Bickford Wesley A (Orcid ID: 0000-0001-7612-1325)
Zak Donald R. (Orcid ID: 0000-0002-9730-7337)

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Title: Plant effects on and response to soil microbes in native and non-native *Phragmites australis*

Authors:

Wesley. A. Bickford[1,2*], Deborah E. Goldberg[2], Donald R. Zak[2,3], Danielle S. Snow[1], and Kurt P. Kowalski[1,3]

[1]U.S. Geological Survey – Great Lakes Science Center, Ann Arbor, MI 48015, USA

[2]Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109, USA

[3]School for Environment and Sustainability, University of Michigan, Ann Arbor, MI 48109, USA

*Corresponding Author: Wesley A. Bickford, wbickford@usgs.gov

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As a publication of the U.S. government, all data are in the public domain and available on USGS ScienceBase, along with R code used for data analysis, at https://doi.org/10.5066/P9ZAEZPD (Bickford et al, 2021). The code is not novel and is released for reproducibility purposes only. All microbial DNA sequences are available in the NCBI Sequence Read Archive under accession number PRJNA719385 at https://www.ncbi.nlm.nih.gov/bioproject/PRJNA719385/.

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Abstract

Plant-soil feedbacks (PSFs) mediate plant community dynamics and may plausibly facilitate plant invasions. Microbially-mediated PSFs are defined by plant effects on soil microbes and subsequent changes in plant performance (responses), both positive and negative. For microbial interactions to benefit invasive plants disproportionately, native and invasive plants must either (1) have different effects on *and* responses to, soil microbial communities or (2) only respond differently to similar microbial communities. In other words, invasive plants do not need to cultivate different microbial communities than natives if they respond differently to them. However, effects and responses are not often explored separately, making it difficult to determine the underlying causes of performance differences. We performed a reciprocal-transplant PSF experiment with multiple microbial inhibition treatments to determine how native and non-native lineages of *Phragmites australis* affect and respond to soil bacteria, fungi and oomycetes. Non-native *Phragmites* is a large, fast-growing, cosmopolitan invasive plant, whereas the North American native variety is comparatively smaller, slower growing, and typically considered a desirable wetland plant. We identified the effects of each plant lineage on soil microbes using DNA meta-barcoding and linked plant responses to microbial communities. Both *Phragmites* lineages displayed equally weak, insignificant PSFs. We found evidence of slight differential effects on microbial community composition, but no significant differential plant responses. Soils conditioned by each lineage differed only slightly in bacterial community composition, but not in fungal composition. Additionally, native and non-native *Phragmites* lineages did not significantly differ in their response to similar soil microbial communities. Neither lineage appreciably differed when plant biomass was compared between those grown in sterile and live soils. Targeted microbial inhibitor treatments revealed both lineages were negatively impacted by soil bacteria,

but the negative response was stronger in non-native *Phragmites*. These observations were opposite of expectations from invasion theory and imply that the success of non-native *Phragmites,* relative to the native lineage, does not result from its interaction with soil microorganisms. More broadly, quantifying plant effects on, and responses to soil microbes separately provides detailed and nuanced insight into plant-microbial interactions and their role in invasions, which could inform management outcomes for invasive plants.

Keywords: biological invasions, DNA metabarcoding, plant-soil feedback, plant-microbial interactions, rhizosphere, soil bacteria, soil fungi

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Introduction

Soil microorganisms may play a critical role in plant performance during range expansion, and they are thought to facilitate plant invasions in some circumstances (Reinhart and Callaway 2006, Maron et al. 2014, Kowalski et al. 2015). Theory suggests that microbially-mediated invasiveness occurs when invasive plants disproportionately benefit from their microbial community over native plants. The disproportionate benefit for invasive plants may emerge through increased interaction with mutualist microorganisms or decreased impact of pathogens, relative to native plants (Reinhart and Callaway 2006).

When a plant alters the soil microbial community in a way that directly affects its own growth, a positive or negative plant-soil feedback (PSF) will be realized over the long term (Bever et al. 1997). PSFs can be measured in a species-specific (measuring absolute performance in conspecific *vs.* heterospecific soils) or pairwise (measuring the relative performance of two plant species in soils from each species) manner (Bever et al. 1997). Under the PSF model for invasiveness, native and invasive plants must have a differential effect on soil microbial communities, and, in turn, display a differential response to the soil microbial community, such that invasive plants exhibit a more positive or less negative PSF than native plants (Reinhart and Callaway 2006, Mangla et al. 2008, Meisner et al. 2014, Inderjit and Cahill 2015). Despite compelling theory and several individual validations of these ideas, multiple meta-analyses suggest that PSFs may not be generally important to invasion (Suding et al. 2013, Meisner et al. 2014, Crawford et al. 2019). Of course, many factors not related to soil biota could drive invasiveness (Parker et al. 2006), but it is also important to note that soil microorganisms can still contribute to invasiveness even without PSFs. A simple and effective framework for modeling complex interactions involving an intermediary (resources, microbes, etc.) is to break them down

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into *effects* and *responses* (Goldberg 1990, Suding et al. 2008). For plant interactions mediated by microorganisms, the components are (1) effect of plants on microorganisms and (2) responses of plants to microorganisms (Fig. 1). Two co-occurring plants can have the same or different effects and responses, and the permutations of differential effects and/or responses determine whether plant performance, in either case, is mediated through the soil microbial community (Fig. 1). Disproportionate benefits for invasive plants may emerge by cultivating a different microbial community with relatively more mutualists or fewer pathogens than native counterparts (differential effects *and* responses, Fig. 1D). Alternatively, plants may respond more positively or less negatively than natives to a common soil microbial community (differential response only, Fig. 1C). Either mechanism leads to patterns of increased growth and expansion by invasive plants, relative to their native counterparts. However, species-specific and pairwise PSFs are only generated when both effects *and* responses differ between the native and invasive plants (Fig. 1D; Bever et al. 1997). Therefore, thorough examination of both plant effects on, as well as responses to, soil microbes is critical for a fuller understanding of drivers of invasiveness. Additionally, isolating the components of the microbial community that are most affected by invasive plants and those that invasive plants respond to holds promise for improving the predictability and management of plant invasions (Kowalski et al. 2015).

Phragmites australis is a cosmopolitan wetland grass with multiple lineages worldwide, and it is often considered a model organism for studying plant invasions (Meyerson et al. 2016, Cesarino et al. 2020). Invasive to North America, the European lineage (*Phragmites australis* haplotype M; hereafter, non-native *Phragmites*) is highly productive, fast growing, large, and often forms dense monocultures supporting a low overall plant species diversity. The native lineage in North America (*Phragmites australis* subsp. *americanus*, hereafter native *Phragmites*) is more

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often found in low nutrient wetlands with high plant diversity and is desirable for wildlife habitat (Price et al. 2014). Most investigations of the possible role of soil microorganisms in *Phragmites* invasions have focused on the effect component of plant-microbe interactions, exploring differential effects of *Phragmites* lineages on rhizosphere communities of soil biota. Whereas some studies have found that native and non-native lineages differed in their communities of rhizosphere bacteria (Bowen et al. 2017), archaea (Yarwood et al. 2016), or oomycetes (Nelson and Karp 2013), the existence and strength of those effects are inconsistent both within and among studies (Yarwood et al. 2016, Bowen et al. 2017, Bickford et al. 2020). Further, *Phragmites* is often considered an ecosystem engineer, altering its surroundings (Saltonstall and Meyerson 2016, Cui et al. 2019). As such, it is plausible that differences in rhizosphere communities between native and non-native *Phragmites* may be a consequence of soil micro-environmental changes (*e.g.,* differential aeration) caused by invasion, rather than the driver of invasion itself (Bickford et al. 2020). Most studies of the effect components have surveyed rhizosphere soil, but recent studies have found that foliar fungi (Allen et al. 2020) and root endophyte communities (Gonzalez Mateu et al. 2020) differed between *Phragmites* lineages along the East Coast of the U.S.; conversely, root endophyte communities of bacteria, fungi, and oomycetes did not differ between lineages of *Phragmites* in the Great Lakes region of North America (Bickford et al. 2018).

In contrast to the many studies of the effect component, fewer studies explore how and why *Phragmites* responds to soil microbial communities. *Phragmites* lineages have displayed differential virulence to belowground oomycete pathogens (Crocker et al. 2015) and differential susceptibility to foliar fungal pathogens (Allen et al. 2020). However, another study focusing on foliar fungi found that pathogen virulence did not differ between native and non-native *Phragmites* lineages (DeVries et al. 2020). Only a single study has combined effect and response components

and explored species-specific PSFs, although effects and responses were not quantified separately (*i.e.*, they tested for as interactions shown in Fig 1d, but not 1c), finding that the native and non-native lineage of *Phragmites* both produced weakly negative PSFs (Allen et al. 2018), suggesting that both lineages may be weakly negatively impacted by their own soil relative to other lineages. The existence of PSFs implies significant plant effects on soil microorganisms, but those effects may not be independently demonstrated within PSF studies, because they generally only measure plant responses in different soils (Bever et al. 1997). Additionally, when PSFs are not strongly different from zero, it is difficult to infer whether differential effects and/or differential responses are actually occurring (Bever et al. 1997). Thus, it remains unclear whether soil microorganisms have significant impacts on invasiveness of *Phragmites* and, if so, which of the aforementioned mechanisms are at play. A thorough understanding of the mechanisms also requires understanding which microbial groups are most directly affected by each lineage of *Phragmites*, which microbial groups each lineage responds to strongly, and if differential effects on and/or responses to microbial groups are likely drivers of invasiveness.

Here, we report the results of a PSF experiments that isolated effects and responses of native and non-native *Phragmites* lineages to specific microbial groups in soil. If microbial communities surrounding non-native *Phragmites* are driving its invasiveness, we expect non-native *Phragmites* to benefit disproportionately from them by either (1) displaying differential effects on, and response to, soil microbes compared to native *Phragmites* (*i.e.* generating PSFs, Fig. 1D) or (2) responding differently to a similar community of microbes (Fig. 1C). First, we explore plant effects on microbial communities by using molecular methods to peer inside the microbial "black-box" and compare microbial communities cultivated by each lineage. We then compare *Phragmites* performance in live and sterile soils to assess each lineage's response to the

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total soil microbiome. Additionally, we apply targeted microbial inhibitors to soils conditioned by each lineage to compare each lineage's response to broad components (bacteria, fungi, oomycetes) of each microbial community. Finally, we look for evidence of species-specific or pairwise PSFs using a traditional reciprocal transplant PSF approach. This combination of approaches enables us to determine which of the four categories in Figure 1 applies to the *Phragmites* system and address whether interactions with soil microbial communities likely drive invasiveness.

Materials and Methods

We implemented a reciprocal transplant PSF experiment to assess the growth response of each *Phragmites* lineage to the particular soil microbial groups that were differentially conditioned (*i.e.*, altered) by each lineage. We also compared plant growth between conditioned soils and sterilized soils to assess the significance of the total microbial community on plant growth. To identify which microbes most strongly influenced plant responses, we applied microbial inhibitors individually and in combinations to decrease bacteria, fungi, and oomycetes in soil conditioned by either plant lineage. The full experiment consisted of two phases: a 120-day soil Conditioning Phase, followed by a 120-day Feedback Phase; in between these phases we applied microbial inhibitor treatments. The full experiment was a partial factorial arrangement with four factors: soil inoculum (live *vs.* sterile), soil conditioning lineage (native *vs.* non-native), microbial inhibitor (7 levels, described below), and seedling lineage (native *vs.* non-native). The microbial inhibitors were only applied to pots containing "live" soil inoculum. Each experimental treatment involving microbial inhibitors was replicated 10 times; treatments involving sterile soil inoculum were replicated 5 times, resulting in 320 total pots used in the full experiment. See Fig. 2 for a full design schematic.

Plant material collection. Plant belowground material and rhizosphere soils were collected throughout Michigan and Ohio, USA (See Appendix S1: Table S1 for precise collection locations). We collected 10 unique genotypes each of native and non-native *Phragmites*. At each sampling location, *Phragmites* stands were identified as native or non-native using morphology; plant material was subsequently collected and analyzed for genetic verification of the field identification after Saltonstall (2003). Belowground plant material, along with rhizosphere soil, was exhumed using a sharpshooter shovel, keeping much of the rhizomes intact. All belowground materials were stored in a cold room at 4 °C prior to use.

Preparing vegetative clones and soils. Rhizomes from each site were thoroughly washed with deionized water and cut into segments up to 50 cm long. Rhizome segments were placed into trays filled approximately half full of deionized water and placed under LED growth lights programmed on a 16 hr light: 8 hr dark cycle within the indoor plant growth facility in the Biological Sciences Building at the University of Michigan, Ann Arbor, MI, USA. Temperature in the growth facility was maintained at approximately 20-22 ºC throughout the entire experiment. After roughly three days, stem sprouts appeared at the nodes. Trays were drained, rinsed, and refilled every three days to prevent contamination and ensure steady moisture conditions. Stems grew for approximately two weeks.

Following Kulmatiski and Kardol (2008), we prepared mixes of 70 % sand, 25 % peat, and 5% field soil inoculum. We triple autoclaved (gravity cycle; exposure time 30 mins; exposure temperature 122 ºC; drying time 25 mins) pre-washed and screened sand and stored it in sterilized 5-gal buckets. We also triple autoclaved Pro-Moss (Premier Tech Horticulture) sphagnum and stored it in sterilized 5-gal buckets. Field soil was gathered from buckets containing rhizomes and was homogenized among all locations to create a generalized inoculum that combined soil beneath

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native and non-native *Phragmites* (Fig. 2A). Given the range of sites where collections occurred and the influence of site on soil microbial communities (Bickford et al. 2020), we chose to homogenize field soils into a general inoculum and then allow each lineage to cultivate its microbial community from the general inoculum. A subset of that inoculum was triple autoclaved for addition into our sterile treatment pots. Sand, peat, and field inoculum were homogenized in large batches in a sterilized portable cement mixer at the proportions listed above. Four-hundred pots (15 cm diameter x 11 cm tall) were each filled with 1 L of the loosely packed soil mixture.

Rhizomes were cut into 3-cm segments, each containing a node with a single stem and roots. Rhizome cuttings and roots were triple rinsed with deionized water prior to planting. Cuttings were not sterilized, because preliminary tests indicated that plants responded negatively to surface-sterilization. Only stems at least 7 cm tall and with at least 2 leaves were transferred to pots. Half of the pots were planted with native cuttings and half with non-native. Due to insufficient material or poor growth of some rhizomes, genotypes were not equally represented in each treatment group, although no genotype was used more than 2x per treatment (see full dataset at https://doi.org/10.5066/P9ZAEZPD for complete details of distribution of genotypes). All cuttings that did not survive for 2 weeks following initial transplantation were replaced.

Soil Conditioning Phase. Pots were randomly distributed among benches and subjected to a 16 hr light: 8 hr dark cycle using 16- 400 W ceiling mounted high intensity growth lights. Plants were fertilized initially with 0.5 $g \cdot L^{-1}$ Fe chelate (Sequestrene 330) and 2.66 $g \cdot L^{-1}$ 15-16-17 NPK fertilizer. Pots were irrigated using a dripline irrigation system with individual drippers in each pot for 4 mins twice daily. Plants were fertilized again after 60 days with the same fertilizer mixture as described above. Plants grew under constant temperature conditions of approximately 27 °C for a total of 120 days (Fig. 2B).

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At the end of the soil Conditioning Phase, one soil sample from each pot was collected from the top 2 cm and placed in a 2 mL cryovial and flash-frozen in liquid N_2. Plants were removed from each pot by pulling out pot-bound below-ground mass and shaking adhering soil into a sterile bucket. Below-ground tissues remained intact and were only broken up to sufficiently remove all soil from roots and rhizomes. Soils were then returned to their original pot. Pots with plants that grew insufficiently or did not survive were not used in the next phase; 320 of the initial 400 pots were used in the subsequent phase of this experiment.

Microbial inhibitor treatments. We randomly assigned pots conditioned by each lineage to one of seven microbial inhibitor treatments: 1. No inhibitor treatment (control), 2. Antibacterial, 3. Antifungal, 4. Anti-oomycete, 5. Antibacterial and Antifungal, 6. Antibacterial and Anti-oomycete, and 7. Antifungal and Anti-oomycete. Pots that received sterilized inoculum prior to the Conditioning Phase also received no microbial inhibitors (See Fig. 2C). The antibacterial treatment consisted of 7.44 $mg \cdot L^{-1}$ streptomycin sulfate (calculated to equal roughly 5 $mg \cdot g^{-1}$ dry soil adapted from Kooijman et al. 2016). The antifungal treatment contained 0.93 mL of 40% pentachloronitrobenzene (PCNB) per L of deionized water (following label recommendation for soil drench). The anti-oomycete treatment was comprised of 0.16 mL of 22.5% Mefenoxam (2-[(2,6-dimethylphenyl) methoxyacetylamino propionic acid methyl ester) per L of deionized water (following label recommendation for soil drench). Mixed inhibitors were combined at the same amount of active ingredient as the single inhibitor treatments. All inhibitors were applied by submerging each individual pot in a separate 2.4 L bucket filled with the assigned inhibitor solution. Pots remained submerged for 60 mins, then drained to approximately field capacity and returned to their randomized locations on benches. After treating a pot, the bucket was rinsed with deionized water and sanitized with 70% EtOH before receiving fresh inhibitor solution to avoid

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any cross-contamination. The no-inhibitor treatment pots were submerged in deionized water. This method minimized air-filled pore space and maximized soil contact with inhibitors.

Because the initially sterilized pots were likely colonized by airborne microbes during the 120-day Conditioning Phase, we re-sterilized (triple autoclaved) 20 of the 40 initially sterile pots prior to planting seedlings. Soils were removed from the pots, triple autoclaved, returned to the pot, and pots returned to their randomized locations on the benches. The subset that were re-sterilized are referred to as the "Post-conditioning Sterile" treatment.

Feedback Phase. Seeds were collected from field-identified and genetically confirmed populations of native and non-native *Phragmites* in Michigan and Ohio USA (See Appendix S1: Table S1 for collection locations). Seeds were cold stratified for 6 weeks, and then surface sterilized following a 2-day procedure to minimize recruitment of microbes through the seeds. On day one, seeds were washed in 97% EtOH for 3 mins, in 1% bleach for 2 mins, triple rinsed in deionized water, and stored overnight in deionized water at 4°C. On day 2, seeds were washed in 1% bleach for 5 mins, triple rinsed with deionized water, and stored in deionized water at 4°C. Sterilized seeds were plated on 1.5 % Gamborg's media with 0.5 $g \cdot L^{-1}$ Sequestrine 330 Fe chelate, parafilmed shut, and placed on edge into a growth chamber set at 37°C and 12 hour light / 12 dark cycle. Germinating seedlings were transplanted into sterilized (triple autoclaved) seed-starting medium (SunGro) and placed under LED growth lights programmed on a 16 hr light: 8 hr dark cycle. Seedling trays were watered with deionized water containing 0.5 $g \cdot L^{-1}$ Fe chelate (Sequestrene 330) and 2.66 $g \cdot L^{-1}$ 15-16-17 NPK solution.

Three days after inhibitor treatments were applied, we transplanted seedlings that had at least 1 leaf (non-cotyledon) into pre-conditioned and treated pots. Conditioning lineage and seedling lineages were crossed, such that each seedling lineage was equally represented in each

conditioning lineage (Fig. 2D). Initial seedling height at the time of planting varied from 0.5 cm to 21.3 cm; due to the faster growth rate of the non-native lineage, the non-native seedlings tended to be larger than native seedlings at the time of planting (non-native med = 7.5 cm, sd = 4.22; native med = 3.8 cm, sd = 2.55). Therefore, we assigned each seedling to one of five size classes corresponding to their initial height. Plants of each size class were evenly disbursed across all treatment combinations to ensure no treatment combination was biased in terms of initial seedling size (See full dataset at https://doi.org/10.5066/P9ZAEZPD). All seedlings were grown in the same growth room under identical conditions to the Conditioning Phase.

All seedlings that did not survive the first week after initial transplantation (< 5 pots affected) were replaced. Seedlings grew under constant conditions for 120 days. Stem heights of each plant were recorded at transplanting and measured weekly. Plants were fertilized with 0.5 $g \cdot L^{-1}$ Fe chelate (Sequestrene 330) and 2.66 $g \cdot L^{-1}$ 15-16-17 NPK fertilizer approximately 60 days after transplanting. Soil was subsampled 10 days after initial transplantation and at the end of the study period (before plants were harvested) using the same methods as at the end of the conditioning phase. Plants were then removed from pots and shaken vigorously to remove all soils from belowground tissues. Plants were separated into aboveground tissues, roots, and rhizomes and then dried at 70 °C for 48 hours and weighed. All analyses reported in this study are based on the sum of these components, i.e., total biomass per plant.

Soil molecular methods

DNA was extracted from 50 mg (wet weight) of soil collected from half of the pots (n = 160) at the end of Conditioning Phase and at the end of the Feedback Phase using Qiagen PowerSoil PowerLyzer DNA extraction kits. We used manufacturer protocols, except for improvements to reduce ethanol contamination (*e.g.,* extra spins, more frequent transfers to sterile

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tubes). 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, CA, USA).

Quantitative polymerase chain reactions (qPCR) were performed to quantify total abundances of all fungi and of all bacteria in the samples from each pot prior to the application of microbial inhibitors at end of Conditioning Phase, as well as at the end of the Feedback Phase. All qPCR reactions were performed using subsamples diluted from the same template genomic DNA samples. DNA standards for qPCR were prepared by cloning amplicons generated using primers 338f/518r (bacteria) and ITS1f/5.8s (fungi) from environmental soil DNA with a TOPO TA 2.1 Cloning Kit (Life Technologies). The inserts were then amplified via polymerase chain reaction (PCR) with M13 primers to create a linear target region (Hou et al. 2010). PCR products were purified using a QIAquick PCR Purification Kit (Qiagen) and quantified using a Quant-iT PicoGreen dsDNA kit. Copy number was then calculated from DNA concentrations and known amplified region length (bacteria or fungi specific insert + M13 region). See Appendix S1: Table S2 for qPCR reagents, conditions, efficiencies, and R^2. To minimize between-run variability, samples collected from the same pot at different time points were analyzed within the same qPCR run. For the same reason, sample order was determined so that the 7 different inhibitor treatments would be evenly distributed among runs to minimize bias. Data obtained from qPCR was normalized to copies / ng of dry soil; for this conversion, soil moisture content was determined by drying a subsample of approximately 0.5 g, taken from each vial used for DNA extraction, and dried at 105°C for 48 hours. Soils were weighed before and after drying to determine percent moisture content.

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To determine taxonomic composition within the fungi and bacteria, polymerase chain reactions (PCR) were performed using subsamples of the same template genomic DNA sample. Genomic DNA was diluted to ensure equimolar concentration of template DNA in each PCR reaction. Bacterial amplicons were generated using primers described in Kozich et al. (2013), which target the V4 region of the 16S rRNA gene. Fungal amplicons were produced using primers described by Taylor et al. (2016), which target the ITS2 region of the 5.8S rRNA gene. See Appendix S1: Table S3 for specific primer sequences and PCR conditions. We did not identify oomycete communities in soils due to the lack of community differences and low phylogenetic resolution obtained from small amplicons of oomycetes, which we have previously documented in these soils (Bickford et al. 2020). All PCR reactions were performed in triplicate using Phusion High Fidelity DNA Polymerase and master mix (New England BioLabs, MA, USA). Libraries were normalized using SequalPrep Normalization Plate Kit (Life technologies A10510-01) following the manufacturer's protocol for sequential elution. The concentration of the pooled samples was determined using Kapa Biosystems Library Quantification kit for Illumina platforms (Kapa Biosystems KK4824). The sizes of the amplicons in the library were determined using the Agilent Bioanalyzer High Sensitivity DNA analysis kit (cat# 5067-4626). The final library consisted of equal molar amounts from each of the plates, normalized to the pooled plate at the lowest concentration. Amplicons were sequenced by the Microbial Systems Molecular Biology Laboratory (MSMBL) at the University of Michigan on the Illumina MiSeq platform, using a MiSeq Reagent Kit V2 500 cycles (Illumina cat# MS102-2003), according to the manufacturer's instructions.

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Bioinformatics analysis

Raw bacterial sequence data were processed using mothur v1.40.1 (Schloss et al. 2009). Operational taxonomic units (OTUs) were clustered at 97% for bacterial sequences and assigned to taxonomy by comparing representative sequences to the taxa found in the SILVA database (Quast et al. 2018). Raw fungal sequences were processed using QIIME2 (Bolyen et al. 2019), which can implement *de novo* sequence clustering that does not require sequence alignment. Fungal sequences were clustered into OTUs at 97% similarity and assigned to taxonomy based on the UNITE database (Nilsson et al. 2013, Abarenkov et al. 2020). Bacterial data were rarefied according to the sample that yielded the fewest number of sequences to ensure equal coverage across all samples (10,019 sequences). Fungi were rarefied to 1700 sequences which required eliminating 7 samples with low sequence depth (Appendix S1: Fig S1). A large proportion of fungal reads were assigned to unknown taxa prompting us to inspect the reads closely to determine if there were any issues that might suggest technical problems. Upon close inspection of a random sample of unknown sequences, all were determined to start at the end of the 28S and end as far as the 5.8S indicating sequences were of the correct length and region. Additionally, samples had BLAST matches confirming their unknown taxonomy, mostly matching environmental sequences.

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Data Analysis

Total biomass per seedling at the end of the Feedback Phase was square root transformed to conform to a normal distribution; transformed biomass values were used for all further analyses. PSFs and response to treatments were calculated using Cohen's d.

$$(1)\ Cohen's\ d\ =\ \frac{x_t - x_c}{pooled\ sd}$$

To calculate species-specific PSFs with the total microbial community present, x_t is heterospecific conditioned soil and x_c is conspecific conditioned soil, using no-inhibitor controls. To assess response to inhibitor treatments, x_t is treated soil and x_c no inhibitor control (within same seedling and conditioning lineage). We calculated pairwise PSFs (I_s) following Bever et al. (1997) and Crawford et al. (2019)

$$(2)\ I_s = \alpha_A - \alpha_B - \beta_A + \beta_B$$

where α_A is plant A's performance in conspecific soil, α_B is plant B's performance in heterospecific soil, β_A is plant A's performance in heterospecific soil, and β_B is plant B's performance in conspecific soil.

To assess response of seedling biomass to the total microbiome, we included only the no-inhibitor pots (n = 60) and performed a 3-way ANCOVA (Type III Sum of Squares) with conditioning lineage (2 levels), seedling lineage (2 levels), and soil inoculum (3 levels: sterilized pre-conditioning, sterilized post-conditioning, live) as main effects. We included initial seedling height as a covariate. To test response to inhibitor treatments, we excluded the pots with sterilized inoculum and used a 3-way ANCOVA (Type III Sum of Squares) with conditioning lineage (2 levels), seedling lineage (2 levels), and inhibitor treatment (7 levels) as main effects, again including initial seedling height as a covariate. Additionally, we used a *post-hoc* Dunnett's test to assess plant response to each inhibitor treatment relative to the control.

All microbial community analyses were performed separately on each time point (end of Conditioning Phase, end of Feedback Phase) to determine the effects of lineage on conditioning soil microbes and the effects of the inhibitor treatments on microbes, respectively. To explore microbial community composition, we calculated Bray-Curtis distances and used permutational multivariate analysis of variance (PERMANOVA) to test whether conditioning lineage, seedling lineage, or inhibitor treatment resulted in significant microbial community differences among our samples at the end of the Conditioning Phase and at the end of the Feedback Phase. Pairwise PERMANOVAs (comparing all possible pairs) were also calculated to identify which inhibitor treatments significantly impacted microbial community composition. *P*-values generated from pairwise comparisons were adjusted using a Benjamini-Hochberg adjustment (Benjamini and Hochberg 1995). Homogeneity of Dispersions (PERMDISP) further assessed whether microbial community samples differed in their degree of dispersion from their centroid. We used Principal Coordinate Analysis (PCoA) to assess and visualize microbial community differences between lineages and inhibitor treatments. All statistical analyses were run in the R environment (R Core Team 2016). All R code, notes, and associated data can be accessed at https://doi.org/10.5066/P99BHLKZ.

Results

Differential effects on the soil microbiome

At the end of the Conditioning Phase, both bacterial and fungal communities differed significantly, albeit modestly between soils conditioned by native and by non-native *Phragmites*; plant conditioning lineage accounted for < 2% of the variance in bacterial and fungal community composition (Fig. 3). In addition, soil fungal communities differed in their dispersion around the centroid, and therefore may not actually differ significantly (Fig. 3). Conditioning lineages also

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had no effect on the relative abundance of the most common bacterial phyla (Appendix S1: Fig. S2), although several fungal phyla did differ significantly: soils conditioned by non-native *Phragmites* were comprised of fewer Chytridiomycota (P = 0.005), but more of the unknown fungal sequences (P = 0.020) than native-conditioned communities (Table 1).

Differential response to total soil microbiome

Seedling biomass was not influenced by sterilization relative to the entire intact microbial community, for either lineage (Table 2, Fig. 4). This holds whether sterilization occurred only pre-conditioning or pre- and post-conditioning. Nor did the seedling lineages respond differently to the sterilization treatments, although even when accounting for initial seedling height, non-native *Phragmites* grew larger than its native counterpart (Table 2, Fig. 4).

Effectiveness of inhibitor treatments

As expected, prior to inhibitor application, soil communities did not differ among the inhibitor treatments, although both fungal and bacterial communities differed between pots sterilized before conditioning and those not sterilized (Fig. 5A; Appendix S1: Table S4, S5). Following application of the microbial inhibitors, total bacterial and fungal gene copy numbers were not impacted (Appendix S1: Fig. S3); however, the community composition of both groups was affected by some of the treatments. All bacterial communities that received the antibacterial agent were similar to each other and different from all other treatments (Fig. 5; Appendix S1: Table S6). Additionally, the bacterial community composition following the treatments containing fungicide significantly differed from the control treatment (Appendix S1: Table S6), indicating a possible interaction between bacteria and fungi in the soil. As expected, the oomycete inhibitor alone had no impact on the bacterial community. Fungal communities that received either antibacterial or antifungal agents significantly differed from sterile and no-inhibitor control (Fig.

5; Appendix S1: Table S7). Having established that the inhibitor treatments were effective in changing soil microbial communities, although not their total abundance, we interpret plant response to the inhibitor treatments as response to different microbial community composition.

Differential response to major microbial groups

Final seedling biomass depended significantly on initial seedling height, and accounting for those differences, total biomass differed among inhibitors and seedling lineages, but not conditioning lineage (Table 3). A post-hoc Dunnett's test comparing each treatment to the no-inhibitor control showed that the antibacterial and antibacterial + anti-fungal treatments were effective at eliciting a positive plant response compared to the control, highlighting the importance of the soil bacterial community to plant growth (Table 4, Fig. 6). The full model shows no interaction between seedling lineage and inhibitor indicating that responses to microbial inhibitors did not differ significantly between *Phragmites* lineages (Table 3). However, it appears the non-native lineage had a slightly more positive response to the antibacterial and antibacterial + antifungal treatments than the native seedlings, which showed no responses to any of the treatments (Fig. 6).

Plant-Soil Feedbacks

In treatments receiving no microbial inhibitors, neither native nor non-native *Phragmites* displayed species-specific PSFs different from zero (Fig. 7A). That is, neither grew significantly differently in the soil conditioned by the other, relative to their own soil. Interestingly however, both lineages trended toward negative PSFs, indicating both lineages tend to do better in soils of the other lineage. Despite the weak strength of species-specific PSFs, the pairwise PSF model predicts coexistence when no inhibitors were applied (Fig. 7B), because both are more limited by their own conditioned soil. The microbial inhibitors did not significantly change the species-

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specific PSFs (*i.e.,* no treatments produced PSFs significantly different from zero); however, some interesting trends emerged implying that targeted microbial inhibition could alter plant responses to soil microbes (Fig. 7A). For instance, inhibitors targeting the oomycete community produced a modest positive PSF for the non-native lineage, meaning that non-native *Phragmites* tended to be more productive in its own soil than in native in the absence of oomycetes. The magnitude and direction of pairwise PSFs also varied in the presence of microbial inhibitors (Fig. 7B). Most microbial disturbances caused by targeted inhibition drove the pairwise PSFs to neutral values, where predictions about maintenance of diversity are ambiguous. Only the inhibitors targeting the fungal community resulted in the pairwise PSF remaining negative, predicting coexistence (Fig. 7B).

Discussion

Based on invasion theory and the framework we propose in Fig. 1, we expected non-native *Phragmites* to generate more positive, or less negative, species-specific PSFs than native *Phragmites* through differential effects and differential responses to soil microorganisms. However, although differential effects existed, they were quite weak (Bacteria: PERMANOVA R^2 = 0.017, P = 0.001. Fungi: PERMANOVA R^2 = 0.019, P = 0.001), and responses did not differ significantly between lineages (Fig. 4, Fig. 6). Species-specific PSFs in native and non-native *Phragmites* were therefore insignificant. Additionally, the pairwise PSF indicated that these two lineages should coexist due to conspecific density dependence, which is inconsistent with field observations of strong invasiveness of non-native *Phragmites*. The PSF models make several simplifying assumptions (*e.g.*, species have identical resources and are competitively equivalent) (Bever et al. 1997); therefore, if the predictions are inconsistent with field observations, it is likely that these underlying assumptions are not met. The inconsistency between our experimental

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evidence, invasion theory, and widespread patterns of *Phragmites* invasion suggest that PSFs are not the primary drivers of performance differences between the native and non-native lineages. Below, we first discuss differences in effect, then response, then how effects and responses combine to influence PSFs. We conclude with a discussion of invasiveness in *Phragmites*, as well as the implications of our results for management.

Differential effects

Consistent with recent field studies, we found evidence of differential lineage effects on soil microbial communities, but the strength of this difference was weak. The strength of *Phragmites* lineage differences on microbial communities vary widely across studies and regions. For instance, Bowen et al. (2017) found clear differences between bacterial communities among lineages of *Phragmites* across distant populations, suggesting that *Phragmites* lineages exhibit strong differential effects on rhizosphere organisms. The strength of differences displayed in our study more closely mirror those found in Bickford et al. (2020), who suggested that slight differences in rhizosphere bacterial communities observed in the field may arise between lineages of *Phragmites* as a consequence of invasion, rather than driving the initial invasion. Experimentally cultivated bacterial communities differed only slightly (Fig. 3A) and fungal communities did not detectably differ between conditioning lineages in our study (Fig. 3B), in further support of this observation. Despite the high percentage of unidentified fungal sequences (likely representing uncultured fungi; see Methods for further justification), the unidentified sequences were dissimilar enough to represent a fair amount of diversity in the fungal communities.

As stated in the methods, we were unable to sterilize field collected *Phragmites* rhizomes prior to the Soil Conditioning Phase, due to adverse effects of sterilization on rhizome and shoot

health. Additionally, the method of clone generation from rhizomes required submersion of rhizomes in water while new shoots developed. The combination of the growth environment and the inability to sterilize the rhizomes may have resulted in an inadvertent "seeding" of the microbial community with organisms not typical of natural environments. For instance, we found that the most prevalent fungal phylum was Chytridiomycota after soil conditioning (Table 1). This fungal phylum is typically associated with aquatic environments, and while present in soils, it is not usually as dominant as Ascomycota and Basidiomycota. For instance, we previously observed (Bickford et al. (2020) that Chytridiomycota made up less than 2% of fungal rhizosphere sequences in field populations of the Great Lakes region. However, the fact that we still found evidence, albeit weak, of microbial community differences following conditioning (Fig. 3) suggests that conditioning effects were not driven entirely by the lack of tissue sterilization prior to soil conditioning.

Differential Responses

Plant-microbial interactions in the soil may re not be the primary driver of performance differences between *Phragmites* lineages. Native and non-native *Phragmites* seedlings responded similarly when compared between live and sterile soils, as well as to the different microbial inhibitor treatments. Non-native *Phragmites* may have responded more positively to antibacterial treatments, but those results were not statistically significant. Even if this were the case, it was the opposite of what would be expected from invasion theory, because it would imply stronger microbially-induced limits on non-native growth than native.

An alternative interpretation for our observations revolves around stress tolerance induced by microbial associations and context dependence. Many microbial mutualists improve stress tolerance of the host plant by protecting against herbivory, pathogens, or improving salt tolerance

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(De Zelicourt et al. 2013, Kumar and Verma 2018, Gonzalez Mateu et al. 2020). Our controlled experiment was, by design, not stressful to the plants. Therefore, microbes inducing stress tolerance would not have provided a benefit but may have had a cost to plants (*e.g.*, carbohydrate transfer). Our anti-bacterial treatment could have removed these would-be mutualists, thereby liberating the plants from parasitism. If this experiment had been conducted in an environment where natural stressors exist, the plant response to microbial inhibition could have been different (*e.g.*, negative response to removal of microbes that induce stress tolerance). A recent meta-analysis found evidence that stress was more likely to induce positive PSF in field experiments than in controlled greenhouse experiments (Beals et al. 2020), potentially because microbial mutualists are providing stress tolerance. The context dependence associated with mutualism via stress tolerance may be an explanation for the finding that PSFs identified in controlled experiments are poor predictors of range expansion in the field (Suding et al. 2013, Schittko et al. 2016, Crawford et al. 2019).

Plant-Soil Feedbacks

Our experimental results are also in agreement with the only other PSF experiment on *Phragmites* (Allen et al. 2018) and previous surveys in the same region (Bickford et al. 2018, 2020), showing weak PSF magnitude and negligible evidence of differential microbial community cultivation (no differences in bacterial, fungal, or oomycete communities in roots or rhizosphere) in field conditions. This agreement gives us confidence that PSFs are not the primary driver of non-native *Phragmites'* advantage over native *Phragmites*, at least in North American Great Lakes populations. It is possible that there is spatial variation in *Phragmites* PSFs (*sensu* Cronin et al. 2015) such that the strength and importance of PSFs may vary by region. Given the spatial extent

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of this study (Great Lakes region), we may find regional patterns exist if the spatial scope was broadened.

The microbial inhibitor treatments revealed interesting trends in their impact on species-specific and pairwise PSFs between native and non-native *Phragmites* (Fig. 7). For instance, the species-specific PSF for non-native *Phragmites* shifted direction when the oomycete community was disturbed, implying that the non-native lineage cultivates an oomycete community that is more virulent towards itself than the oomycete community cultivated by the native lineage. This finding is consistent with Crocker et al. (2015) who found differential susceptibility to oomycetes cultivated by native and non-native *Phragmites* lineages. Although these trends were not statistically significant, it is striking that targeted microbial disturbances were more likely to affect PSFs in the non-native lineage than the native, suggesting that non-native *Phragmites* may be more responsive to microbial interventions than the native lineage.

Microbial drivers of invasiveness

In the context of the four models of plant-microbial interactions we outline in Fig. 1, our results indicate interactions between soil microorganisms and two lineages of *Phragmites* most closely resemble model B. We found evidence of slight differential effects on microbes, but no significant differential responses. We have no evidence that these interactions drive invasiveness of the non-native lineage relative to the native. In fact, all the interactions that we observed impacted plant performance in the opposite direction from what we would expect from invasion theory. Specifically, (1) native and non-native *Phragmites* generated weak PSFs of the same magnitude, (2) the total microbiome had a negative impact on non-native productivity and did not affect productivity of the native lineage, (3) bacteria negatively impacted non-native *Phragmites*, (4) the pairwise PSF for native and non-native *Phragmites* was negative. Taken together, these

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data suggest that the non-native lineage is more vulnerable to belowground pathogen pressure, specifically that it is more consistently hampered by bacterial pathogens.

Evidence for the importance of mutualists for either lineage in this study is minimal (Appendix S1: Table S8, Fig. S4). However, indirect mutualisms may play an important role in natural populations. As mentioned above, microbial mutualism via stress tolerance may not have been a factor in our experiment due to the artificially stress-free conditions. In fact, we found negative responses to many microbes (*e.g.,* bacteria in the non-native lineage) that could be mutualistic in a more stressful context. While we do not know whether this occurred in our experiment, it is notable that disease protection by endophytic bacteria has been identified in *Phragmites* (White et al. 2018) and thus may play a role in natural populations. Additionally, in a similar PSF study, Allen et al. (2018) found that while native and non-native *Phragmites* lineages exhibited weak negative PSFs of similar magnitude, pathogens cultivated by *Phragmites* were more virulent to neighboring native competitors than to themselves. Thus, pathogen spillover to other native plants may be a mechanism by which *Phragmites* expansion occurs (although Schroeder et al. 2020 did not find evidence of spillover), and the impact of pathogens on *Phragmites* alone may be less informative than the relative impact on surrounding native species.

Our results suggest that factors other than interactions with soil biota may be the main drivers of performance differences between native and non-native *Phragmites*. Importantly, non-native *Phragmites* exhibits greater rates of photosynthesis, stomatal conductance, a larger photosynthetic canopy, higher specific leaf area, and greater leaf N content than the native lineage (Mozdzer and Zieman 2010, Mozdzer et al. 2013), which may underlie its invasiveness more than interactions with soil microbial communities. These disparities show up in both glasshouse-grown plants and naturally occurring populations, verifying the heritability of these differences (Mozdzer

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and Zieman 2010). Therefore, given inherent performance advantages in the non-native lineage, soil microbiota may play an insignificant role in *Phragmites'* invasiveness, at least as it pertains to differences between the native and non-native lineages. However, the impact of the microbial inhibitor treatments on responses and PSFs (Fig 6 & 7) suggested that plant responses, particularly in the non-native lineage could be sensitive to targeted microbial disturbance, which may be important for management efforts. As interest grows in microbially-mediated biocontrol of non-native *Phragmites* (Kowalski et al. 2015), differential responses to microbial manipulation by each lineage could allow biocontrol efforts to target non-native *Phragmites* and leave the native lineage unaffected. While our results did not identify significant differential negative responses to broad microbial groups, there was a modest differential response to bacterial pathogens, suggesting that testing specific bacterial pathogens as biocontrol agents could prove successful.

Conclusions

We found little evidence that native and non-native *Phragmites* exhibit differential effects on, and responses to, soil microorganisms; consequently, there is no evidence that those small differences provide advantages to non-native *Phragmites* over the native lineage. Disentangling plant-microbial interactions into effects and responses enabled us to obtain fuller insight into which specific soil microbes matter to each lineage of *Phragmites* and how each lineage responds to them. Given that we have also previously found minimal differences in the effect component from field surveys (Bickford et al. 2018, 2020), the data reported here likely reflect conditions in the field. We conclude that interactions with soil microbes play a minor role in the performance advantage of the non-native *Phragmites* lineage compared to the native lineage, and that other physiological factors are likely to be the primary drivers of invasiveness. Our results further challenge the widespread importance of PSFs to the monodominance of invasive species (Suding

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et al. 2013). Given the assumptions of the PSF model (Bever et al. 1997), and the results presented here, PSFs may play an insignificant role in invasions when plant species differ substantially in resource uptake and competitive abilities.

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Author Contributions

WB, DG, DZ, and KK conceived of the ideas and designed the experiment; WB and DS collected the data; WB and DS analyzed the data; WB led the writing of the manuscript; All authors contributed critically to the drafts and gave final approval for publication.

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Tables

Table 1: Mean relative abundance of the most common fungal phyla found in soils cultivated by each *Phragmites* lineage at the end of the Conditioning Phase. *P*-values result from one-way ANOVA (Type III sum of squares) with a Bonferroni correction for multiple comparisons. Bold values indicate significant different between lineages at the α = 0.05 level.

	Native Mean Rel. Abundance	Non-native mean Rel. Abundance	P-value
Unidentified Fungi	0.596	0.668	**0.020**
Chytridiomycota	0.213	0.131	**0.005**
Ascomycota	0.090	0.127	0.105
Basidiomycota	0.055	0.039	0.980
Glomeromycota	0.025	0.018	0.295

Table 2: ANCOVA table (Type III sum of squares) comparing square root transformed seedling biomass between inoculum types, soil conditioning lineages, and seedling lineages taking into account the initial seedling height. Model included only data from subset of pots that did not receive chemical microbial inhibitors. Bold values indicate a significant difference between lineages at the $\alpha = 0.05$ level. Italic values indicate marginal significance ($0.05 < P < 0.10$).

	Sum Sq	Df	F-value	*P*-value
Initial Seedling Height	**3.13**	**1**	**4.79**	**0.032**
Conditioning Lineage	0.66	1	1.00	0.320
Seedling Lineage	**9.20**	**1**	**14.08**	**<0.001**
Inoculum	0.26	2	0.20	0.822
Conditioning X Seedling	*1.92*	*1*	*2.94*	*0.091*
Conditioning X Inoculum	0.87	2	0.67	0.516
Seedling X Inoculum	1.26	2	0.96	0.388
Conditioning X Seedling X Inoculum	0.03	2	0.02	0.981

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Table 3: Comparisons of square root transformed total seedling biomass across inhibitor treatments, soil conditioning lineages, and seedling lineages taking into account initial seedling height using an ANCOVA (Type III sum of squares). Bold values indicate a significant difference between lineages at the α = 0.05 level.

	Sum Sq	Df	F-value	P-value
Conditioning Lineage	1.09	1	1.71	0.192
Seedling Lineage	**8.00**	**1**	**12.59**	**<0.001**
Inhibitor	**11.22**	**6**	**2.94**	**0.009**
Initial Seedling Height	**16.77**	**1**	**26.37**	**<0.001**
Conditioning X Seedling	0.07	1	0.12	0.733
Conditioning X Inhibitor	2.31	6	0.61	0.726
Seedling X Inhibitor	1.8	6	0.47	0.828
Conditioning X Seedling X Inhibitor	2.32	6	0.61	0.724

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Table 4: Comparisons of square root transformed total seedling biomass between inhibitor treatments and non-inhibitor controls using a post-hoc Dunnett's Test. Bold values indicate a significant difference between lineages at the α = 0.05 level. Italic values indicate marginal significance (0.05 < *P* < 0.10).

Treatment	Estimate	St. Error	t-value	P-value
Antibacterial + Antifungal	*0.599*	*0.249*	*2.37*	*0.085*
Antibacterial	**0.669**	**0.247**	**2.71**	**0.036**
Antibacterial + Anti-oomycete	0.576	0.254	2.27	0.109
Anti-oomycete	0.138	0.250	0.55	0.987
Anti-oomycete + Antifungal	-0.280	0.247	-1.14	0.733
Antifungal	-0.455	0.250	-1.82	0.277

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Fig. 1: Conceptual model of plant effects on and responses to soil microbes, the impacts on plant performance, and invasions. Yellow and blue arrows indicate individual effect or response; green arrows indicate shared effect or response. **A:** Native and invasive plants cultivate similar microbial communities (N+I) and respond similarly to those microbes. **B:** Native and invasive plants affect the microbial communities differently, selecting for distinct microbial communities (I: selected by invasive plant, N: selected by native plant). However, those different soil microbial communities do not differentially alter plant performance. **C:** Native and invasive plants cultivate similar microbial communities (N+I), but each plant's response to the community of microbes is different such that performance is impacted differentially. **D:** Native and invasive plants affect the microbial communities differently, selecting for distinct microbial communities and those communities generate a differential plant response such that performance is impacted differentially. Invasion is impacted only if plants respond differently to microbes (as in C and D).

Fig. 2: Conceptual model of experimental design. **A:** Soil inoculum was obtained by homogenizing rhizosphere soils from field populations of both *Phragmites* lineages. A subset of the soil inoculum was triple autoclaved to sterilize. **B: Conditioning Phase:** Pre-sprouted rhizomes of native and non-native *Phragmites* were transplanted in pots with sterile soil plus live or sterile inoculum. Rhizome cuttings grew for 120 days to condition soil microbiota. Plants were harvested and soil samples were taken to assess conditioned microbial community. **C: Soil Treatments:** Microbial inhibitors were applied to all "live" soils. A subset of the sterile soils was re-sterilized. **D: Feedback Phase:** Plants and soils were reciprocally crossed so that each lineage was grown in each soil type. Seedlings grew for 120 days. Treatments with live inoculum were replicated 10 times and those with sterile inoculum were replicated 5 times resulting in 320

pots. At the end of the Feedback Phase, plant biomass was harvested and measured in each pot and microbial communities were analyzed in soils.

Fig. 3: Principal coordinate analysis of Bray-Curtis distances showing composition of **(A)** soil bacterial and **(B)** fungal communities in pots following soil conditioning phase (pre-treatment). Bacterial communities were slightly, but significantly different by conditioning lineage (PERMANOVA $R^2 = 0.017$, $P = 0.001$, PERMDISP $P = 0.054$) Fungal communities were dispersed differently, likely driving slight differences in centroid locations (PERMANOVA $R^2 = 0.019$, $P = 0.001$, PERMDISP $P = 0.004$).

Fig. 4: *Phragmites* biomass response to soil microbes. Plots show biomass in soils **(A)** sterilized prior to the Conditioning Phase or **(B)** sterilized after the Conditioning Phase subtracted from biomass in live soils. Negative effect size indicates growth was worse in live soils relative to sterile. Effect sizes calculated using Cohen's d. Error Bars indicate 95 % confidence intervals.

Fig. 5: Principal coordinate analysis (PCoA) of Bray-Curtis distances representing **(A)** bacterial and **(B)** fungal community composition pre-inhibitor treatment and at the end of the study. Both communities shift in response to inhibitor treatments. Plots show centroids of points grouped by inhibitor treatment. Error bars indicate 95 % confidence intervals around the centroid. PERMDISP indicated that dispersion from the centroid did not differ among treatments for either bacteria or fungi. The two conditioning lineages were combined in this figure because the magnitude of difference between conditioning lineages was much smaller than differences resulting from inhibitors. Sterile indicates "Pre-conditioning Sterile" treatment.

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Fig. 6: Response of native and non-native *Phragmites* seedlings to microbial communities affected by inhibitor treatments. Values indicate microbial inhibitor effects when compared to controls calculated using Cohen's d. Error bars indicate 95% confidence intervals. Values with error not overlapping zero are considered statistically significantly different from control. Inhibitor treatments are abbreviated B+F: Antibacterial & Antifungal, B: Antibacterial, B+O: Antibacterial & Anti-oomycete, O: Anti-oomycete, F+O: Antifungal & Anti-oomycete, F: Antifungal.

Fig. 7: A) Species-specific PSFs indicating growth in conspecific relative to heterospecific soil. PSF calculated using Cohen's d. **B)** Pairwise PSF indicating relative performance of two plant species in soils from each species. Negative values indicate that coexistence is predicted. Error bars represent 95% confidence intervals. PSFs calculated separately by soil microbial inhibitor treatment group. None: No inhibitor, B+F: Antibacterial & Antifungal, B: Antibacterial, B+O: Antibacterial & Anti-oomycete, O: Anti-oomycete, F+O: Antifungal & Anti-oomycete, F: Antifungal.

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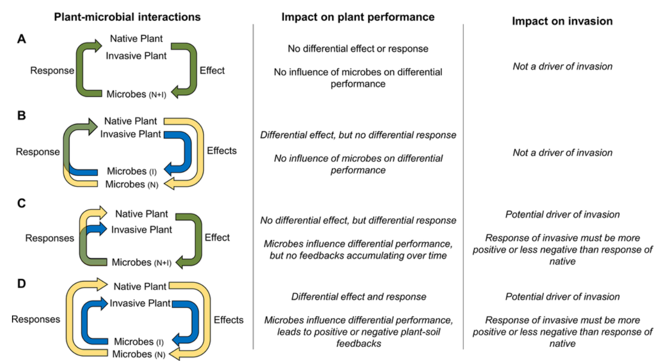

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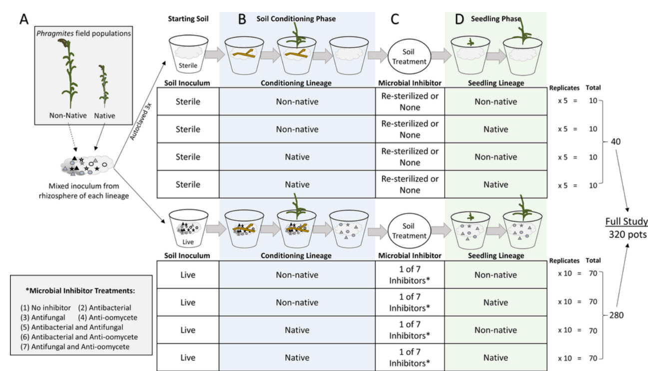

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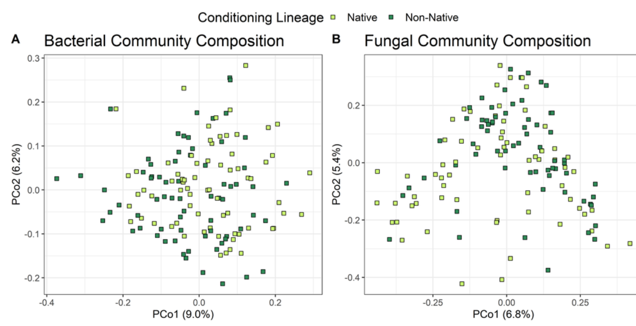

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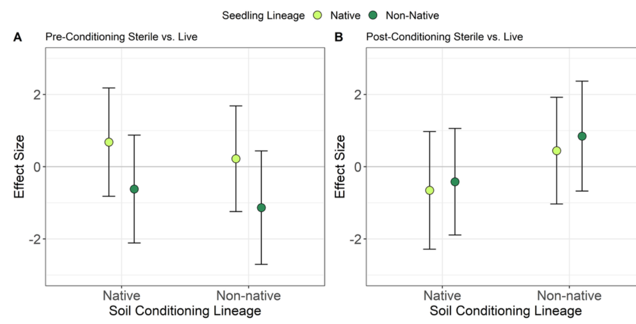

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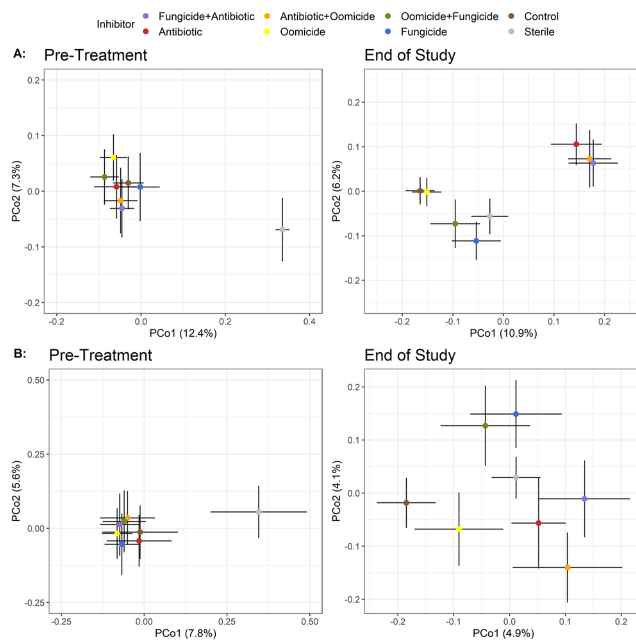

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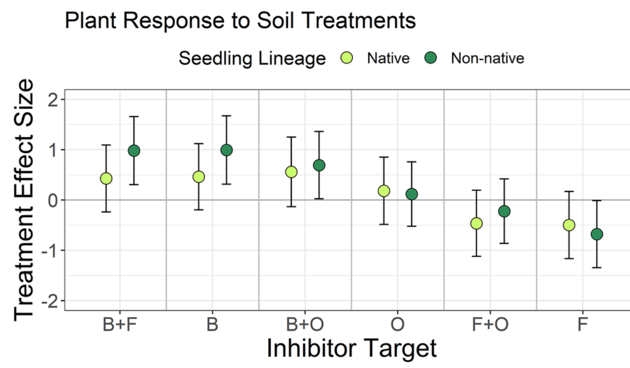

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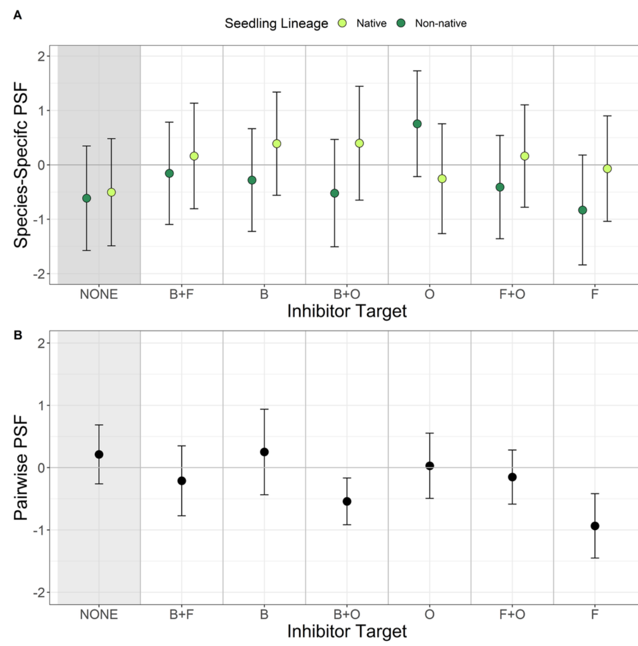

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