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Root endophytes and invasiveness: no difference between native and non-native *Phragmites* in the Great Lakes Region

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Abstract. Microbial interactions could play an important role in plant invasions. If invasive plants associate with relatively more mutualists or fewer pathogens than their native counterparts, then microbial communities could foster plant invasiveness. Studies examining the effects of microbes on invasive plants commonly focus on a single microbial group (e.g., bacteria) or measure only plant response to microbes, not documenting the specific taxa associating with invaders. We surveyed root microbial communities associated with co-occurring native and non-native lineages of *Phragmites australis,* across Michigan, USA. Our aim was to determine whether (1) plant lineage was a stronger predictor of root microbial community composition than environmental variables and (2) the non-native lineage associated with more mutualistic and/or fewer pathogenic microbes than the native lineage. We used microscopy and culture-independent molecular methods to examine fungal colonization rate and community composition in three major microbial groups (bacteria, fungi, and oomycetes) within roots. We also used microbial functional databases to assess putative functions of the observed microbial taxa. While fungal colonization of roots was significantly higher in non-native *Phragmites* than the native lineage, we found no differences in root microbial community composition or potential function between the two *Phragmites* lineages. Community composition did differ significantly by site, with soil saturation playing a significant role in structuring communities in all three microbial groups. The relative abundance of some specific bacterial taxa did differ between *Phragmites* lineages at the phylum and genus level (e.g., *Proteobacteria, Firmicutes*). Purported function of root fungi and respiratory mode of root bacteria also did not differ between native and non-native *Phragmites*. We found no evidence that native and non-native *Phragmites* harbored distinct root microbial communities; nor did those communities differ functionally. Therefore, if the trends revealed at our sites are widespread, it is unlikely that total root microbial communities are driving invasion by non-native *Phragmites* plants.

Key words: bacteria; endophytes; fungi; invasive plants; microbes; mutualists; oomycetes; pathogens; plant-microbial interactions; roots; soil saturation.

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INTRODUCTION

The intimate association of plants and their microbiome has significant impacts on plant performance and thereby may be an important driver of invasion success. Recent studies have focused on the plant-associated microbiome of invasive plants, because it could provide a mechanism for their colonization and recruitment, as well as for decreased performance of native

species (Thorpe and Callaway 2006, Salles and Mallon 2014, Kowalski et al. 2015). More specifically, if invasive plants cultivate a microbial community with stronger mutualistic effects or less intense pathogenic effects than native plants, then the net effect of their microbiome will be more positive than for native plants (Reinhart and Callaway 2006).

Evidence linking belowground microbial communities to invasive plant success is mixed. For instance, invasive grasses can have stronger positive or less negative plant–soil feedbacks than natives in monoculture (Klironomos 2002) and in mixtures (Kulmatiski 2018). However, a meta-analysis covering many different habitat types from forest to wetland did not find a consistent trend supporting feedbacks as a mechanism for invasion success (Suding et al. 2013), suggesting that belowground microbial community effects on plant performance may be taxon- or habitat-specific. In addition, plant–soil feedback experiments typically do not identify which microbes are cultivated such that specific microbial mechanisms are unclear. Moreover, microorganisms are phylogenetically diverse and can affect plant performance in many ways, ranging from direct pathogenesis to alteration of site nutrient availability. However, studies that examine plant-associated microbes often target one specific group of taxa (e.g., bacteria or fungi), thereby ignoring the potential interactions among these diverse organisms that could have important outcomes for plant performance.

One of the most noxious and widespread wetland plant invaders is the common reed, *Phragmites australis* (Cav.) Trin. ex Steud. (Meyerson et al. 2016). This clonal plant occurs widely throughout North American wetlands and includes both native (hereafter native *Phragmites*) and exotic, invasive haplotypes (hereafter non-native *Phragmites;* Saltonstall 2002, Saltonstall et al. 2004). In many Atlantic Coast and Great Lakes wetlands, non-native *Phragmites* is much more productive, and in some cases, displaces native *Phragmites* (Martin and Blossey 2013, Mozdzer et al. 2013, Price et al. 2013). Due to their close phylogenetic relationship, comparison between the non-native and native lineages of *Phragmites* offers a unique opportunity to understand how microbial communities may impact invasive success.

As a species considered invasive in most of North America, *Phragmites* has been well studied, including some exploration of select microbial groups. Previous studies have examined leaf, root, and seed fungal endophytes of non-native *Phragmites* (Clay et al. 2016, Soares et al. 2016, Shearin et al. 2018), characterized and compared soil oomycete communities (Nelson and Karp 2013, Crocker et al. 2015, Cerri et al. 2017), and compared soil bacterial (Bowen et al. 2017) and archaeal (Yarwood et al. 2016) communities between *Phragmites* lineages. However, the functional significance of *Phragmites*-associated microbiomes remains unknown. To date, no comprehensive survey of root microbial communities and their interactions with native and non-native plant lineages has been performed. A comparison of the microbial communities associated with the two lineages may lend mechanistic insights into the invasiveness of non-native *Phragmites*. For example, associating with relatively more mutualists or fewer pathogens would give the non-native lineage a performance advantage over the native lineage.

Here, we report on an intensive field survey of bacterial, fungal, and oomycete communities associated with the roots of native and non-native *Phragmites* in sites where both lineages co-occur throughout the state of Michigan, USA. We focus on roots as the main interface between the plant and the soil environment and where nutritional mutualisms as well as pathogen attack are common. If performance differences between native and non-native plant lineages are driven by their root microbial communities, then we hypothesize that plant lineage would be a stronger predictor of microbial community differences than environmental variables. Furthermore, mutualist microbes should be more abundant and/or pathogen microbes should be less abundant in the non-native lineage.

Materials and Methods

Study sites

In August 2015, we sampled roots from native and non-native *Phragmites* individuals at 8 sites distributed across Michigan, USA (Appendix S1: Table S1). We sought sites that had at least 3 distinct patches of native and non-native *Phragmites* in close proximity to one another growing under

similar environmental conditions (i.e., soil type, hydrology) with no recent history of invasive plant management (e.g., herbicide, burning). Due to the rarity of co-occurring native and non-native *Phragmites* populations that met these criteria (non-native is rare and well-managed in northern Michigan; native is rare in southern Michigan), patch size varied considerably among sites (1–100 m^2) and micro-environmental differences among patches within sites and sample size differences among sites were unavoidable. To account for this variation, we used environmental characteristics as co-variables in our analyses.

At each site, we morphologically identified all *Phragmites* patches as native or non-native. Size of *Phragmites* clones can be quite variable, wherein patches can consist of a single clone or multiple clones. While clones can differ morphologically (Křiváčková-Suchá et al. 2007), such differences are not consistent or distinct enough to reliably use for field identification. To maximize probability that patches represented distinct clones, we considered patches that were separated by at least 10 m to be separate clones. We classified the degree of soil saturation as either unsaturated, saturated, or saturated with surface water, recorded height of water table (if over the surface), and nature of surrounding vegetation. Within each patch, a ramet near the center of the clone was randomly selected. Roots were collected by cutting with a serrated knife in a 10 cm diameter circle around the chosen ramet, exhuming subtending roots, and then placing them in a plastic bag. Leaf samples from the same stem were collected for nutrient analysis. Soil samples (5 cm depth) were collected from the area adjacent to the selected ramet. All samples were kept on ice until returned from the field.

Sample preparation

Roots were washed with deionized water to remove all soil particles and litter and placed into a sample bag. A subset of the fine roots (<1 mm diameter) was removed and placed into biopsy cassettes for microscopic analysis, and the remaining roots were stored for molecular analysis. Biopsy cassettes containing fine roots were submerged in 10% KOH at 20°C for 24–48 h or until roots appeared clear, and then submerged in a mixture of 5% black ink (Sheaffer Pen and Art Supply, Providence, Rhode Island) in 5% acetic acid at 95°C for 2 min (modified from Kosuta et al. 2005). Cassettes were then immediately rinsed with deionized water and a few drops of acetic acid to remove excess stain. Cassettes containing stained roots were stored at 4°C until microscopic analysis. Stained roots were cut into 1-cm sections, mounted on microscope slides, and examined at 40× magnification for the presence of fungal hyphae following the line-intersection method (McGonigle et al. 1990). While we looked for distinct fungal structures, no vesicles or arbuscules were found. Therefore, counts were restricted to presence/absence of hyphae only.

Fine roots used for molecular analysis were surface sterilized by submerging in 70% ethanol for 1 min, 0.5% sodium hypochlorite for 3 min, and 95% ethanol for 30 s. A subset of sterilized roots was pressed onto petri plates containing Malt Extract Agar for 30 s and incubated at room temperature for 24 h. No growth on the agar was used as an indicator of successful surface sterilization. After surface sterilization, roots were placed into a sterile whirl pack bag and lyophilized in preparation for DNA extraction.

Soils were passed through a 2-mm sieve and oven dried at 60°C for 48 h. Dried samples were ground with a mortar and pestle, and subsamples from each (0.5 g) were processed in duplicate in a Leco CNS2000 Analyzer (LECO, St. Joseph, Michigan, USA) to measure carbon and nitrogen. Soil phosphorus was determined colorimetrically following the Bray P1 extraction method (Bray and Kurtz 1945).

Molecular analysis

To prepare for DNA extractions, approximately 50 mg of freeze-dried fine roots was ground in a mortar and pestle with approximately 250 mg autoclaved sand and approximately 1 mL liquid N. The finely ground root sample was then further homogenized with the lysis buffer from Macherey-Nagel Nucleospin Plant II DNA extraction kits (Macherey-Nagel, Bethlehem, Pennsylvania, USA). All genomic DNA extracts were verified by electrophoresis. Extracts were checked for quality on a NanoDrop UV/Vis spectrophotometer and concentration using a Quant-iT PicoGreen dsDNA kit (Invitrogen, Carlsbad, Carlsbad, USA).

All polymerase chain reactions (PCRs) for each taxon of interest (fungi, bacteria, oomycetes) were performed using subsamples of the same template genomic DNA. Genomic DNA was diluted to ensure equimolar concentration of template DNA in each PCR product. For fungal amplification, ITS1F and ITS4 forward and reverse primers were used (White et al. 1990). For bacterial amplification, the 16s region was amplified using primers 27F and 519R (Lane 1991). Oomycete DNA was amplified using the Oom1F and Oom1R (Arcate et al. 2006). See Appendix S1: Table S2 for all PCR conditions, primer sequences, and master mixes. PCR products were purified using the Qiagen MinElute PCR kit and quantified using a Quant-iT PicoGreen dsDNA kit (Invitrogen, Carlsbad, California, USA).

Sequencing was performed on a PacBio-RS II system utilizing circular consensus technology, which can generate 99.5–99.9% sequence accuracy for DNA fragments ranging from 150 to 500 bp (Travers et al. 2010). Seventeen barcoded samples, pooled in equimolar concentration, were multiplexed per SMRT chip. Three SMRT chips were sequenced per microbial group (9 total SMRT chips) at the University of Michigan Sequencing Core.

Bioinformatics analysis

Raw sequence data were processed using mothur v1.39.5 (Schloss et al. 2009). Operational taxonomic units (OTUs) were clustered at 97% for bacterial sequences. Fungal and oomycete sequences were binned into phylotypes because ITS does not allow for sequence alignment over large datasets and mothur requires a sequence alignment to cluster OTUs. Bacterial taxonomy was determined by comparing representative sequences to the taxa found in the SILVA database (Quast et al. 2018). Fungal taxonomy was assigned based on the UNITE database (Nilsson et al. 2013). For oomycete taxonomy, we created a custom oomycete-specific database from NCBI records. Sequences were compared to this database using the blastn toolkit. Each microbial group was rarefied according to the sample that yielded the fewest number of sequences to ensure equal sampling across all samples. Fungi were rarified to 200 sequences per sample, bacteria to 459 sequences, and oomycetes to 468 sequences. Sequencing coverage of fungal and bacterial communities was estimated using Good's coverage estimator (Good 1953). Although rarefaction was carried out at a relatively low number of sequences, Good's coverage indicated strong sampling effort at each sample (Appendix S1: Fig. S1–S3). Sequences were uploaded to the NCBI Sequence Read Archive under SRA accession number SRP160913.

Functional assignment

To evaluate functional potential of fungal OTUs, we used FUNGuild (Nguyen et al. 2015) which parses fungal communities by trophic mode and functional guilds. We analyzed outputs at the trophic mode level to understand the proportion of the root communities composed of mutualists (symbiotrophs), pathogens (pathotrophs), and likely commensalists (saprotrophs). For bacterial functional potential, we used the BugBase (Ward et al. 2017) tool that groups organisms into functional groups based on KEGG pathways (Ogata et al. 1999) compiled by PICRUSt (Langille et al. 2013). This tool allowed us to view bacterial communities by their oxygen requirements and potential for stress tolerance. Use of this tool required that we reclassify our OTUs using the Greengenes taxonomic database (Desantis et al. 2006).

Data analysis

We used principle component analysis (PCA) to visualize environmental gradients at our sample sites and select uncorrelated variables to use in subsequent analysis. We used a two-phased analysis to account for the fact that environmental variables were not balanced with respect to site and lineage. First, we tested differences by site and lineage for root colonization, microbial alpha diversity, and relative sequence abundance using 2-way ANOVA (Type III Sum of Squares). Second, we ignored site and assessed the impact of environmental variables (soil nutrients and saturation) on the response variables, including potential interactions with lineage using analysis of co-variance (ANCOVA). Permutational multivariate analysis of variance (PerMANOVA) tested whether plant lineage or site predicted significant microbial community differences among our samples. Homogeneity of dispersions (PermDISP) tested whether microbial community

samples differed in their degree of dispersion from their centroid. Additionally, we used principle coordinate analysis (PCoA) with fitting of environmental data to visualize microbial community differences between lineages and potential environmental drivers. All statistical analyses were run in the R environment (R Core Team 2016) with the exception of PerMANOVA and PermDISP, which were conducted in PRIMER-E with PerMANOVA+ (Clarke and Gorley 2006). All R code, notes, and associated data can be accessed on GitHub at https://github.com/wesbick/Root_paper.

Results

Environmental characteristics

A PCA of our environmental variables revealed that soil saturation, soil nitrogen, and soil phosphorus were uncorrelated. Soil carbon and nitrogen were strongly correlated, so soil carbon is not reported (Appendix S1: Fig. S4). Soil nitrogen and phosphorus did not differ significantly by lineage, but patch saturation level was non-random with respect to lineage at our sites ($\chi^2 = 11.99$, $P = 0.005$), wherein patches of non-native *Phragmites* were more likely to be unsaturated and patches of native were more likely to be saturated. Both were equally likely to occur in high water (Appendix S1: Table S3), suggesting that differences in growth habitat between lineages at our sites do not represent differential water tolerances, but instead is an artifact of our sampling locations.

Fungal colonization

Evidence from microscopy revealed that fungal root colonization was significantly greater in non-native *Phragmites* (Fig. 1a; ANOVA, $F = 24.57$, $P < 0.001$). Fungal colonization also differed significantly among sites (ANOVA, $F = 6.52$, $P < 0.001$), but site and plant lineage did not interact (Appendix S1: Table S4). Of all environmental variables measured, only soil saturation was a significant predictor of fungal root colonization (ANCOVA, $F = 23.47$, $P < 0.001$, Appendix S1: Table S5). In fact, the magnitude of the differences between root colonization between native and non-native *Phragmites* depended on the degree of soil saturation (Fig. 1b), although the interaction

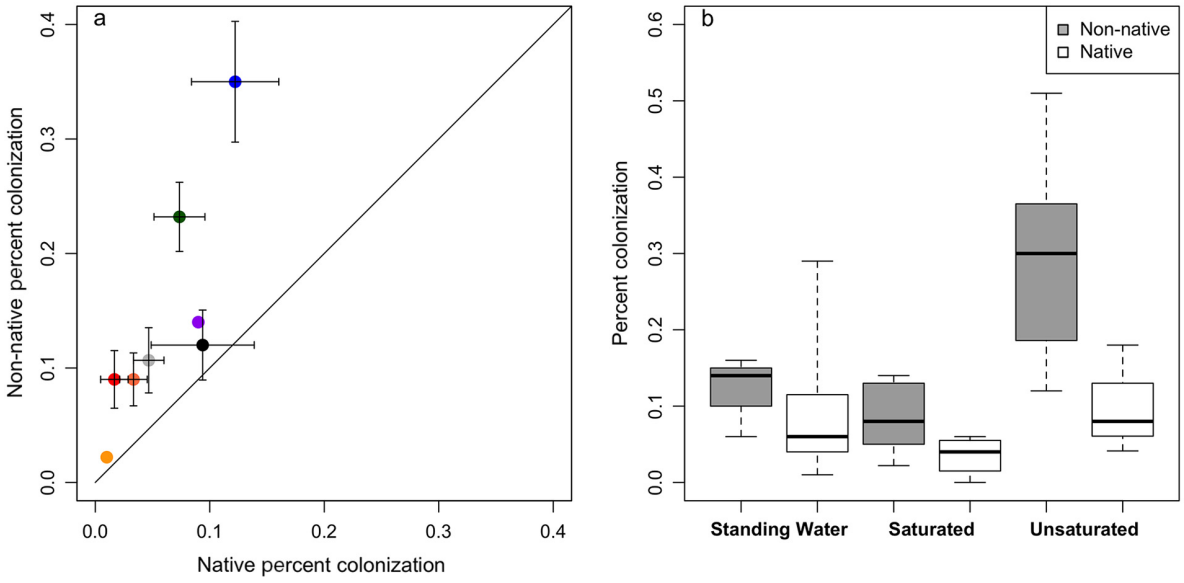

Fig. 1. (a) Percent fungal colonization of roots of Native and Non-native *Phragmites*. Each point represents the average colonization at a site in the non-native patches relative vs. the native patches. Error bars indicate standard error. Diagonal line represents equal colonization among plant lineages. Colors represent sites (blue, BL; red, CB; green, CH; coral, CM; black, CR; brown, PLB; gray, Rt2; purple, SB). (b) Effects of soil saturation on fungal colonization. Black lines indicate the median; bars indicate the full spread of data.

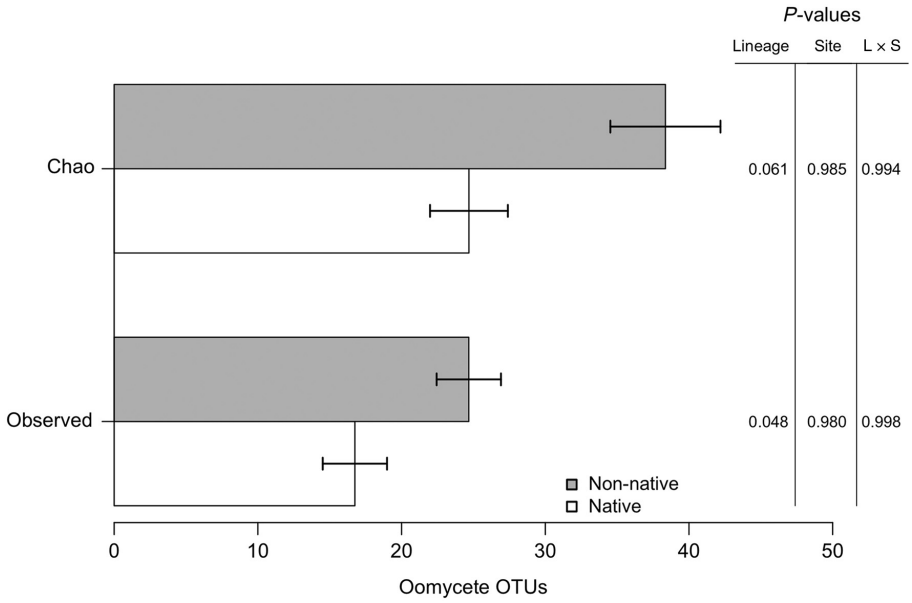

Fig. 2. Oomycete Chao Richness and Observed OTUs on Native and Non-Native Phragmites Roots. Bars indicate standard error. Significance calculated using ANOVA with Type III Sum of Squares.

between lineage and saturation was not significant (Appendix S1: Table S5, Fig. S5). Fungal root colonization was most different between native and non-native lineages in unsaturated conditions and was not different when soil was saturated or submerged. We observed no structures characteristic of arbuscular mycorrhizal fungi.

Alpha diversity

As one measure of microbial community differences, we analyzed differences in alpha diversity among sites and between lineages. Bacterial and fungal alpha diversity (richness, Shannon-Weiner diversity) differed among sites but not by plant lineage (Appendix S1: Table S6) or with any of the measured environmental variable (Appendix S1: Table S7, Fig. S6). Oomycete community richness differed modestly between the two lineages, with the non-native roots hosting a greater number of species and higher diversity than the native roots (Fig. 2; ANOVA, $F = 4.22$, $P = 0.048$, Appendix S1: Table S8). Environmental variables did not affect oomycete alpha diversity (Appendix S1: Table S9, Fig. S7).

Community composition

Host lineage was not a significant predictor of community composition for any microbial group based on PerMANOVA (Table 1). However, site was a significant predictor of variation for all three microbial groups and significantly interacted with lineage to shape bacterial communities (Table 1). PermDISP revealed a marginally significant difference in the dispersion patterns among sites in bacterial (pseudo-$F = 5.76$, $P = 0.072$) and fungal (pseudo-$F = 4.56$, $P = 0.064$) communities, which indicates that the difference in community detected by PerMANOVA may be due to heterogeneity of

Table 1. Community differences by site and lineage in all microbial groups via PerMANOVA.

	Fungi		Bacteria		Oomycetes	
Predictor variable	Pseudo-F	*P*	Pseudo-F	*P*	Pseudo-F	*P*
Lineage	1.06	0.438	1.24	0.298	1.10	0.389
Site	**1.68**	**<0.001**	**2.43**	**<0.001**	**1.24**	**0.088**
Lineage × site	0.96	0.616	**1.14**	**0.050**	1.22	0.109

Note: Bold values indicate significance at the a = 0.10 level.

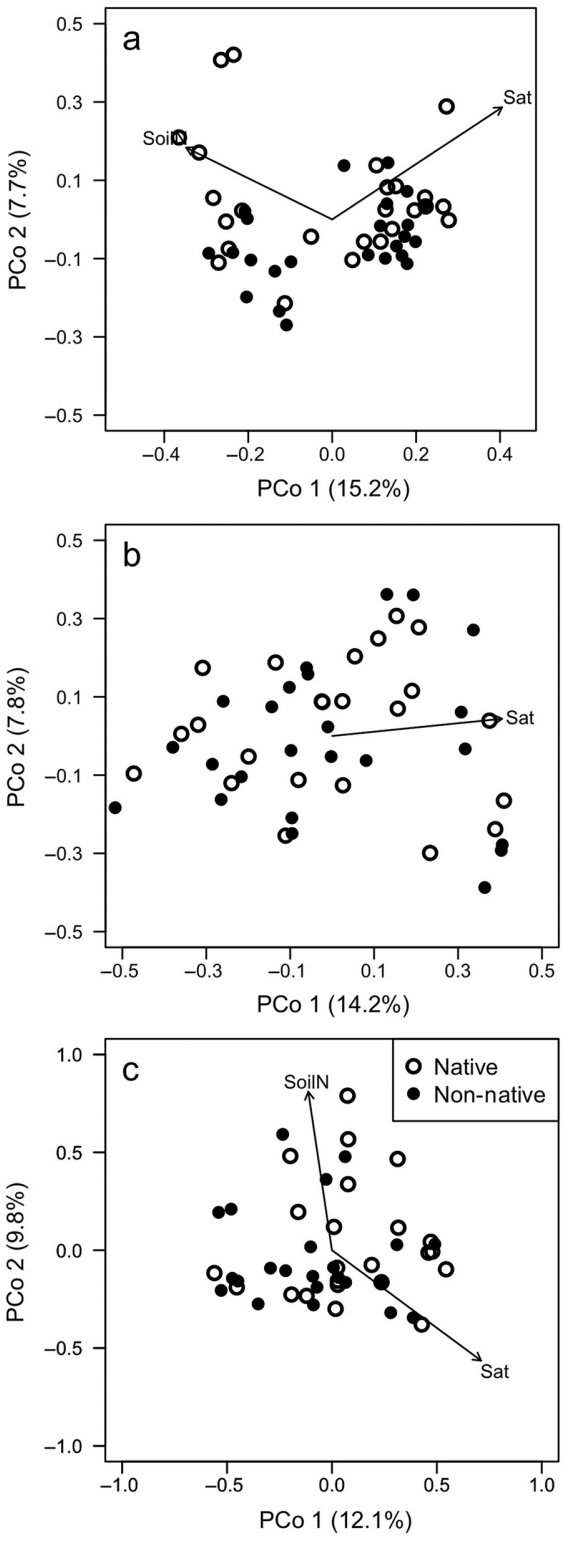

Fig. 3. Principle coordinate analyses (PCoA) of (a) bacterial, (b) fungal, and (c) oomycete communities. (Fig. 3. *Continued*)
Points represent the microbial community within a single root sample. Vectors displayed are environmental fit of variables (Soil N, Soil P, and Soil Saturation) to ordination space significant at the $\alpha = 0.10$ level (See Table 2 for P-values).

dispersion around the centroids of the sites. Given the importance of site for community composition, we explored which environmental variables could account for this spatial variation with PCoA. While soil nitrogen marginally explained community variation in two of the three microbial groups, degree of saturation more consistently explained substantial variation in ordination space for all three groups (Fig. 3, Table 2).

Taxonomic analyses

Despite the lack of difference in overall community composition according to the multivariate analyses, relative abundance of some bacterial phyla on roots did differ by plant lineage (Fig. 4; Appendix S1: Table S6). Relative sequence abundance of the most common bacterial phyla was associated with plant host lineage and not site (Fig. 4). While the majority of all bacterial sequences recovered in both lineages were Proteobacteria, non-native *Phragmites* roots host relatively more Proteobacteria, but fewer Firmicutes and Bacteroidetes taxa. The Bacteroidetes were also influenced by site (Appendix S1: Table S6). Major bacterial genera are listed in Appendix S1: Tables S6–S7, Fig. S8.

Relative sequence abundance of fungi did not differ by site or host lineage at the phylum level (ANOVA; Appendix S1: Table S4). Over 90% of recovered fungal sequences were from Ascomycota. Minor phyla included Basidiomycota (5.7%), Zygomycota (0.6%), Glomeromycota (0.2%), and unclassified fungi (2.7%; Appendix S1: Fig. S9). We expected that Ascomycetes would make up the majority of sequences as most root endophytes are from this phylum. The extremely low relative abundance of Glomeromycota, the dominant phylum of AMF, was consistent with the lack of AMF structures found via microscopy. Relative abundances of recovered genera differed significantly by site, but not by lineage (Appendix S1: Table S4). The dominant genera recovered in roots were *Gibberella* (19.5%), *Tetracladium* (13.4%),

Table 2. Environmental fit of Soil Parameters to PCoA ordinations of microbial community compositions.

Predictor variable	Fungi		Bacteria		Oomycetes	
	r^2	P	r^2	P	r^2	P
Soil N	0.096	0.179	**0.309**	**0.004**	**0.135**	**0.097**
Soil P	0.067	0.334	0.049	0.338	0.100	0.193
Saturation	**0.399**	**0.001**	**0.492**	**0.001**	**0.166**	**0.046**

Note: Bold values indicate significance at the a = 0.10 level.

Microdochium (11.0%), and *Stagonospora* (7.6%; Appendix S1: Fig. S10). Environmental variables did not affect relative sequence abundance at the phylum level, but saturation level was a strong determinant of relative abundance in all dominant fungal genera (Appendix S1: Table S5). The majority of our recovered oomycete sequences matched most closely to unclassified uncultured oomycete strains (59.4%). The dominant classified genus was *Pythium* which made up 40% of the sequences recovered. *Phytophthora*, *Saprolegnia*, and *Lagena* were recovered in very low abundance. Oomycete genera did not differ in relative abundance by site or lineage (Appendix S1: Table S8, Fig. S11), or saturation level (Appendix S1: Table S9, Fig. S7).

Functional classification

Because soil saturation was a strong driver of bacterial community differences, we focused on respiratory mode as a trait that could differentiate groups based on saturation. Additionally, differences in respiratory mode could drive rates of nutrient cycling and have the potential to feedback to plant productivity. We used BugBase to make respiratory mode determinations based on Greengenes taxonomy of our 16S sequences. While most OTUs were from aerobic bacteria (Fig. 5), the relative abundance of anaerobes tended to be higher in the native lineage and interacted with site. Soil saturation was also a predictor of relative abundance of facultative anaerobes (ANCOVA, $F = 3.34$, $P = 0.077$, Appendix S1: Table S7). However, plant lineage and soil saturation did not significantly interact in determining relative abundance of bacteria based on respiratory mode, suggesting that plants hosted similar communities with respect to oxygen requirements in all saturation levels (Appendix S1: Table S7, Fig. S6).

Using functional assessment from FUNguild, we compared the relative sequence abundance of the dominant trophic modes of fungi inhabiting

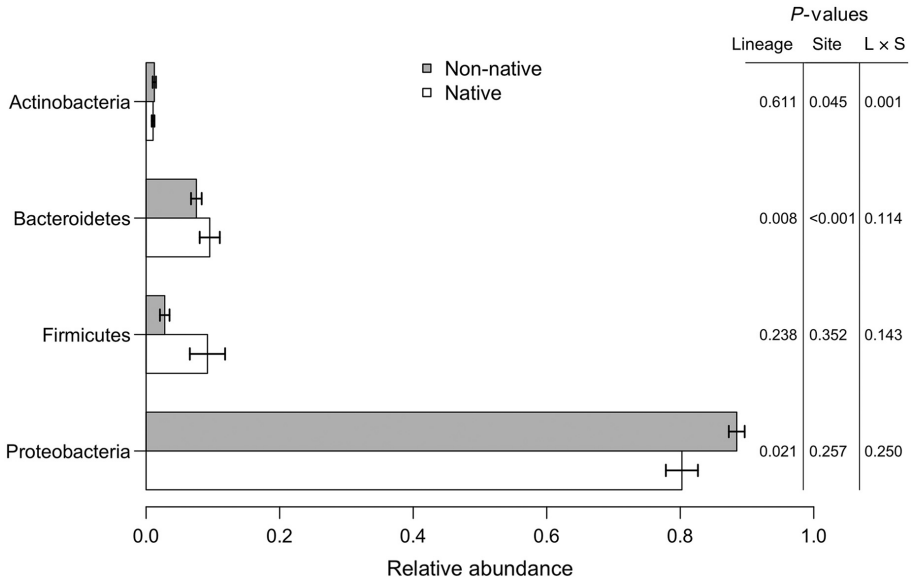

Fig. 4. Relative sequence abundance of major bacterial phyla in native and non-native samples. All samples were rarified to a consistent number of sequences (459) making relative abundances comparable. Error bars represent standard error. Significance calculated using ANOVA with Type III Sum of Squares.

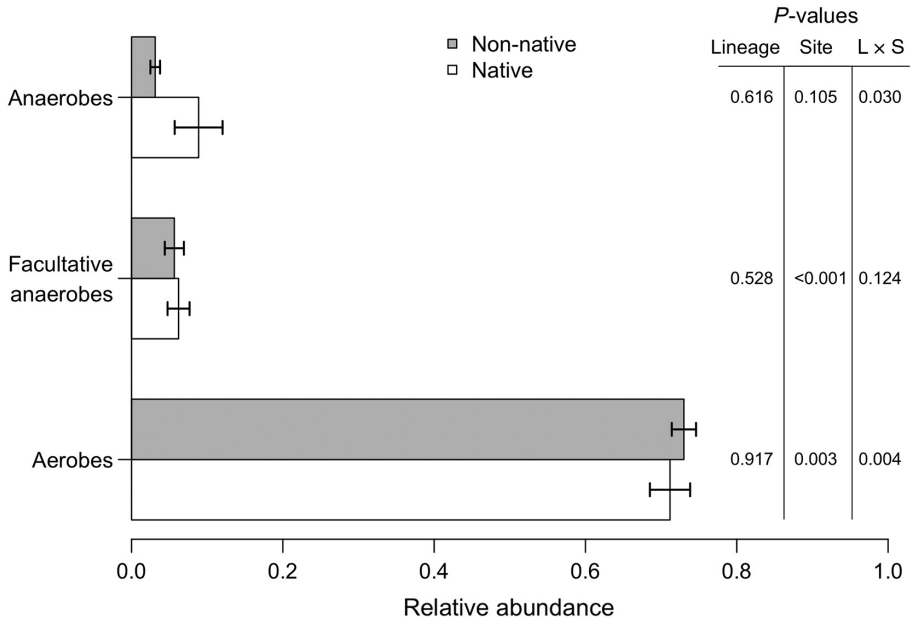

Fig. 5. Relative abundance of bacterial classes based on oxygen requirements compared by plant lineage. Oxygen-use classes determined using BugBase. Error bars indicate standard error of the mean. Significance calculated using ANOVA with Type III Sum of Squares.

Phragmites roots (Fig. 6). Most taxa were categorized as pathotrophs or saprotrophs, with no difference in the relative abundance of either group between native and non-native lineages (Appendix S1: Table S4). Symbiotrophs (mutualists) and pathotroph-symbiotrophs (a hybrid group consisting mostly of Dark Septate Endophytes) also did not differ by site or lineage. Pathotrophs (ANCOVA, $F = 9.13$, $P = 0.005$) and saprotrophs (ANCOVA, $F = 14.63$, $P < 0.001$) did differ based on soil saturation (Appendix S1: Table S5, Fig. S5), perhaps reflecting the oxygen demands of these functional groups under various saturation levels. In the fungal communities, there was no evidence for higher mutualist or lower pathogen load on the non-native *Phragmites*.

Discussion

If performance differences between native and non-native plant lineages (e.g., growth rate, productivity) are primarily driven by their root microbial communities, then we would expect that (1) bacterial, fungal, and oomycete communities will be differentially assembled, comprised of different taxa abundances, OTU abundances, or colonization rates in native and non-native *Phragmites* roots and (2) non-native *Phragmites* roots will associate with relatively fewer microbial taxa capable of pathogenesis and relatively more microbial taxa capable of mutualistic interactions than native *Phragmites* roots. Despite a thorough examination of the root microbial community encompassing three of the major microbial groups (bacteria, fungi, and oomycetes), we could find no evidence to support the hypothesis that performance differences between native and non-native *Phragmites* are driven by their root microbial communities alone. With the exception of relative abundance of a few bacterial genera and the extent of fungal colonization, plant lineage did not predict root microbial community structure or composition in our study. Instead, site, and specifically soil saturation level, was a stronger predictor of the identity of microorganism that endophytically colonized roots. Additionally, there was no evidence for functional differences in the root microbial communities that could explain performance advantages observed in the non-native lineage. Consequently, it is unlikely that the invasive capacity of non-native *Phragmites* arises due to a greater

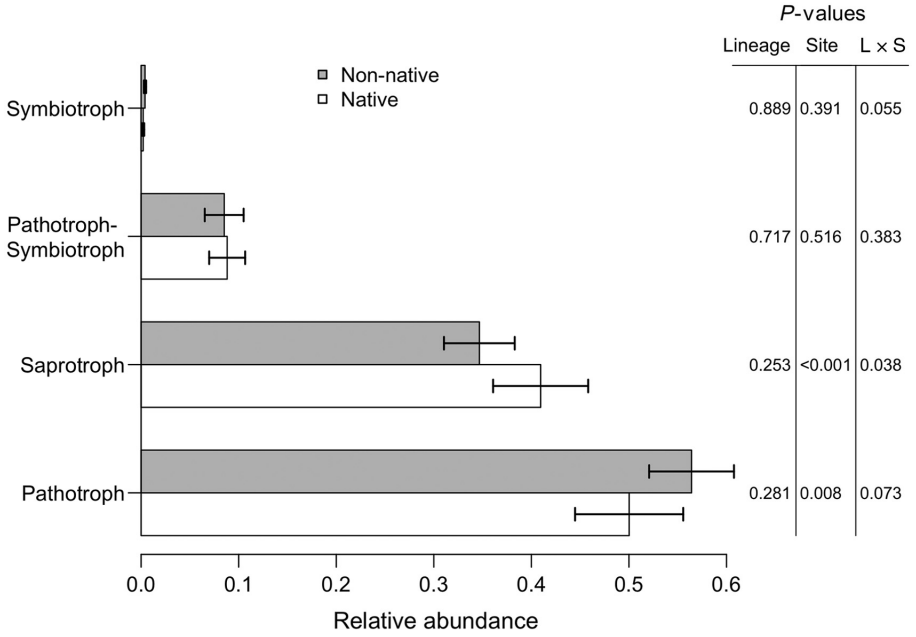

Fig. 6. Relative abundance of trophic modes based on FUNguild determinations. Error bars represent standard error of the mean. Significance calculated using ANOVA with Type III Sum of Squares.

abundance of mutualists or a lower abundance of pathogens in roots.

For bacterial root endophytes, soil saturation level was a strong predictor of community composition wherein we observed different bacterial communities under saturated and unsaturated conditions. However, soil saturation only modestly affected the relative abundance of bacterial groups by respiratory requirement or taxonomy. *Pseudomonas*, the most common genus recovered, were slightly less abundant in high water than in unsaturated patches (Appendix S1: Fig. S6). Most of the sequences recovered were from aerobic taxa, and the abundance of aerobes did not differ with saturation. Facultative anaerobes were affected by soil saturation level, but they made up a much smaller proportion of bacterial sequences (Appendix S1: Table S7, Fig. S6). Given that ventilation efficiency differs dramatically between native and non-native *Phragmites* (Tulbure et al. 2012), one might expect to see differences in microbial communities based on respiratory mode. However, the discrepancies in ventilation did not appear to affect endophytic colonization. It may be that, while rhizosphere oxygen concentrations likely depend on ventilation and differ in the two lineages, internal root tissues can remain sufficiently aerobic due to the existence of aerenchyma, despite the differences in surrounding soil. Bacterial endophytes mainly colonize roots from the rhizosphere (Hardoim et al. 2008) or plant litter (Ryan et al. 2008). Thus, while there may be differences in the rhizosphere bacterial communities between the two lineages (Bowen et al. 2017) and those differences may be in part influenced by ventilation, the broadly habitable, aerobic internal root may select for aerobes among the community of bacteria in the rhizosphere.

For fungal endophytes, soil saturation was also important, influencing colonization rates, community composition, alpha diversity, and the relative abundance of many common genera (Appendix S1: Table S5). As with bacteria, the soil environment may act as a barrier for colonization. High water levels creating anaerobic soil conditions would make it difficult for germinating spores of AMF or other aerobic filamentous fungi to reach the root surface. While fungal colonization was higher in non-native *Phragmites* roots overall, colonization rate did differ by soil saturation with the largest differences found in unsaturated conditions (Fig. 1). Fungal root colonization in native and non-native *Phragmites* lineages has been investigated previously

(Holdredge et al. 2010) with no consistent or statistical difference appearing between lineages. That study was conducted in a coastal tidal marsh that likely experiences a high degree of saturation, which may have affected the colonization rates similarly to those in our study. No arbuscules or vesicles were identified in any samples and the relative abundance of Glomeromycota taxa in our samples was extremely low. Although we note the deficiencies of ITS primers in detecting Glomeromycota (Stockinger et al. 2010), given the low levels of AMF sequences recovered in our samples and the results of the FUNGuild analysis, the fungal hyphae observed in our microscopy analysis were likely endophytes or root pathogens rather than AMF mutualists.

The only consistent microbial community difference between native and non-native *Phragmites* was found in oomycete diversity, with the non-native *Phragmites* roots hosting more diverse oomycete pathogens. While pathogen diversity is not the same as pathogen load, we might expect that the trend would be in the opposite direction to agree with classical enemy release dynamics. Additionally, we did not observe any difference in overall communities of oomycetes between plant lineages that would suggest native and non-native *Phragmites* roots are differentially colonized by oomycete pathogens. Nelson and Karp (2013) compared oomycete communities between native and non-native *Phragmites* and found no overall difference in oomycete richness between plant lineages but discovered higher richness in the native lineage at two of their four sites. Although our results are thus inconsistent with previous findings (Nelson and Karp 2013), they are consistent with the suggestion by Allen et al. (2018) that non-native *Phragmites* attracts more generalist soil pathogens than the native *Phragmites*. Importantly, richness reveals very little about pathogen load or pressure and it is still possible that the two lineages are experiencing different levels of pathogen pressure given differences in pathogen virulence on each plant lineage or surrounding plants (Crocker et al. 2015, Allen et al. 2018).

Functional potential of endophytic bacteria is difficult to determine (Hardoim et al. 2008). While others have found specific endophytic bacteria that provide stress tolerance to invasive *Phragmites* (White et al. 2018), functional determinations often come from culture studies that can measure plant growth response to individual inoculations. Without using an experimental approach, functional potential can be approximated by looking at specific genes (Hardoim et al. 2008), but that requires mapped genomes of OTUs found in the study, which can be difficult to obtain. We instead relied on community and respiratory mode differences between lineages to infer functional differences. We found no such differences between the native and non-native lineages within the roots.

While our study found no differences in root microbial communities of native and non-native *Phragmites*, the complete role of microbes in *Phragmites'* invasiveness is likely much more complex. For example, important microbial community differences driving plant performance differences could exist elsewhere in the phyllosphere or in the rhizosphere. In fact, Bowen et al. (2017) found strong rhizosphere bacteria differences between *Phragmites* lineages, and rhizosphere fungal and oomycete community differences have not yet been studied on a large scale. Additionally, the plant response to microbes could be lineage specific. We know that some oomycetes have lineage-specific virulence in *Phragmites* (Crocker et al. 2015), and that the roles of many endophytic fungi and bacteria are plant and genotype specific (Schulz and Boyle 2005, Hardoim et al. 2008). Therefore, plant responses to microbial communities as well as community differences are important to definitively understand if microbial communities are driving invasiveness in *Phragmites*. Finally, we recognize the limitations of this study in that it relies heavily on publicly available databases of microbial taxonomy (SILVA, NCBI, UNITE) and function (FUNguild, BugBase). While these databases are improving, analyses derived from them are not definitive, but should provide useful trends in functional potential (Nguyen et al. 2015).

Conclusions

We found little evidence of differences in the *Phragmites* root microbiome between lineages at our sites. There was also no evidence that invasive *Phragmites* associated with relatively more mutualist or relatively fewer pathogen microbes

than the native *Phragmites*. Thus, it is unlikely that root microbial communities are driving *Phragmites* invasions at these sites. Future studies should explore microbial communities in the rhizosphere or elsewhere in the phyllosphere, as well as differential response to microbes in native and non-native *Phragmites* as potential mechanisms for invasiveness.

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Supporting Information

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