Received: 9 December 2020 | Accepted: 31 January 2021

DOI: 10.1111/1365-2745.13629

RESEARCH ARTICLE

Journal of Ecology | BRITISH ECOLOGICAL SOCIETY

Plant and microbial impacts of an invasive species vary across an environmental gradient

Emily C. Farrer[1] | Christina Birnbaum[1,2,3] | Paweł Waryszak[1,3] | Susannah R. Halbrook[1] | Monica V. Brady[1] | Caitlin R. Bumby[1] | Helena Candaele[1] | Nelle K. Kulick[1] | Sean F. H. Lee[1] | Carolyn S. Schroeder[1] | McKenzie K. H. Smith[1] | William Wilber[1]

[1]Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA, USA

[2]Applied Chemistry and Environmental Science, School of Science, RMIT University, Melbourne, VIC, Australia

[3]Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Burwood, VIC, Australia

Correspondence
Emily C. Farrer
Email: efarrer@tulane.edu

Funding information
Louisiana State Board of Regents, Grant/Award Number: LEQSF(2017–20)-RD-A-14

Handling Editor: Brajesh Singh

Abstract

1. Invasive plants often successfully occupy large areas encompassing broad environmental gradients in their invaded range, yet how invader dominance and effects on ecological communities vary across the landscape has rarely been explored. Furthermore, while the impacts of invasion on plant communities are well studied, it is not well understood whether responses of above-ground (plant) and below-ground (microbial) communities are coupled.
2. Here we test patterns in *Phragmites australis* (common reed) invasion in a field survey of eight sites situated across a salinity gradient, ranging from freshwater to saline marsh, in Southeast Louisiana. At each site, we surveyed plant composition and used metabarcoding methods to assess soil fungal and bacterial composition in plots within the dense *Phragmites* stand, in a transition zone of ~50:50 *Phragmites*:native plants, and in native-only areas. We hypothesized that *Phragmites*' abundance and impact on above- and below-ground communities would vary across the salinity gradient and that the responses of above- and below-ground communities to invasion would be coupled.
3. We found weak evidence that invasion varied across the gradient: *Phragmites* stem densities increased slightly with salinity, and *Phragmites* increased above-ground litter accumulation more in fresh and saline areas compared to brackish. We found stronger evidence that plant and microbial responses to invasion varied with salinity. *Phragmites* strongly reduced native plant density across the gradient, with slightly greater reductions in fresh and saline areas. Plant species richness displayed consistent decreases with invasion across the salinity gradient; however, fungal and bacterial richness increased sharply with invasion only in brackish sites. Furthermore, the effect of *Phragmites* on plant and microbial community composition became stronger as salinity increased. Plants and microbes exhibited coupled responses to invasion in the magnitude of compositional shifts brought on by *Phragmites*, but *Phragmites*' effects on richness were not coupled.
4. *Synthesis*. Overall, the variability in *Phragmites* impacts across the gradient, particularly soil microbial impacts, suggests that it may be difficult to generalize

invader effects from single-site or single-ecosystem studies. However, above- and below-ground communities showed some coupled responses to *Phragmites*; thus understanding plant community responses to invasion gives insight into impacts occurring below-ground.

KEYWORDS

16S, diversity, Gulf Coast, haplotype, ITS, marsh, *Phragmites australis*, plant–soil interactions

1 | INTRODUCTION

Invasive plants often successfully occupy large areas encompassing broad environmental gradients and habitat types in their invaded range. For example, *Bromus tectorum* (cheatgrass) dominates dry sagebrush steppe in the southern Great Basin and moist coniferous forests of the Rocky Mountains (Mosely et al., 1999), *Phalaris arundinacea* (reed canarygrass) dominates wetlands and riparian areas from Southern California to Alaska (Lavergne & Molofsky, 2004), and invasive lineages of *Phragmites australis* (common reed) dominate coastal saltmarshes in the humid subtropics of the Southeastern United States as well as inland freshwater marshes in the Northern United States and Canada (Eller et al., 2017). However, because the abiotic and biotic conditions vary substantially across invasive species' ranges, it is likely that the degree of invasive species dominance as well as invader effects on ecological communities varies across environmental gradients.

Invasive species have abiotic tolerances and optima that, in part, determine their range limits and abundance (Louthan et al., 2015). Abiotic factors like temperature, precipitation and nutrients can affect invader success and dominance (Dukes & Mooney, 1999). However, despite these limitations, many invaders can achieve high abundance across diverse habitats in their range (Sexton et al., 2009). A well-developed literature suggests that an invader's ability to dominate diverse habitats is due to high levels of plasticity (Davidson et al., 2011; Geng et al., 2016; Liao et al., 2016) and/or high genetic diversity (Estoup et al., 2016; Roman & Darling, 2007). Biotic factors, such as competitors, herbivores and microbial communities can also enhance or limit invasion across diverse landscapes (Inderjit & van der Putten, 2010; Sexton et al., 2009). Communities often vary in their biotic resistance, which can affect both the presence and abundance of invaders (Leffler et al., 2014; Levine et al., 2004).

Despite much work on the determinants of invasive species' range limits and how invader abundance varies across its invaded range, little work has explored how the *impacts* of an invasive species may vary across its invaded range. Invader abundance can influence its impact on native species, in sometimes nonlinear ways (Bradley et al., 2019; Sofaer et al., 2018). The ability of an invader to reduce plant diversity can also be contingent on environmental context like N availability or disturbance (Wilson & Pinno, 2013). Different types of communities may also differ in their susceptibility to the invader. For example, a native community comprised of tall plant species may be less impacted by a tall invader compared to a native community comprised of short-statured species.

Generally, the measurement of impact of invasive plant species is native plant diversity or above-ground measures of plant abundance (Vilà et al., 2011). However, invasive species can also profoundly alter microbial communities below-ground. Invasive species often have high root biomass (Jo et al., 2017), different root and exudate chemistry (Callaway et al., 2008; Macel et al., 2014), and in general cultivate distinct microbial communities compared to native plants (Bunn et al., 2015; Coats & Rumpho, 2014; Dawson & Schrama, 2016). Indeed, many of these changes in pathogens, mutualists and saprotrophs lead to feedbacks that have been found to enhance invasion though mechanisms such as pathogen spillover (Flory & Clay, 2013), disruption of mutualisms (Hale & Kalisz, 2012) and enhanced nutrient cycling (van der Putten et al., 2007). Considering the heterogeneity in abiotic and biotic conditions across space, we do not know if these below-ground, microbial changes are consistent across the landscape or whether above-ground plant communities and below-ground microbial communities exhibit coupled responses to plant invasion.

We focus on invasion patterns in the invader, *Phragmites australis* (Cav.) Trin. ex Steud. (common reed), which successfully invades marshes across a salinity gradient in the Gulf Coast. *Phragmites* is a cosmopolitan species that is emerging as a model organism for studying plant invasions (Meyerson et al., 2016). Several lineages of *Phragmites* co-occur in North America, and the Gulf Coast in particular has a unique assemblage of *Phragmites* lineages. While the most well-studied invasive lineage in North America, Haplotype M, is not common in the Gulf Coast (Saltonstall, 2002), two other lineages are restricted to the Gulf Coast and are abundant and putatively invasive in the region: the Delta lineage (haplotype M1) and the Gulf lineage (or Land type, haplotype I; Bhattarai & Cronin, 2014; Meyerson et al., 2010, see Supporting Information for more information on these haplotypes). Haplotypes M1 and I exhibit characteristics of invasive species; they are rapidly spreading in the region (Bhattarai & Cronin, 2014; Hauber et al., 2011; Williams et al., 2012) and are anecdotally associated with reduced native plant diversity and negative ecosystem consequences such as poor habitat quality for migratory birds (Hauber et al., 2011; Kettenring et al., 2012). Because of the well-documented invasiveness of haplotype M across North America, managers are concerned about the recent increase in abundance of other *Phragmites* haplotypes in Gulf Coast wetlands (Hauber et al., 2011).

Here we test how patterns of *Phragmites* invasion vary across a salinity gradient in Southeastern Louisiana, from freshwater to saline marshes. While haplotypes M, M1 and I are all present in Louisiana (and had potential to occur in our study sites), nearly all previous research on *Phragmites* concerns haplotype M. Research on *Phragmites* (haplotype M) expansion on the East Coast of the United States has found that *Phragmites* has similar rates of spread (Chambers et al., 1999) and abundance (Meyerson et al., 2000) in freshwater and brackish marshes. However, higher salinity tends to limit haplotype M growth (Amsberry et al., 2000; Hellings & Gallagher, 1992). Furthermore, *Phragmites* (haplotype M) has the strongest impact on native richness in the highly diverse freshwater marshes of the East Coast (Chambers et al., 1999; Meyerson et al., 2000). Few previous studies have investigated the below-ground microbial impacts of *Phragmites*. Studies on the East Coast have found that *Phragmites* (haplotype M) alters community composition of archaeal but not bacterial rhizosphere communities in both freshwater and low salinity marshes (Yarwood et al., 2016) but that its effect on oomycete richness is site dependent (Nelson & Karp, 2013). Like many previous studies (Meyerson et al., 2000; Nelson & Karp, 2013; Uddin & Robinson, 2017; Yarwood et al., 2016), we take a survey approach to assess impacts of *Phragmites* on marsh communities. While direction of causality of invader-community relationships in a survey cannot be known with certainty, our careful selection of sites and plot locations ensured that *Phragmites* was highly likely to be the driver of community changes (see Section 2 for more details). In each of eight marsh sites, we measured plant and soil microbial communities in dense *Phragmites* stands, in transition zones (~50:50 *Phragmites*:native plants), and in native-only areas. We haplotyped *Phragmites* at each site, since haplotype distributions in the state are still fairly uncertain. We hypothesize that (a) *Phragmites* invasion [measured by its abundance and its associated ecosystem-level effects, standing biomass and litter (Rooth et al., 2003; Windham, 2001)] varies across a salinity gradient, and (b) the effect of *Phragmites* on plant and microbial communities (i.e. differences in communities between native and invaded areas) varies across the salinity gradient. We also hypothesize that above-ground (plant) and below-ground (fungi, bacteria) communities exhibit coupled responses to *Phragmites* across the salinity gradient, such that areas of high plant response to invasion correspond to areas of high microbial response.

2 | MATERIALS AND METHODS

2.1 | Study sites

Samples were collected from eight freshwater to saline marsh sites in southeast Louisiana (Barataria Preserve, Turtle Cove Research Station, Pearl River WMA, Fontainebleau State Park, Big Branch NWR, Bayou Sauvage NWR, and two sites at the Louisiana Universities Marine Consortium, LUMCON, Figure 1). We classified

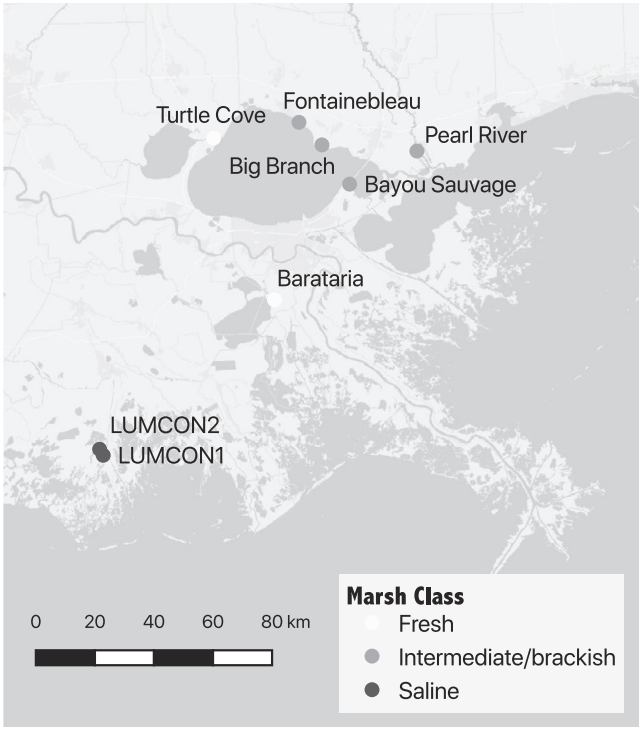

FIGURE 1 Map of study sites in SE Louisiana, USA

sites based on dominant vegetation. Barataria and Turtle Cove were classified as freshwater marsh, because they were dominated by *Sagittaria lancifolia* with mean annual soil salinities (from nearby Coastwide Reference Monitoring System sites, 10 cm depth, 2010–2017) of <2 ppt (Coastal Protection and Restoration Authority (CPRA) of Louisiana, 2020). Pearl River, Fontainebleau, Big Branch and Bayou Sauvage were classified as intermediate/brackish marsh (hereafter 'brackish'), as they were dominated by *Spartina patens* and *Schoenoplectus americanus* with mean annual salinities between 1 and 4 ppt. LUMCON 1 and 2 were classified as saline marsh, because they were dominated by *Spartina alterniflora* with mean annual salinities of ~15 ppt. While we refer to the 'salinity gradient' because salinity is the dominant factor in determining the distribution of plant species along the estuarine gradient (Odum, 1988), we recognize that marsh classes vary in many environmental factors other than salinity (some are listed in Table S1).

All sites had substantial stands of *Phragmites*. Using available aerial imagery, we estimated that for all but one site the stands ranges from at least 5–13 years old when sampled, and all are expanding over time (Table S2). For one site (LUMCON1), the stand is much more recent, having invaded 1–2 years prior to sampling (Table S2).

In 2017 at each site, 21 permanent 1 m^2 plots were established in three transects based on vegetation type: *Phragmites* stand, transition and native, each with seven plots. The *Phragmites* stand transect plots were located entirely within an area where *Phragmites* was highly dominant. The native plots ran parallel to, but outside of, the *Phragmites* stand and contained only native plants representing the native marsh community. The transition plots ran along the edge of

the *Phragmites* stand, capturing the interface of the native community and the invading *Phragmites* front. Plots were spaced approximately 10 m apart.

From site observations and aerial imagery, it is likely that *Phragmites* colonized 'normal' marsh areas and thus was the driver of any observed changes in community structure. For the majority of sites (Barataria, Pearl River, Big Branch, Bayou Sauvage, LUMCON2) the *Phragmites* stands were located in the middle of the marsh with no apparent other driver of heterogeneity that could be causing, for example, low plant diversity or altered composition, and historical aerial imagery showed that *Phragmites* colonized marsh area. At two sites, the *Phragmites* stand was on the border of some woody vegetation at the edge of a natural waterway (Turtle Cove) and on the border of a forested area (Fontainebleau), so that *Phragmites* plots were at a slightly higher elevation than native marsh. At these sites, aerial imagery suggests that *Phragmites* colonized transitional areas of native marsh with scattered woody vegetation, and that these areas have been disturbed due to the building of a nearby house and a hurricane, respectively; however, at the time of sampling, native marsh communities also existed at these higher elevations abutting the woody areas. At LUMCON1, the *Phragmites* stand was at the water's edge in slightly deeper water; however, native plant communities also occur in similar areas at the water's edge under a range of water depth conditions, and areal imagery indicates that *Phragmites* plots were located in areas that were formerly native marsh but were sometimes under water during high water years. Overall, Turtle Cove, Fontainebleau and LUMCON1 represent one each of freshwater, brackish and saline marsh; thus combining these sites with the other sites above, we believe we can extract the consistent effects that *Phragmites* has on plants and microbes.

2.2 | Sample collection

Plant species composition was sampled from September to November 2017 by performing stem counts of all plant species rooted in each 1 m^2 plot. For bunch grasses, each ramet (culm) was counted separately. Live biomass and litter mass per m^2 were estimated for each plot by harvesting from a 20 cm × 20 cm area, drying at 60°C for 48 hr, and weighing.

Soil samples for microbial analysis were collected in October and November 2017. Soils were collected with a sterilized soil corer (5 cm diameter, approximately 10 cm depth), homogenized in a plastic bag, and a subsample collected in a sample tube. Samples were placed directly into a liquid nitrogen container in the field and transferred to a −80°C freezer upon returning to the laboratory.

Fresh leaf tissue was collected from one *Phragmites* individual for haplotyping at each site (except for LUMCON 1 and 2 for which only one sample was taken due to the close proximity of sites and similarity of morphology). In the field, each sample was placed in a separate plastic bag with silica gel in the cooler and stored the same day at −20°C upon return to the laboratory.

2.3 | Molecular methods

DNA was extracted from the soil samples with a Qiagen DNeasy PowerSoil Kit following the manufacturer's protocol. Extractions were standardized to 10 ng/ml prior to amplification. We used a dual-index, two-step PCR approach (U'Ren & Arnold, 2017). We first amplified the fungal ITS region (primers ITS1f/ITS2) and the bacterial 16S region (primers 515F/806R). PCR was done in duplicate and duplicates were pooled. We then performed a second PCR to attach barcodes and Illumina adaptors. Samples were purified and standardized with a SequalPrep kit (Invitrogen Inc.), and pooled into ITS and 16S libraries. Libraries were sequenced on two lanes of an Illumina Miseq v3 (300bp PE) by Duke Sequencing Core, Duke University, NC.

Phragmites leaf samples for haplotyping were crushed using a sterilized mortar and pestle, and DNA was extracted using a DNeasy PowerPlant Pro DNA extraction kit (Qiagen) following the manufacturer's protocol. Following the detailed protocol by Saltonstall (2003), two non-coding chloroplast regions were amplified using PCR: (a) the *trn*T-*trn*L chloroplast intergenic spacer (Taberlet et al., 1991) and (b) the *rbc*L-*psa*I chloroplast intergenic spacer (Saltonstall, 2001, 2003). The amplified products were Sanger sequenced at Genewiz (South Plainfield, NJ, USA).

2.4 | Bioinformatics

Microbial sequence data were processed using the amplicon sequence variants (ASV) method in QIIME2 (Bolyen et al., 2019) and DADA2 (Callahan et al., 2016). We first trimmed reads where the median quality score fell below ~30, then quality-filtered the reads (no N's, max expected errors 2, truncated at quality score 2, minimum read length 50) and denoised the data and joined paired reads using DADA2. We assigned taxonomy using a pre-trained Naïve Bayes classifier. The classifier was trained on the UNITE 8.2 database (Abarenkov et al., 2020) for ITS and Greengenes 13.8 (DeSantis et al., 2006) for 16S. Prior to analyses, both datasets were rarefied to an even sampling depth (fungi to 5,334 reads and bacteria rarefied to 9,316 reads per sample).

Sequences for haplotyping were aligned using Mesquite and edited using Sequencher. Following methods in Saltonstall (2016), locus haplotypes were identified for both the trnT-trnL and rbcL-psaI regions. Sample haplotype identity was determined by combining the locus haplotype assignments. We distinguished between M1 and M haplotypes by comparing our sequences to known variation in microsatellite regions of *Phragmites* cpDNA locus haplotypes following methods and reference material in Saltonstall (2016).

2.5 | Statistical analysis

The effect of invasion stage (transition/*Phragmites* stand), marsh class (fresh/brackish/saline) and their interaction on *Phragmites* density was tested using linear mixed effects models with site as a random effect and accounting for spatial autocorrelation with a spherical model using the NLME package (Pinheiro et al., 2019) in R (R Core Team, 2019). Heterogeneous variances were included in the model if significant using a likelihood ratio test. Significance of explanatory variables was assessed with *F*-statistics and Type III ANOVAs. Tukey post hoc tests were performed using the MULTCOMP package (Hothorn et al., 2008). The effect of invasion (native/transition/*Phragmites* stand), marsh class and their interaction on live biomass and litter mass was also tested using similar linear mixed effects models.

The effect of invasion (native/transition/*Phragmites* stand), marsh type and their interaction on native plant abundance, native plant richness, fungal chao1 richness and bacterial chao1 richness was tested using linear mixed effects models as above.

The effect of invasion, marsh class and their interaction on plant composition, fungal composition and bacterial composition was tested using ordinations. For simplicity of interpretation and visualization, we only included native and *Phragmites* stand plots (not transition plots) in these analyses. We performed two types of distance-based RDA ordinations, full models capturing all marsh classes and models run separately for each marsh class. For full models, we used permutation tests (PERMANOVA) to assess significance of marsh class and invasion stage using packages VEGAN (Oksanen et al., 2019) and PHYLOSEQ (McMurdie & Holmes, 2013). For models run separately within marsh class, we included site as a conditioning variable in the analysis and used PERMANOVA to test the effect of invasion. For plants, we included all plant species, including *Phragmites*, as our dependent variables; we had to include *Phragmites* because too many plots within the dense *Phragmites* stand had no other species in them. For plants, we used the Jaccard (presence/absence) dissimilarity metric because visualizations were clearer compared to Bray–Curtis (on relative abundance) due to extreme dominance by species in some plots; however, statistical results were the same based on either method. For fungi and bacteria, we used Bray–Curtis dissimilarity (on rarefied count data).

We also used FUNGuild (Nguyen et al., 2016) to classify fungal taxa into functional guilds to assess invasion and marsh class impacts on fungal functional composition. We focused on taxa classified as 'plant pathogen' and 'arbuscular mycorrhizae' as there were sufficient taxa classified as such for these two guilds. For each of these two guilds, we summed the relative abundance of taxa classified as plant pathogens and arbuscular mycorrhizal fungi (AMF) to yield total pathogen and AMF relative abundance per sample. We tested the effect of invasion (native/transition/*Phragmites* stand), marsh class and their interaction on relative abundance of plant pathogens and AMF using linear mixed effects models and ANOVA as above. Log transformation was used to correct for non-normal residuals.

Our final hypothesis was whether plant and microbial responses to invasion were coupled, and we assessed whether the magnitude of response to invasion of richness and composition at the site level were correlated for plants, fungi and bacteria. For this analysis, we also only used native and *Phragmites* stand (not transition) plots. Because the native and *Phragmites* stand transects in each site were roughly parallel to one another, we paired each native plot with its corresponding closest *Phragmites* stand plot ($n = 7$ pairs). For richness, we calculated the difference in richness between each of the seven pairs of plots, then averaged the richness difference for each site. For composition, we calculated Jaccard (plant) or Bray–Curtis (fungi, bacteria) dissimilarity between each of the seven pairs of plots, then averaged the dissimilarity values for each site. We then used Deming regressions ($n = 8$, one mean and one standard deviation for each of the eight sites) to assess whether responses of plants and fungi, plants and bacteria, and fungi and bacteria to *Phragmites* were correlated using the R package DEMING (Therneau, 2018). Deming regressions take into account the error for observations in both the *x* and *y* variables by minimizing the sum of squared distances from the regression line in both the *x* and *y* direction; the relationship was deemed significant if the 95% confidence intervals of the regression slope did not overlap zero.

For creating figures, we used ggplot2 (Wickham, 2016). For all figures except the coupled response to invasion regressions, we plotted estimated marginal means (i.e. least squares means) and standard error calculated with the EMMEANS package (Lenth, 2020) so that we take into account pseudo-replication of having multiple measurements within multiple sites in each marsh class category. Lastly, we found two different haplotypes at our sites (see Section 3.1); however, we could not include haplotype as a covariate in the models because haplotype was confounded with marsh class. Thus, we redid all analyses including only the dominant haplotype (see Table S3); this did not change the results appreciably. All code can be found on GitHub: https://github.com/ecfarrer/LAmarshGradient2.

3 | RESULTS

3.1 | Haplotyping

We found that the Gulf haplotype (haplotype I) was present at the freshwater (Barataria, Turtle Cove) and brackish (Pearl River, Fontainebleau, Big Branch, Bayou Sauvage) sites along the gradient, whereas the Delta haplotype (haplotype M1) was present in the saline marshes (LUMCON1/LUMCON2). Despite differences in haplotypes among marshes, we included all marshes in our analyses below, because we were asking questions about how patterns in invasion change across a realistic landscape in Southeastern Louisiana (but see Table S3 for analyses only including haplotype I). In other words, differences in invasion or response to invasion in saline marshes versus brackish or freshwater marshes may be due to the salinity gradient per se or the fact that a different haplotype invades saline marshes.

3.2 | Phragmites density and above-ground biomass and litter

The effect of marsh class on *Phragmites* density depended on invasion stage (significant marsh class × invasion stage interaction, $F_{2,101} = 3.53$, $p = 0.033$), such that *Phragmites* density in transition plots did not vary with marsh class, but plots in the dense *Phragmites* stand had three times higher density in saline compared to freshwater marshes (Figure 2a). Above-ground live biomass did not vary across marsh classes or invasion (Figure 2b). However, *Phragmites* increased above-ground litter mass ($F_{2,151} = 8.23$, $p < 0.001$); and this effect varied by marsh class (marsh class × invasion stage interaction, $F_{4,151} = 2.83$, $p = 0.027$), such that brackish marshes were not as affected by *Phragmites* because they had high levels of litter in native plots (Figure 2c).

3.3 | Native plant density

Phragmites significantly affected the density of native plants ($F_{2,154} = 26.34$, $p < 0.0001$), and the strength of the effect varied by marsh class (marsh class × invasion stage interaction, $F_{4,154} = 2.94$, $p = 0.022$), with brackish marshes experiencing less reduction in density compared to freshwater and saline marshes (Figure 2d).

3.4 | Plant, fungal and bacterial richness and diversity

Phragmites invasion strongly reduced native plant richness ($F_{2,154} = 36.99$, $p < 0.0001$), with plant richness being similar in the native and transition plots, but much lower in the dense *Phragmites* stand (Figure 3a). Diversity metrics (Shannon, Simpson, inverse Simpson) all showed that the transition plots had higher diversity than either native or *Phragmites* plots (Figure S1), likely due to the co-dominance of *Phragmites* and the native dominant in the transition zone. There was also a trend that freshwater marshes had higher plant richness ($F_{2,5} = 5.08$, $p = 0.062$) and diversity (Figure S1) compared to brackish or saline marshes.

The effect of *Phragmites* on fungal Chao1 richness (Figure 3b) and diversity (Figure S1) depended on marsh class (marsh class × invasion stage interaction, for richness $F_{4,148} = 4.99$, $p = 0.0008$), with *Phragmites* increasing richness and diversity in brackish marshes but not strongly affecting richness or diversity in freshwater or saline marshes.

The effect of *Phragmites* on bacterial Chao1 richness (Figure 3c) and diversity (Figure S1) was also dependent on marsh class (marsh class × invasion stage interaction, for richness $F_{4,146} = 3.75$, $p = 0.006$), such that *Phragmites* increased bacterial richness and diversity in brackish marshes but not in freshwater or saline marshes. Marsh classes also differed in their bacterial richness ($F_{2,5} = 6.33$, $p = 0.043$) and diversity (Figure S1), with saline marshes having

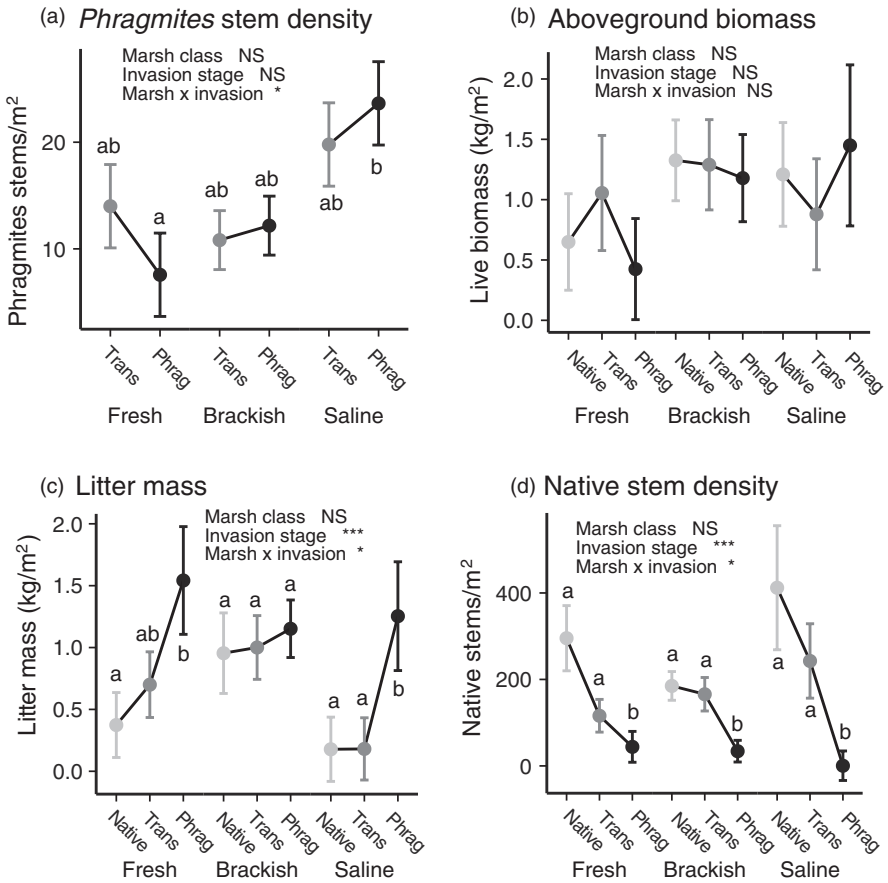

FIGURE 2 Effect of invasion stage (native, transition, *Phragmites* stand) and marsh class (fresh, brackish, saline) on *Phragmites* stem density (a), above-ground biomass (b), litter mass (c) and native stem density (d). Error bars represent ±1 *SE* of the estimated marginal mean (least squares mean). Statistical results shown are from an ANOVA: $^{**}p < 0.01$, $^{***}p < 0.001$; NS, non-significant. Results of Tukey post hoc tests for comparing multiple treatments are shown as letters; for *Phragmites* stem density the Tukey test compares across all treatment combinations, for other panels, the Tukey tests represent within-marsh class comparisons

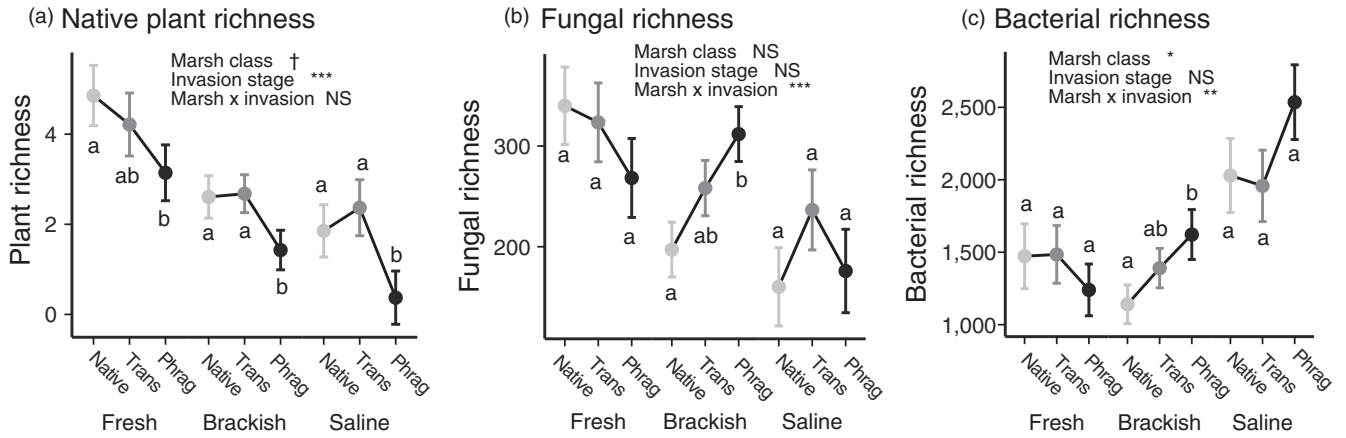

FIGURE 3 Effect of invasion stage (native, transition, *Phragmites* stand) and marsh class (fresh, brackish, saline) on richness of native plants (a), soil fungi (b) and soil bacteria (c). Error bars represent ±1 *SE* of the estimated marginal mean (least squares mean). Statistical results shown are from an ANOVA: $^{\dagger}p < 0.1$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; NS, non-significant. Results of Tukey post hoc tests for comparing multiple treatments are shown as letters and represent within-marsh class comparisons

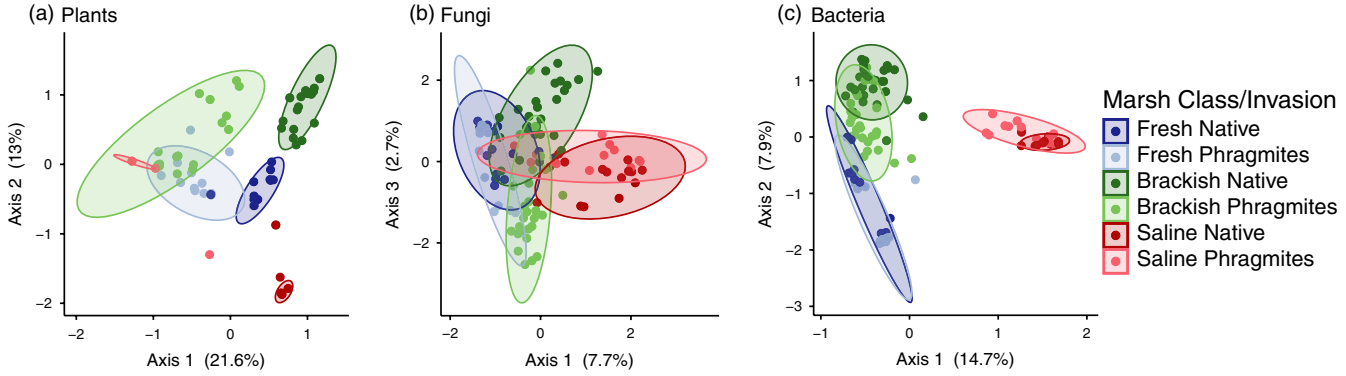

FIGURE 4 Distance-based RDAs showing the effect of invasion (native, *Phragmites* stand) and marsh class on community composition of plants (a), soil fungi (b) and soil bacteria (c). For plants and bacteria, axes 1 and 2 are shown; for fungi, axes 1 and 3 are shown because axis 3 illustrated the effect of invasion. See Table S4 for permutation test (PERMANOVA) results for these full models. See Figure S2 and Table 1 for ordinations and permutation test results within each marsh class separately

TABLE 1 Results from dbRDA permutation tests (PERMANOVA), testing the effect of invasion on plant, fungal and bacterial community composition for each marsh class separately. Site was used as a conditioning variable in all ordinations. See Figure S2 for ordination visualizations. See Table S4 and Figure 4 for ordinations and visualizations of full models

Taxon	Marsh type	Variance explained by invasion	Pseudo-*F* (*df*)	*p*
Plants	Fresh	11.5%	4.61 (1, 25)	<0.001
	Brackish	34.3%	28.56 (1, 51)	<0.001
	Saline	62.5%	49.19 (1, 25)	<0.001
Fungi	Fresh	5.3%	1.85 (1, 24)	0.002
	Brackish	6.1%	4.30 (1, 51)	<0.001
	Saline	7.5%	1.98 (1, 23)	0.044
Bacteria	Fresh	3.2%	1.67 (1, 23)	0.005
	Brackish	6.6%	5.60 (1, 50)	<0.001
	Saline	11.0%	3.53 (1, 23)	<0.001

higher richness than freshwater/brackish marshes and brackish marshes having the lowest diversity.

3.5 | Plant, fungal and bacterial community composition

Ordination analyses showed that both marsh class and *Phragmites* invasion had strong impacts on plant composition, and the effect of *Phragmites* depended on marsh class such that *Phragmites* effects on plant composition increased with salinity (see Table S4 and Figure 4a for full models, see Table 1 and Figure S2 for models by marsh class). Marsh class strongly affected both fungal and bacterial community composition, and there was a weak but significant interaction similar to the pattern with plants indicating that *Phragmites*' effect was stronger in more saline marshes (see Table S4 and Figure 4b,c for full models, see Table 1 and Figure S2 for models by marsh class).

Marsh class and *Phragmites* invasion affected fungal pathogen abundance but not AMF abundance (Figure S3). Pathogens were

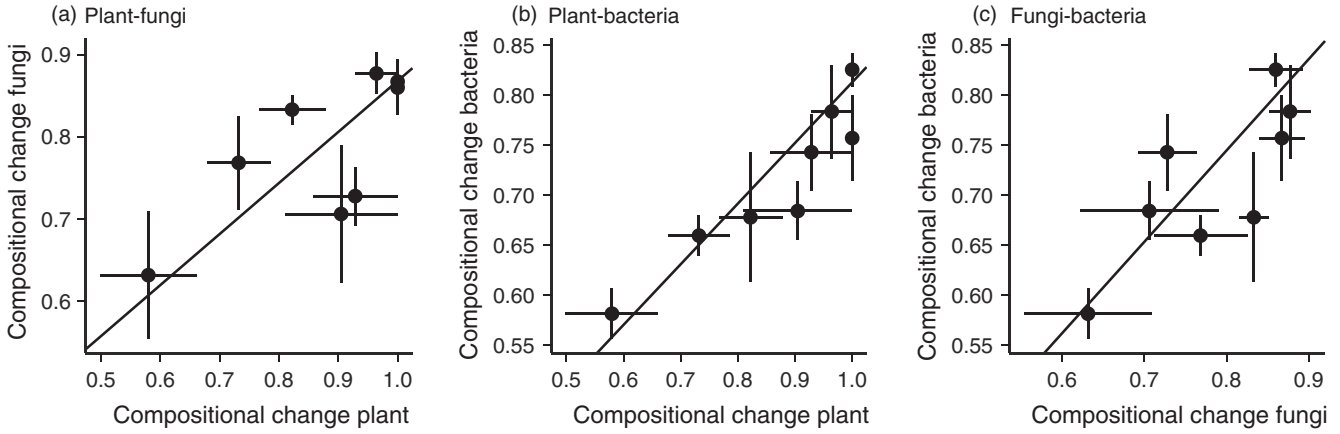

FIGURE 5 Compositional responses of plants, fungi and bacteria to *Phragmites* invasion are correlated; sites with large compositional shifts in plant communities also had large compositional shifts in fungi and bacterial communities, (a) plant and fungal responses, (b) plant and bacterial responses, (c) fungal and bacterial response. Values shown are means and standard errors of Jaccard (plants) and Bray–Curtis (fungi, bacteria) dissimilarities in community composition between native and *Phragmites*-invaded plots at each of eight sites. Deming regressions (which take into account error in both the *x* and *y* variables) indicated that all relationships were significant

more abundant in transition and dense *Phragmites* stands compared to native communities ($F_{2,146} = 4.72$, $p = 0.010$), and brackish marshes had lower pathogen abundances compared to fresh and saline marsh ($F_{2,5} = 10.60$, $p = 0.016$).

3.6 | Coupled responses

Richness responses of plants, fungi and bacteria were not correlated with one another across the landscape (95% CI for slopes in the Deming regressions overlapped zero). For example, sites where *Phragmites* greatly reduced plant richness were not the same sites in which *Phragmites* greatly reduced fungal or bacterial richness (data not shown). However, compositional shifts among plants, fungi and bacteria were all correlated (Figure 5); sites with large responses in plant composition corresponded to sites with large responses in fungal and bacterial composition (plant–fungi slope 0.61, 95% CI 0.31–0.92; plant–bacteria slope 0.61, CI 0.25–0.96; fungi–bacteria slope 0.93, CI 0.35–1.51).

4 | DISCUSSION

Understanding how invasive species abundance and impacts vary across environmental gradients and whether above-ground (plant) and below-ground (soil microbial) communities exhibit coupled responses to invasion is important for invasive species management. Here, we found weak evidence that invasion varied across a salinity gradient: *Phragmites* stem densities increased slightly with increasing salinity, and *Phragmites* increased above-ground litter accumulation more in fresh and saline areas compared to brackish. There was strong evidence that plant and microbial community responses to invasion varied with salinity: brackish marshes were most responsive to invasion, with all measured variables (abundance, richness, composition of plants and microbes) shifting due to invasion. Saline marshes were also responsive to invasion, but lacked shifts in fungal and bacterial richness. Freshwater marshes were the least responsive to invasion, primarily experiencing plant rather than microbial changes. We found that plants and microbes exhibited coupled responses in the magnitude of compositional shifts brought on by *Phragmites*, but changes in richness of plants and microbes were not coupled.

4.1 | Haplotype distribution

Many invasive species have broad environmental tolerances in their invaded range, which can, in part, be due to intraspecific genetic diversity and adaptation to environmental conditions (Estoup et al., 2016; Roman & Darling, 2007). Here we find haplotype I is dominant in freshwater and brackish marshes, while haplotype M1 is dominant in saltmarsh. This is consistent with other studies that have found haplotype I to be distributed in the interior of SE Louisiana while European haplotypes (M/M1) are present on the southern coast (Howard et al., 2008; J. Cronin, unpublished data). Interestingly, haplotype M1 is thought to be concentrated in the Mississippi River Delta (Meyerson et al., 2012); however, we observed numerous patches of M1 at our saline study sites in Terrebonne Basin, 150 km away. A study of haplotypes M1 and I2 (closely related to I) in the Mississippi River Delta suggests salinity tolerance aligns with haplotype distribution patterns; haplotype M1 is saline tolerant and more coastal and haplotype I2 is saline intolerant and more inland (Achenbach & Brix, 2014). The salinity partitioning we find among *Phragmites* haplotypes is similar to that of cattail species in which the invasive hybrid *Typha x glauca* dominates freshwater sites and the invasive *T. angustifolia* dominates brackish areas (Bansal et al., 2019; Grace & Harrison, 1986). Overall, intra-species or intra-genus variation may be a widespread mechanism to allow invading taxa to extend their range.

4.2 | Invasion across a salinity gradient

While some invasive species can achieve high dominance across environmental gradients, many wetland invaders are limited in salinity tolerance. For example, freshwater marshes by far host the greatest number and cover of invasive species (Birnbaum et al., in review), and many notable wetland invaders (e.g. water hyacinth, purple loosestrife, alligatorweed) are restricted to or have highest abundances in fresh areas (Konisky & Burdick, 2004; Penfound & Earle, 1948; Birnbaum et al., in review). Previous research on *Phragmites* invasion and performance across salinity gradients has exclusively been conducted on haplotype M. Haplotype M is primarily an invader of freshwater and brackish marshes (Lambert et al., 2010), and it has similar rates of spread (Chambers et al., 1999), biomass and stem densities (Meyerson et al., 2000) in freshwater and brackish areas on the East Coast. Here we find that haplotype I, likewise, has similar stem densities in freshwater and brackish areas of the Gulf Coast. In the saline areas in our study where haplotype M1 was present, the stem densities in *Phragmites* stands were three times higher than stands in freshwater marshes. This suggests that these outlying saline marshes are more highly invaded and may be associated with greater impacts on native biota compared to interior marshes (Bradley et al., 2019). This is partially borne out in our response data; for example, saline marshes had much lower native stem densities (only 0–2 stems of native plants per m^2) in the dense *Phragmites* stand compared to brackish (0–246 stems) and freshwater marshes (1–196 stems).

While *Phragmites* did not affect above-ground biomass in any marshes in our study, *Phragmites* increased above-ground litter mass in fresh and saline marshes by four times and seven times respectively. *Phragmites* did not increase litter mass in brackish marshes due to high litter production by *Spartina patens*. Work on haplotype M also shows that *Phragmites* increases litter by 2–10 times compared to native areas (Rooth et al., 2003), suggesting that haplotypes M1 and I operate similarly to M in this respect. The increase in litter likely contributes to the competitive dominance of haplotype M1 and I, as it has been found to do in haplotype M (Holdredge & Bertness, 2011) and more broadly in other invasive wetland macrophytes like cattails (Farrer & Goldberg, 2009) and reed canarygrass (Eppinga et al., 2011) and grassland and shrubland invaders like wild oats and medusahead (Mariotte et al., 2017; Wainwright et al., 2017). Furthermore, our study demonstrates that an invader's ability to accumulate litter may vary across different habitats, which has not been shown before.

4.3 | Impacts of invasion across a salinity gradient

Much experimental work has shown that invasive species' competitive effects on native plants are contingent on environmental conditions, particularly disturbance and nitrogen availability (Besaw et al., 2011; Broadbent et al., 2018; Daehler, 2003; Wilson & Pinno, 2013). However, few studies have investigated field patterns in invader impacts across environmental gradients. We found that native plant density and richness were the most consistently negatively affected by *Phragmites* over the salinity gradient, which is comparable with other research on *Phragmites* haplotype M on the East Coast (Meyerson et al., 2000; Silliman & Bertness, 2004). However, we found that *Phragmites* had the greatest effect on plant composition in saline marshes, where invaded areas typically contained *Iva fructescens* (a shrub) rather than the native dominant grass *Spartina alterniflora*. *Phragmites* also had a large effect on brackish marshes, in which invaded plots contained *Ipomoea sagittata* (a vine), rather than the native graminoid dominants, *Spartina patens* and *Schoenoplectus americanus*. The major effects that we observed *Phragmites* haplotypes I and M1 to have on native abundance, richness and composition of plants suggest that even though *Phragmites* is sometimes not considered to be an invader in the Gulf Coast, it does appear to have qualities of an invasive species.

Overwhelming evidence, primarily from the plant–soil feedback literature, suggests that invasive plants modify soil microbial communities (for reviews see Coats & Rumpho, 2014; van der Putten et al., 2007; Reinhart & Callaway, 2006). However, to our knowledge only two studies have tested whether an invader's microbial effects change across environmental gradients, and they found that invader impact depends on site nutrient availability (Kao-Kniffin & Balser, 2008) and forest type (Lorenzo et al., 2013). A growing number of studies are investigating the below-ground microbial impacts of *Phragmites*. Consistent with our finding that *Phragmites* had weak effects on microbes in freshwater marshes, work from freshwater marshes on the Midwest and East Coast has shown minimal effects of haplotype M on bacteria, fungi and oomycetes (Bickford et al., 2020) and effects of haplotype M on oomycete pathogen composition but mixed effects on oomycete richness (Nelson & Karp, 2013). Another study in freshwater to low salinity marshes on the East Coast showed that haplotype M altered composition of soil archaea, but not bacteria (Yarwood et al., 2016). Less comparable work has been done in brackish marshes (and none in saline marshes), but *Phragmites* was shown to increase methanogen functional gene abundance but not total fungi, laccase or denitrifier abundance in brackish marsh on the East Coast (Kim et al., 2018) and reduce AMF spore densities in Australia (Uddin & Robinson, 2017). Our results show that *Phragmites* increased pathogen abundances across marshes, only affected fungal and bacterial richness in brackish marshes, and that the effects of *Phragmites* on fungal and bacterial composition were stronger in more saline areas. Our work and work in other systems (Kao-Kniffin & Balser, 2008; Lorenzo et al., 2013) generally suggests that below-ground invader impacts are variable across environmental gradients, which makes it difficult to generalize invader effects from single-site or single-ecosystem studies. We recommend more multi-site, gradient and regional studies be performed to provide power to generalize where and when invaders alter microbial communities.

4.4 | Coupling of above- and below-ground response to invasion

Although the responses of above-ground plant and below-ground microbial communities to invasive species have been

well tested in the literature, most of these studies are done in isolation, leaving it an open question whether plant and microbial responses are coupled. Plant and microbial response may be directly linked, because invaders can alter microbial taxa (pathogens, mutualists) that impact plant performance (Inderjit & van der Putten, 2010) and because plant and microbial diversity in general are often coupled (Eisenhauer et al., 2011; Lange et al., 2015; Porazinska et al., 2018; Wagg et al., 2014). However, plants and microbes also respond to distinct changes associated with invasion; for example, plants are impacted by light competition and microbes are impacted by carbon substrate quality, which may not necessarily change in concert or to the same degree. In *Phragmites*, one study found correlated responses of plant (richness) and microbial (AMF spore density) properties across a *Phragmites* density gradient in brackish marshes in Australia (Uddin & Robinson, 2017). In a grassland system, plant diversity and soil fungal composition were directly linked during invasion, because native plant root loss drove fungal community change (Mamet et al., 2017). In contrast to expectations, we did not find that plant and microbial richness were coordinated. However, we found strong coupling between plant and microbial composition, such that sites that experienced the greatest shifts in plant composition also exhibited the greatest shifts in bacterial and fungal composition. Interestingly, this is the same pattern found in a global survey of plant and microbial communities: alpha diversity of plants and microbes did not correlate but beta diversity (compositional dissimilarity) did (Prober et al., 2015).

4.5 | Limitations

The main limitation in this study and any non-manipulative survey is that we cannot be sure of the direction of causality in invader-community relationships. Based on what is known about *Phragmites*, it is likely that observed patterns represent effects of the invader on plant and microbial communities, as similar patterns have been found with other haplotypes in other areas (Meyerson et al., 2000; Uddin & Robinson, 2017; Yarwood et al., 2016). Furthermore, historical aerial image analysis (see Section 2) showed that, in most of our sites, *Phragmites* colonized native marsh areas that did not differ from uncolonized areas. In three of our sites, the *Phragmites* stand was on the border between the marsh and more woody vegetation (Turtle Cove, Fontainebleau) and the water's edge (LUMCON1); thus elevation differences, for example, may have caused compositional differences between invaded and native plots. However, our finding that areas with *Phragmites* in the Gulf Coast are associated with highly altered plant and microbial communities compared to uninvaded areas is an important first step in understanding invasion in this region. Future experimental work should focus on confirming the direction of causality and testing whether removal efforts would restore native communities.

4.6 | Implications and conclusion

Our work has implications for the restoration and management of widespread invaders. The most important takeaway message from our research is that invader impacts on plant and microbial communities are not consistent across environmental gradients. Very little research exists on how the effects of invaders vary across the landscape, thus this challenges an implicit assumption that invader impacts are generalizable from site to site. Understanding how invader effects change across environmental gradients is important for prioritizing management across heterogeneous landscapes. For example, we found that brackish and saline marshes are the most susceptible to plant and microbial impacts of *Phragmites*, thus they could be selected over freshwater areas for limited management funds and restoration research. Furthermore, knowledge of whether invaders affect soil microbial communities is important for restoration. We found that soil fungal and bacterial composition is much altered underneath *Phragmites*, suggesting that soil legacies could affect native plant restoration even if *Phragmites* itself is removed from an area. Lastly, the coupling of plant and soil microbial compositional response to invasion suggests that shifts in above-ground communities may be used as a proxy for invasive impact, which is useful as they are much easier to sample.

More specific to our study system, there is little representation of the Gulf Coast in the vast amount of research being done on *Phragmites*. Due to the extensive nature of Gulf Coast marshes—40% of the wetlands in the continental United States are in Louisiana alone (Williams, 2016)—this represents a huge gap in our understanding of *Phragmites* and its potential impacts. In particular, the unique haplotypes of *Phragmites* in the Gulf Coast (i.e. M1, I) may have different ecologies compared to the well-studied haplotype M. Here we find that these haplotypes are, in fact, associated with reductions in plant abundance and diversity and shifts in plant and soil microbial composition, which echoes some of the observed impacts of haplotype M. It is important to note that M1, I and M haplotypes differ substantially in their leaf and rhizosphere microbiomes (Allen et al., 2020; Bowen et al., 2017, Bumby and Farrer in review); thus future work investigating similarities and differences among haplotypes is warranted.

Despite its invasive properties, or perhaps because of them, *Phragmites* may be a last line of defence against the rapid land loss occurring in Louisiana. In the outer marshes of the Mississippi River Delta, *Phragmites* is considered critical for reducing erosion, promoting vertical accretion and protecting the interior marsh from wave action and storms (Horppila et al., 2013; Rooth & Stevenson, 2000). It is even intentionally being planted for land-building efforts in the Delta. Understanding the native plant and microbial consequences of *Phragmites* is important when weighing the costs and benefits of using *Phragmites* in restoration.

Overall, understanding how invasive dominance and above- and below-ground impact shift across environmental gradients is important in understanding how environmental context might limit invasive dominance and whether the effects of invasive species are generalizable over large areas. The more we learn about widespread

impacts of invasive species, the better we can manage them at a landscape level and reduce their negative consequences for biodiversity and ecosystem function.

ACKNOWLEDGEMENTS

We thank the undergrads who helped in the field and laboratory: Liana Bethala, Isabella Donnell, Kasandra Scholz, Helen Weierbach and Claire Willis. We also thank the many scientists and managers at our field sites who helped with logistics: Julie Whitbeck at Barataria Preserve, Robert Moreau at Turtle Cove, Brian Roberts and Craig McClain at LUMCON, Jessica Dixon at Fontainebleau State Park, Shelley Stiaes at Bayou Sauvage NWR, Daniel Breaux at Big Branch NWR, and Jeffrey Duguay at Pearl River WMA. Keith Clay and Quynh Quach gave thoughtful comments on the manuscript. Funding was provided by the Louisiana State Board of Regents grant LEQSF(2017–20)-RD-A-14 to EF.

AUTHORS' CONTRIBUTIONS

E.C.F., C.B., P.W., S.R.H. and M.S. conceived the ideas and designed methodology; C.B., P.W., H.C., S.R.H., N.K.K., C.S.S. and W.W. collected the data; E.C.F. and M.K.H.S. analysed the data; E.C.F. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/1365-2745.13629.

DATA AVAILABILITY STATEMENT

The raw 16S and ITS sequence data are available in the NCBI GenBank Sequence Read Archive (SRA): BioProject PRJNA646524. Plant data and processed microbial data are available in the Dryad Digital Repository https://doi.org/10.5061/dryad.3j9kd51h6 (Farrer et al., 2021).

ORCID

Emily C. Farrer https://orcid.org/0000-0001-8003-8831
Christina Birnbaum https://orcid.org/0000-0002-2511-1845
Paweł Waryszak https://orcid.org/0000-0002-4245-3150
Sean F. H. Lee https://orcid.org/0000-0003-4869-6652

REFERENCES

Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, R. H., & Kõljalg, U. (2020). *UNITE general FASTA release for Fungi 2. Version 04.02.2020*. UNITE Community. https://doi.org/10.15156/BIO/786369

Achenbach, L., & Brix, H. (2014). Can differences in salinity tolerance explain the distribution of four genetically distinct lineages of *Phragmites australis* in the Mississippi River Delta? *Hydrobiologia*, *737*, 5–23. https://doi.org/10.1007/s10750-013-1601-y

Allen, W. J., DeVries, A. E., Bologna, N. J., Bickford, W. A., Kowalski, K. P., Meyerson, L. A., & Cronin, J. T. (2020). Intraspecific and biogeographical variation in foliar fungal communities and pathogen damage of native and invasive *Phragmites australis*. *Global Ecology and Biogeography*, *29*, 1199–1211.

Amsberry, L., Baker, M. A., Ewanchuk, P. J., & Bertness, M. D. (2000). Clonal integration and the expansion of *Phragmites australis*. *Ecological Applications*, *10*, 1110–1118.

Bansal, S., Lishawa, S. C., Newman, S., Tangen, B. A., Wilcox, D., Albert, D., Anteau, M. J., Chimney, M. J., Cressey, R. L., DeKeyser, E., Elgersma, K. J., Finkelstein, S. A., Freeland, J., Grosshans, R., Klug, P. E., Larkin, D. J., Lawrence, B. A., Linz, G., Marburger, J., … Windham-Myers, L. (2019). Typha (Cattail) invasion in North American Wetlands: Biology, regional problems, impacts, ecosystem services, and management. *Wetlands*, *39*, 645–684. https://doi.org/10.1007/s13157-019-01174-7

Besaw, L. M., Thelen, G. C., Sutherland, S., Metlen, K., & Callaway, R. M. (2011). Disturbance, resource pulses and invasion: Short-term shifts in competitive effects, not growth responses, favour exotic annuals. *Journal of Applied Ecology*, *48*, 998–1006. https://doi.org/10.1111/j.1365-2664.2011.01988.x

Bhattarai, G. P., & Cronin, J. T. (2014). Hurricane activity and the large-scale pattern of spread of an invasive plant species. *PLoS ONE*, *9*, e98478. https://doi.org/10.1371/journal.pone.0098478

Bickford, W. A., Zak, D. R., Kowalski, K. P., & Goldberg, D. E. (2020). Differences in rhizosphere microbial communities between native and non-native *Phragmites australis* may depend on stand density. *Ecology and Evolution*, *10*, 11739–11751.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., … Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*, 852–857. https://doi.org/10.1038/s41587-019-0209-9

Bowen, J. L., Kearns, P. J., Byrnes, J. E. K., Wigginton, S., Allen, W. J., Greenwood, M., Tran, K., Yu, J., Cronin, J. T., & Meyerson, L. A. (2017). Lineage overwhelms environmental conditions in determining rhizosphere bacterial community structure in a cosmopolitan invasive plant. *Nature Communications*, *8*, 433. https://doi.org/10.1038/s41467-017-00626-0

Bradley, B. A., Laginhas, B. B., Whitlock, R., Allen, J. M., Bates, A. E., Bernatchez, G., Diez, J. M., Early, R., Lenoir, J., Vilà, M., & Sorte, C. J. B. (2019). Disentangling the abundance–impact relationship for invasive species. *Proceedings of the National Academy of Sciences of the United States of America*, *116*, 9919–9924. https://doi.org/10.1073/pnas.1818081116

Broadbent, A., Stevens, C. J., Peltzer, D. A., Ostle, N. J., & Orwin, K. H. (2018). Belowground competition drives invasive plant impact on native species regardless of nitrogen availability. *Oecologia*, *186*, 577–587. https://doi.org/10.1007/s00442-017-4039-5

Bunn, R. A., Ramsey, P. W., & Lekberg, Y. (2015). Do native and invasive plants differ in their interactions with arbuscular mycorrhizal fungi? A meta-analysis. *Journal of Ecology*, *103*, 1547–1556. https://doi.org/10.1111/1365-2745.12456

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*, 581–583. https://doi.org/10.1038/nmeth.3869

Callaway, R. M., Cipollini, D., Barto, K., Thelen, G. C., Hallett, S. G., Prati, D., Stinson, K., & Klironomos, J. (2008). Novel weapons: Invasive plant supresses fungal mutualists in America but not in its native Europe. *Ecology*, *89*, 1043–1055.

Chambers, R. M., Meyerson, L. A., & Saltonstall, K. (1999). Expansion of *Phragmites australis* into tidal wetlands of North America. *Aquatic Botany*, *64*, 261–273. https://doi.org/10.1016/S0304-3770(99)00055-8

Coastal Protection and Restoration Authority (CPRA) of Louisiana. (2020). *Coastwide reference monitoring system-wetlands monitoring data*. Retrieved from Coastal Information Management System (CIMS) database. http://cims.coastal.louisiana.gov

Coats, V. C., & Rumpho, M. E. (2014). The rhizosphere microbiota of plant invaders: An overview of recent advances in the microbiomics of invasive plants. *Frontiers in Microbiology*, *5*, 00368. https://doi.org/10.3389/fmicb.2014.00368

Daehler, C. C. (2003). Performance comparisons of co-occurring native and alien invasive plants: Implications for conservation and restoration. *Annual Review of Ecology, Evolution, and Systematics*, *34*, 183–211. https://doi.org/10.1146/annurev.ecolsys.34.011802.132403

Davidson, A. M., Jennions, M., & Nicotra, A. B. (2011). Do invasive species show higher phenotypic plasticity than native species and if so, is it adaptive? A meta-analysis. *Ecology Letters*, *14*, 419–431. https://doi.org/10.1111/j.1461-0248.2011.01596.x

Dawson, W., & Schrama, M. (2016). Identifying the role of soil microbes in plant invasions. *Journal of Ecology*, *104*, 1211–1218. https://doi.org/10.1111/1365-2745.12619

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., Huber, T., Dalevi, D., Hu, P., & Andersen, G. L. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology*, *72*, 5069–5072. https://doi.org/10.1128/AEM.03006-05

Dukes, J. S., & Mooney, H. A. (1999). Does global change increase the success of biological invaders? *Trends in Ecology & Evolution*, *14*, 135–139. https://doi.org/10.1016/S0169-5347(98)01554-7

Eisenhauer, N., Migunova, V. D., Ackermann, M., Ruess, L., & Scheu, S. (2011). Changes in plant species richness induce functional shifts in soil nematode communities in experimental grassland. *PLoS ONE*, *6*, e24087. https://doi.org/10.1371/journal.pone.0024087

Eller, F., Skálová, H., Caplan, J. S., Bhattarai, G. P., Burger, M. K., Cronin, J. T., Guo, W.-Y., Guo, X., Hazelton, E. L. G., Kettenring, K. M., Lambertini, C., McCormick, M. K., Meyerson, L. A., Mozdzer, T. J., Pyšek, P., Sorrell, B. K., Whigham, D. F., & Brix, H. (2017). Cosmopolitan species as models for ecophysiological responses to global change: The common reed *Phragmites australis*. *Frontiers in Plant Science*, *8*, 01833. https://doi.org/10.3389/fpls.2017.01833

Eppinga, M. B., Kaproth, M. A., Collins, A. R., & Molofsky, J. (2011). Litter feedbacks, evolutionary change and exotic plant invasion. *Journal of Ecology*, *99*, 503–514. https://doi.org/10.1111/j.1365-2745.2010.01781.x

Estoup, A., Ravigné, V., Hufbauer, R., Vitalis, R., Gautier, M., & Facon, B. (2016). Is there a genetic paradox of biological invasion? *Annual Review of Ecology, Evolution, and Systematics*, *47*, 51–72. https://doi.org/10.1146/annurev-ecolsys-121415-032116

Farrer, E. C., Birnbaum, C., Waryszak, P., Halbrook, S. R., Brady, M. V., Bumby, C. R., Candaele, H., Kulick, N. K., Lee, S. F. H., Schroeder, C., Smith, M., & Wilber, W. (2021). Data from: Plant and microbial impacts of an invasive species vary across an environmental gradient. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.3j9kd51h6

Farrer, E. C., & Goldberg, D. E. (2009). Litter drives ecosystem and plant community changes in cattail invasion. *Ecological Applications*, *19*, 398–412. https://doi.org/10.1890/08-0485.1

Flory, S. L., & Clay, K. (2013). Pathogen accumulation and long-term dynamics of plant invasions. *Journal of Ecology*, *101*, 607–613. https://doi.org/10.1111/1365-2745.12078

Geng, Y., van Klinken, R. D., Sosa, A., Li, B., Chen, J., & Xu, C.-Y. (2016). The relative importance of genetic diversity and phenotypic plasticity in determining invasion success of a clonal weed in the USA and China. *Frontiers in Plant Science*, *7*, 00213. https://doi.org/10.3389/fpls.2016.00213

Grace, J. B., & Harrison, J. S. (1986). The biology of Canadian weeds. 73. *Typha latifolia* L., *Typha angustifolia* L. and *Typha x glauca* Godr. *Canadian Journal of Plant Science*, *66*, 361–379.

Hale, A. N., & Kalisz, S. (2012). Perspectives on allelopathic disruption of plant mutualisms: A framework for individual- and population-level fitness consequences. *Plant Ecology*, *213*, 1991–2006. https://doi.org/10.1007/s11258-012-0128-z

Hauber, D. P., Saltonstall, K., White, D. A., & Hood, C. S. (2011). Genetic variation in the common reed, *Phragmites australis*, in the Mississippi River delta marshes: Evidence for multiple introductions. *Estuaries and Coasts*, *34*, 851–862. https://doi.org/10.1007/s12237-011-9391-9

Hellings, S. E., & Gallagher, J. L. (1992). The effects of salinity and flooding on *Phragmites australis*. *Journal of Applied Ecology*, *29*, 41–49. https://doi.org/10.2307/2404345

Holdredge, C., & Bertness, M. D. (2011). Litter legacy increases the competitive advantage of invasive *Phragmites australis* in New England wetlands. *Biological Invasions*, *13*, 423–433. https://doi.org/10.1007/s10530-010-9836-2

Horppila, J., Kaitaranta, J., Joensuu, L., & Nurminen, L. (2013). Influence of emergent macrophyte (*Phragmites australis*) density on water turbulence and erosion of organic-rich sediment. *Journal of Hydrodynamics. Ser. B*, *25*, 288–293.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, *50*, 346–363. https://doi.org/10.1002/bimj.200810425

Howard, R. J., Travis, S. E., & Sikes, B. A. (2008). Rapid growth of a Eurasian haplotype of *Phragmites australis* in a restored brackish marsh in Louisiana, USA. *Biological Invasions*, *10*, 369–379. https://doi.org/10.1007/s10530-007-9136-7

Inderjit, & van der Putten, W. H. (2010). Impacts of soil microbial communities on exotic plant invasions. *Trends in Ecology & Evolution*, *25*, 512–519. https://doi.org/10.1016/j.tree.2010.06.006

Jo, I., Fridley, J. D., & Frank, D. A. (2017). Invasive plants accelerate nitrogen cycling: Evidence from experimental woody monocultures. *Journal of Ecology*, *105*, 1105–1110. https://doi.org/10.1111/1365-2745.12732

Kao-Kniffin, J., & Balser, T. C. (2008). Soil fertility and the impact of exotic invasion on microbial communities in Hawaiian Forests. *Microbial Ecology*, *56*, 55–63. https://doi.org/10.1007/s00248-007-9323-1

Kettenring, K. M., de Blois, S., & Hauber, D. P. (2012). Moving from a regional to a continental perspective of *Phragmites australis* invasion in North America. *AoB PLANTS*, *2012*, pls040. https://doi.org/10.1093/aobpla/pls040

Kim, S., Kang, J., Megonigal, J. P., Kang, H., Seo, J., & Ding, W. (2018). Impacts of *Phragmites australis* invasion on soil enzyme activities and microbial abundance of tidal marshes. *Microbial Ecology*, *76*, 782–790. https://doi.org/10.1007/s00248-018-1168-2

Konisky, R. A., & Burdick, D. M. (2004). Effects of stressors on invasive and halophytic plants of New England salt marshes: A framework for predicting response to tidal restoration. *Wetlands*, *24*, 434–447.

Lambert, A. M., Dudley, T. L., & Saltonstall, K. (2010). Ecology and impacts of the large-statured invasive grasses *Arundo donax* and *Phragmites australis* in North America. *Invasive Plant Science and Management*, *3*, 489–494.

Lange, M., Eisenhauer, N., Sierra, C. A., Bessler, H., Engels, C., Griffiths, R. I., Mellado-Vázquez, P. G., Malik, A. A., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B. C., Trumbore, S. E., & Gleixner, G. (2015). Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications*, *6*, 6707. https://doi.org/10.1038/ncomms7707

Lavergne, S., & Molofsky, J. (2004). Reed canary grass (*Phalaris arundinacea*) as a biological model in the study of plant invasions. *Critical Reviews in Plant Sciences*, *23*, 415–429.

Leffler, A. J., Leonard, E. D., James, J. J., & Monaco, T. A. (2014). Invasion is contingent on species assemblage and invasive species identity in experimental rehabilitation plots. *Rangeland Ecology & Management*, *67*, 657–666. https://doi.org/10.2111/REM-D-13-00140.1

Lenth, R. (2020). *emmeans: Estimated marginal means, aka least-squares means*. R package version 1.4.6.

Levine, J. M., Adler, P. B., & Yelenik, S. G. (2004). A meta-analysis of biotic resistance to exotic plant invasions. *Ecology Letters*, *7*, 975–989. https://doi.org/10.1111/j.1461-0248.2004.00657.x

Liao, H., D'Antonio, C. M., Chen, B., Huang, Q., & Peng, S. (2016). How much do phenotypic plasticity and local genetic variation contribute to phenotypic divergences along environmental gradients in widespread invasive plants? A meta-analysis. *Oikos*, *125*, 905–917. https://doi.org/10.1111/oik.02372

Lorenzo, P., Pereira, C. S., & Rodríguez-Echeverría, S. (2013). Differential impact on soil microbes of allelopathic compounds released by the invasive Acacia dealbata Link. *Soil Biology and Biochemistry*, *57*, 156–163. https://doi.org/10.1016/j.soilbio.2012.08.018

Louthan, A. M., Doak, D. F., & Angert, A. L. (2015). Where and when do species interactions set range limits? *Trends in Ecology & Evolution*, *30*, 780–792. https://doi.org/10.1016/j.tree.2015.09.011

Macel, M., de Vos, R. C. H., Jansen, J. J., van der Putten, W. H., & van Dam, N. M. (2014). Novel chemistry of invasive plants: Exotic species have more unique metabolomic profiles than native congeners. *Ecology and Evolution*, *4*, 2777–2786. https://doi.org/10.1002/ece3.1132

Mamet, S. D., Lamb, E. G., Piper, C. L., Winsley, T., & Siciliano, S. D. (2017). Archaea and bacteria mediate the effects of native species root loss on fungi during plant invasion. *The ISME Journal*, *11*, 1261–1275. https://doi.org/10.1038/ismej.2016.205

Mariotte, P., Spotswood, E. N., Farrer, E. C., & Suding, K. N. (2017). Positive litter feedbacks of an introduced species reduce native diversity and promote invasion in Californian grasslands. *Applied Vegetation Science*, *20*, 28–39. https://doi.org/10.1111/avsc.12291

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE*, *8*, e61217. https://doi.org/10.1371/journal.pone.0061217

Meyerson, L. A., Cronin, J. T., & Pyšek, P. (2016). *Phragmites australis* as a model organism for studying plant invasions. *Biological Invasions*, *18*, 2421–2431. https://doi.org/10.1007/s10530-016-1132-3

Meyerson, L. A., Lambert, A. M., & Saltonstall, K. (2010). A tale of three lineages: Expansion of common reed (*Phragmites australis*) in the U.S. Southwest and Gulf Coast. *Invasive Plant Science and Management*, *3*, 515–520.

Meyerson, L. A., Lambertini, C., McCormick, M. K., & Whigham, D. F. (2012). Hybridization of common reed in North America? The answer is blowing in the wind. *AoB PLANTS*, *2012*, pls022. https://doi.org/10.1093/aobpla/pls022

Meyerson, L. A., Saltonstall, K., Windham, L., Kiviat, E., & Findlay, S. (2000). A comparison of *Phragmites australis* in freshwater and brackish marsh environments in North America. *Wetlands Ecology and Management*, *8*, 89–103.

Mosely, J. C., Bunting, S. C., & Manoukian, M. E. (1999). Cheatgrass. In R. L. Sheley & J. K. Petroff (Eds.), *Biology and management of noxious rangeland weeds* (pp. 175–188). Oregon State University Press.

Nelson, E. B., & Karp, M. A. (2013). Soil pathogen communities associated with native and non-native *Phragmites australis* populations in freshwater wetlands. *Ecology and Evolution*, *3*, 5254–5267.

Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., Schilling, J. S., & Kennedy, P. G. (2016). FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*, *20*, 241–248. https://doi.org/10.1016/j.funeco.2015.06.006

Odum, W. E. (1988). Comparative ecology of tidal freshwater and salt marshes. *Annual Review of Ecology and Systematics*, *19*, 147–176. https://doi.org/10.1146/annurev.es.19.110188.001051

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2019). *vegan: Community ecology package*. R package version 2.5-5.

Penfound, W. T., & Earle, T. T. (1948). The biology of the water hyacinth. *Ecological Monographs*, *18*, 447–472. https://doi.org/10.2307/1948585

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team. (2019). *nlme: Linear and nonlinear mixed effects models*. R package version 3.1-141.

Porazinska, D. L., Farrer, E. C., Spasojevic, M. J., Bueno de Mesquita, C. P., Sartwell, S. A., Smith, J. G., White, C. T., King, A. J., Suding, K. N., & Schmidt, S. K. (2018). Plant diversity and density predict belowground diversity and function in an early successional alpine ecosystem. *Ecology*, *99*, 1942–1952. https://doi.org/10.1002/ecy.2420

Prober, S. M., Leff, J. W., Bates, S. T., Borer, E. T., Firn, J., Harpole, W. S., Lind, E. M., Seabloom, E. W., Adler, P. B., Bakker, J. D., Cleland, E. E., DeCrappeo, N. M., DeLorenze, E., Hagenah, N., Hautier, Y., Hofmockel, K. S., Kirkman, K. P., Knops, J. M. H., La Pierre, K. J., … Fierer, N. (2015). Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. *Ecology Letters*, *18*, 85–95. https://doi.org/10.1111/ele.12381

R Core Team. (2019). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Reinhart, K. O., & Callaway, R. M. (2006). Soil biota and invasive plants. *New Phytologist*, *170*, 445–457. https://doi.org/10.1111/j.1469-8137.2006.01715.x

Roman, J., & Darling, J. A. (2007). Paradox lost: Genetic diversity and the success of aquatic invasions. *Trends in Ecology & Evolution*, *22*, 454–464. https://doi.org/10.1016/j.tree.2007.07.002

Rooth, J. E., & Stevenson, J. C. (2000). Sediment deposition patterns in *Phragmites australis* communities: Implications for coastal areas threatened by rising sea-level. *Wetlands Ecology and Management*, *8*, 173–183.

Rooth, J. E., Stevenson, J. C., & Cornwall, J. C. (2003). Increased sediment accretion rates following invasion by *Phragmites australis*: The role of litter. *Estuaries*, *26*, 475–483. https://doi.org/10.1007/BF02823724

Saltonstall, K. (2001). A set of primers for amplification of noncoding regions of chloroplast DNA in the grasses. *Molecular Ecology Notes*, *1*, 76–78. https://doi.org/10.1046/j.1471-8278.2001.00031.x

Saltonstall, K. (2002). Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 2445–2449. https://doi.org/10.1073/pnas.032477999

Saltonstall, K. (2003). A rapid method for identifying the origin of North American *Phragmites* populitons using RFLP analysis. *Wetlands*, *23*, 1043–1047.

Saltonstall, K. (2016). The naming of Phragmites haplotypes. *Biological Invasions*, *18*, 2433–2441. https://doi.org/10.1007/s10530-016-1192-4

Sexton, J. P., McIntyre, P. J., Angert, A. L., & Rice, K. J. (2009). Evolution and ecology of species range limits. *Annual Review of Ecology, Evolution, and Systematics*, *40*, 415–436. https://doi.org/10.1146/annurev.ecolsys.110308.120317

Silliman, B. R., & Bertness, M. D. (2004). Shoreline development drives invasion of *Phragmites australis* and the loss of plant diversity on New England salt marshes. *Conservation Biology*, *18*, 1424–1434. https://doi.org/10.1111/j.1523-1739.2004.00112.x

Sofaer, H. R., Jarnevich, C. S., & Pearse, I. S. (2018). The relationship between invader abundance and impact. *Ecosphere*, *9*, e02415. https://doi.org/10.1002/ecs2.2415

Taberlet, P., Gielly, L., Pautou, G., & Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, *17*, 1105–1109. https://doi.org/10.1007/BF00037152

Therneau, T. (2018). *deming: Deming, theil-sen, passing-bablock and total least squares regression*. R package version 1.4.

Uddin, M. D. N., & Robinson, R. W. (2017). Responses of plant species diversity and soil physical-chemical-microbial properties to *Phragmites australis* invasion along a density gradient. *Scientific Reports*, *7*, 11007. https://doi.org/10.1038/s41598-017-11205-0

U'Ren, J. M., & Arnold, A. E. (2017). Illumina MiSeq dual-barcoded two-step PCR amplicon sequencing protocol. *Protocols io*, https://doi.org/10.17504/protocols.io.fs9bnh6

van der Putten, W. H., Klironomos, J. N., & Wardle, D. A. (2007). Microbial ecology of biological invasions. *The ISME Journal*, *1*, 28–37. https://doi.org/10.1038/ismej.2007.9

Vilà, M., Espinar, J. L., Hejda, M., Hulme, P. E., Jarošík, V., Maron, J. L., Pergl, J., Schaffner, U., Sun, Y., & Pyšek, P. (2011). Ecological impacts of invasive alien plants: A meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters*, *14*, 702–708. https://doi.org/10.1111/j.1461-0248.2011.01628.x

Wagg, C., Bender, S. F., Widmer, F., & van der Heijden, M. G. A. (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, 5266–5270. https://doi.org/10.1073/pnas.1320054111

Wainwright, C. E., Dwyer, J. M., & Mayfield, M. M. (2017). Effects of exotic annual grass litter and local environmental gradients on annual plant community structure. *Biological Invasions*, *19*, 479–491. https://doi.org/10.1007/s10530-016-1303-2

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag.

Williams, D. A., Hanson, M., Diaz, R., & Overholt, W. A. (2012). Determination of common reed (*Phragmites australis* (Cav.) Trin. ex Steudel) varieties in Florida. *Journal of Aquatic Plant Management*, *50*, 69–74.

Williams, S. J. (2016). Louisiana coastal wetlands: A resource at risk. *US Geological Survey: Coastal and Marine Geology Program*. Retrieved from http://pubs.usgs.gov/fs/la-wetlands/

Wilson, S. D., & Pinno, B. D. (2013). Environmentally-contingent behaviour of invasive plants as drivers or passengers. *Oikos*, *122*, 129–135. https://doi.org/10.1111/j.1600-0706.2012.20673.x

Windham, L. (2001). Comparison of biomass production and decomposition between *Phragmites australis* (common reed) and Spartina patens (salt hay grass) in brackish tidal marshes of New Jersey, USA. *Wetlands*, *21*, 179–188.

Yarwood, S. A., Baldwin, A. H., Gonzalez Mateu, M., & Buyer, J. S. (2016). Archaeal rhizosphere communities differ between the native and invasive lineages of the wetland plant *Phragmites australis* (common reed) in a Chesapeake Bay subestuary. *Biological Invasions*, *18*(9), 2717–2728. https://doi.org/10.1007/s10530-016-1144-z

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Farrer EC, Birnbaum C, Waryszak P, et al. Plant and microbial impacts of an invasive species vary across an environmental gradient. *J Ecol*. 2021;109: 2163–2176. https://doi.org/10.1111/1365-2745.13629