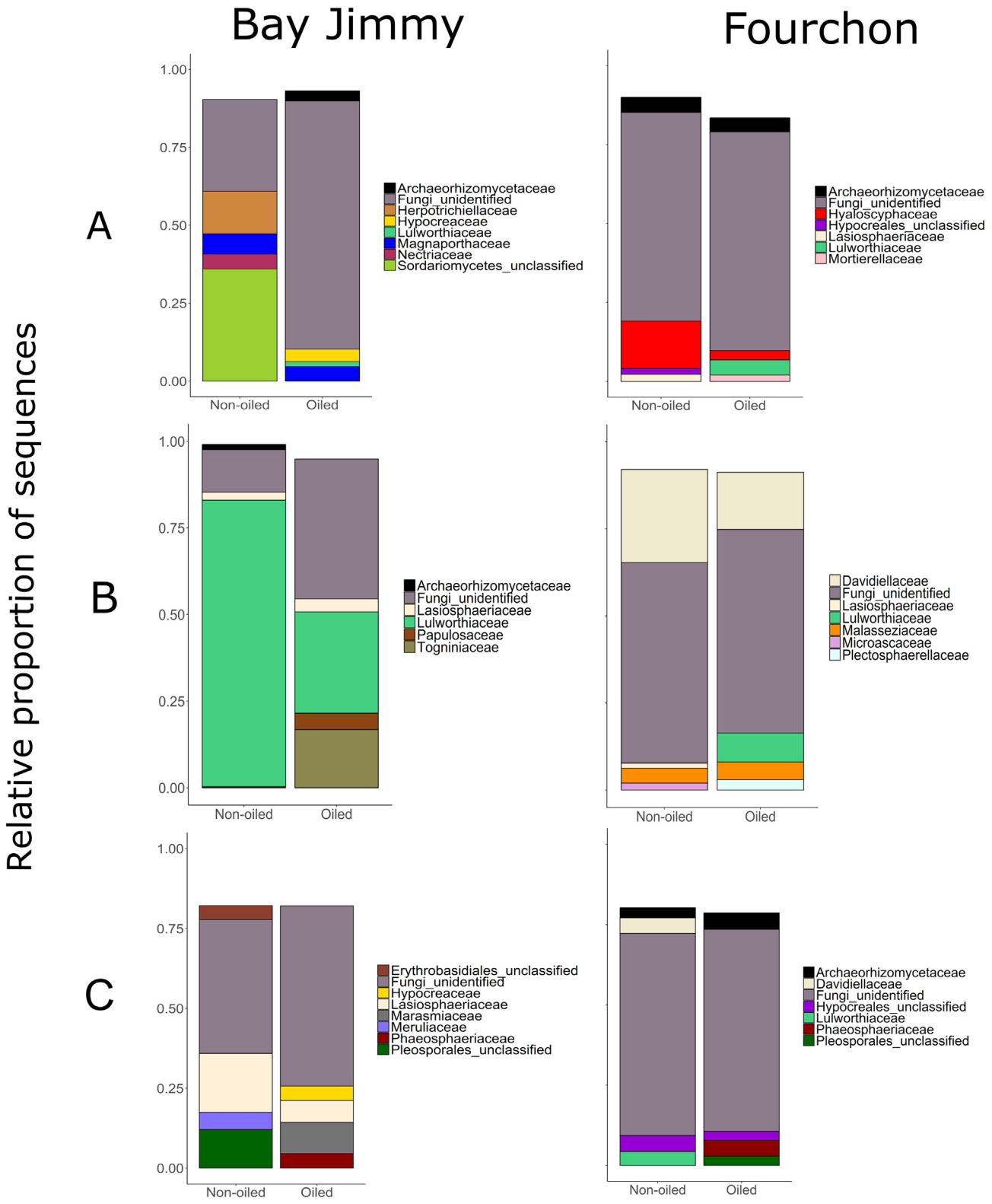

Fig. 2. Family assignment. Relative abundance by proportion of assigned families across all samples in (A) leaf, (B) root and (C) rhizosphere soil fungal communities between oiled and non-oiled areas in Bay Jimmy and Fourchon. Proportions were calculated as the number of sequences of assigned family divided by sum of all sequences within the group.

areas in Bay Jimmy than in Fourchon (Fig. 3). Patterns of within-community Shannon diversity, however, did not correspond to any local environmental factors (Table 2), though model averaging analysis indicated that fungal richness varied with soil pH (Table 2).

Results from PERMANOVAs based on Bray-Curtis dissimilarity index values also showed that the structure of leaf communities corresponded to oiling ($R^2 = 0.052$, $p = 0.050$, Fig. 4). We detected inconsistent effects of oiling across sites and PAH concentrations, however, as indicated by their significance of interactions ($R^2 = 0.100$, $p = 0.026$). NMDS ordination across all pairwise leaf datasets recovered a stronger clustering by oiling history within each site than between sites (Fig. 5), although hierarchical clustering by group showed less distinct grouping either by

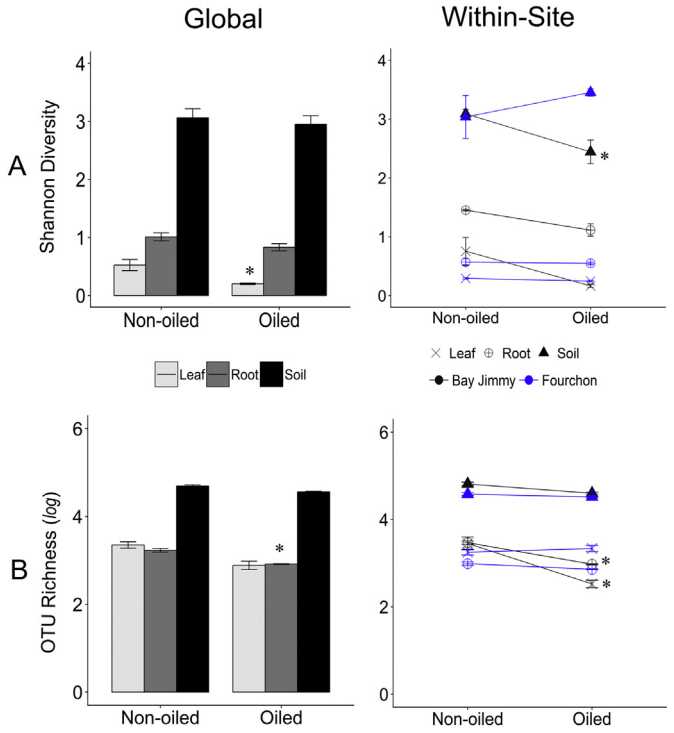

Fig. 3. Fungal diversity. Comparison of mean within-community diversity in leaf, root and rhizosphere soil between non-oiled and oiled areas measured as rarefied (A) Shannon diversity and (B) OTU richness (log) across the whole dataset (left panel) and within each site (right panel) (*$p < 0.05$). Lines in within-site panel (left) correspond to change in diversity values from non-oiled to oiled areas. Error bars represent standard errors.

oiling history or by site (Fig. S3). Further evidence of leaf endophyte community shifts comes from observed changes in the most dominant class-level (Fig. S3) and family-level (Fig. 2) taxonomic delineations between oiled and non-oiled areas within each site.

3.3. Influence of oiling history on belowground endophyte communities

Oiling history appeared to exert little influence on root endophyte communities. Global analyses found that oiling correlated with significant reduction in endophyte richness (non-oiled = 27.500 ± 32.847, oiled = 18.85 ± 3.443, $p = 0.003$) but not Shannon diversity (non-oiled = 1.010 ± 0.070, oiled = 0.831 ± 0.062, $p = 0.304$, Fig. 3). However, we detected evidence of site-dependency as the global decline in richness appeared to be driven by the oiling effects in Bay Jimmy (Fig. 3). Analyses controlling for site effects showed that neither oiling history nor any environmental co-variates exerted a strong influence on diversity, though oiling had a negative effect on root endophytic richness (Table 2).

Although we detected associations with both PAH concentration and P_{WR} (Fig. 4), stronger correlations were found between root community variation and site differences ($R^2 = 0.222$, $p = 0.001$) than with oiling history. Substantial compositional shifts between sites- regardless of oiling history- were also illustrated by NMDS and hierarchical clustering analyses, which recovered clusters according to site rather than oiling history (Figs. 5 and S3).

3.4. Influence of oiling history on rhizosphere communities

Responses of rhizosphere communities to oiling differed from leaf and root endophytic community responses. Global analyses across all data showed that diversity (non-oiled = 3.063 ± 0.156, oiled = 2.950 ± 0.148, $p = 0.668$) and richness (non-oiled = 115 ± 341.640, oiled = 99 ± 169.476, $p = 0.139$, Fig. 3) of rhizosphere communities were similar between oiled and non-oiled areas. However, we detected

Table 2
Significant factors explaining within-community diversity measured as OTU (Shannon) diversity and OTU richness.

Factors	Shannon diversity		OTU richness	
	Impt[a]	Est (adj S.E.)	Impt	Est (adj S.E.)
Leaf				
(Intercept)		−1.213 (0.470)**		3.258 (0.193)**
Oil spill history	0.930	−0.783 (0.522)	0.630	−0.398 (0.212)
PAH	0.180	−0.344 (2.788)	0.160	−0.086 (0.115)
Oil spill history × PAH	0.020	2.214 (9.179)	–	–
P_{WR}	0.110	0.008 (0.213)	0.130	0.022 (0.138)
D_{WR}	0.410	0.274 (0.181)	0.470	0.181 (0.116)
Conductivity	0.150	−0.205 (0.779)	0.160	−0.482 (0.747)
TDS	0.230	0.314 (0.506)	0.470	0.309 (0.386)
pH	0.310	−0.223 (0.161)	0.890	−0.237 (0.107)**
Root				
(Intercept)		−0.338 (0.543)		3.210 (0.175)**
Oil spill history	0.090	−0.106 (0.248)	0.970	−0.283 (0.106)**
PAH	0.240	0.101 (0.149)	0.110	−0.006 (0.060)
P_{WR}	0.460	0.227 (0.131)	0.110	0.023 (0.055)
D_{WR}	0.300	0.212 (0.145)	0.110	0.024 (0.062)
PAH × P_{WR}	0.020	−0.076 (0.076)	–	–
Conductivity	0.200	0.138 (0.152)	0.320	−0.007 (0.223)
TDS	0.220	0.134 (0.128)	0.360	0.123 (0.149)
pH	0.110	−0.079 (0.134)	0.160	−0.058 (0.057)
Soil				
(Intercept)		1.056 (0.096)**		4.65 (0.071)**
Oil spill history	0.110	−0.043 (0.112)	0.330	−0.141 (0.111)
PAH	0.240	−0.059 (0.064)	0.050	0.014 (0.054)
P_{WR}	0.260	−0.079 (0.058)	0.430	−0.074 (0.054)
D_{WR}	0.080	−0.049 (0.063)	0.100	−0.035 (0.069)
PAH × P_{WR}	0.020	−0.034 (0.035)	–	–
Conductivity	0.170	−0.050 (0.160)	0.540	0.105 (0.074)
TDS	0.270	0.106 (0.106)	0.210	−0.004 (0.113)
pH	0.220	−0.052 (0.057)	–	–

Est are model averaged estimates with adjusted standard error (S.E.). Only those terms that appeared in models within the δ = 4 have estimates.

[a] Impt (Importance) values are weighted proportion of models that a factor appeared in the best models within δ = 4 AIC units.

** $p < 0.01$.

evidence of site-dependency. Site-level analyses demonstrated that Shannon diversity was significantly lower in oiled areas of Bay Jimmy, though diversity and richness did not correspond to local environmental variables (Table 2).

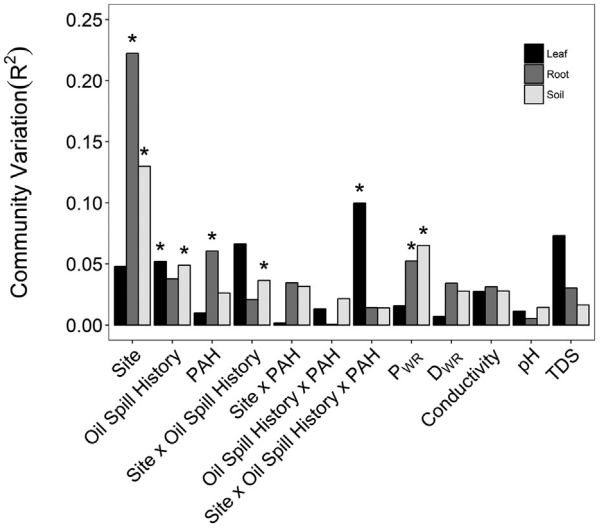

Fig. 4. Sources of community variations. PERMANOVA partitioning variation among fungal communities, showing the effects of site, oiling history and other environmental factors. R-square values from PERMANOVA were plotted to compare the relative effects of environmental factors on turnover among fungal endophyte communities in leaf, root and rhizosphere soil (*$p < 0.05$).

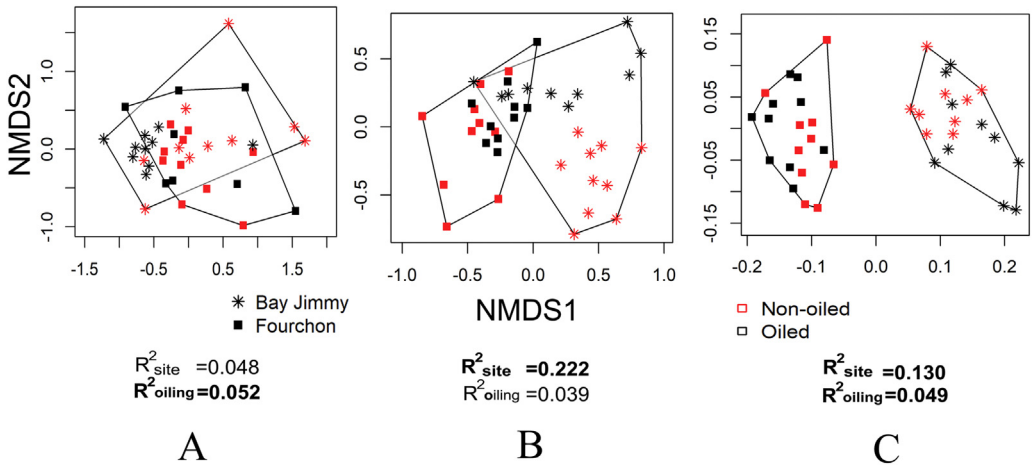

Fig. 5. Non-Metric Multidimensional Scaling (NMDS) based on pairwise, abundance-weighted Bray-Curtis distance values for (A) leaf, (B) root and (C) rhizosphere soil communities. Ellipses were based on site clustering. R^2 values for site and oiling history were from the PERMANOVA analyses, with significant terms in bold text.

We detected an association between oiling history and rhizosphere community structure. PERMANOVA analyses showed that rhizosphere communities differed between oiled and non-oiled areas ($R^2 = 0.049$, $p = 0.001$, Fig. 4), though the effect of oiling differed between the two sites, as we recovered a significant interaction between site and oiling history ($R^2 = 0.037$, $p = 0.019$, Fig. 4). Shifts in the relative abundance of family-level delineations provided supporting evidence of turnover. While *Davidiellaceae* was abundant in both oiled and non-oiled areas in Bay Jimmy, *Pleosporales*, *Meruliaceae* and *Erythrobasidiales* were only dominant in non-oiled areas (Fig. 2). In addition, P_{WR} had an effect on rhizosphere community structure ($R^2 = 0.065$, $p = 0.001$, Fig. 4). Rhizosphere communities also exhibited strong spatial structuring within sites ($R^2 = 0.130$, $p = 0.001$, Fig. 4), which was also evident in NMDS and hierarchical clustering analyses (Figs. 5 and S3).

3.5. Associations between aboveground and belowground fungal communities

Pairwise similarities between each tissue (i.e., leaf-root, leaf-soil, root-soil) within an individual host plant did not show convergence or divergence with increasing PAH concentrations (Fig. 6, Table S1). The weak or absent effect of oiling for disrupting potential associations among aboveground and belowground communities was further supported by the lack of correlations between the diversity and composition of aboveground and belowground communities. According to Spearman rank correlation tests, within-community (alpha) diversity was not significantly correlated between pairwise leaf-root, leaf-soil and root-soil communities regardless of oiling history at both sites. Furthermore, in oiled areas of Bay Jimmy, analyses of the association in among-community dissimilarity for corresponding community pairs showed that increased dissimilarity in endophytic root communities was significantly correlated to increased dissimilarity in soil communities within individual host plants (Mantel r = 0.333, $p = 0.048$, Table S1). No significant correlations between pairwise leaf-root, leaf-soil, and root-soil communities were found in other comparisons regardless of oiling history at both sites (Table S2).

4. Discussion

We examined the nature and magnitude of endophyte and rhizosphere fungal community responses to oil exposure six years after the Deepwater Horizon oil spill. We found that response to oiling differed among fungal communities according to host plant tissue and site. Foliar endophytic fungal communities in plants from oiled areas consistently exhibited lower diversity accompanied by substantial shifts in community composition. The richness of root endophytic communities also was lower in oiled areas, but overall differences appeared to be driven by shifts in Bay Jimmy communities, which experienced greater oil deposition than Fourchon. We also detected site-dependent shifts in rhizosphere community structure associated with oiling, without significant loss of overall diversity. Furthermore, the similarity of aboveground and belowground fungal communities within a host plant did not shift relative to oiling. These results indicate that responses to stressor exposure vary between aboveground and belowground communities, and that site-specific differences in microbial diversity and composition can constrain outcomes of perturbations.

Our results are consistent with prior reports that perturbations - including oil exposure - can alter the diversity of plant-associated microbial communities (e.g., Giauque and Hawkes, 2013; Kandalepas et al., 2015; Iffis et al., 2017). Our study extends prior findings by showing that aboveground and belowground fungal communities exhibited distinct responses to the same perturbation. The stronger response of foliar communities to oiling than root and rhizosphere communities might be a consequence of PAH accumulation acting as a strong selection force on foliar fungal communities. Although there is limited information on the ability of *S. alterniflora* to accumulate PAHs in leaves, there is evidence that phenanthrene and other low molecular weight PAHs can partition directly into leaf tissue through the cuticle from contaminated air masses around the leaf tissue of wetlands plants, including *S. alterniflora* (Wild

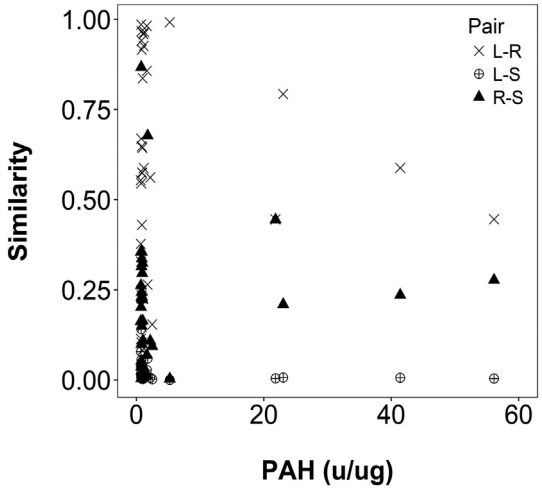

Fig. 6. Patterns and associations of fungal communities across plant tissues. Random pattern in pairwise community similarities (1 - Bray-Curtis dissimilarity) between leaf-root (L-R), leaf-soil (L-S) and root-soil (R-S) within individual host plants across PAH concentrations.

et al., 2005; Watts et al., 2006; Wang et al., 2012; Li and Chen, 2014). PAH accumulation might have reduced diversity by promoting the growth of only a few taxa or by limiting recruitment of endophytes through penetration of leaf surfaces. Notably, we did not find a significant association with PAHs, P_{WR} or D_{WR} and leaf endophyte diversity, however, our measures of contamination and weathering were estimated from rhizosphere soil rather than leaves. Thus, we might have detected an association had measurements been taken from leaves. It is also possible that oiling effects on litter decomposition might have reduced the diversity of foliar endophyte communities by limiting potential sources of spore inoculum.

Contrary to other studies (e.g., Su et al., 2016; Iffis et al., 2017), we did not find evidence that oiling consistently reduced the diversity of root and rhizosphere communities. Our findings possibly reflect the recovery of both communities following initial exposure. Alternatively, oiling may exert less influence on (alpha) diversity of belowground fungal communities associated with *S. alterniflora* in salt marshes. Support for this inference comes from other studies showing that the diversity of aboveground and belowground communities exhibit varying responses to environmental changes (e.g., Coince et al., 2014).

Our results indicate that environmental disturbances can result in site-dependent community variations. Oiling altered the composition of foliar endophyte and rhizosphere fungal communities but not root endophyte communities, suggesting that the nature and magnitude of response to oiling varies across plant-associated fungal communities. This parallels evidence from other studies indicating that microbial communities located in different plant organs exhibit unique responses to environmental conditions (e.g., Bodenhausen et al., 2013; Wagner et al., 2016). Additionally, we found that the influence of oiling on community composition differed between our two sites, most likely due to differences in the amount of residual oil present at each site. Oiled areas in Bay Jimmy had significantly higher PAH concentrations than in Fourchon (Fig. S1, Michel et al., 2013; Rodrigue, 2014). Though our results were equivocal, it remains possible that the effects of oiling were compounded by differences in local biotic or abiotic conditions. Thus the potential for complex, site-level interactions might preclude the possibility of deriving predictable, generalizable trends in responses of plant microbiomes to perturbation.

Our results nonetheless allude to a possible trend in site-dependent variation among fungal communities. As illustrated by measures of community turnover, site-specific differences in responses to oiling were more apparent in rhizosphere communities than in foliar and root endophytes. The observed differences in site-dependency might be due to differences in propagule pools. Rhizosphere fungal communities at each site appear to be drawn from distinct, local pools of microbes, which suggests that rhizosphere communities are not well-mixed across sites. For instance, *Sordariomycetes* were the most abundant class of rhizosphere fungi in oiled areas of Bay Jimmy while *Dothideomycetes* was dominant in oiled areas of Fourchon. Consequently, the nature and magnitude of rhizosphere community responses to contaminant exposure are also likely contingent on area-dependent propagule pools.

Though prior studies have found that disturbance can drive community convergence or divergence (Houseman et al., 2008; Greer et al., 2011), we found that oiling history did not alter community similarity or influence potential associations between fungal communities in different plant tissues. Evidence of distinct root and rhizosphere communities parallels evidence that root and rhizome microbial communities form through different assembly 'rules' (e.g., Gottel et al., 2011; Edwards et al., 2015 but see, e.g., Broeckling et al., 2008). Our results suggest that assembly likely differs in part because the physiology of plant organs outranks the influence of environmental perturbations on microbial communities. Accordingly, while the diversity and composition of both communities might be affected by contaminant exposure, the trajectory of resulting shifts (including recovery) should consequently be distinct.

5. Conclusion

Our study offers novel and timely perspectives on outcomes after severe environmental perturbations. Although it is unclear how endophytic and rhizosphere microbes influence *S. alterniflora* physiology, exposure-driven loss of associated microbiomes can potentially reduce organismal integrity, immunity to disease and overall performance. If severe, loss of function in *S. alterniflora* could very well threaten the stability and persistence of salt marsh ecosystems. By demonstrating that perturbations differentially impact plant-associated microbiomes, this study offers a framework for assessing and accounting for likely losses of function and outcomes (Kardol and Wardle, 2010). It also illustrates that consideration of host-microbiome interactions (i.e., shifts, feedbacks, etc.) could improve prognostic models of ecosystem and Earth system responses to global environmental change.

Data statement

Illumina sequences are available in NCBI SRA (BioProject ID: PRJNA475039). Other data, including sample information and scripts are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org (https://doi.org/10.7266/N7ZP44P4).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.06.077.

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