POTENTIAL ROLES OF SOIL PATHOGENS IN THE SUCCESS OF WETLAND PLANT SPECIES

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POTENTIAL ROLES OF SOIL PATHOGENS IN THE SUCCESS OF WETLAND PLANT SPECIES

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Pathogenic soil fungi and oomycetes are known to play key roles in the distribution and abundance of plant species in natural systems. Soil pathogens are thought to be important both in maintaining the diversity of plant communities and in facilitating the dominance of invasive plant species. However, much of what we know about soil pathogens in natural systems comes from "black box" soil experiments in which the roles of soil fungi and oomycetes are inferred by either sterilizing soils or treating them with fungicides. While this genre of experiments has repeatedly established relationships between soil pathogens and plant dominance in the landscape, they fail to provide mechanistic insight. In addition, very little is known about the impacts of pathogens on wetland plant species as most research has focused on forest and grassland systems, despite the fact that wetlands are among the most highly invaded ecosystems.

Through my dissertation research I sought to investigate soil fungal and oomycete communities with regard to wetlands and lay the groundwork for better understanding the roles of pathogens in the success of both native and non-native plant species. First, I determined if soil legacy effects of one plant species of interest, *P. australis,* negatively impact the performance of other plant species, a prerequisite if pathogen mediated plant-soil feedbacks are contributing to its invasive success. Second, I looked at the host range and virulence of the oomycetes associated with *P. australis* and other marsh plant species in an attempt to understand the potential of

pathogenic species to affect marsh seedling establishment. Third, I characterized the fungal communities associated with seeds of various plant species overwintering in marsh wetland field soils to determine the effect of those fungi on seed viability and survival. Fourth, I coupled a reciprocal plant-soil feedback experiment with the characterization of soil fungal communities associated with locally dominant native and non-native plant species that displayed different feedback responses to better understand the potential relationships of specific fungal groups to invasive success.

BIOGRAPHICAL SKETCH

Ellen Veronica Crocker was born on April 27, 1984 to Lina and Kelly Crocker in Lexington, Kentucky. After graduating from Lafayette Senior High School, she studied biology and history at Williams College in Williamstown, Massachusetts. She completed a senior thesis in plant-insect interactions with Dr. Joan Edwards and graduated with a B.A. in 2006. She worked as a staff research associate in the laboratory of Dr. Matteo Garbelotto at UC Berkeley from 2007 to 2009, studying and conducting extension activities in the area of forest pathology. In the fall of 2009 she began a Ph.D. program at Cornell University in the department of Plant Pathology and Plant-Microbe Biology in the laboratory of Dr. Eric Nelson.

Dedicated to my mom and dad.

Thank you for your love and support.

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Chapter 1: Soil legacy effects of *Phragmites australis* on native wetland plant survival

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Abstract

Interactions between introduced plants and soils they colonize are central to invasive species success in many systems. Below ground biotic and abiotic changes can influence both success of introduced species as well as their native competitors. All plants alter soil properties after colonization but, in the case of many invasive plant species, it is unclear if the strength and direction of soil legacy effects are due to plant traits, plant origin or to local population characteristics and site conditions in the invaded range. *Phragmites australis* in North America exists as a mix of populations of different evolutionary origin. Populations of endemic native *P. australis americanus* are declining, while introduced European lineages are one of the most important wetland invaders rapidly expanding across much of the continent. We assessed soil legacy effects of native and non-native *P. australis* genotypes on early and late seedling survival of wetland plants. We further used soil biocide treatment to assess the role of soil fungi on seedling survival. Survival of seedlings in soils colonized by *P. australis* was either unaffected or negatively affected; no species showed improved survival in *P. australis* conditioned soils. Population or genotype of *P. australis* was a significant factor explaining the response of seedlings but origin (native or non-native) was not a significant factor. Our results highlight the importance of phylogenetic control when assessing impacts of invasive species to avoid conflating general plant traits with mechanisms of invasive success. Both native (non-invasive) and non-native (invasive) *P. australis* haplotypes alter soil conditions that reduce seedling survival of competing native plant species. Because the soil legacy effects of native and non-native *P. australis* are similar, this study suggests that the close phylogenetic relationship between the two plants, and not the invasive status of *P. australis*, is more relevant to their soil mediated impact on other plant species.

Introduction

Interactions between introduced plant species and the soils they colonize are increasingly being recognized for their central role in determining the success and failure of plants to establish, grow and become invasive (Hierro & Callaway 2003; Wolfe & Klironomos 2005; Reinhart & Callaway 2006; Mitchell *et al.* 2006; Inderjit & Van Der Putten 2010). Although all plants have species-specific effects on soil they colonize (Berg & Smalla 2009; Bardgett & Van Der Putten 2014) invasive plants often appear to alter soils to their advantage creating positive plant-soil feedback and promoting dominance (Bever 1994; Klironomos 2002; Kowalchuk *et al.* 2002; van Grunsven *et al.* 2007; Peterman *et al.* 2008; Berg & Smalla 2009; Diez *et al.* 2010; Fitzsimons & Miller 2010; Van Der Putten *et al.* 2013; Flory & Clay 2013). Soil biota contribute strongly to these negative plant-soil feedbacks and seedling survival appears a critical demographic stage in determining invasive success (Packer & Clay 2000; Blaney & Kotanen 2001; Reinhart & Clay 2009; Reinhart *et al.* 2010b). Sometimes invasive plant seedlings are less susceptible to soil pathogens (Reinhart *et al.* 2010b), alternatively, they are able to condition soil resulting in increased disease incidence on competing seedling (Beckstead *et al.* 2010).

Understanding effects of soil legacies is of considerable importance for conservation and management of plant invasions. Removal of introduced species may alleviate their impact on resource competition above and below ground, but if their soil conditioning legacies continue, they may impede successful site restoration (Suding, Gross & Houseman 2004; Suding & Hobbs 2009; Yelenik & Levine 2010). Furthermore, recent studies establish the importance of genotypes and plant functional traits as strong influences on soil biota (Van Der Putten *et al.* 2013), and their effect on associated consumers, such as amphibians (Martin & Blossey 2013).

The importance on intraspecific variation on effects of plant species and genotypes on soils can select for soil biota community composition, which, in turn, can affect above ground plant community composition (Van Der Putten *et al.* 2013). The ecological and evolutionary dynamics and impacts of these interactions have only recently become the focus of investigations in non-agricultural systems. Further evaluation of impacts and mechanisms would greatly enhance the ability of societies to address the purposeful and accidental movement of introduced and potentially invasive species, as well as the recovery and conservation of rare or declining species that appear to suffer the most from negative soil feed backs.

We were interested in assessing how wetland plant communities in North America are affected by introduced genotypes of *Phragmites australis* and the role soil microbial communities may have in determining the outcome of these interactions. Introduced from Europe, *P. australis* is one of the most important invasive plants spreading through North America forming dense monocultures along roadsides, in tidal areas and in wetlands (Chambers, Meyerson & Saltonstall 1999; Saltonstall 2002). The spread of *P. australis* is unique since endemic native haplotypes, subspecies *Phragmites australis americanus* (Saltonstall, Peterson & Soreng 2004), are widespread on the continent and are being replaced by the advancing European genotypes (Saltonstall 2002; 2003; Meadows & Saltonstall 2007). There are large overall similarities in growth pattern and other traits between native and introduced genotypes (Park & Blossey 2008; Martin & Blossey 2013), but native genotypes are generally considered non-invasive although certain populations can rapidly expand (Lynch & Saltonstall 2002). Observational evidence suggests that floristic diversity is higher in *P. a. americanus* stands whereas dense near monospecific stands of *P. australis* are of great concern to wetland managers. These concerns have led to widespread and extensive herbicide control campaigns

that are largely unsuccessful (Martin & Blossey 2013) given rise to an attempt to develop a biological control program (Tewksbury *et al.* 2002).

The advancement of *P. australis* involves long distance dispersal via seed and rhizome fragments as well as local clonal spread through rhizomes (Chambers *et al.* 1999; 2003; Jodoin *et al.* 2008; McCormick *et al.* 2010a; Belzile *et al.* 2010; McCormick *et al.* 2010b). In contrast, native wetland plants commonly regenerate from long-lived seedbanks often on exposed mudflats after water draw downs (van der Valk 1981; van Grunsven *et al.* 2007). Seedling establishment is therefore key to the success of *P. australis* (Belzile *et al.* 2010)*, P. a. americanus* and other native plant species. Suggestions that *P. australis* may produce root-secreted allelopathic gallic acid inhibiting other plant species (Galatowitsch, Anderson & Ascher 1999; Zedler & Kercher 2004; Rudrappa *et al.* 2007; Bains *et al.* 2009) are contested (Weidenhamer *et al.* 2013), yet overall soil legacy effects of introduced *P. australis* on germination and seedling recruitment of native competitors remain unclear. We established a common garden and a field transplant experiments to assess soil legacy effects that may contribute to the success of introduced *P. australis* on early life history (first 4 weeks including germination) and late (two months after germination) seedling survival of native plant species. We tested the following hypotheses: 1) successful germination and early seedling survival will be higher in soils conditioned by native *P.a. americanus* than in soils conditioned by introduced *P. australis;* 2) fungicide application will eliminate negative soil legacy effects of *P. australis*; and 3) in the field, soils conditioned by introduced *P. australis* will reduce seedling survival compared to seedling survival in the surrounding wetland species matrix.

Materials and Methods:

Germination and early seedling survival

In summer of 2008 we established a common garden at the Cornell Resource Ecology and Management Facility (REM) in Ithaca, New York growing *P. australis* and *P. a. americanus* genotypes in 10m-long, 50cm wide and 50cm deep trenches lined with pond-liner (45 mil EPDM [Ethylene Propylene Diene Monomer], Pondliner.com, Shawnee, Oklahoma) and filled with Cornell compost mix (Cornell University, Ithaca NY). We propagated plants from rhizome cuttings obtained from Maine (ME), Minnesota (MN), New York (NY), South Dakota (SD) and Washington (WA) (see Table S I.1 in Supporting Information). Within each state we were able to pair collection locations because populations of native *P. a. americanus* and introduced *P. australis* existed within a short distance from each other allowing us to reduce effects of longitudinal and latitudinal influences on our results. To reduce potential maternal effects, we propagated initial field collected rhizome cuttings for 2 years in a common garden in 100L tree pots (BFG Supply, Lancaster, New York, USA) filled with commercial potting soil (Farfard Canadian growing mix No. 1-P, Agawam, Massachusetts, USA) before obtaining fresh rhizome cuttings in 2008 for our trench experiment. We completely randomized planting locations within our common garden and established 5 replicate trenches for each population and allowed plants to expand through clonal growth within their trenches (other plants were regularly removed) until they were well established.

Two years after planting, we sampled soil from the rhizosphere of 3 - 5 trenches per population on 11 August 2010 and homogenized samples for each population. We also collected control soils in trenches without *P. australis* growth but otherwise treated in an identical manner. After homogenizing, we filled 107 ml individually labelled plastic containers (Ray Leach Cone-tainer SC7U, Tangent, Oregon) with soil from each population, and arranged them randomly in

plastic trays (98 containers/tray) that kept containers 5 cm off the ground and 2 cm apart. On 12 August 2010, we treated half of the containers from each population and half of the unconditioned control containers with a broad-spectrum non-systemic fungicide, Daconil Weather Stik® (active ingredient chlorothalonil, Syngenta, Greensboro, North Carolina), at the highest recommended single field application rate (0.125 mg active ingredient/cm^2). We randomly arranged trays outdoors in a walk-in field cage (Lumite® screening, shade 15%, porosity 1629CFM, Synthetic Industries, Gainesville, GA, USA; exposing seeds to outside fluctuating summer conditions but preventing bird or mammal disturbances) and rearranged containers every week.

We purchased seed of *Asclepias incarnata* "swamp milkweed", *Astragalus canadensis* "Canadian milkvetch"*, Calamagrostis canadensis* "bluejoint grass", *Carex lacustris* "lake sedge", *Epilobium glandulosum* "northern willowherb", *Eupatorium maculatum* "spotted Joe-Pye weed"*, Euthamia graminifolia* "grass-leaved goldenrod", and *Juncus effusus* "common rush" from Prairie Moon, Winona, Minnesota and *Phalaris arundinacea* "reed canarygrass" from River Source Botanical, Taos, New Mexico. Where necessary, we cold stratified seeds according to grower's directions. All species show high seed viability and very rapid germination under suitable conditions (Baskin 2001). On 15 August 2010 we planted 5 or 20 seeds per species (Table S I.2) into each container. The difference in number of seeds was based on expected early seedling size. As designed, this experiment integrates the combined effects of potential suppression of germination, very early death upon germination but before emergence of cotyledon, as well as very early seedling mortality before seedlings can be called established. Except for the death of seedlings with established above ground shoots, this mortality is often difficult to observe. We established 10 replicate containers for each combination of species/ soil

type/ fungicide treatment for a total of 1980 containers [11 soil types (5 *P. a. americanus*, 5 *P. australis*, 1 control) x 2 soil treatments (none, fungicide) x 9 plant species x 10 replicate containers]. We watered containers every 3-5 days, recorded the number of surviving seedlings every 5 days and scored final seedling survival on 15 September 2010, when we terminated the experiment.

Transplant survival

We assessed *P. australis* effects on wetland seedling survival and growth at four field sites in the Montezuma Wetlands Complex, Savannah NY (see Table S I.3). We were unable to include *P. a*. *americanus* impacts in this experiment because existing stands in the study area are small and growing intermixed with other plant species preventing us from isolating *P. a. americanus* -specific effects. We selected locations with dense *P. australis* populations adjacent to mixed wetland plant communities. At each site we located four 3x3 m plots, two in the interior (at least 5 m from the edge of the *P. australis* stand) and two in diverse marsh vegetation at least 5 m away from the edge of the *P. australis* invasion front. Plots at each site were within 50 m of each other and all sites were within 10 km of each other. At each plot, we removed all above ground vegetation using clippers and cleared the area of leaf litter to expose the wetland soil surface. This treatment kept below-ground rhizomes and roots intact but eliminated potentially confounding effects of light competition on seedling survival. We continued to weed experimental areas weekly by hand to minimize soil disturbance until the termination of the experiment.

We propagated seedlings of seven plant species of which five (*A. incarnata, A. canadensis, C. canadensis, E. glandulosum,* and *E. graminifolia* were also used for the early

seedling growth experiment (Table S I.2), We purchased seed of *Elymus riparius* "riverbank wildrye," *Mimulus ringens* "monkeyflower," and *Muhlenbergia glomerata* "marsh muhly" from Prairie Moon, Winonoa, Minnesota, USA). These plants span a wide phylogenetic range yet are easy to propagate. We followed species-specific germination requirements and grew plants until they were approximately 2 months old in a greenhouse at Cornell University in a potting mix/sand media.

Before field transplanting, we established a grid (1.8 x1.8 m, cell size 20 x 20 cm) in June 2011 in each cleared plot and randomly planted 1120 individuals (10 individuals x 7 plant species x 4 plots per site x 4 sites). For each plot we selected similar sized individuals of each plant species and randomly assigned them to specific cells. Sites were planted on consecutive days to minimize drying while waiting to be transplanted. We watered plants weekly due to an extended drought at all field sites in summer 2011 and assessed plant survival at 4 weeks after transplanting.

Analyses

To test influence of soil properties on both early-seedling common garden and late-seedling field survival we employed generalized linear mixed models (GLMM) with binomial distribution. For early seedling survival in common garden soil we tested main effects of *P. australis* conditioning, origin (*P. a. americanus* or *P. australis*) and soil fungicide on seedling survival at four weeks. We included collection location as a random variable and ran separate analysis for each plant species. We evaluated differences between *P. australis* and *P. a. americanus* a posteriori by aggregating both levels (Crawley 2013). Starting with the full model [which included *P. australis* soil conditioning (control*, P. australis* or *P. a. americanus),* soil

fungicide application and their interaction] we reduced models in a backwards-stepwise process to determine the best model and significance via log-likelihood tests at P<-0.05. For late-seedling survival we tested main effects of *P. australis* colonization (invaded and non-invaded), with site as a random variable, and followed the same backwards-stepwise process to determine the best model. We used R version 3.0.1 (R Development Core Team 2013) and the add-on package 'lme4' (Bates *et al.* 2013).

Results

Germination and early seedling survival

Germination and early seedling survival was extremely variable among the different plants species ranging from >80% for *Ph. arundinacea* to <20% for *C. lacustris* (Fig. I.1) and there was a strong effect of population (Fig. S I.1). Soil conditioning by native and introduced, *P. australis* reduced survival for four of nine species (*C. canadensis, C. lacustris, E. graminifolia,* and *J.* effusus) while the others (*A. incarnata, A. canadensis, E. glandulosum, E. maculatum,* and *Ph. arundinacea)* remained unaffected (Fig. I.1, Table I.1). Effects of *P. australis* were either negative or neutral but never increased survival of any tested wetland species (Fig. I.1, Table I.1). For two species, *E. graminifolia* and *J. effusus*, we fund a significant origin effect, but reductions in survival were larger when soils were conditioned by native *P. a. americanus* compared to introduced *P. australis* or control soils (Fig. I.1, Table I.1). Fungicide application had no impact on seedling survival in control soils but increased survival in conditioned soils for all species except for *A. canadensis* and *Ph. arundinacea* (Fig. I.1, Table

I.1). We found a significant interaction of fungicide application and origin for *J. effusus* with fungicide increasing the survival of *P. australis* more than *P. a. americanus* (Fig. I.1, Table I.1).

Transplant survival

Transplant survival varied dramatically among species and locations (Fig. I. 2) with all species showing 60 - >80% survival when transplanted into a wetland matrix. In contrast, transplant survival was greatly reduced for all species when growing inside *P. australis*, but differences in survival were only significant for *A. canadensis* and *C. canadensis* (Fig. I.2, Table I.2). Transplant survival within *P. australis* stands differed widely by site, with the lowest survival at Carncross (9%, mean of all species) but very high at Teal Pond (94%, mean of all species) (Fig. S I.2).

Discussion

We designed our common garden and field experiments to assess potential mechanisms contributing to invasiveness of introduced *P. australis* in North America using plant-soil feedback theory (PSF). PSF theory predicts that invasive plant species can engineer a competitive advantage over native plants through soil legacy effects (Hierro & Callaway 2003; Saltonstall 2003; Wolfe & Klironomos 2005; Eppinga *et al.* 2006; Reinhart & Callaway 2006; Mitchell *et al.* 2006; Mangla & Callaway 2007; Beckstead *et al.* 2010). Our results demonstrate that, consistent with PSF theory, *P. australis* amplifies abundance, or facilitates colonization, of fungi that reduce survival of competing wetland plant species. This is clearly a soil conditioning effect in response to active plant growth since control soils without *P. australis* growth do not

show this effect (Fig. I.1). However, this effect is not restricted to introduced *P. australis* and its native congener, *P. a. americanus*, has similar negative impacts on survival of other wetland plants (Figs. I.1, S I.1). In contrast to our hypothesis, the strength of suppression does not depend on origin. Furthermore, even for those species for which origin was a significant factor (*E. graminifolia* and *J. effusus*), effect sizes in the common garden study were small questioning their ecological relevance in affecting plant community dynamics. While we were unable to incorporate origin effects into our transplant study, lack of significance in survival between individuals growing inside and outside of *P. australis* patches suggest that PSF theory and soil legacy effects alone appear to have little power to explain invasiveness of introduced *P. australis* genotypes.

While our experiments covered only the very early life history of wetland species, and the potential for effects to change or magnify over time does exist, our results suggest that invasive success of *P. australis* does not depend on unique soil conditioning mechanisms that distinguish it from native *P. a. americanus*. These results support other studies reporting trait similarities between *P. a. americanus* and *P. australis* (Saltonstall 2002; Park & Blossey 2008) as well as effects on consumers (Martin & Blossey 2013; Larochelle *et al.* 2014). However, field evidence clearly shows rapidly expanding populations and development of near monocultures of introduced *P. australis* across many coastal and inland wetlands in North America (Saltonstall 2002; 2003). In our attempt to assess PSF mechanisms, we eliminated effects of resources competition, for example for light and nutrients by cutting above ground vegetation, yet particularly plant height and clonal extent are important factors in determining competitive hierarchies in wetland plant communities (Gaudet & Keddy 1988; Keddy & Shipley 1989; Keddy, Twolan-Strutt & Wisheu 1994). Various other factors, including superior photosynthetic

capacity (Mozdzer & Zieman 2010) suppression of competitors by shade and litter (Haslam 1971a; b; Minchinton, Simpson & Bertness 2006; Holdredge & Bertness 2010), shoreline development and eutrophication (Bertness, Ewanchuk & Silliman 2002; Holdredge, Bertness & Wettberg 2010), as well as effect of consumers such as herbivorous crabs (Holdredge, Bertness & Altieri 2008) are frequently mentioned to explain the invasive success of introduced *P. australis*. In contrast, some experimental evidence suggests that functional group identity and diversity of resident plant communities may represent a form of biotic resistance (Byun, de Blois & Brisson 2012).

Our results establish the importance of soil fungal communities, the importance of *P. australis* or *P. a. americanus* population (but not origin) and growing location on survival of different wetland competitor species. This was especially prominent in our transplant experiment where seedlings survival at one site was unaffected by *P. australis* invasion. While this may be a function of site-specific conditions, *P. australis* colonization at this site may also be more recent and not have accumulated negative soil feedbacks (Packer & Clay 2004; Meadows & Saltonstall 2007; Diez *et al.* 2010). How soil fungal communities may interact with other factors reported to facilitate introduced *P. australis* invasion will require more detailed, and more long-term investigations that go beyond the typical experimental investigations of a few years but may offer some intriguing potential for invasion management.

Our results of fungicide treatments suggesting the importance of soil fungi in seedling establishment are consistent with many other studies that point to soil pathogens as key contributors to soil legacy effects (Klironomos 2002; Lynch & Saltonstall 2002). However, identities of soil fungi that may contribute to reduced seedling survival remain unknown. Although *P. australis* is known to host diverse assemblages of fungi (Wirsel *et al.* 2001; Neubert

et al. 2006; Angelini *et al.* 2012; Fischer & Rodriguez 2013) their specific roles in limiting seedling survival are largely unknown. Similarly, *P. australis* also associates with diverse communities of oomycetes (Chambers *et al.* 1999; 2003; Nechwatal, Wielgoss & Mendgen 2005; Nechwatal & Mendgen 2006; Nechwatal, Wielgoss & Mendgen 2008; Jodoin *et al.* 2008; McCormick *et al.* 2010a; Belzile *et al.* 2010; McCormick *et al.* 2010b; Nelson & Karp 2013) that are known to affect plant survival and would have been inhibited by the broad spectrum fungicide used.

The responses of plants to *P. australis* soil conditioning, especially to decrease in soil fungi from fungicide application, varied dramatically among species. Among out other wetland competitors was only one other invasive species, *P. arundinacea*, which was not affected by the fungicide treatment. Others have suggested that invasive success may be conveyed by resistance to soil pathogens (Belzile *et al.* 2010; Reinhart *et al.* 2010a). However, *A. canadensis*, a native North American plant species, also was not affected changes in soil fungal communities as a result of fungicide treatment, suggesting that soil legacy are species-specific and do not follow plant origin.

Our experiments show that the soil legacy of *P. australis* has a significant negative impact on the seedling survival of many plant species. Because of their impact on seedling survival, soil legacy effects, caused by changes to soil biota or to other abiotic soil properties, likely contribute to plant population dynamics. However, our results also suggest that these soil legacy effects are not necessarily determined by the invasive nature of the plant, indicating that invasive status alone is not sufficient to explain its soil-mediated impact on other plant species at the seedling stage.

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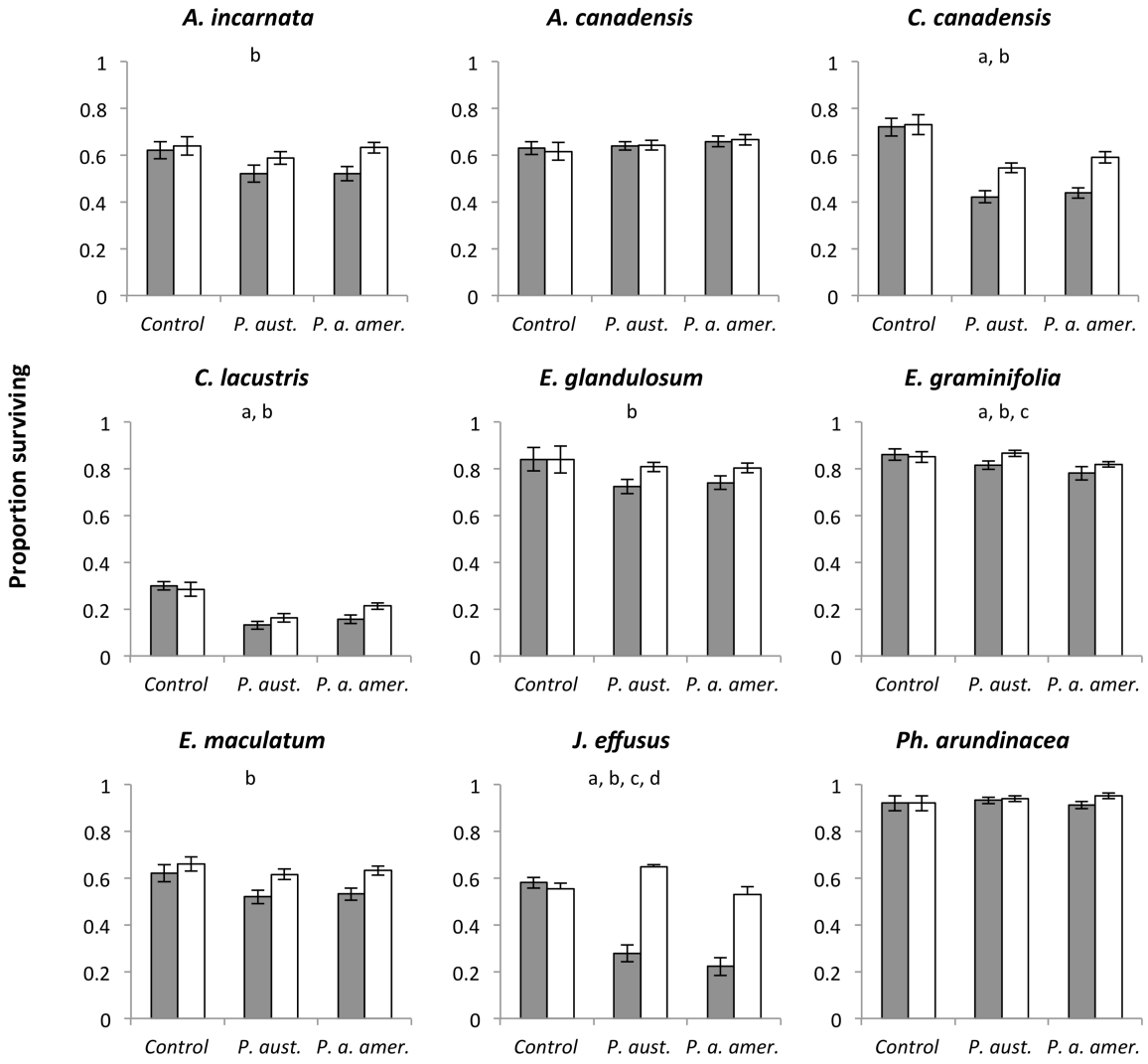
Figure Legends:

Figure I.1. Proportion early seedling survival of nine plant species when sown onto experimental soils (Soil types: control, conditioned by *P. australis* , conditioned *P. a. americanus*, fungicide untreated soil (grey bars) and fungicide treated soil (white bars). Data are means ± 1SE with either 10 (control soils) or 50 (all other treatments) replicates.

Figure I.2. Probability of survival when transplanted into *P. australis* (grey bars) or the adjacent wetland plant community (white bars) for seven different species. Data are means of each plant species tested (n=4 sites). Asterisk (*) indicate significant differences (GLMM model, $p < 0.05$).

Fig. I.1.



[a] significant *P. australis* soil conditioning effect (control vs. *P. australis/P. a. americanus*)

[b] significant fungicide effect

[c] significant origin effect (*P. a. americanus* vs. *P. australis*)

[d] significant fungicide and origin interaction

Fig. I. 2.

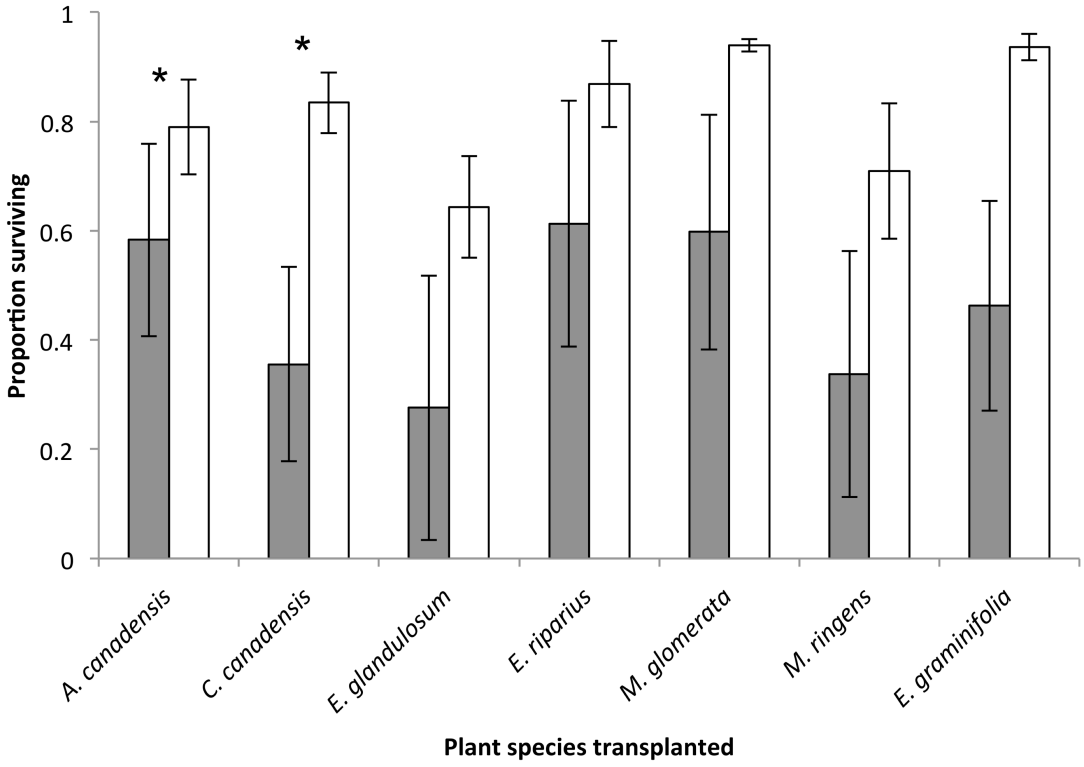

1
0.8
0.6
0.4
0.2
0
Proportion surviving
*
*
A. canadensis
C. canadensis
E. glandulosum
E. riparius
M. glomerata
M. ringens
E. graminifolia
Plant species transplanted

Table I.1. Model results for effect of *Phragmites* presence on seedling survival for nine plant species in *P. australis* conditioned soil analyzed using a generalized linear mixed model (GLMM) with binomial distribution. Models included fungicide treatment and soil conditioning (control or *P. australis*[†]) as fixed effects and collection location as a random effect[‡].

Species	**Intercept**	**Fungicide (F)**	**Soil conditioning (S)** ***P. australis* (EU) and/or *P. a. americanus* (NA)**	**F x S interaction**
Asclepias incarnata	*0.13 ± 0.15*	*0.35 ± 0.12* **		
Astragalus canadensis	*0.61 ± 0.05*			
Calamagrostis canadensis	*0.73 ± 0.29*	*0.53 ± 0.09***	-0.99 ± *0.31***	
Carex lacustris	*-1.03 ± 0.61*	*0.28 ± 0.08***	*-0.87 ± .66***	
Epilobium glandulosum	*1.12 ± 0.19*	*0.40 ± 0.15***		
Eupatorium maculatum	*0.14 ± 0.09*	*0.38 ± 0.12***		
Euthamia graminifolia	*1.66 ± 0.11*	0.27 ± *0.08****	*-0.1 ± 0.24**** (NA)	
			*-0.39 ± 0.24**** (EU)	
Juncus effusus	*0.32 ± 0.59*	*-0.10 ± 0.20*	*-1.36 ± 0.65* (EU)	*1.80 ± 0.23**** (EU)
			*-1.68 ± 0.65*** (NA)	*1.60 ± 0.23* *** (NA)
Phalaris arundinacea	*2.63 ± 0.12*			

[†] *P. australis* and *P. a. americanus* combined unless origin is significant

[‡] Empty cells denote parameters that were not part of the best model.

*, **, *** Asterisks indicate P-values from log-likelihood tests between a model without the term and a model with all terms included (* P < .05; ** P < .01, *** P < .001).

Table I.2. Model results for effect of *P. australis* presence on seedling survival for seven plant species analyzed using a generalized linear mixed model (GLMM) with binomial distribution. Models included *P. australis* presence (P) as fixed effects and site as a random effect. We determined significance of each factor in a best model by log likelihood comparisons of best model and model missing each factor (* P < .05; ** P < .01, *** P < .001).

Species	**Intercept**	***P. australis* presence**
Astragalus canadensis	*0.33 ± 0.49*	*1.58 ± 0.52 **
Calamagrostis canadensis	*-0.91 ± 0.75*	*2.85 ± 0.74 **
Epilobium glandulosum	*-0.35 ± 1.16*	
Elymus riparius	*1.56 ± 0.87*	
Euthamia graminifolia	*1.46 ± 0.86*	
Muhlenbergia glomerata	*1.83 ± 0.95*	
Mimulus ringens	*0.16 ± 0.94*	

CHAPTER 1 SUPPORTING INFORMATION

Table S I.1. Collection locations of the *P. australis* populations.

Table S I.2. Plant species and number of seeds used in early seedling common garden (1) and late seedling field transplant (2) experiments.

Table S I.3. Site location and vegetation community composition for field transplant survival experiment.

Figure S I.1. Early seedling survival (%) of different plant species in control soils and soils conditioned by different populations of *P. australis* and *P. australis americanus.*

Figure S I.2. Proportion late-seedling survival when transplanted a) at each site outside and within *australis* patches

Chapter 2: Virulence of oomycete pathogens from *Phragmites australis*-invaded and non-invaded soils to seedlings of wetland plant species

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Abstract:

Soil pathogens affect plant community structure and function through negative plant-soil feedbacks that may contribute to the invasiveness of non-native plant species. Our understanding of these pathogen-induced soil feedbacks has relied largely on observations of the collective impact of the soil biota on plant populations, with few observations of accompanying changes in populations of specific soil pathogens and their impacts on invasive and non-invasive species. As a result, the roles of specific soil pathogens in plant invasions remain unknown. In this study, we examine the diversity and virulence of soil oomycete pathogens in freshwater marsh wetland soils invaded by non-native *Phragmites australis* (European common reed) to better understand the potential for soil pathogen communities to impact a range of native and non-native species and influence invasiveness. We isolated oomycetes from four sites over a two-year period, collecting nearly 500 isolates belonging to 36 different species. These sites were dominated by species of *Pythium* and many decreased seedling survival of a range of native and invasive plants. Despite any clear host specialization, many of the *Pythium* species were differentially virulent to the native and non-native plant species tested. Isolates from invaded and non-invaded soils were equally virulent to given individual plant species and no apparent differences in susceptibility were observed between the collective groups of native and non-native plant species.

Introduction:

Plant pathogens often have significant impacts on plant populations, where they may influence the diversity and structure of plant communities (Mangla & Callaway 2007; Beckstead *et al.* 2010; Mordecai 2011). This is particularly true for populations of non-native plant species for which plant pathogens are increasingly believed to play key roles in invasiveness (Inderjit & Van Der Putten 2010). Many invasive species are thought to experience reduced negative impacts from pathogens in introduced ranges relative to their native ranges (Callaway *et al.* 2011; Flory & Clay 2013; Maron *et al.* 2013b), in part because the composition and relative abundance of pathogens in introduced ranges differ from those in native ranges as a result of geographic isolation and local evolution (Rout & Callaway 2012). This leads to pathogen interactions in the introduced range that could potentially contribute to invasiveness through a number of different mechanisms including 1) the inhibition of pathogens in the introduced range by the invading plant species (Zhang *et al.* 2009; 2011), 2) reduced frequency or abundance of virulent taxa in the invaded range (Reinhart *et al.* 2010b; 2011), and 3) decreased susceptibility of introduced invasive plants to pathogens endemic to the invaded range (Klironomos 2002; Beckstead *et al.* 2010; Mordecai 2011). Each of these potential mechanisms would allow invasive plant species to serve as reservoirs for pathogen multiplication and spillback to native plant populations at local and regional scales (Flory & Clay 2013; Li *et al.* 2014).

Some have hypothesized that introduced plants should accumulate increasing populations and/or richness of pathogenic species with longer residence time in the invaded range (Mitchell *et al.* 2010; Flory, Kleczewski & Clay 2011). However, there is limited empirical support for this phenomenon (Mangla & Callaway 2007; Reinhart & Clay 2009; Hawkes, Douglas & Fitter 2010; Flory *et al.* 2011; Rout & Callaway 2012) and often interpretations of pathogen

accumulation are based on observations of plant impacts rather than quantitative changes in pathogen populations or species richness (Flory *et al.* 2011; Rout & Callaway 2012). Rarely have the soil pathogens associated with invasive plant species been described (Packer & Clay 2000; Zhang *et al.* 2009; Reinhart *et al.* 2010a; 2011; Callaway *et al.* 2011; Zhang *et al.* 2011; Nelson & Karp 2013; Li *et al.* 2014). Even for those invasive species where the plant-associated microbiota has been studied (e.g., *Prunus serotina* (Reinhart & Clay 2009; Reinhart *et al.* 2010b; 2011) and *Bromus tectorum* (Beckstead *et al.* 2010)), we have little understanding of the species composition, dynamics, and impacts of these pathogen communities on plant performance in their native and introduced ranges. From the few studies that have focused on pathogen populations, it is becoming clear that species within the oomycete genus *Pythium* can be significant regulators of native plant communities (Mills & Bever 1998; Packer & Clay 2000; 2003; 2004; Augspurger & Wilkinson 2007; Gómez-Aparicio *et al.* 2012) and, at the same time, facilitate invasiveness of a number of plant species (Reinhart *et al.* 2005; Reinhart & Clay 2009; Reinhart *et al.* 2010b; a; 2011; Butof & Bruelheie 2011).

Phragmites australis (Cav.) Trin. ex Steudel (European common reed) has emerged as one of the most significant invasive plant species in North America (Chambers, Meyerson & Saltonstall 1999; Saltonstall 2002). Over the past century, a European haplotype of *P. australis* has spread throughout North America into roadsides and wetland plant communities (Saltonstall 2002; Tulbure, Johnston & Auger 2007; Plut *et al.* 2011). Rapid long-distance dispersal of *P. australis* is largely the result of abundant and widespread seed production (Belzile *et al.* 2010) and subsequent dispersal through transportation corridors (Lelong *et al.* 2007; Jodoin *et al.* 2008). Although it is commonly believed that rhizome fragments contribute to long-distance dispersal based on anecdotal accounts, there is no experimental or theoretical evidence for this.

Once established, *P. australis* grows in characteristically dense stands (Saltonstall 2002). In contrast, there are over 14 native North American haplotypes (*P. australis* subsp. *americanus* [hereafter referred to as *P. a. americanus*]) (Saltonstall 2003a; b) that do not exhibit the same rapid range expansion or high stand density despite their phenotypic and genotypic similarity to *P. australis*.

Although many pathogenic fungi have been described from *P. australis* in both its native and introduced ranges (Ban, Engloner & Viranyi 2000; Nechwatal, Wielgoss & Mendgen 2005; Neubert *et al.* 2006; Nechwatal, Wielgoss & Mendgen 2008a; b; Wielgoss *et al.* 2009; Kurokawa & Tojo 2010), the impacts of these pathogens on *P. australis* and other native and non-native plant species have not been well studied. In both its native and non-native ranges, *P. australis* patches are dominated by diverse oomycete pathogens, especially of the genus *Pythium* (Nechwatal *et al.* 2008a; Nelson & Karp 2013). In the European native range of *P. australis*, the dominant *Pythium* (*Py*) species include *Py. phragmitis, Py. litorale*, and *Py. dissotocum*. These species are commonly recovered from submerged *P. australis* leaves and rhizosphere soils (Nechwatal *et al.* 2005) and are highly virulent to *P. australis* seedlings (Nechwatal *et al.* 2008a; b) and rhizomes (Nechwatal & Mendgen 2009; Nechwatal & Lebecka 2014). Similarly, a diversity of oomycetes have been identified from both *P. australis*-invaded and non-invaded soils (Nelson & Karp 2013), suggesting that multiple potential interactions between pathogens and both native and non-native plants could potentially influence *P. australis* invasiveness. Although many of the oomycetes previously detected from invaded and non-invaded soils are known to be pathogens of agricultural plants, their virulence to native and non-native wetland species is unknown.

To better understand these host-pathogen relationships, we isolated oomycetes from wetland soils colonized by either mixed non-native and native species including *P. a. americanus* but excluding *P. australis* (hereafter referred to as non-invaded soils) or dense stands of *P. australis* (hereafter referred to as *P. australis*-invaded soils). We determined the virulence of these oomycetes to a range of native and non-native wetland plant species. We sought to answer the following questions:

1) Can the pathogenic oomycete taxa known to be present in *P. australis*-invaded and non-invaded soils be isolated and grown in culture?
2) Are these oomycete species pathogenic to *P. australis* and other wetland plant species, including *P. a. americanus*?
3) Are pathogens from *P. australis*-invaded soils more virulent to a range of plant species than those from non-invaded soils?

Materials and Methods:

Study site and soil sampling:

We identified four sites within and near the Montezuma National Wildlife Refuge with *P. australis* populations (Supporting Table S II.1). These sites were chosen because of the immediate proximity of non-invaded sites that supported populations of *P. a. americanus* along with other native and non-native plant species. Non-invaded sites were characterized by mixed native and non-native plant communities separated by ≤ 100 m from *P. australis*-invaded sites, increasing the likelihood of similar microclimates, soil characteristics, and pre-invasion community composition. All sites were intermittently flooded, with the exception of the *P. australis*-invaded area at the Carncross site.

Rhizosphere soils were sampled (~40 g/sample) at 2-month intervals beginning mid May 2009 and ending in mid May 2010. Soils were collected to a depth of 15 cm within *P. australis* patches and immediately adjacent (≤ 10 cm) to individual *P. a. americanus* plants as described previously (Nelson & Karp 2013). *P. australis* and *P. a. americanus* populations were distinguished based on a number of morphological characteristics (Blossey 2002). Individual soil samples were pooled for each population (five soil samples were taken from each site to make up ~200 g soil from each population), placed in plastic bags, and transported in a cooler back to the laboratory for oomycete isolations.

Oomycete isolation and identification:

At each sampling time, oomycetes were isolated from soils using a *P. australis* leaf disk baiting method that was modified from commonly-used oomycete baiting procedures (Arcate, Karp & Nelson 2006). Approximately 5 g of rhizosphere soil collected at each time point from each of the *P. australis* populations were placed in petri plates and flooded with sterile distilled water. Leaf disks (5 mm-diam) were excised from *P. a. americanus* and *P. australis* leaves and floated on the surface of each of the flooded soils. In preliminary experiments no differences were detected in the oomycete taxa recovered from either *P. a. americanus* or *P. australis* leaf disks so we did not distinguish these isolates in our analyses. Baited soil samples were incubated at 18˚C in the dark for both 7 and 21 days at which time leaf disks were removed from the flooded soils, rinsed, and placed into fresh Petri dishes containing 10ml of sterile H_2O. After 7 or 21 days, leaf disks were plated onto a selective medium containing water agar amended with 50mg/mL rifampicin and penicillin G and incubated at 18˚C in the dark for 1 to 2 days until mycelium was visible. As mycelia emerged from baits, portions were transferred to clarified V8

juice agar (CV8A) (containing 200ml V8™ juice [centrifuged at 7000 rpm for 10 min to remove solids, then filtered through a glass fiber filter], 800ml Milli Q H_2O, 3g CaCO3, and 17g agar) then hyphal-tip transferred for subsequent experiments. One isolate was collected from each leaf disk. In preliminary experiments, the number and identity of species recovered from leaf disks incubated for 7 or 21 days did not differ and, although the bulk of the distribution of oomycete taxa is based on cultures obtained from baits incubated for 7 days, we do not distinguish these isolates here.

For isolate identification, we sequenced the internal transcribed spacer (ITS) 1 and 2 region of the rRNA operon. Mycelia from 5-day-old cultures (grown in 100 mm Petri dishes on top of a layer of cellophane) were scraped from the surface of the cellophane, lyophilized overnight, and kept at -20°C under argon in 2.2 ml micro centrifuge tubes. DNA was then extracted from 0.5g of ground mycelium using standard procedures. PCR reactions for DNA extracted from live cultures were carried out using the ITS1 and ITS4 primer pair (White *et al.* 1990; Arcate *et al.* 2006). Raw sequences were trimmed and edited in Sequencher 4.8 (Gene Codes Corp.), imported into MEGA 5.0 (Tamura *et al.* 2011) and aligned using the Clustal W algorithm (Chenna 2003) under default settings. After initial alignments, sequences were manually edited using MEGA 5.0 to correct misaligned sequences and ambiguous base designations. During this final editing, all alignments were further trimmed to a fixed length ranging from 511-576 bp (gaps included), depending on the alignment grouping. Species identity of each isolate was then assigned using the best BLAST match from the NCBI database. We consolidated isolates from species complexes that could not be differentiated (Levesque & Cock 2004) to reduce confusion in species assignment: sequences matching *Py. dissotocum, Py. dicilinum* or *Py. lutarium* could not be resolved and these isolates were all designated as *Py.*

dissotocum. Similarly, *Py. folliculosum, Py. catenulatum* and *Py. torulosum* sequences could not be resolved and isolates were all designated as *Py. torulosum.* We excluded from further analyses those *Pythium* species for which no clear species designation could be inferred.

Virulence bioassay procedure:

We evaluated the impacts of oomycete inoculation on seedling survival for several native and non-native plant species: *P. australis* ("Common reed"), *P. a. americanus* ("Common reed"), *Epilobium glandulosum* ("Northern Willowherb"), *Muhlenbergia glomerata* ("Marsh muhley"), *Euthamia graminifolia* ("Grass-leaved goldenrod"), *Lythrum salicaria* ("Purple loosestrife"), and *Phalaris arundinacea* ("Reed canarygrass"). Not all plant species/ *Pythium* species combinations were conducted, due to limited availability of some seeds (Table 1). The plant species we tested were chosen because they represent a phylogenetic range of wetland species and because all are well suited to the bioassay technique we use (i.e., they exhibited high germination rates, no stratification period required, and seeds of all species were available). Seeds of *P. australis, P. a. americanus, L. salicaria,* and *Ph. arundinacea* were collected from nearby field locations whereas *E. glandulosum, E. graminifolia*, and *M. glomerata* were obtained from Prairie Moon Nursery, Winona, Minnesota. All seeds were surface sterilized prior to use in bioassays by dipping seeds sequentially for 2 min each in 70% ethanol, 0.25% sodium hypochlorite, and 70% ethanol. A 10 sec water rinse followed each of the sterilizing solutions.

We conducted two sets of bioassay experiments. First, we compared the virulence of different *Pythium* species by selecting one representative isolate for each of 20 different *Pythium* species isolated from our wetland sites. We selected these species because they represented a broad phylogenetic range within the genus *Pythium* (Levesque & Cock 2004; Uzuhashi,

Kakishima & Tojo 2010). Second, we compared the virulence of isolates of a given *Pythium* species from *P. australis*-invaded soils with isolates of that same species from non-invaded soils. We chose six different *Pythium* species to test because we recovered at least 3 isolates of each from both *P. australis*-invaded and non-invaded soils. We selected an equal number of isolates of a given species from each soil.

For both sets of bioassays, isolates were grown for 10-20 days on CV8A and the cultures were allowed to completely colonize the plate surface. Ten surface-sterilized seeds of a given plant species were placed on the surface of each of 7-10 replicate plates for each isolate/plant species combination and allowed to germinate and grow in an incubator alternating between 12-hours light at 30° C and 12-hours dark at 10° C (Ekstam & Forseby 1999). Non-inoculated plates containing only sterile CV8A were used as controls for seedling survival (Fig. II.1). Plates containing seedlings were regularly monitored and their position in the incubator was reassigned weekly. Seedling survival was assessed after 3 weeks. Both sets of bioassays were conducted in temporally staggered blocks by *Pythium* species.

Because oomycete pathogens are known to affect very early stages of plant development, we assessed both seed germination and seedling survival. For our purposes, we define seed germination as the emergence of the radicle whereas seedling survival represents plants that develop healthy cotyledons over the 3-week period of our bioassay.

Statistical analyses:

Statistical analyses of virulence bioassays were conducted using the statistical package JMP (SAS Institute Inc.). For the purpose of our assays we defined pathogenic isolates as those that significantly decreased seedling survival relative to non-inoculated control seeds. Virulence

was defined as the degree of this decrease in survival relative to other isolates tested on the same plant species. Because seedling survival was normally distributed, our analyses compared the continuous variable of percentage seedling survival, instead of binomial survival/ death. The pathogenicity of each individual isolate to a given plant species was determined using a Dunnett's test to compare mean percent seedling survival at 3 weeks when seeds were grown on inoculated plates versus control plates. The virulence differences between isolates from invaded and non-invaded soils was determined using a Tukey's HSD test to compare the mean percentage of seedlings alive at 3 weeks. Significant differences were determined at a *P*-value of less than 0.05. For each inoculation treatment, replicates were the percentage of seedlings surviving within a given Petri dish. We selected 3-weeks for survival assessments because this period allowed sufficient time for all viable seeds to germinate, but avoided the point where seedlings began to show signs of distress due to the limited space and nutrients of our bioassays. We also assessed seed germination rates but do not report them here because we observed no significant differences within plant species by inoculation.

Results:

From both *P. australis*-invaded and non-invaded soils, we collected a total of 496 oomycete isolates representing 36 species. Nearly all of these 36 species were members of the genus *Pythium* (Fig. II.2). The only other oomycete genera isolated were *Phytopythium* and *Saprolegnia*, each with low isolation frequencies of <0.01%. Some *Pythium* species were recovered at relatively high frequencies, whereas a number of other species were found only at a single invaded site. Because of the wide variation in the relative frequency and diversity of *Pythium* species and relatively small sample sizes, isolation frequencies of individual *Pythium*

species from non-invaded and *P. australis*-invaded soils could not be compared (Supporting Figure S II.1 and S II.2). Some species were found exclusively in *P. australis*-invaded soils whereas a number of other species were found only in non-invaded soils (Fig II.2) or at a single non-invaded site (Supporting Figure S II.2). No single *Pythium* species was recovered from all soils however, some species, such as *Py. inflatum, Py. dissotocum, Py. heterothallicum*, and *Py. monospermum,* were found in soils across all non-invaded sites.

None of the *Pythium* species tested reduced the germination of seeds. However, despite the lack of direct seed infection, seedlings were subsequently infected. All *Pythium* species that we tested significantly reduced seedling survival of at least one of the plant species and many were pathogenic to nearly all (Table II.1). Although virulence of individual *Pythium* species varied greatly, some, such as *Py. aquatile, Py. citrinum, Py. heterothallicum*, and *Py. perplexum* only moderately decreased survival in a few host plants while others, such as *Py. phragmitis, Py. volutum, Py. pyrilobum, Py. irregulare*, and *Py. dissotocum* were highly virulent pathogens of nearly all plant species tested.

Seedling survival following inoculation with *Pythium* species varied greatly by plant species. *E. glandulosum* and *L. salicaria* were the most susceptible plant species, exhibiting the largest decrease in survival when inoculated with different *Pythium* species (Supporting Figure S II.4). *Ph. arundinacea*, on the other hand, was the only plant species we tested where none of the *Pythium* isolates reduced seedling survival. The susceptibilities of seedlings of the two *Phragmites* haplotypes were similar. However, *P. a. americanus* but not *P. australis* was susceptible to *Py. angustatum* and *Py. heterothallicum* whereas *P. australis* but not *P. a. americanus* was susceptible to *Py. aquatile, Py. attrantheridium, Py. oopapilum,* and *Py. perplexum* (Table II.1). Collectively, *Pythium* species recovered from *P. australis*-invaded soils

were more virulent to *P. australis*, *P. a. americanus*, and *M. glomerata* than those recovered from non-invaded soils (Figure II.3). However, no differences in virulence were observed between *Pythium* species from invaded and non-invaded soils for any of the other plant species tested.

The virulence of individual isolates of *Py. angustatum, Py. attrantheridium, Py. dissotocum, Py. heterothallicum* and *Py. inflatum* collected from non-invaded soils did not differ from the virulence of isolates of the same species collected from *P. australis*-invaded soils (Supporting Figure S II.4). However, isolates of *Py. dissimile* from *P. australis*-invaded soils (which came from only one site) were significantly more virulent to *M. glomerata, P. a. americanus,* and *P. australis* than isolates from non-invaded soils and this difference in the virulence of *Py. dissimile* was responsible for an overall higher virulence of isolates from *P. australis*-invaded soils. Although *Py. dissimile* was highly virulent to both *E. glanulosum* and *L. salicaria,* no differences in virulence to these species were observed between isolates from non-invaded and *P. australis*-invaded soils.

Discussion

The major goal of our work was to assess the pathogenicity and virulence of oomycete species isolated from *P. australis*-invaded and non-invaded soils to native and non-native wetland plants. While our results have confirmed the high prevalence of *Pythium* species in both invaded and non-invaded soils, they have also revealed the wide differential virulence of many of these generalist pathogens to a range of wetland plant species. Perhaps among the more significant findings from our work was the observation that isolates of most *Pythium* species did not differ in their virulence to individual plant species regardless of whether they were isolated

from invaded or non-invaded soils. Additionally, the observation that the susceptibility of native species as a group to a given *Pythium* species was no different than the susceptibility of non-native plants suggests that the invasiveness is likely to be context specific. Communities of particular plant species may provide more or less biotic resistance to invasion, depending not only on pathogen prevalence, but also on the collective susceptibilities of the plants in invaded communities (Kardol *et al.* 2007; Harrison & Bardgett 2010; Van de Voorde, Van Der Putten & Bezemer 2011).

We chose to focus on oomycete pathogens because our previous work had identified diverse communities of *Pythium* species associated with *P. australis* and *P. a. americanus* populations (Nelson & Karp 2013). Pathogens within this genus are largely generalists with broad host ranges of plant species (Supporting Table S II.2), commonly attacking juvenile tissues where they impact seedling performance (Martin & Loper 1999) and recruitment (Augspurger & Wilkinson 2007). Additionally, this group of soil pathogens is recognized for contributing to negative soil feedbacks and potentially to invasiveness (Reinhart *et al.* 2010b; Callaway *et al.* 2011). Of the most prevalent species of *Pythium* from invaded soils, only *Py. dissotocum* and *Py. heterothallicum* are widespread and commonly recognized as generalist pathogens (van der Plaats-Niterink 1981), in part because there have been few previous reports of the distribution, virulence, or host ranges of many of the other abundant *Pythium* taxa, with perhaps the exception of *Py. arrhenomanes* and *Py. torulosum*, both of which are common pathogens of the Poaceae (Sprague, 1950).

The culture-based oomycete community characterization used in our current study revealed the presence of different *Pythium* species from those we previously detected using a DNA-based characterization (Nelson & Karp 2013). Although a number of *Pythium* species were

detected by both methods, some species were not detected previously whereas other previously-detected species were not isolated in our current study. While such findings point to the importance of multiple approaches for assessing pathogen communities associated with plants, it also reveals that the actual diversity of *Pythium* species in these wetland soils may be considerably greater than what we describe here. The reasons for the differential detection are unclear but may relate, in part, to the selectivity of our isolation method, which favors those species that are able to produce zoospores under the temperature and flooding conditions during isolation, enabling them to more readily colonize baits (Arcate *et al.* 2006; Nechwatal *et al.* 2008a). Since zoospores are the key developmental stage of *Pythium* species that leads to plant infection (Deacon & Donaldson 1993), it is likely that culture-based studies may provide the most meaningful assessments of the important pathogenic species. However, the isolation conditions would need to be varied to include a broader range of environmental conditions for zoospore production, since this has been shown to influence the species isolated (Fuller & Jaworski 1987).

There have been a number of mechanistic explanations for how the interactions of soil pathogens with native and non-native plants might facilitate invasiveness of an introduced plant species (Catford, Jansson & Nilsson 2009). Currently, most of the proposed pathogen-mediated mechanisms involve variations of Janzen-Connell phenomena (Nijjer, Rogers & Siemann 2007; Peterman *et al.* 2008), differential plant-soil feedbacks (Inderjit & Van Der Putten 2010; Eviner & Hawkes 2012; Suding *et al.* 2013), or spillover/spillback phenomena (Eppinga *et al.* 2006; Mangla & Callaway 2007; Beckstead *et al.* 2010; Flory *et al.* 2011; Flory & Clay 2013; Li *et al.* 2014). Nearly all proposed mechanisms are based on observations of above-ground plant responses and assume either 1) differential pathogen distributions (i.e., greater pathogen species

richness in the native non-invaded ranges than in the invaded range) (Mitchell & Power 2003), 2) differential host specialization among pathogens affecting the introduced species in the invaded range compared to the native non-invaded range allowing for pathogen escape (Keane & Crawley 2002; Colautti *et al.* 2004; Halbritter *et al.* 2012), 3) differential virulence of pathogens from invaded and non-invaded ranges to the introduced species (i.e., pathogens in the native range more virulent than those in the invaded range) (Reinhart *et al.* 2010b; 2011; Callaway *et al.* 2011) or 4) pathogen-mediated apparent competition between native and introduced plants in the invaded range (i.e., greater susceptibility of native plants than the introduced invader to pathogens in the introduced range) (Klironomos 2002; van Grunsven *et al.* 2007; Gilbert & Parker 2010; Zuppinger-Dingley *et al.* 2011).

If the invasiveness of *P. australis* was due solely to escape from the impacts of key pathogens present in the native range of *P. australis* (point 1 and 2 above), we would expect to find either specific taxa in the native range that would be absent in the introduced range or a greater abundance of key pathogens in the introduced range than in the native range. Since *P. australis* populations at our test sites have been present for some time (probably for decades), it is unlikely that we would observe pathogens absent in the invaded range that were present in the non-invaded range, especially given that all of the *Pythium* species recovered from our wetland sites, regardless of invasion history, represent globally-distributed species (Farr & Rossman, 2015). In fact, many of the taxa detected in both our current and previous studies (Nelson & Karp 2013) have also been found in the native European range of *P. australis* (Nechwatal *et al.* 2008a; Wielgoss *et al.* 2009). Since we have no data on the relative abundance or virulence of pathogens in the native European range of *P. australis* [with the exception of *Py. phragmitis* (Nechwatal *et al.* 2005; b; a; 'Evidence for the occurrence of natural hybridization in reed-

associated Pythium species' 2009; Wielgoss *et al.* 2009; Mazurkiewicz-Zapalowicz 2010) and *Py. litorale* (Nechwatal & Mendgen 2006)] (point 3 above), we can only make inferences about how pathogens influence invasiveness based solely on relative abundance and virulence of various taxa to *P. australis* and their differential virulence to other native plant species (point 4 above).

It is commonly assumed that some level of host specialization is required for pathogens to be able to regulate plant species dominance or coexistence (e.g., Janzen-Connell phenomena; negative plant-soil feedbacks) (Bever, Platt & Morton 2012). However, nearly all of the pathogens identified in this study are generalists with broad host ranges (Supporting Table S II.2). This suggests that other biotic or abiotic factors in the field may contribute to the effective specialization (Benítez *et al.* 2013) of generalist *Pythium* species that leads to differential plant responses. Others have also observed that generalist pathogens such as *Pythium* spp. may elicit host-specific responses (Augspurger & Wilkinson 2007; Halbritter *et al.* 2012). These responses are often due not only to the inherent differences in host susceptibility, but also to other biotic and abiotic interactions with hosts and pathogens that ultimately determine plant performance and influence competitive outcomes (Scholthof 2007; Perkins, Johnson & Nowak 2011). Since our virulence assays were designed to eliminate these biotic and abiotic interactions so as to determine the absolute potential to induce disease, we cannot yet make field predictions about the role of pathogens in invasiveness from these laboratory assays alone. However, the insights gained from this study about the species present and their corresponding virulence, can better inform the design of experiments to test more specific hypotheses about the relationships of sets of pathogenic species to competitive outcomes between *P. australis* and native species.

One of the more important observations from our study was the differential virulence of various *Pythium* species to a range of native and non-native plant species. Differential pathogen impacts are necessary for apparent competition between two plant species sharing a common community of pathogens (Holt 1977; Holt & Hochberg 1998). These differential pathogen impacts could arise either from different levels of virulence to *P. australis* and other plant species, differences in the relative abundance of specific pathogenic species associated with *P. australis* and native plant species, or differences in the relative contribution of other biotic and abiotic factors that may regulate host responses. Of the species of *Pythium* tested, *P. australis* seedlings were the least susceptible to *Py. angustatum*. Yet, the greatest differential virulence between *P. australis* and *P.* a. *americanus* was with *Py. angustatum* (40.6% greater seedling survival of *P. australis* than of *P. a. americanus*). Although other species such as *Py. inflatum* and *Py. heterothallicum* did not display differential virulence between *P. australis* and *P.* a. *americanus* as great as that of *Py. angustatum*, their relative isolation frequencies (a function of relative abundance) coupled with their slightly lower differential virulence leads us to hypothesize that *Py. inflatum* and *Py. heterothallicum* represent the two species most likely to influence competitive outcomes between *P. australis* and *P. a. americanus*. This assumes that seedlings of both species interact spatially and temporally with these *Pythium* species in the field. In similar logic, the greatest differential virulence between *P. australis* and *E. graminifolia* was with *Py. sylvaticum* (51.9% greater seedling survival of *P. australis*) and *Py. attrantheridium* (46.9% greater). These observations coupled with isolation frequencies, allow us to hypothesize that *Py. attrantheridium* should be the most likely *Pythium* species to influence competitive outcomes between *P. australis* and *E. graminifolia*.

Collectively, when following this line of reasoning for all of the plant species tested, four *Pythium* species (*Py. inflatum*, *Py. heterothallicum*, *Py. angustatum*, and *Py. attrantheridium*) emerge from our study that represent the most likely species to facilitate apparent competition between *P. australis* and the other native plants in our study. This may not be surprising for *Py. attrantheridium* since this species has been implicated previously in limiting the dominance of other plant species (Packer & Clay 2004; Reinhart *et al.* 2010b). However, nothing is known about the impact of *Py. inflatum*, *Py. heterothallicum*, and *Py. angustatum* on invasions given that they have rarely been described. *Py. sylvaticum* (not isolated frequently in our current study but very abundant based on DNA-based characterizations (Nelson & Karp 2013)), is also potentially significant because of its demonstrated role in limiting the dominance of *Prunus serotina* in its native range (Reinhart & Clay 2009; Reinhart *et al.* 2010b; 2011).

Our interpretation of the role of particular *Pythium* species in facilitating invasiveness of *P. australis* is complicated by the observation of other highly virulent pathogens that are abundant in non-invaded soils but were either absent or greatly reduced in abundance in invaded soils. Some of these species were highly virulent to *P. australis* and nearly all plant species tested. If these species contribute indirectly to invasiveness, *P. australis* would need to somehow reduce or avoid the negative impacts of these pathogens during initial stages of invasion. Although this could be accomplished through the production of antimicrobial compounds in root exudates (Li & Hu 2005; Hong, Hu & Li 2008; Bains *et al.* 2009), some of which are known to inhibit some *Pythium* species (Dix 1979), it is more likely that *P. australis* could avoid the negative impacts of pathogens during seedling establishment by recruiting microbes from the environment to assist in plant defense (Philippot *et al.* 2013). This principle is well recognized in agricultural systems and often exploited for the biological control of pathogens of agriculturally-

important plant species (Berendsen, Pieterse & Bakker 2012). *P. australis* is known to recruit endophytic and epiphytic microbes from the soil (Ernst, Mendgen & Wirsel 2003; Fischer & Rodriguez 2013; Wu *et al.* 2014) that are able to protect plants from pathogen infection. Unpublished data from our lab suggests that such interactions with epiphytically-recruited microbes may allow *P. australis* to avoid infection by *Pythium* species under laboratory conditions (Windstam & Nelson, *unpublished*).

While we have focused on oomycete pathogens, in part, because of their ubiquity in wetlands and other aquatic habitats (Apinis 1964; Nechwatal *et al.* 2008a; Nelson & Karp 2013), it must be recognized that fungal pathogens are likely to play equally important roles in influencing invasiveness (Power & Mitchell 2004; Maron *et al.* 2013a; Xiao *et al.* 2014). Future microbe-centric studies with a focus on fungal pathogens will be equally important in determining the role of soil pathogens on invasiveness. A focus on the dynamics of pathogens within complex plant communities and in and on the root systems of selected native and non-native plants will better reveal the host-pathogen associations likely to facilitate competitive interactions between *P. australis* and non-invasive native plant species.

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Figure Legends:

Figure II. 1: Oomycete species isolated from *P. australis*-invaded (white) and non-invaded (grey) soils. Isolation frequency reflects the number of isolates obtained of a given species relative to the total number of isolates. Species names represent best BLAST matches to the NCBI database.

Figure II. 2: Virulence bioassay setup. Ten seeds of a given plant species (here *P. australis*) were added to the surface of CV8 agar plates inoculated with a *Pythium* isolate. Top box (A) displays a non-inoculated control plate and examples of two low-virulence (high seedling survival) *Pythium* isolates. Bottom box (B) displays three examples of high-virulence (low seedling survival) isolates.

Figure II. 3: Seedling survival of non-inoculated and inoculated seedlings following inoculation by *Pythium* isolates from *P. australis*-invaded and non-invaded soils. Black bars indicate non-inoculated control seedlings, grey bars indicate seedlings inoculated with *Pythium* species from *P. australis*-invaded soils, and white bars indicate seedlings inoculated with *Pythium* species from non-invaded soils. Error bars indicate standard error from the mean and asterisk (*) indicates a significant difference between seedlings inoculated with *P. australis*-invaded and non-invaded *Pythium* isolates (Dunnett's method, $P \leq 0.05$).

Figure II. 1: Oomycete species isolated from *P. australis*-invaded (white) and non-invaded (grey) soils.

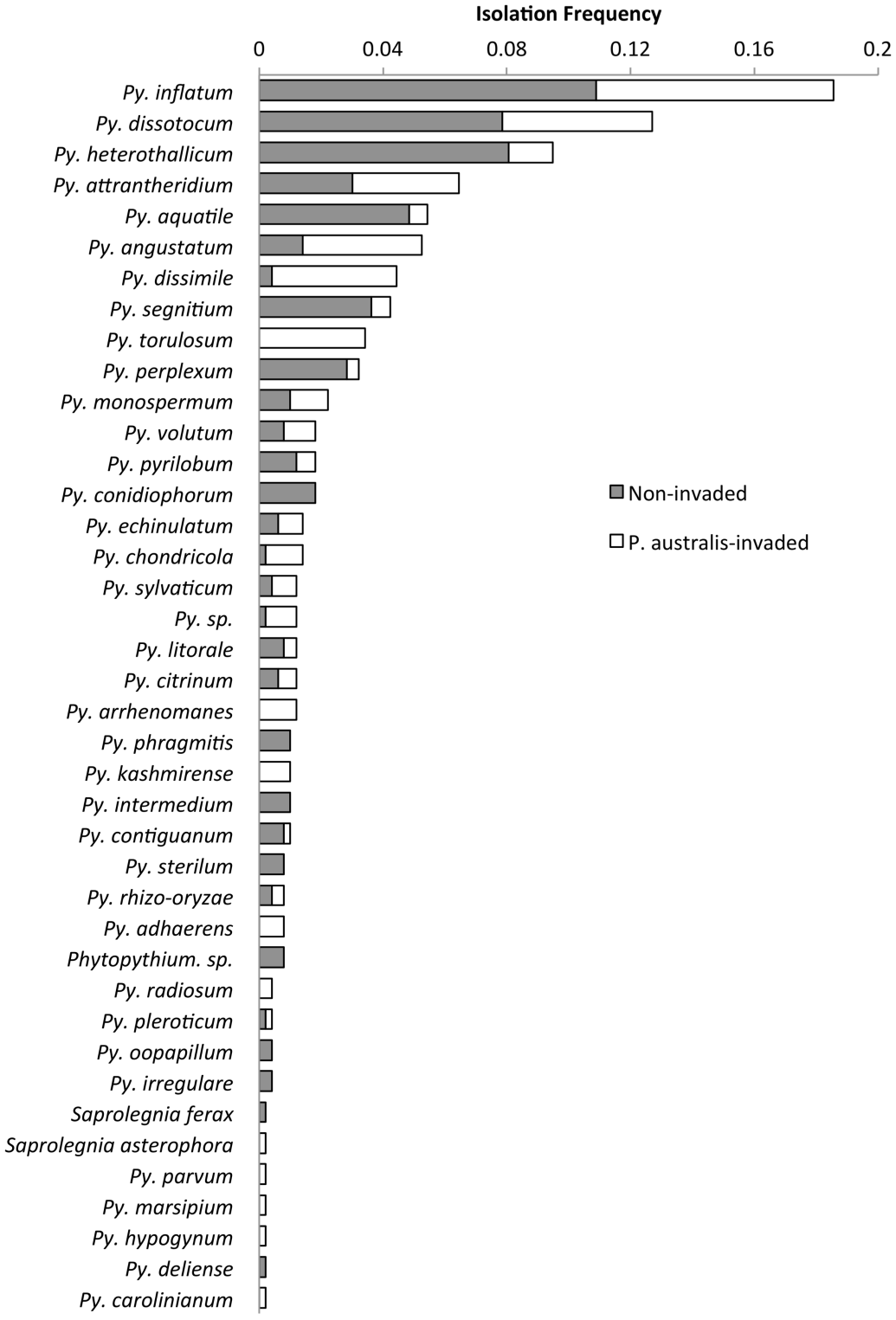

Figure II.2: Virulence bioassay setup.

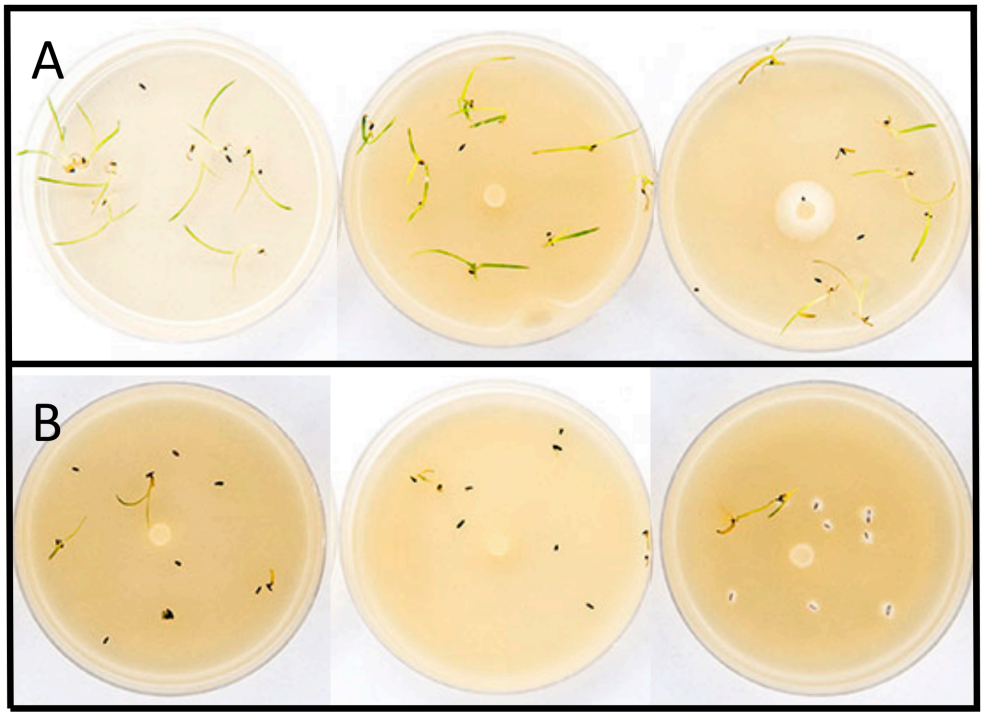

Figure II.3: Seedling survival of non-inoculated and inoculated seedlings following inoculation by *Pythium* isolates from *P. australis*-invaded and non-invaded soils.

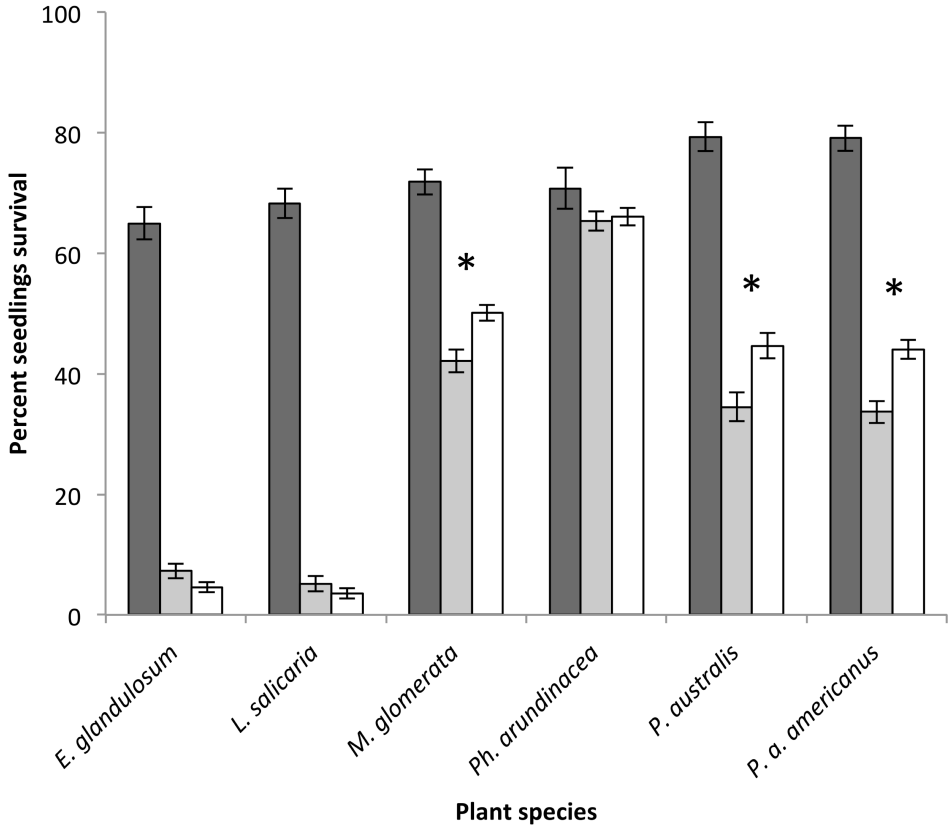

Table II.1: Seedling survival of native and non-native plant species following inoculation with different *Pythium* species.*

Pythium species	Plant species						
	P. australis	*P.a. americanus*	*E. glandulosum*	*M. glomerata*	*E. graminifolia*	*L. salicaria*	*Ph. arundin-acea*
Py. aquatile	0.543	0.785	0.972	0.764	1.314	/	/
Py. angustatum	0.975	0.570	0.000	0.833	/	0.090	1.015
Py. attrantheridium	0.469	0.785	0.125	1.097	0.000	0.000	1.090
Py. citrinum	0.926	0.772	0.333	0.944	/	/	/
Py. conidiophorum	0.543	0.709	0.097	0.722	0.667	/	/
Py. dissimile	0.346	0.620	0.000	0.597	/	0.015	0.970
Py. dissotocum	0.309	0.532	0.000	0.625	0.373	0.194	0.776
Py. echinulatum	0.593	0.722	0.014	0.917	/	/	/
Py. torulosum	0.370	0.658	0.264	0.778	1.196	/	/
Py. heterothallicum	0.840	0.797	0.347	0.694	/	/	0.806
Py. inflatum	0.531	0.405	0.028	0.750	/	0.090	1.090
Py. irregulare	0.346	0.620	0.125	0.625	0.000	/	/
Py. litorale	0.457	0.468	0.125	0.681	/	/	/
Py. oopapilum	0.667	0.835	0.028	0.889	/	/	/
Py. perplexum	0.728	0.861	0.139	0.778	1.216	/	/
Py. phragmitis	0.000	0.000	0.111	0.000	0.980	/	/
Py. pyrilobium	0.284	0.241	0.417	0.444	/	/	/
Py. rhizo-oryzae	0.346	0.367	0.028	0.667	/	/	/
Py. sylvaticum	0.519	0.595	0.319	0.764	0.000	/	/
Py. volutum	0.025	0.025	0.000	0.056	0.980	/	/

*Numbers represent the mean proportion of seedlings surviving three weeks after inoculation relative to non-inoculated control seeds. Shaded numbers represent pathogenic interactions for which inoculation significantly ($P \leq 0.05$) reduced seedling survival relative to the control. Inoculations that resulted in <10% seeding survival are indicated with **dark grey shading**. All other significant decreases in seedling health are indicated with **light grey shading**. "/" = not tested.

CHAPTER 2 SUPPORTING INFORMATION

Table S II.1. Field sites at the Montezuma National Wildlife Refuge, Savannah NY for soil collection.

Table S II.2. Reported plant host ranges of *Pythium* species isolated from *P. australis*-invaded and non-invaded soils.

Figure S II.1. Twenty-five most frequently isolated oomycete species recovered from *P. australis*-invaded and non-invaded soils at different sampling dates.

Figure S II.2. Distribution of oomycete species across sampling sites

Figure S II.3. Overall seedling survival following inoculation by a single isolate of a given *Pythium* species.

Figure S II.4. Seedling survival of *E. glandulosum* (black bars), *P. australis* (dark grey bars), *P. a. americanus* (medium grey bars), *M. glomerata* (light grey bars) and *L. salicaria* (white bars) following inoculation with isolates recovered from *P. australis*-invaded and non-invaded soils.

Chapter 3: Overwintering seeds as reservoirs for seedling pathogens of wetland plant species

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Abstract

Seed germination and seedling establishment are central to the distribution and abundance of plant species in wetlands. While fungal and oomycete pathogens are known to affect seed viability and emergence, relatively little is known about which fungi and oomycetes are associated with seeds in the soil or how these species affect seeds and seedlings. We characterized the fungi and oomycetes associated with overwintering seeds in wetlands and determined their potential to influence seed germination and subsequent seedling mortality. Fungi and oomycetes did not affect seed germination, despite the isolation of high frequencies of known seed and seedling pathogens in the fungal genera *Alternaria*, *Peyronellaea*, *Epicoccum* and *Fusarium*. However, many of the most frequently isolated fungal species from overwintering seeds were highly virulent to seedlings. The high seedling virulence of fungi from overwintering seeds coupled with the differential abundance of some of the more pathogenic fungi among seeds of different plant species, lead us to the conclusion that fungal pathogens are likely to strongly impact seedling establishment in wetlands despite not impacting overwintering seed germination in the seed bank.

Introduction

Seed germination and seedling establishment play important roles in the subsequent distribution and abundance of plant species in many ecosystems (Fenner and Thompson 2005). Most seeds eventually come in contact with soil, forming a transient to persistent seed bank population, which represents the sum of all viable seeds, dormant and non-dormant, in and on the soil. The success of seedlings emerging and establishing from the seed bank may greatly determine plant community composition following disturbances (Templeton and Levin 1979, Csontos 2007). Wetlands, in particular, have diverse and long-lived seed banks where seeds remain dormant until appropriate conditions trigger their germination and growth (Heerdt and Drost 1994, Zedler and Kercher 2004).

Despite the importance of seed banks in wetland plant community dynamics (Wang et al. 2009, Baldwin et al. 2010), relatively little is known about how soil microbes in the seed bank affect germination and subsequent seedling establishment. While in the soil, seeds encounter and acquire a rich microbial community that includes saprobic, mutualistic and pathogenic bacteria, fungi and oomycetes (Wagner and Mitschunas 2008). Previous research into seed bank dynamics suggests that pathogenic soil fungi are especially important in reducing the persistence of seeds (Blaney and Kotanen 2001a, 2001b, Schafer and Kotanen 2003, O'Hanlon-Manners and Kotanen 2004, Schafer and Kotanen 2004, Kotanen 2007, Orrock and Hoisington-Lopez 2009, Gallery et al. 2010, Orrock et al. 2011), degrading the seed in its dormant state and/or directly infecting germinating seeds and developing seedlings (Crist and Friese 1993). While these interactions may play a role in seedling establishment of particular plant species, little is known about the specific seed-pathogen interactions that impact seed germination and subsequent seedling establishment, with several exceptions (Crist and Friese 1993, Leishman et al. 2000, Kluger et al.

2008, Gallery et al. 2010, Mordecai 2012).

Such interactions with pathogens may be of particular importance to plant invasions since seed bank dynamics have been implicated in the invasiveness of introduced plant species (Klironomos 2002, Meyer et al. 2008, Reinhart and Clay 2009, Meyer et al. 2010, Orrock et al. 2011, Mordecai 2013). Invasive plant species are known to change not only the richness and evenness of seed bank plant communities at the sites they colonize (Gioria et al. 2014), but they also can alter soil and rhizosphere microbial communities (Van Der Putten 1997, Nelson and Karp 2013). In some cases, introduced invasive plants encounter fewer virulent pathogens (Reinhart et al. 2010, Callaway et al. 2011) and are less likely than native plant species to experience negative feedbacks at the seedling stage (Diez *et al.* 2010). Although soil microbes have the potential to influence interactions between native and non-native species, our understanding remains incomplete with regard to the plant-microbe interactions and plant developmental stages (infection in the seed-bank to post-germination seedling infection) for which such interactions are most influential.

A more thorough understanding of the relative virulence of specific pathogens to plant species in seed bank communities is necessary to better understand how such pathogens potentially influence plant demography and community dynamics in freshwater wetlands. To that end, experiments were designed to test the following hypotheses: 1) Specific fungal and oomycete pathogens significantly reduce the germination of overwintering seeds. 2) Invasive plant species experience higher rates of seed germination than native species following overwintering. 3) Unique fungal and oomycetes species associate with seeds of different plant species. 4) Seeds of invasive plant species are less susceptible to fungi and oomycetes than seeds of native species.

Materials and Methods

Seed container set-up

Ten plant species were selected for seed burial: *Asclepias incarnata*, *Calamagrostis canadensis*, *Carex comosa*, *Carex frankii*, *Epilobium glandulosum*, *Muhlenbergia glomerata*, *Lythrum salicaria*, European (non-native, invasive) haplotype of *Phragmites australis* (hereafter referred to as *P. australis*), North American (native, non-invasive) haplotype of *Phragmites australis* (hereafter referred to as *P. a. americanus*), and *Phalaris arundinacea.* Seeds of some plant species (*P. australis, P. a. americanus, Ph. arundinacea*) were collected locally whereas all others were ordered from Prairie Moon Nursery, Winona MN. These species were selected because they reflect a phylogenetic range of native and non-native plant species as well as a range of species frequencies (common to rare). All seeds were surface sterilized by dipping in a 70% EtOH solution followed by a sterile water rinse prior to burial.

Seeds were prepared for burial by filling conical containers (Stewe and Sons, Ray Leach "Cone-tainer"™ SC7U) with ~50 ml sterile, sieved 0.25-0.5 mm quartz sand (see Fig. S III.1 in Supplemental Information). Prior to filling containers with sand, sterile cheesecloth squares were placed over the drainage holes to prevent the loss of the container contents. A sterile 5.5 cm diameter filter paper was placed on the sand surface upon which seeds (number varied from ~25 to 100 depending on plant and seed size) were placed. An additional ~50 ml sand was then added to nearly fill containers. Sterile cheesecloth squares were affixed over the top of containers to exclude granivores and detritus. Nine containers were established for each of the 10 plant species at each of 10 different wetland sites (for a total of 900 containers). These burial sites reflected a range of local wetland conditions and vegetation types (see Table S III.1). For each plant species, three of the nine containers at each site were non-treated (dipped in water), three were treated by

drenching with the fungicide (anti-fungal) difenoconazole (1.18 mL/gallon), and the remaining three were treated by drenching with the stramenicide (anti-oomycete) mefenoxam (0.07mL/ gallon).

Seed container burial, overwintering, retrieval and germination assessment

Three quadrats were established at each field site, each within three meters of the other two. Each container was buried in its own 20 x 20 cm square of the quadrant in a randomized design. Seeds were buried in January and collected the following April. Container recovery was staggered by site to reflect the order in which they were buried. Recovered containers were transported to the lab and stored at ~ 4° C prior to processing. At the time of container retrieval, one site was flooded making it impossible to retrieve containers until after the experiment concluded. These were excluded from subsequent analyses.

Seeds were extracted from each individual container by emptying the top layer of sand, containing the filter paper with seeds, into a beaker of water. The seeds were then elutriated under a stream of running water until seeds either floated or were released into the supernatant. Seeds were then collected by decanting the water and vacuum-filtering through a Watman #1 filter paper. Seed retrieval efficiency using this method was unexpectedly high and essentially all seeds buried were eluted from containers following overwintering. Some of the retrieved seeds from plots at a few sites were already germinated upon collection and these were excluded from further analysis. After seeds were isolated they were stored at 4° C (generally 1-2 days) on sterile filter paper in sterile glass petri dishes until placed into germination assays or plated on isolation media.

Seed germination was assessed by placing a subsample of 12 seeds from each container onto water agar (WA) in wells of a 96-well microtiter plate (1.5 ml WA per well). For the purposes of our study, seed germination is defined as seeds with a clearly emerged radicle. Plates were covered, sealed with Parafilm®, and placed in a growth chamber set to alternating light (12 hours at 30° C) and dark (12 hours at 10° C), conditions (Ekstam and Forseby 1999). At three weeks, plates were examined and germination assessed by counting the number of seeds with emerged radicles out of the total seeds plated.

Isolate collection and pathogenicity bioassays

Fungi and oomycetes were collected from 6 seeds per container for each plant species/ treatment combination at 8 different sites, one plots at each (~432seeds in total). Each seed was placed in one of six wells of a 12-well tissue culture plate filled with an anti-bacterial selective medium (Nelson & Hsu, 1994). Fungal and oomycete mycelia were allowed to emerge from seeds at which time mycelia were hyphal-tipped and transferred to WA plates. The hyphal tip transfers were repeated several times to reduce contamination and ensure that each isolate reflected a single fungus or oomycete strain. Isolates were then stored on WA at 18° C until use in pathogenicity assays.

To test the pathogenicity and virulence of fungi and oomycetes, seedling survival (defined as plants that developed healthy cotyledons over the 3-week period of our assay) was assessed following inoculation with each of the collected isolates. Pathogenic isolates were defined as those that significantly decreased seedling survival relative to non-inoculated (control) seeds. Virulence, on the other hand, was defined as the degree of this decrease in survival relative to other isolates tested on the same plant species. Four plant species were selected for

these bioassays: *P. australis*, *L. salicaria*, *M. glomerata* and *E. glandulosum*. These plants were chosen because of their ease of growth, high seed germination and because they reflect a range of plant species (native and non-native, grasses and forbs). Due to a failure in germination of some *L. salicaria* seeds, several pathogen interactions with this plant species are missing. For each isolate, a small (~1 mm diameter) colonized agar disk was placed in the center of each of four plates of WA and allowed to grow for approximately one week at 18° C until hyphae had covered nearly all of the agar surface. Ten surface-sterilized seeds of a given plant species were then placed on the surface of one plate for each isolate, as well as on the surface of non-inoculated WA plates to act as a control for seedling survival. Plates were sealed in Parafilm® and placed in a growth chamber set to the previously described conditions. Three weeks later, plates were examined and percent seedling survival assessed.

Isolate sequencing and identification

Fungal and oomycete isolates were identified by sequencing the internal transcribed spacer (ITS) region. To prepare fungi and oomycetes for DNA extraction, isolates were grown on 10 cm diameter petri dishes containing Potato Dextrose Agar (PDA). The agar surface was covered with a sterile cellophane membrane to facilitate hyphal removal once colonies had reached the edge of plates. Hyphae were then placed in microcentrifuge tubes, and lyophilized. DNA was extracted from lyophilized hyphae using a QIAGEN® DNeasy Plant Mini Kit following the manufacturer's protocols. DNA was stored at -20° C until subsequent processing. For PCR amplifications, we used ITS 1 and ITS 4 primers to amplify the ITS region of each isolate (White *et al.* 1990). All PCR reactions contained 10 mmol/L Trizma HCl, pH 8.3, 50 mmol/L KCl, 2.5 mmol/L $MgCl_2$, 0.2 lmol/L of each primer, 200 lmol/L of each dNTP, 1unit of

Sigma REDTaq Genomic Polymerase, and 0.5 µl of template DNA per 25 lL reaction. DNA was amplified using a Bio-Rad T100^{TM} (Bio-Rad Laboratories, Hercules, CA) thermal cycler. PCR amplicons were purified using the Promega® SV 96 PCR Purification Kit.

Sequencing was performed at the Cornell University Life Sciences Core Laboratories Center on an Applied Biosystems Automated 3730 DNA Analyzer (Life Technologies) using Big Dye Terminator chemistry and AmpliTaq-FS DNA Polymerase. Sequences were compiled and edited in Sequencher 5.2.4 (Gene Codes Corp., Ann Arbor, MI). Sequence affinities to known taxa were determined by BLAST searches of the NCBI GenBank database on Nov. 3, 2014.

All sequences were aligned in MAFFT {Katoh:vi} trimmed in gblocks and converted to PHYLIP format in readseq. A maximum likelihood phylogenetic tree was built using raxML blackbox to determine how well this region resolved sequences. Along with our isolate sequences, GeneBank voucher sequences for the ITS region were added to alignments for each species we detected in our preliminary BLAST search and we included these in the tree. The sequenced ITS region resolved most of these genera to species very well, with the exception of *Alternaria alternata, A. tenuissima, A. s*p., and *A. brassica*. To minimize confusion, these were grouped as *A. alternata,* whereas *A. rosea* and *A. infectoria* were grouped as *A. infectoria.*

Statistical analysis

Generalized linear mixed models (GLMM) with a binomial distribution were employed to test whether biocide treatment influenced seed germination. For each plant species the main effect of biocide application on seed germination was tested with site as a random variable. Differences between biocides were evaluated *a posteriori* by aggregating biocide treatments

unless significant differences were present (Crawley 2013). Starting with the full model [which included soil treatment (non-treated, fungicide-treated or stramenicide-treated)] models were reduced in a backwards-stepwise process to determine the best model and significance via log-likelihood tests at $P<0.05$. R version 3.0.1 (R Development Core Team 2013) and the add-on package "lme4" (Bates *et al.* 2013) was used for all mixed models.

To better understand the effects of the seed colonizing fungi and oomycetes on seedling survival, the pathogenicity and virulence among selected fungal and oomycete isolates was examined at 3 weeks by comparing seedling survival in the presence of the pathogens with the non-inoculated control seedlings. The ten most frequently isolated fungal species were selected and effects calculated as mean percentage seedling survival of species "y" when inoculated with fungal species "x" divided by mean percentage seedling survival of species "y" in non-inoculated control plates. Seedling survival when inoculated with isolates of these species was also compared to non-inoculated seedlings using a one-way ANOVA followed by Student's T-test ($P<0.05$) in JMP to determine significant differences between means. Percent seedling germination was normally distributed and thus we treated this as a linear rather than binomial term indicating survival or death of each seedling. The same procedure was used to analyze the cumulative effects of all isolates from each source plant species on seedling survival. Isolates were grouped by the plant species from which they were isolated and effects values were calculated as mean percentage seedling survival of species "y" when inoculated with isolates from plant species "x" divided by mean percentage seedling survival of species "y" in non-inoculated controls.

Results

Effect of soil fungi and oomycetes on seed germination

Overall germination following overwintering was variable across plant species. *C. comosa* exhibited the lowest seed germination (2%) whereas *L. salicaria* exhibited the highest (89%). No clear trends in germination were observed between forbs and grasses or between native and non-native species. Furthermore, little site-to-site variation in germination rates was observed, despite large differences in soil flooding status and plant community composition at each of the different sites (Fig. S III.2).

Germination of most plant species was unaffected by fungicide or stramenicide treatment (Fig. III.1). However, for the three plant species where seed germination was significantly altered by treatment, it was lower for *E. glandulosum* when treated with fungicide, higher for *L. salicaria* when treated with fungicide, and lower for *Ph. arundinacea* when treated with stramenicide. Although statistically significant, effect sizes were very small.

Diversity of seed-isolated fungi and oomycetes

Over 200 fungi and only 3 oomycetes were isolated and identified from seeds across all plant species and sites (Table S III.4). Nearly half of the fungal isolates (104) were collected from stramenicide-treated seeds (no oomycetes were isolated from stramenicide-treated seeds as predicted) whereas 98 and 11 fungal isolates came from non-treated and fungicide-treated seeds, respectively. The most frequently isolated fungal genera were species of *Alternaria* (80 isolates), *Peyronellaea* (57 isolates), *Epicoccum* (22 isolates), and *Fusarium* (13 isolates). The remaining 26% of isolates were distributed among 32 different species, each of which accounted for less

than 2% of the total number of isolates (see Table S III.4). All oomycetes isolated were species of *Pythium* (*P. citrinum, P. logandrum*, and *P. heterothallicum*).

Some fungal species were more commonly isolated from particular plant species. For example, nearly all isolates of *Peyronellaea glomerata* were from *P. a. americanus* and *P. australis* seeds (Fig. III.2). More than 50% of the *Alternaria alternata* isolates were from the *Carex* species (*C. comosa* and *C. frankii*). All of the other fungal species were isolated at relatively low frequencies from all the plants tested.

Virulence of fungal isolates

Many of the dominant fungal species isolated from seeds were pathogenic not only to the plant species from which they were isolated but also to the other plant species we tested (Table III.1) however, there were wide variations in virulence. Among the most virulent pathogens were *Fusarium sporotrichiodes, Alternaria infectoria* and *A. alternata* (Table III.1). *Cadophora luteo-olivacea*, *Phaeosphaeria* species, *Pilidium concavum,* and *Stagonospora* were not pathogenic to any of the plant species tested.

The general susceptibility of each of the plant species to multiple pathogens varied. The mean seedling survival for each species when inoculated was 51% for *E. glandulosum*, 61% for *L. salicaria*, 56% for *P. australis* and 77% for *M. glomerata.* These variations reflect different susceptibilities of the different plant species to individual isolates. For example, inoculation with *Fusarium sporotrichiodes* decreased seedling survival of *E. glandulosum* to 6% and *P. australis* to 0% but only decreased seedling survival of *M. glomerata* to 44%. Some pathogens such as *Alternaria alternata* and *Fusarium sporotrichiodes* significantly decreased seedling survival of all plant species tested, whereas others exhibited a narrower host range. For example, *Epicoccum*

nigrum decreased seedling survival only of *P. australis* whereas *Peyronellaea glomerata* decreased seedling survival of *M. glomerata, P. australis* and *L. salicaria* but not *E. glandulosum*.

In general, the source plant species from which fungal pathogens were originally isolated did not affect their virulence (Table III.2). The susceptibility of *P. australis, M. glomerata,* and *L. salicaria* to fungal isolates did not differ regardless of the source plant. However, *E. glandulosum* was significantly more susceptible to fungi that came from seeds of *C. frankii*, *A. incarnata, C. comosa* and *L. salicaria* than those from *P. australis*, *P. a. americanus,* and *Ph. arundinacea*. Additionally, plant species that served as source hosts for the isolates were no more susceptible to those isolates than non-source host species. Fungal isolates from non-native hosts were no more virulent to different plant species than those isolated from all but one of the native hosts. Instead, *E. glandulosum* was more susceptible to fungal isolates from native plant species than isolates from non-native plant species.

Discussion

In this study, we determined the identity and pathogenicity of the fungi and oomycetes that colonize seeds of wetland plant species during overwintering in soil. Whereas previous studies have examined the potential roles of fungal and oomycete pathogens on the persistence of seeds in seed banks (Crist and Friese 1993, Leishman et al. 2000, Blaney and Kotanen 2001b, 2001a, Schafer and Kotanen 2003, 2004, Gallery et al. 2010, Mordecai 2012), our work focused primarily on the impacts of those pathogens that colonize seeds in the soil on subsequent seed germination and seedling survival. This work is especially significant for freshwater wetland ecosystems where the prevalence and impacts of plant pathogens are rarely acknowledged and,

with the exception of only a few studies (Blaney and Kotanen 2001a, Schafer and Kotanen 2004, Nechwatal et al. 2008, Nelson and Karp 2013), have not been examined.

While fungi and oomycetes have little to no impact on the germinability of seeds overwintered in the seed bank in our study, individual species may have significant impacts on subsequent seedling mortality. Many of the fungi and oomycetes colonizing seeds during the overwintering period were generalist pathogens similar to those described previously (Crist and Friese 1993, Kluger et al. 2008). These same pathogens have also been described from many other agricultural and non-agricultural ecosystems, as well as wetland soils (Ellis et al. 1951a, 1951b, Stenton 1953, Schafer and Kotanen 2004, Nelson and Karp 2013). Some of the most dominant genera of fungi (*Alternaria, Peyronellaea,* and *Fusarium*) were also among the most virulent to seedlings of a wide range of plant species, further highlighting their likely importance to wetland seedling establishment.

Often in studies of seed bank viability, the initial germination of seeds and the subsequent mortality or survival of seedlings are conflated (Blaney and Kotanen 2001a), making it challenging to determine the stages of early plant development where significant pathogen interactions occur. However, given that seed viability often decreases with increasing time in the seed bank (Schafer and Kotanen 2003), it is possible that any potential declines in seed germination due to colonization by fungi and oomycetes would not be realized in the relatively short (4-months) residence time of our study. Nonetheless, the observation that the fungi recovered from apparently healthy seeds could lead to high seedling mortality suggests that these fungi may persist on healthy seeds yet have large impacts on subsequent seedling establishment in the field.

Of the pathogenic fungi detected from overwintering seeds, species of *Alternaria,*

Peyronellaea, and *Fusarium* comprised ~55% of all fungal isolates. These observations indicate that populations of lethal seedling pathogens can be maintained over the winter months in the seed bank with little or no impact on seed viability and germinability. This could support sufficient pathogen populations to allow for high seedling infection the following spring when seedlings are extremely susceptible and mortality rates can be high (Gilbert 2002). That different plant species varied in their susceptibility to a given fungal pathogen but also altered the relative abundance of pathogens suggests that these differential responses may have strong impacts on seedling establishment and possibly recruitment of different plant species.

With the limited number of native and non-native plants tested, our results fail to support the more common hypotheses to explain invasiveness (i.e., spillover, spillback, or enemy release), consistent with the findings of others (Blaney and Kotanen 2001a). Non-native plant species did not experience higher rates of seed germination than native species during the overwintering process. Neither were the fungal pathogens associated with seeds of non-native plant species more virulent than those from seeds of native plant species, which is inconsistent with observations in forest and grassland systems (Reinhart et al. 2010, Diez et al. 2010, Callaway et al. 2011) or where non-native plants increase populations of seed bank pathogens (Beckstead *et al.* 2010).

Instead, our results support a growing body of research indicating fewer differences between native and non-native plants with regard to plant traits (Park and Blossey 2008), their suitability as animal habitat (Martin and Blossey 2013) or in their associated microbial communities (Nelson and Karp 2013) than were previously assumed. For example, there were no major differences in the isolation frequency or virulence of fungal pathogens associated with seeds of the non-native invasive plant *P. australis* compared to its native non-invasive

conspecific *P. a. americanus*.

We often isolated fungal species at different frequencies depending on the particular plant species. For example, *Peyronllaea glomerata,* one of the more frequently isolated fungal species (~25% of all isolates), was almost exclusively isolated from both *P. australis* and *P. a. americanus*. There were many other fungal species isolated exclusively from specific plant species, however these were all very low in abundance and we cannot determine if there is specialization to any particular host plant. Furthermore, there are many other wetland plant species that we did not test and for which specialization could occur. *Peyronellaea glomerata* has previously been associated with *P. australis* (Wong and Hyde 2001, Wirsel et al. 2001) and *L. salicaria* (Nyvall 1995), both common wetland plant invaders. However, it is somewhat surprising that it was so infrequently isolated from other plant species, especially having been described as a ubiquitous fungus with a broad host range (Farr and Rossman 2013) that includes crayfish (Dörr *et al.* 2011).

In contrast, *Alternaria alternata* was isolated from many plant species, but most commonly isolated from either *Carex frankii* or *Carex comosa*. Given that the germination of *C. frankii* seeds was very low, it is possible that seeds were already infected with *A. alternata* and were non-viable when we received them (Nguyen *et al.* 2015). However, it is unlikely that seeds were the dominant source of *Alternaria* because of the high isolation frequencies from other plant species with higher germination rates.

Several of the fungal isolates we recovered from seeds were non-pathogenic but it is still possible that they, as well as other soil microbes, play important direct or indirect roles in seedling mortality or cause disease only under certain conditions. For example the fungi *Cadophora luteo-olivacea, Pilidium concavum* and *Phaeosphaeria sp*. did not reduce seedling

survival of the plant species we tested. However each genera contains many pathogenic as well as non-pathogenic endophytic species (Farr and Rossman 2013) and may establish significant interactions with other wetland plant species that were not tested.

This research increases our understanding of the diversity of soil fungi associated with wetland seed banks and how particular seed bank fungal species affect seedling mortality. Our results suggest that the differential responses of seedlings to specific seed-associated pathogens have the potential to impact seedling establishment and possibly subsequent seedling recruitment in wetlands. These results further highlight the importance of studying specific pathogen interactions at specific stages of plant development to better understand the full impacts of pathogens on plant dynamics.

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Figure legends

Figure III.1. Overwintering seed viability as influenced by biocide treatment. Non-treated seeds =dark grey bars, fungicide-treated seeds=light grey bars, stramenicide-treated seeds=white bars. Data are means (+1SE) with significant differences (GLMM) represented by an asterisk (*).

Figure III.2. Dominant fungal genera isolated from seeds of each plant species. Genera included: *Alternaria*= black, *Peyronellaea*= dark grey, *Epicoccum*= medium grey, *Fusarium*= light grey, *Cadophora*= white.

Figure 1.

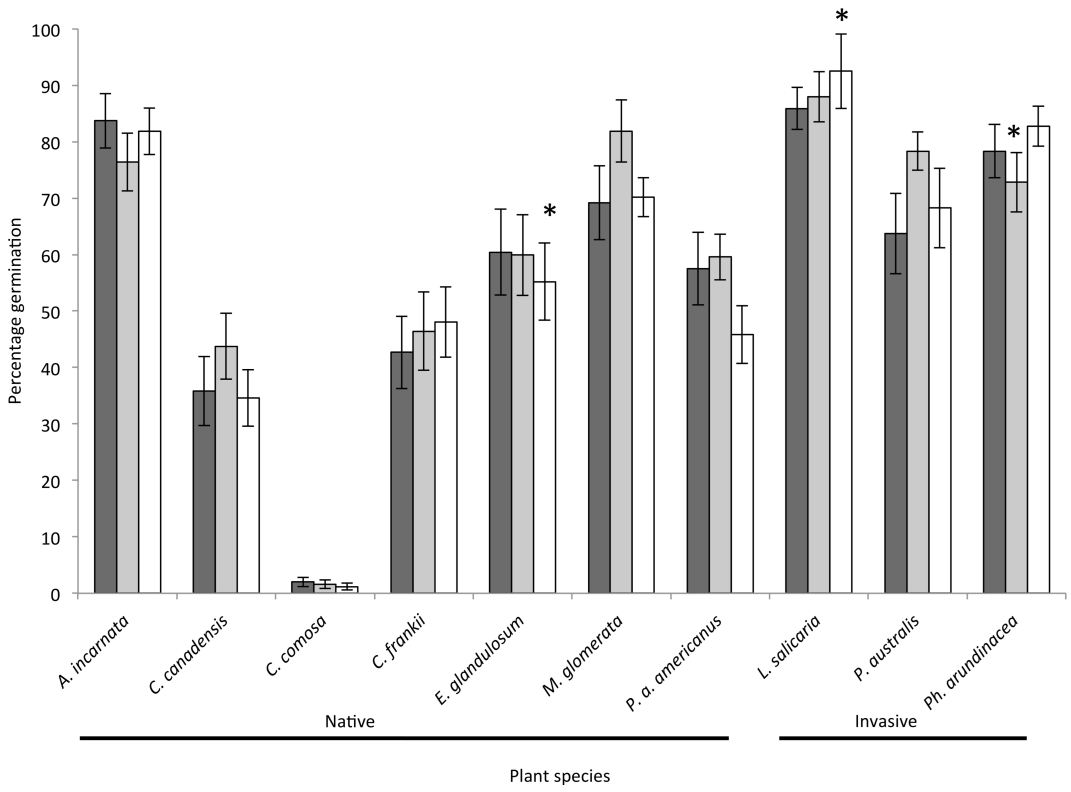
Percentage germination
100
90
80
70
60
50
40
30
20
10
0
*
*
*
A. incarnata
C. canadensis
C. comosa
C. frankii
E. glandulosum
M. glomerata
P. a. americanus
L. salicaria
P. australis
Ph. arundinacea
Native
Invasive
Plant species

Figure 2.

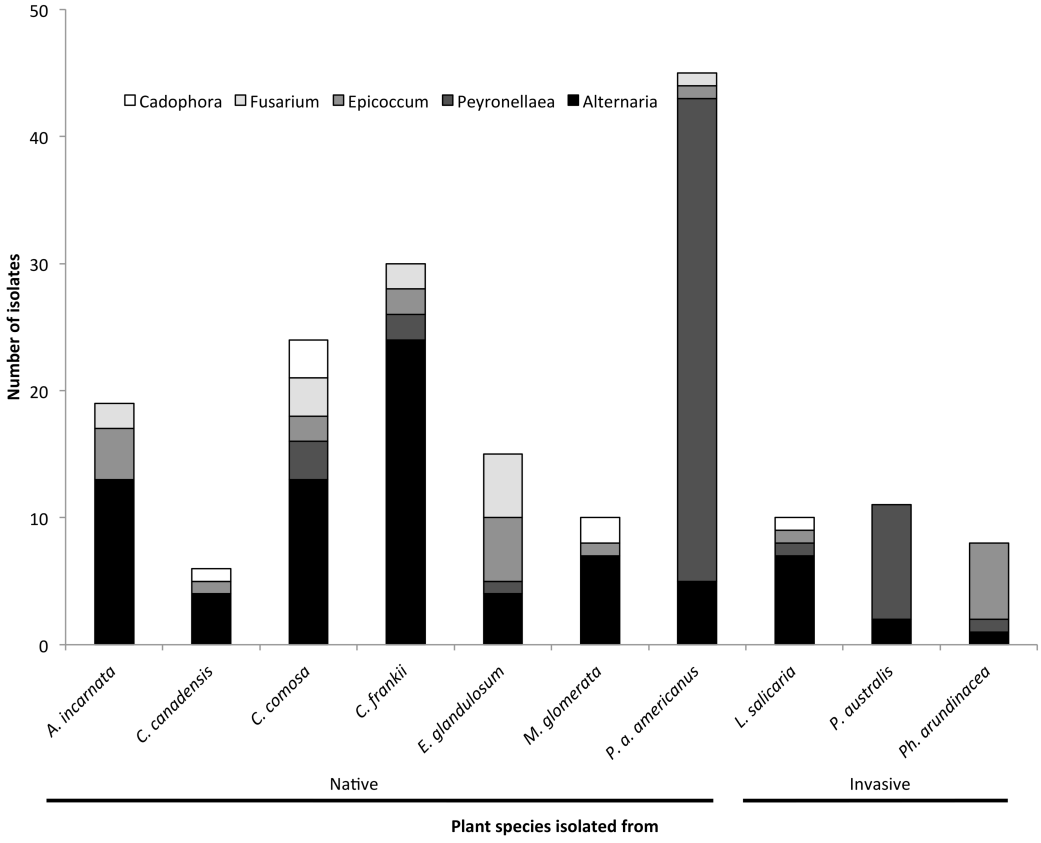
50
40
30
20
10
0
Number of isolates
Cadophora
Fusarium
Epicoccum
Peyronellaea
Alternaria
A. incarnata
C. canadensis
C. comosa
C. frankii
E. glandulosum
M. glomerata
P. a. americanus
L. salicaria
P. australis
Ph. arundinacea
Native
Invasive
Plant species isolated from

Table 1. Virulence of nine most frequently isolated fungal species to wetland plant species.

	Plant species inoculated			
Fungal species	*E. glandulosum*	*M. glomerata*	*P. australis*	*L. salicaria*
Fusarium sporotrichiodies	**0.06***	**0.44***	**0.00***	nd
Alternaria alternata	**0.09***	**0.80***	**0.42***	**0.52***
Alternaria infectoria	**0.20***	**0.54**	**0.25***	0.59
Peyronellaea glomerata	0.80	**0.79***	**0.54***	**0.36***
Epicoccum nigrum	0.92	0.79	**0.49***	1.03
Phaeosphaeria sp.	0.99	0.79	0.50	nd
Stagnospora sp.	0.84	0.66	0.51	0.93
Cadophora luteo-olivacea	0.71	0.89	0.82	0.61
Pilidium concavum	0.64	0.87	0.68	0.98

Note: Virulence was calculated as (mean % seedling survival of plant species "y" when inoculated with isolates of fungal species "x") / (mean % seedling survival of plant species "y" in non-inoculated control). Significant decreases in seedling survival when inoculated with a given species (Student's T-test $p<0.05$) are indicated with an asterisk (*) and bolded. Fungal species are ordered by overall virulence, "nd" indicated no data for fungus/plant combination

Table 2. Virulence of fungi isolated from seeds of specific host plant species to different wetland plant species.

	Plant species inoculated			
Plant species as source of isolates	*E. glandulosum*	*M. glomerata*	*P. australis*	*L. salicaria*
A. incarnata	0.316 [d]	0.760[b]	0.610[b]	0.866[a]
C. canadensis	0.493 [b,c,d]	0.798[a,b]	0.679[a,b]	0.669[a,b]
C. comosa	0.473 [c,d]	0.741[b]	0.535[b]	0.640[a,b]
C. frankii	0.233 [d]	0.753[b]	0.488[b]	0.826[a,b]
E. glandulosum	0.535 [b,c,d]	0.736[b]	0.515[b]	0.634[a,b]
L. salicaria	0.315 [c,d]	0.839[a,b]	0.536[b]	0.572[a,b]
M. glomerata	0.465 [b,c,d]	0.799[a,b]	0.674[b]	0.734[a,b]
P. a. americanus	0.697 [a,b,c]	0.726[b]	0.534[b]	0.429[b]
P. australis	0.902 [a,b]	0.868[a,b]	0.674[b]	0.360[b]
Ph. arundinacea	0.754 [a,b,c]	0.806[a,b]	0.500[b]	0.596[a,b]

Note: Virulence was calculated as (mean % seedling survival of host species "y" when inoculated with isolates from plant species "x") / (mean % seedling survival of host species "y" in non-inoculated control).

Numbers in each column followed by the same letter are not significantly different (Tukey's Test $p<0.05$)

CHAPTER 3 SUPPLEMENTAL INFORMATION

Chapter 4: Plant-soil feedbacks of dominant native and non-native wetland plant species

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Abstract:

Plant-soil feedbacks (PSFs) are known to influence the abundance and distribution of plant species. Differential PSFs are thought to drive the success of non-native invasive plants and the rarity of uncommon native plants but less is known about how PSFs affect locally dominant native and non-native plant species. In addition, the identity of soil fungal species associated with PSFs is rarely known. We conducted a completely reciprocal PSF soil conditioning experiment, using 10 congenerically paired native and non-native plants species able to become locally dominant in our region. We also characterized soil fungal communities using Illumina sequencing. Some, but not all, species we tested experienced negative PSFs when grown in conspecific soil relative to heterospecific soil. For two plant families in particular (Apocynaceae and Poaceae), the level of negative feedback was inversely related to the phylogenetic distance of the conditioning plant species. Analysis of fungal communities in conditioned soils revealed a large number of species, dominated by Ascomycota in the class Eurotiomycetes. Most of the conditioned soils we examined had similar species compositions but abundance of rare fungal groups varied by plant species. We did not find consistent differences in PSFs of native and non-native plant species. The contrast between our results and those found previous is likely due to our un-pairing of native origin and local dominance in that all plant species are dominant and highly successful in wetland soil. We observed that both native and non-native wetland plants can experience strong negative plant soil feedbacks, however the direction and magnitude of feedbacks were not determined by plant origin.

Introduction:

Plant-soil feedbacks (PSFs) are processes whereby plants alter abiotic and biotic soil properties that subsequently influence plant survival, growth or fitness. These processes are believed to be key factors determining distribution and abundance of plants (Kulmatiski *et al.* 2009; Van Der Putten *et al.* 2013). PSFs can contribute to plant community dynamics in several ways, including affecting diversity of plant communities by favouring rare plant species through negative density-dependent processes (Packer & Clay 2000; Brinkman *et al.* 2010; Mangan *et al.* 2010; Fitzsimons & Miller 2010).

While both biotic and abiotic soil properties are known to play roles in PSFs, soil biota are thought to be particularly important determinants of plant species distribution and abundance (Reinhart *et al.* 2005; Ehrenfeld, Ravit & Elgersma 2005; Kotanen 2007; Brinkman *et al.* 2010; Fitzsimons & Miller 2010; Bever, Platt & Morton 2012). Different plant species attract unique rhizosphere microbiomes (Arcate, Karp & Nelson 2006; Nelson & Karp 2013; Lankau & Lankau 2014) that may then influence their performance as well as the performance of neighbouring plants. For example, negative PSFs are thought to arise from increased proliferation and transmission of soil pathogens when plant species are at high density. Plant species may also compete indirectly with each other through soil biota-mediated processes such as amplification of soil pathogens or acquisition of a beneficial microbiome (Beckstead *et al.* 2010; Merrild *et al.* 2013).

Differential PSFs between native (typically rare) and non-native (typically invasive) species are believed to contribute to the demographic success of invasive plant species (Wolfe & Klironomos 2005; Mitchell *et al.* 2006; Kulmatiski *et al.* 2009; Inderjit & Van Der Putten 2010; Maron *et al.* 2013), although few attempts have been made to compare the PSFs of native and

non-native locally dominant species. While most plants experience negative PSFs (i.e., lower growth, survival or fitness in their native range or when grown in soil conditioned by that the same plant species), some invasive plants experience neutral or positive PSFs in their invaded ranges or when grown in conspecific soil, despite experiencing negative PSFs in their native range (Callaway *et al.* 2004; Reinhart *et al.* 2010b; Callaway *et al.* 2011; Maron *et al.* 2013). While the PSFs experienced by invasive species become more negative over time as plants encounter and acquired more soil antagonists with increased residence time (Diez *et al.* 2010), the advantage that these plant species experience from their initial lack of soil pathogens enables them to establish in new areas and rapidly displace native species.

In addition to the differential PSFs experienced by native and invasive plant species, other studies suggest that the strength and direction of PSFs are a function of plant traits (Baxendale *et al.* 2014), plant community structure (Kulmatiski *et al.* 2009), and environmental conditions (Harrison & Bardgett 2010). For example, annual plant species are more affected by negative PSFs than perennials while grasses and forbs are more affected by negative PSFs than trees (Kulmatiski *et al.* 2009). In addition, the PSF responses of plants grown in soil conditioned by a single species have been observed to be weaker than those of plants grown in soils conditioned by two or more plant species (Hendriks et al, 2013). While this points to the potential importance of plant community structure in the strength and direction of PSFs, the mechanisms remain unclear.

Despite the presumed importance of soil biota in PSFs, the identity and functions of key microbial species driving PSFs remain a mystery (Brinkman *et al.* 2010). This is due, in part, to the fact that research on PSFs has focused largely on net effects of soil properties on plant species responses with less attention to changes in soil microbial communities that drive those

responses. In one of the few PSF studies to couple soil microbial and plant dynamics, differences in virulence of soil pathogens between invaded and native ranges could explain success of *Prunus serotina* (Reinhart *et al.* 2010b).

Experimental studies of PSFs have largely focused on grassland (Wilsey *et al.* 2009; Suding *et al.* 2013) and forest systems (Klironomos 2002; Reinhart *et al.* 2010b; a), but less is known about the potential importance of PSFs in wetland plant communities. Wetlands are highly invaded systems, often dominated by non-native plants (Galatowitsch, Anderson & Ascher 1999; Moorhouse & Macdonald 2014). Even native wetland plant species often occur and in dense clonal stands. Furthermore, wetland soils are known to host abundant and diverse soil fungal and oomycete communities (Ellis, Ellis & Ellis 1951a; b; Nechwatal, Wielgoss & Mendgen 2008; Nelson & Karp 2013) and high soil moisture of wetlands may increase relative abundance and virulence of fungal and oomycete pathogens (Schafer & Kotanen 2003; Mordecai 2012).

To better understand mechanisms driving wetland plant dynamics we examined direction, strength and potential cause of PSFs. We selected native and non-native plant species from a range of families using phylogenetic controls and conducted a completely reciprocal PSF experiment where seedling growth was assessed for each plant species in its own (conspecific) soil and that of each other plant species (heterospecific soils). We also characterized and compared fungal communities present in soils conditioned by each plant species. We tested the following hypotheses:

1) Native species experience greater net negative PSFs than introduced species.
2) Plants experience greater negative PSFs from more closely related than from distantly related species.

3) The direction of PSFs experienced by a given plant species correlate with differential abundances of plant-specific groups of soil fungi.

Materials and methods

Soil Conditioning Phase

We conditioned soil by growing each plant species in monoculture for three growing seasons, 2011-2013. For each plant species, we filled five 100-L pots with ~99-L of peat mix and 1-L dried, sifted and homogenized field soil, collected from wetlands at 20 nearby sites in Upstate New York. (This field soil served as a source of microbial inoculum, providing soil pathogens potentially necessary for PSFs to accelerate soil conditioning).

We grew the following paired locally dominant native and non-native plant species: native *Asclepias incarnata* and non-native *Cynanchum louiseae,* native *Epilobium glandulosum* and non-native *Epilobium hirsutum*, native *Iris versicolor* and non-native *Iris pseudacorus*, native *Phragmites australis americanus* and non-native *Phragmites australis*, native *Calamagrostis canadensis,* and non-native *Phalaris arundinacea* (Table S IV.1). The source (collected or purchased from Prairie Moon Nursery, Winona MN) and type (rhizomes or seeds) of plant material depended on availability: Rhizomes and shoots were collected from field sites near Ithaca, New York for *P. a. americanus, P. australis, P. arundinacea. I. versicolor, I. pseudacorus, E. hirsutum* and *C. louiseae*. Due to their low frequency at our field sites, the following species were grown from seed and transplanted to pots: *C. canadensis, E. glandulosum* and *A. incarnata*.

Either rhizomes or seedlings of each plant were planted at high density in each pot. Pots

were arranged in a randomized grid at the Resource Ecology and Management Center at Cornell University in an area protected from wind but receiving full sunlight. Pots were regularly weeded and watered to maintain high soil moisture. In spring 2012 and 2013, some species were replanted to compensate for overwintering mortality. In November of 2013, the soil was removed from each pot and sifted through a 1-cm metal mesh screen to remove roots and homogenize soil. Soil from all replicate pots of each plant species was mixed, covered and stored frozen until Spring 2014. At this time we also collected ~100ml soil from each pot for characterization of soil fungal communities.

Soil Feedback Stage

All seeds were surface sterilized prior to planting and species specific stratifications followed where needed. (Table IV.1) Seeds were germinated in a greenhouse and allowed to grow for ~ 30 days whereupon they were transplanted to soil from the conditioning phase, which had been thawed and placed in 656 mL containers (Ray Leach Dee-pot, Stuewe and Son, Tangent OR). Each seedling was grown in soil conditioned by the same plant species (conspecific) as well as each of the other nine plant species (heterospecific). Ten replicate containers were planted for each seedling/conditioning - species combination. In addition, seedlings of each species were also planted in containers of potting mix (Cornell Mix peat mix) and in sterile conspecific soil (autoclaving on three consecutive days). There were a total of 1200 containers (10 seedling plant species x 12 soil treatments (10 conditioning plant species plus sterile conspecific soil and potting mix) x 10 replicates), which were arranged in racks of 25 containers on greenhouse benches. Seedlings were regularly watered, fertilized, weeded and their position on the greenhouse bench rotated.

Depending on seedling growth, we harvested above and below ground parts of all individuals of each species within one week when they appeared to become close to be pot bound. The exceptions were individuals that had been transplanted later due to initial mortality, all of which occurred within the first two weeks after initial transplant. These individuals were allowed to grow as long as other individuals of their species.

Leaf and shoot biomass was weighed immediately after harvest. Roots were inspected for potential lesions and thoroughly rinsed of soil particles. Both leaves and roots were dried in ovens at ~45° C for up to 10 days until no further changes in dry weight were observed. These final weights were used to estimate both above and below-ground dry biomass (summed for total dry biomass).

Root lesions were observed on *I. versicolor* and *I. pseudacorus*. Fungi were isolated from these lesions by surface sterilizing roots in 70% ethanol and progressively transferring fungal hyphal tips to plates containing water agar media. This enabled the isolation of individual fungal isolates which were stored at 18° C on water agar until DNA extraction for isolate identification (Crocker *et al.* 2015).

DNA extraction and sequencing of soil fungi and oomycetes

To characterize soil fungal communities, DNA was extracted from soil using the MoBio Soil Kit (MP Biomedicals). For each plant species DNA was extracted three times from ~0.25g soil each time and then further purified using the MoBio PowerClean® Pro PCR Clean-Up Kit. We adapted a Illumina sequencing protocol (Nguyen *et al.* 2014) to amplify the internal transcribed spacer (ITS) region of our soil DNA. Each PCR reaction contained 21.6 µl ddH2O, 3.5 µl 10x PCR Buffer with MgCl2, 1 µl 10x dNTP mix, 3.5 µl 25mM MgCl2, 0.7 µl forward

primer (10μM), 0.7 μl barcoded reverse primer (10 μM), 0.5 μl HotStarTaq® polymerase, 3.5 μl template DNA (25ng). DNA was amplified with a Bio-Rad MyCycler^{TM} (Bio-Rad Laboratories, Hercules, CA) thermal cycler using PCR conditions appropriate for our Illumina primers. Samples were then pooled, purified again and sequenced by Cornell University Life Sciences Core Laboratories Center using an Illumina Miseq sequencer.

To identify isolates collected from lesions on plants grown during the feedback experiment (only pertinent to *Iris* species) we extracted DNA from each isolate and sequenced the ITS region. To prepare isolates for DNA extraction, they were each grown on 60 mm diameter petri dishes with Potato Dextrose Agar (PDA) covered with sterile cellophane sheets until hyphae grew to the edge of plates. From each isolate we then scrapped hyphae off of the surface of the cellophane, placed hyphae in microcentrifuge tubes and then lyophilized each. The QIAGEN DNeasy Plant Mini Kit was used to extract DNA using the manufacture's protocol. Extracted DNA was subsequently stored at -20° C until further used. The ITS region was amplified using the ITS 1 and ITS 4 primer pair (White *et al.* 1990). DNA was amplified with a Bio-Rad MyCycler^{TM} (Bio-Rad Laboratories, Hercules, CA) thermal cycler using previously described PCR recipes and appropriate PCR conditions (Nelson & Karp 2013; Crocker *et al.* 2015). Species identities were determined by matching the resulting sequences to their closest likely species in the NCBI database using the BLAST algorithm.

Statistical Analysis

To determine the strength and direction of plant-soil feedbacks for each plant species we compared biomass produced in conspecific and heterospecific soil. Feedback response was calculated as [(mean dry biomass in conspecific soil minus the mean dry biomass in

heterospecific soil)/ mean dry biomass in heterospecific soil] to facilitate comparisons of feedback magnitude across plant species. Statistical significance of feedbacks was determined by a one-way ANOVA followed by Student's T-test ($p < 0.05$) in the statistical package JMP Pro 10 to determine significant differences in mean dry biomass when grown in conspecific soil for each plant species. To compare the mean biomass of seedlings grown in conspecific and sterile conspecific soils, we also used a one-way ANOVA followed by Student's T-test ($p < 0.05$). To compare the mean biomass of seedlings grown in each soil type, we also used a one-way ANOVA followed by Tukey's HSD ($p < 0.05$).

The relationship between phylogenetic distance and plant-soil feedback was calculated by generalized linear regressions for each plant family tested. Phylogenetic distance was estimated by computing pairwise distances in MEGA 6.06 from aligned ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene (*rbcL*) sequences of selected plant species. Feedback of each particular soil-conditioning plant species to each seedling was measured as log [dry biomass in conditioned soil/ dry biomass in potting mix]. To determine the impact of phylogenetic distance in soil feedback magnitude, we conducted generalized linear model (GLM) analyses, with a normal distribution, of log (feedback) with the fixed effect of phylogenetic distance in JMP Pro 10. We compared the effect of phylogenetic distance for each plant family, with significant interactions determined by $p > 0.05$.

Illumina sequencing fungal metagenomic analysis

Analyses of all fungal soil sequences were performed in QIIME and forward (R1) reads were used for our analyses (Nguyen *et al.* 2014; Smith & Peay 2014). Sequencing reads were demultiplexed and quality filtered using the split_libraries_fastq.py functions of QIIME. All base

calls were filtered at a Phred score of Q20 or better (-q19). Operational taxonomic units (OTUs) were assigned using the pick_open_reference_otus.py function and compared with the UNITE v6 dynamic database (Kõljalg *et al.* 2013) as a reference using the default settings of UCLUST (Edgar 2010). A large percentage of OTUs could not be mapped to any reference, and these were excluded from further analysis using the filter_otus_from_otu_table.py function. OTUs were summarized using the summarize_taxa_through_plots.py function and a Principal Coordinate Analyses (PCoA) was conducted to distinguish fungal communities in soil conditioned by each plant species. To do this, beta diversity was calculated using beta_diversity.py with the Euclidean method parameter, a principal coordinate table was generated using principal coordinates.py, and plotted using make_2d_plots.py.

Results

Dry biomass of *C. canadensis, E. glandulosum, C. louiseae* and *I. pseudacorus* were all significantly lower when grown in conspecific as opposed to heterospecific soil (Fig. IV.1). While all other plant species trended towards negative PSFs, these differences were not statistically significant. We did not find consistent or significant differences in the PSFs of native and non-native plant species (mean cumulative feedbacks were -0.40 and -0.36, respectively).

The biomass of several plant species, *C. canadensis*, *E. glandulosum* and *I. pseudacorus* was significantly greater when grown in sterilized conspecific soil than in non-sterile conspecific soil (Fig. IV.2). In addition, two other plant species, *E. hirsutum* and *australis*, also exhibited significantly higher biomass when grown in sterilized relative to non-sterilized conspecific soil, despite a lack of significant PSFs for these species. *C. louiseae, I. versicolor, americanus,* and *P. arundinacea* did not experience higher growth in sterilized soils.

Plant species in the families Poaceae and Apocynaceae experienced stronger negative PSFs in soils conditioned by more closely related species than in soils conditioned by more distantly related plants (Figs S IV.1 and S IV.2). Although species in the Onagraceae and Iridaceae also varied in biomass depending on which plant species conditioned the soil, no significant relationship between phylogenetic distance and the strength of negative PSFs was observed (Fig. S IV.2).

Illumina sequencing detected nearly 8 million reads for all combined soil samples (Table S IV.2a). However, after quality control, demultiplexing and mapping sequences to a reference database, we were left with a combined total of 281,222 fungal reads representing over 60 operational taxonomic units (OTUs) total (Table S IV.2b). The number of OTUs varied by plant species, ranging from 30 for *A. incarnata* to 12 for *P. australis*. Similar dominant fungal species were observed in most of the conditioned soils and no consistent differences in soil fungal communities of native versus non-native plant species were observed. Neither did fungal communities correlate with the magnitude of PSF (Fig. S IV.3).

The most frequently and widely detected group of soil fungi were Ascomycota in the class Eurotiomycetes, (Table IV.1, Table S IV.3). Far less frequently detected, but still dominant, were members of the Ascomycota in the class Dothideomycetes (genus *Pseudogymnascus*), Basidiomycota in the class Agaricomycetes (genera *Ceratobasidiaceae, Minimedusa*, and *Ganodermataceae*), Ascomycota in the class Leotiomycetes (genus *Tetracladium*), Zygomycota (genera *Mortierella* and *Mucor*) and unknown Ascomycota (Dothideomycetes family) (Table S IV.3). The only plant species whose soil was not dominated by Eurotiomycetes were *A. incarnata* (fewer Ascomycota and more Basidiomycota than any

other soil tested), *E. glandulosum* (more *Pseudeurotiaceae* than any other soil tested) and *E. hirsutum* (more unknown fungi in the Dothideomycetes family than any other soil tested).

Eight fungal groups were common in all conditioned soils, mostly representing ubiquitous soil saprobes such as *Phialosimplex, Asperigillus* and *Penecillium* (Fig. S IV.4). There were also many unique fungal taxa, only detected in the soil of a particular plant species. Soils conditioned by *E. glandulosum, E. hirsutum and A. incarnata* contained the highest numbers of unique fungal taxa (8, 5, and 5 respectively). On the other hand some plant species (*P. australis, C. canadensis* and *I. pseudacorus*) did not exhibit any unique fungi in their conditioned soils, however the abundance of their common fungal taxa did vary and their fungal communities were distinct (Fig. S IV.3).

Observations of the roots of most species failed to detect any clear disease symptoms with the exceptions of *I versicolor* and *I. pseudacorus* from which we isolated a number of fungi. Of the 15 fungal isolates we obtained from the roots of both species, *Fusarium* species were dominant, especially *F. verticillioides* (6 isolates) and *F. proliferatum* (4 isolates). Both of these fungi are known to be pathogens with a wide host range (Farr & Rossman 2013). Additionally, we detected *Ceratobasidium* sp (2 isolates) and one isolate each of *Fusarium oxysporum, Fusarium* sp., and *Ophiosphaerella korrae*, all of which are also known plant pathogens.

Discussion:

Among the hypotheses we set out to test in this study were that native wetland species experience greater net negative PSFs than introduced species. Contrary to this hypothesis, both native and non-native plant species experienced a range of negative PSFs, although not all plant

species of either group exhibited statistically significant decreases in biomass when grown in conspecific relative to heterospecific soil. Such responses are inconsistent with observations from forests and grasslands where invasive plant species commonly exhibit neutral or positive feedbacks (Klironomos 2002; Kulmatiski *et al.* 2009; Wilsey *et al.* 2009; Zuppinger-Dingley *et al.* 2011; Maron *et al.* 2013). Often, such differential PSFs have been proposed as a likely explanation for success of invaders (Kulmatiski *et al.* 2009; Inderjit & Van Der Putten 2010; Bever *et al.* 2012; Suding *et al.* 2013). However, many studies conflate native/ non-native with local abundance and do not offer a true comparison on native and non-native plant species but rare and dominant plant species. Based on the strong negative PSFs we observed among non-native plant species, these differential feedbacks do not appear to different consistently between dominant native and non-native species, as long as those species are similarly dominant and successful at our local scale.

How might plant species experience strong negative PSFs yet still dominate a landscape? One explanation for such a paradox may be the issue of temporal scale. For example, it is increasingly clear that invasive plant species can accumulate negative PSFs over time (Diez *et al.* 2010; Flory, Kleczewski & Clay 2011; Flory & Clay 2013). While we know that increasing negative PSFs may limit the long-term success of some plant invaders (Nijjer, Rogers & Siemann 2007), it is possible that our short-term experiments failed to capture the full spectrum of PSFs experienced by a given plant species or enable more differences between native and non-native plant species to develop.

Another explanation may be an issue of experimental approach. Plants experience other abiotic and biotic variables in the field, some of which are known to modulate PSF responses (Van de Voorde, Van Der Putten & Bezemer 2011; de Vries *et al.* 2012) and it is possible that

plants do not realize as negative an impact of PSFs as would be expected from our common garden experiments. For example, the timing and speed of seed germination and seedling growth of some dominant plant species may limit or prevent their exposure to and interaction with potentially pathogenic soil fungi (Beckstead *et al.* 2010), allowing them to establish and expand. Because many dominant wetland plant species persist clonally, negative PSFs that limit seedling establishment may be less likely to decrease plant growth and survival at a site once plants are locally established.

Another hypothesis that we tested in this work was that plants experience greater negative PSFs from more closely related than from more distantly related plant species. Although we observed this to be true for some plant families (Apocynaceae and Poaceae), it did not apply to others (Onagraceae and Iridaceae). This indicates that the phylogenetic composition of the plant community coupled with the specific traits of plants within those communities are more likely to be determinants of PSF direction and magnitude than their native or non-native status. This is consistent with a growing awareness that PSFs are context specific and that predicting community responses requires an understanding of community effects as opposed to the effects of individual plant species (Bezemer *et al.* 2006; Harrison & Bardgett 2010; Bezemer *et al.* 2013; Hendriks *et al.* 2013; Baxendale *et al.* 2014; Anacker *et al.* 2014). Although our findings are consistent with some studies identifying phylogenetic signals related to PSFs (Anaker et al, 2014), they conflict with others that failed to find a phylogenetic signal linked to the strength or direction of PSFs (Dostal et al, 2011, Mehrabi & Tuck, 2015, Miller & Menalled, 2015). It is clear that analysis at both the plant species and community levels will be necessary to understand how interactions with soil microbes within a plant community give rise to these negative PSFs.

This research supports a growing literature suggesting that native and non-native plant

species may have fewer consistent differences than was previously believed. Several studies have failed to find the anticipated broad differences between native and non-native invasive plant species in terms of impact on animal habitat (Cohen, Maerz & Blossey 2012; Martin & Blossey 2013), plant traits (Thompson, Hodgson & Rich 1995; van Kleunen, Weber & Fischer 2010), interaction with soil microbial communities (Butof & Bruelheie 2011), and PSFs (Suding *et al.* 2013; Callaway *et al.* 2013; Schittko & Wurst 2013). We predict that soil biota of plant species are differ less by their native or non-native status and more by the local dominance of the given plant species and that if similar PSFs studies were conducted in grassland and forest systems in which a broad range of dominant native and non-native plant species were paired, we may also find that some non-native experience neutral feedbacks while others do not. It is safe to assume that even though PSFs can be a factor in the invasive success of some non-native plant species, they do not impact all non-native species to the same degree.

A final hypothesis we tested in this work was that PSFs correlate with plant-specific groups of soil fungi that accumulated in soil during the plant growth. Our rationale for choosing to look at fungal communities was based on the knowledge that the majority of plant diseases are caused by fungi and oomycetes (Agrios 2004) and that previous studies have linked these groups to negative PSFs (Klironomos 2002; Bever *et al.* 2012; Lankau & Lankau 2014) and differences between invasive plants and soil in their home and invaded ranges (Callaway *et al.* 2004; 2011; Maron *et al.* 2013). In addition, many invasive plants are known to change soil fungal and oomycete communities when they colonize (Nelson & Karp 2013; Lankau & Lankau 2014) but few studies identify the particular fungal taxa that change in abundance.

Given the similarities we observed among the fungal communities in soil conditioned by different plant species, regardless of their PSF magnitude and direction, it is likely that relatively

rare pathogenic fungi play more important roles in PSFs than dominant saprobic fungi. Most of the conditioned soils we tested were dominated by the same group of fungi (Eurotiomycetes) and there were no clear trends in fungal communities by plant invasive status, family or PSF. This may be because we started with the same peat mix for all plants or because this group of predominantly saprobic soil fungi is less sensitive to the plant species colonizing the soil.

The relative rarity of known pathogenic fungal taxa in the conditioned soils also suggests that microbial communities relevant in PSFs likely localize very close to plants and that bulk soil microbial communities are insufficient to explain feedbacks. For example, although we isolated known pathogenic *Fusarium* species from *I. pseudacorus* roots, we did not detect any through soil sequencing. Instead, pathogenic species, which tend to be more specialized than saprobes, are likely to spatially select for the rhizosphere, area immediately surrounding roots, and the spermosphere, area immediately surrounding seeds.

E. glandulosum, *C. canadensis*, and *I. pseudacorus* experienced soil-biota related negative PSFs, as reflected by their recovery from negative PSFs when conspecific soils were sterilized. Although we can't know which shifts in the soil fungal community play significant roles in their feedbacks, we can posit several hypotheses. While *C. canadensis* and *I. pseudacorus* have fungal community profiles similar to other species, dominated by fungi in the class Eurotiomycetes, *E. glandulosum*-conditioned soil was instead dominated by fungi in the class Dothideomycetes. In addition, *E. glandulosum* soils hosted more unique and relatively rare soil fungi than other plant species. These include the known pathogenic fungal groups *Phoma, Taphrinaceae*, and *Sclerotiniaceae*. It is possible that these pathogenic fungi are responsible for the negative PSFs we observed for *E. glandulosum* in conspecific soil and that investigating the pathogenicity and virulence of these fungi to *E. glandulosum* and other wetland plant species

may shed further light onto the mechanism behind *E. glandulosum* PSFs. However, the soil biota key to the negative PSFs of *C. canadensis* and *I. pseudacorus* remain a mystery.

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Figure legends

Figure IV.1. Plant-soil feedbacks among plant species grown in conspecific relative to heterospecific soil. Feedback measured as difference in biomass when grown in conspecific and heterospecific soils divided by biomass in heterospecific soil. Asterisks represent significant differences in the mean biomass in conspecific and heterospecific soil for each plant species using a Student's t-test, $p<0.05$.

Figure IV.1. Effect of conspecific soil-sterilization on plant biomass. Data reflect mean biomass of seedlings grown in conspecific soil (white bars) compared with the mean biomass of seedlings grown in sterile-conspecific soil (grey bars). Asterisks indicate significant differences in biomass using a Student's t-test, $p<0.05$. (*A. incarnata* excluded because number grown in sterile soil was too low for meaningful comparisons.)

Fig. IV.1.

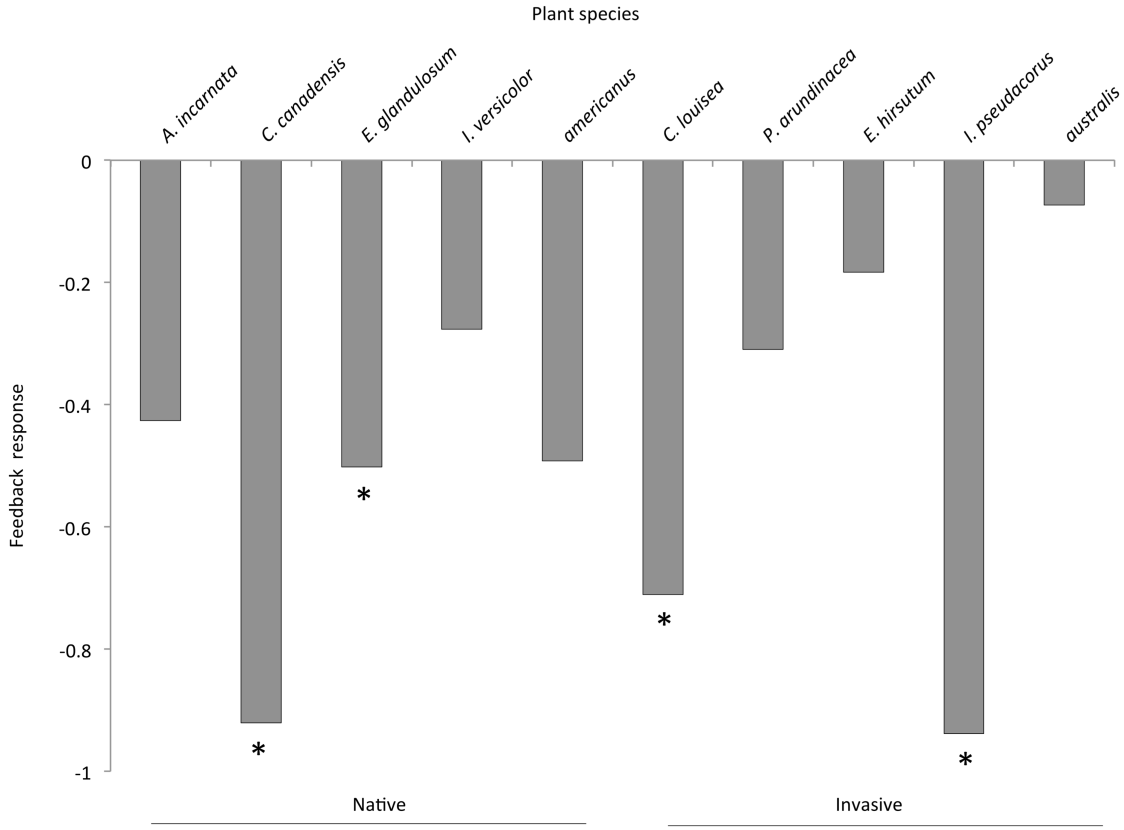

Plant species
A. incarnata
C. canadensis
E. glandulosum
I. versicolor
americanus
C. louisea
P. arundinacea
E. hirsutum
I. pseudacorus
australis
0
-0.2
-0.4
-0.6
-0.8
-1
Feedback response
*
*
*
*
Native
Invasive

Fig. IV.2.

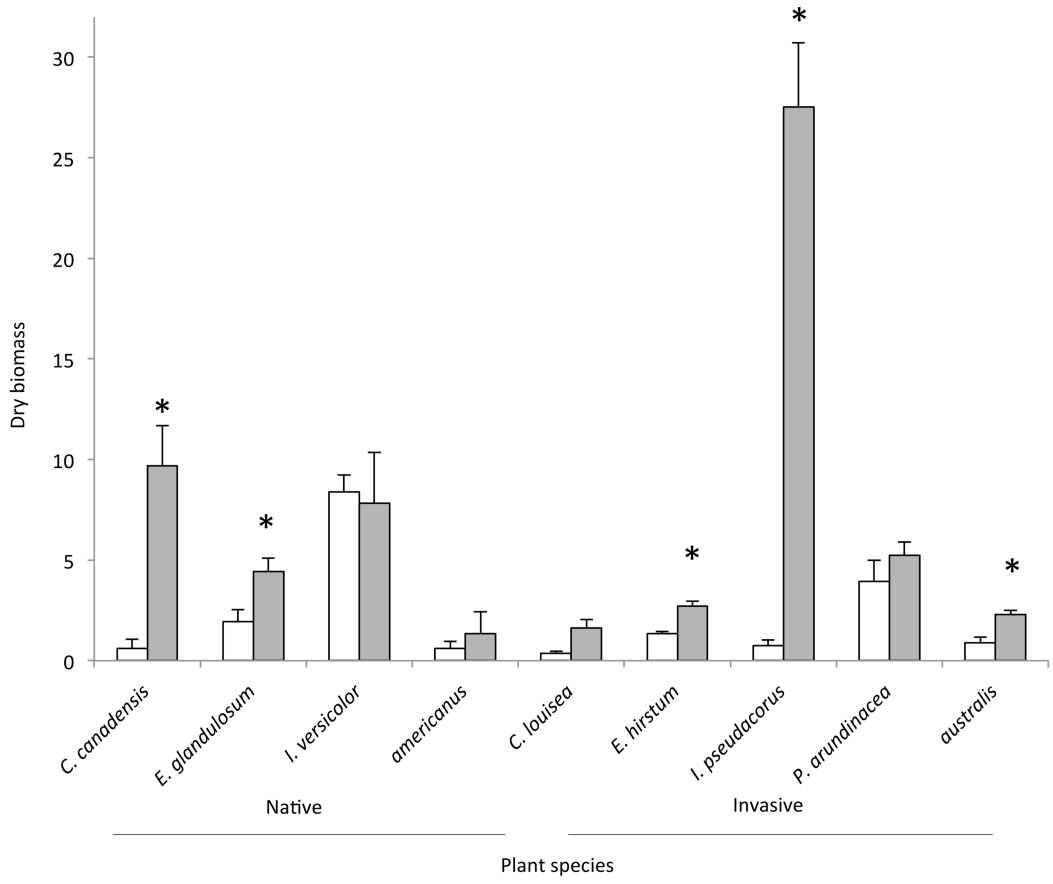

Dry biomass
30
25
20
15
10
5
0
*
*
*
*
*
C. canadensis
E. glandulosum
I. versicolor
americanus
C. louisea
E. hirstum
I. pseudacorus
P. arundinacea
australis
Native
Invasive
Plant species

Table IV.1. Dominant fungi in soil conditioned by each plant species. Based on OTU assignment of Illumina soil sequencing. Only those fungal groups comprising ≥ 0.1% are listed

Fungal group	*Plant species*									
	I. ver	***P. aru***	***aust-ralis***	***A. inc***	***E. gla***	***E. hir***	***I. pse***	***ameri-canus***	***C. can***	***C. lou***
Ascomycota										
Dothideomycetes	6.0%	5.4%	1.3%	8.2%	41.2%	1.8%	0.1%	0.4%	0.2%	1.6%
Eurotiomycetes	84.2%	90.2%	96.2%	14.8%	43.2%	48.9%	96.0%	95.4%	97.7%	90.4%
Incertae sedis	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
Leotiomycetes	2.2%	0.5%	0.9%	7.4%	4.7%	1.8%	2.1%	1.2%	0.6%	0.4%
Pezizomycetes	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%
Saccharomycetes	0.1%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
Sordariomycetes	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%
Taphrinomycetes	0.1%	0.1%	0.0%	0.2%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%
Unidentified	0.2%	0.1%	0.0%	0.3%	0.1%	39.4%	0.0%	0.0%	0.0%	2.7%
Other	0.3%	0.9%	0.6%	1.1%	4.4%	1.1%	0.3%	0.5%	0.2%	0.8%
Totals	**93.1%**	**97.2%**	**99.0%**	**32.0%**	**93.9%**	**93.1%**	**98.5%**	**97.8%**	**98.7%**	**95.9%**
Basidiomycota										
Agaricomycetes	5.1%	1.4%	0.7%	66.6%	4.4%	3.7%	1.4%	0.9%	0.9%	3.5%
Microbotryomy-cetes	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%
Tremellomycetes	0.0%	0.0%	0.0%	0.0%	0.1%	0.1%	0.1%	0.0%	0.0%	0.0%
Totals	**5.1%**	**1.4%**	**0.7%**	**66.6%**	**4.5%**	**3.9%**	**1.5%**	**0.9%**	**0.9%**	**3.5%**
Zygomycota										
Incertae sedis	1.7%	1.2%	0.2%	1.2%	1.5%	2.8%	0.1%	1.2%	0.2%	0.4%
Totals	**1.7%**	**1.2%**	**0.2%**	**1.2%**	**1.5%**	**2.8%**	**0.1%**	**1.2%**	**0.2%**	**0.4%**

CHAPTER 4 SUPPORTING INFORMATION

Supporting Information Chapter 1

Table S I.1: Population, origin (EU = introduced *P. australis*; NA = native *P. australis americanus*) and collection location (Lat/Long) of *P. australis* populations established to in a common garden. Two-letter code indicates US state (ME = Maine; MN = Minnesota; NY = New York; SD = South Dakota; WA = Washington).

Population	State	Origin	Latitude	Longitude
Libby River	ME	EU	43.55	-70.32
Libby River	ME	NA	43.55	-70.32
Forest Lake	MN	EU	45.28	-92.99
Pipewort	MN	NA	37.00	-101.89
Rochester	NY	EU	43.11	-77.73
Bergen Swamp	NY	NA	43.09	-77.98
Davison County	SD	EU	43.60	-98.11
Clark County	SD	NA	44.81	-97.72
Moses Lake	WA	EU	47.12	-119.29
Sun Lake	WA	NA	30.76	-85.69

Table S I.2: Plant species and number of seeds used in early-seedling common garden (1) and species used in field transplant experiments (2).

Species	Experiment	Seeds/ Container
Asclepias incarnata	1, 2	5
Astragalus canadensis	1, 2	20
Calamagrostis canadensis	1, 2	10
Carex lacustris	1	20
Elymus riparius	2	-
Epilobium glandulosum	1, 2	5
Eupatorium maculatum	1	5
Euthamia graminifolia	1, 2	20
Juncus effusus	1	20
Mimulus ringens	2	-
Muhlenbergia glomerata	2	-
Phalaris arundinacea	1	5

Table S I.3: Site location and vegetation community composition for field transplant survival experiment.

Location	Dominant vegetation outside *P. australis* stand	Latitude and longitude
Martin's Marsh 1	Plot 1: *Equistetum sp* 40%, *Phalaris arundinacea* 20%, *Apocynum cannabinum* 15%, <5% *Lythrum salicaria*, *Carex cristallela*, *Typha*, unknown herbaceous, unknown grass Plot 2: *Equistetum* sp 30%, *Phalaris arundinacea* 30% *Spartina pectinata* 20%, *Lythrum salicaria* 10%, <5% *Carex cristallela*, *Carex vulpinoides*, *Eupatorium maculatum, Typha*, unknown herbaceous, unknown grass	43° 5'1.69" N 76°42'36.66"W
Martin's Marsh 2	Plot 1: *Carex lacustris* 30%, *Solidago sp*. 15%, <5% *Lysimachia terrestris* , *Spartina pectinata*, *Juncus flexus*, *Carex cryptolepis*, *Asclepias incarnata*, *Lythrum salicaria, Alnus serrulata, Eupatorium maculosum, Equisetum, Vitus riparius, Phalaris arundinacea*, unknown grasses, unknown herbaceous	43° 5'2.86"N 76°42'23.78"W
Carncross	Plot 1: *Eupatorium maculosum* 70%, *Phalarus arundinacea* 20%, <5% *Carex lacustris*, <5% *Solanum dulcamera* Plot 2: *Carex lacustris* 50%, *Phalaris arundinacea* 20%, *Eupatorium maculosum* 10%, <5% *Vitus raparius, Apocynum cannabinum, Gallium, Solanum dulcamera, Parthenocissus quinquefolia*, unknown herbaceous	43°04´56.624´´ N 76°42´38.241´´ W
Teal Pond	Plot 1: *Persicaria maculosa* 50%, *Lythrum salicaria* 40%, <5% *Carex comosa, Apocynum cannabinum, Phalaris arundinacea*, unknown grasses Plot 2: *Persicaria maculosa* 40%, *Lythrum salicaria* 40%, <5% *Carex cristallela Apocynum cannabinum, Phalaris arundinacea, Spartina pectinata, Scirpus cf. cyperinus, Typha sp*.	43° 5'9.95"N 76°42'18.26"W

Figure S I.1. Early seedling survival (%) of different plant species in control soils and soils conditioned by different populations of either *Phragmites australis* (I) or native *P. australis americanus* (N). Two letter code following origin notation indicates US state of original field collection location (see Table S I.1)

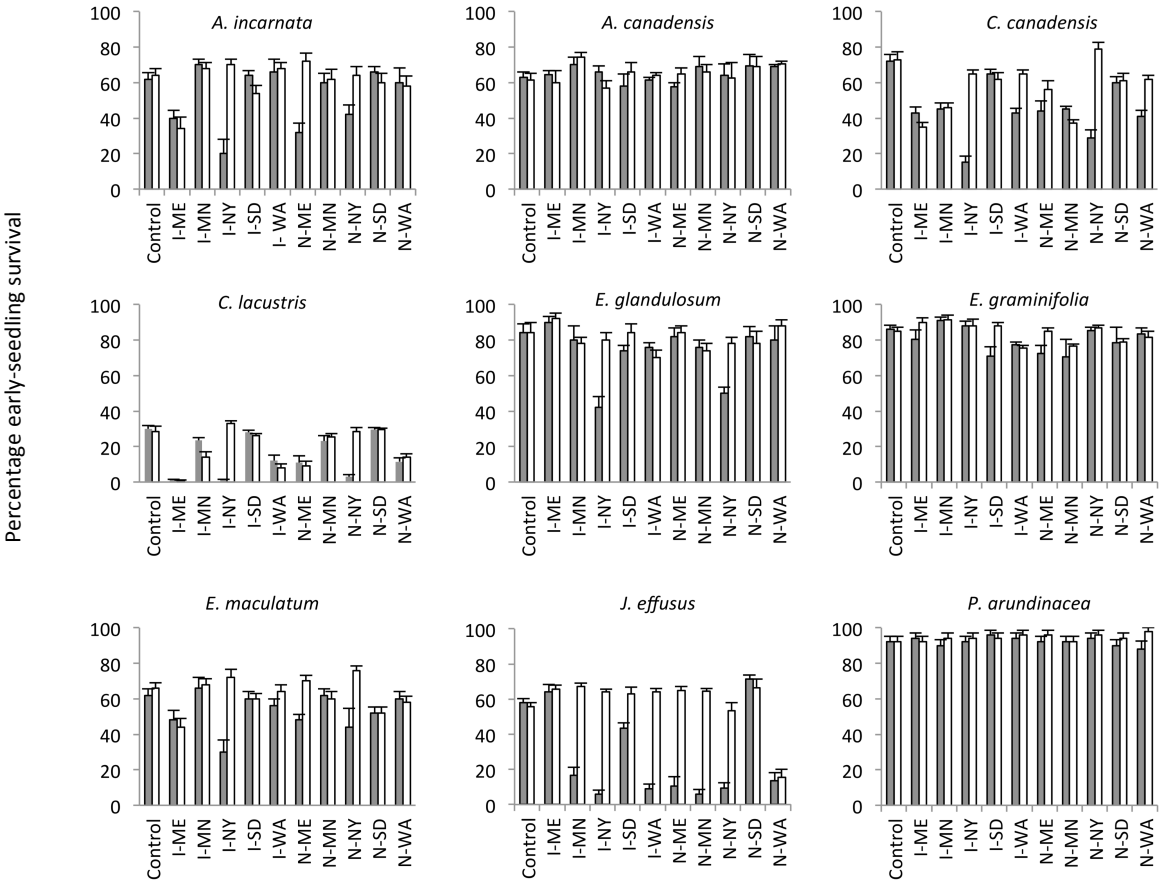
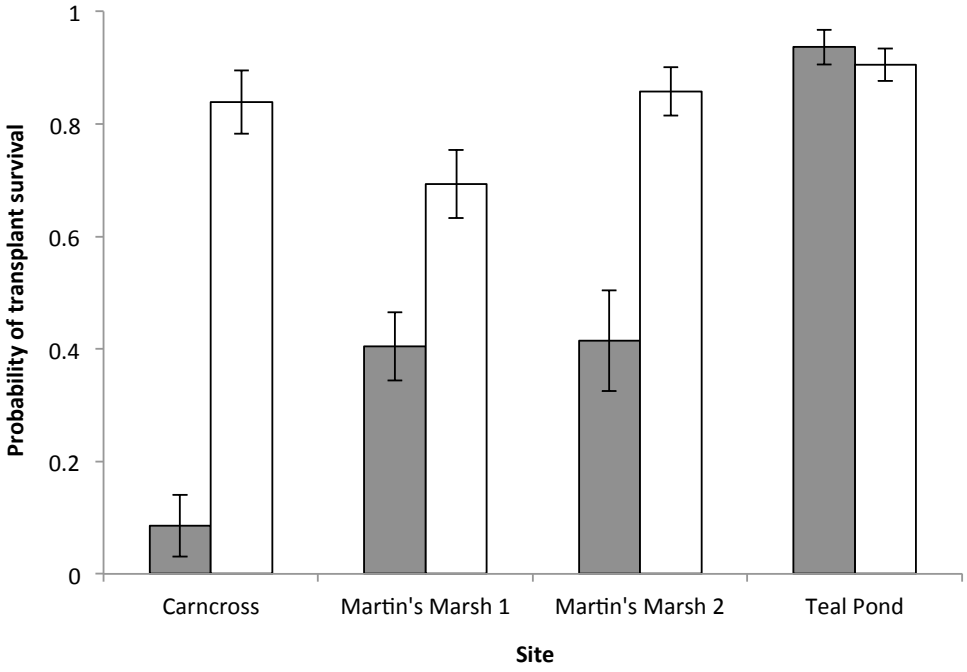


Figure S I.2. Probability of transplant survival inside *P. australis* patches (grey bars) vs in the surrounding mixed wetland plant community (white bars) at four research sites. Data are means ± 1SE of 7 different plant species, each with 20 replicates per site.



Supporting Information Chapter 2

Table SII.1: Field sites at the Montezuma National Wildlife Refuge, Savannah NY for soil collection.

Site	Location	Latitude	Longitude	Plant species present in non-invaded area
CC	Carncross	43°04′56.624′′ N	76°42′38.241′′ W	*P. a. americanus, Ph. arundinacea, Eupatorium maculatum, Lythrum salicaria, Spartina pectinata, Typha sp., Solanum dulcamera, Carex lacustris, Apocynum cannabinum, Vitus riparius*
EP	Eagle Point	43°10′16.123′′ N	76°47′34.704′′ W	
RR	Railroad	43° 3'22.98" N	76°42'37.46" W	
RT	Rt 31	43° 0'58.87" N	76°42'0.19" W	

Table S II.2: Reported plant host ranges of *Pythium* species isolated from *P. australis*-invaded and non-invaded soils.

***Pythium* species**	**Host range (plant families)**	**References**
Py. adhaerens	Chenopodiaceae, Cucurbitaceae, Fabaceae, Poaceae	(Sparrow 1932)
Py. angustatum	Poaceae	(Sparrow 1932)
Py. aquatile	Brassicaceae, Solanaceae	(Robertson 1973; Uzuhashi *et al.* 2008)
Py. arrhenomanes	Poaceae	(Vanterpool 1942; Sprague 1950)
Py. attrantheridium	Fabaceae, Poaceae, Rosaceae, Umbelliferae	(Allain-Boule *et al.* 2004; Packer & Clay 2004; Broders *et al.* 2007; Reinhart *et al.* 2010b)
Py. carolinianum	Malvaceae, Proteaceae, Haloragaceae, Rosaceae, Pinaceae, Cucurbitaceae, Amaranthaceae	(Bazan De Segura 1970; Watanabe, Hashimoto & Sato 1977; Bernhardt & Duniway 1984; Watanabe 1988; Abdelzaher & Elnaghy 1998)
Py. chondricola	None known	
Py. citrinum	None known	(Paul 2004)
Py. conidiophorum	Fabaceae	(Nzungize *et al.* 2011)
Py. contiguanum	None known	
Py. deliense	Poaceae, Fabaceae, Solanaceae, Amaranthaceae	(Raftoyannis & Dick 2006)
Py. dissimile	Poaceae, Fabaceae, Solanaceae, Amaranthaceae	(Vestberg 1990; Raftoyannis & Dick 2006)
Py. dissotocum	Very broad	(Spencer 2004a)
Py. echinulatum	Poaceae, Rosaceae, Fabaceae	(Watanabe, Hashimoto & Sato 1977; Braun 1995; Higginbotham, Paulitz & Kidwell 2004; Broders *et al.* 2007)
Py. heterothallicum	Caprifoliaceae, Chenopodiaceae, Fabaceae, Geraniaceae, Poaceae, Rosaceae	(Spencer 2004b)
Py. hypogynum	Poaceae	(Middleton 1941)
Py. inflatum	Poaceae, Fabaceae, Solanaceae	(Robertson 1973; Broders *et al.* 2007; Van Buyten & Hofte 2013)
Py. intermedium	Rosaceae, Umbelliferae, Brassicaceae, Cucurbitaceae, Onaceae	(Long & Cooke 1969; Stanghellini *et al.* 1988; Mazzola *et al.* 2002; Hermansen *et al.* 2007; Suffert & Guibert 2007)
Py. irregulare	Very broad	(Spencer 2004c; Farr & Rossman 2014)
Py. kashmirense	None known	
Py. litorale	Cucurbitaceae, Rosaceae	(Tewoldemedhin *et al.* 2011; Parkunan & Ji 2013)

Py. marsipium	None known	
Py. monospermum	Poaceae, Solanaceae	(Sprague 1950; Robertson 1973)
Py. oopapillum	Very broad	(Bala *et al.* 2010)
Py. parvum	None known	
Py. perplexum	Myrtaceae	(Mwanza & Kellas 1987)
Py. phragmitis	Poaceae	(Nechwatal, Wielgoss & Mendgen 2005)
Py. pleroticum	Zingiberaceae	(Dohroo, Bhardwaj & Shyam 1987)
Py. pyrilobum	Poaceae, Myrtaceae, Annonaceae	(Ward & Shipton 1984; Cother & Gilbert 1993; Abad, Shew & Lucas 1994; Linde, Kemp & Wingfield 1994)
Py. radiosum	None known	
Py. rhizo-oryzae	None known	(Bala, Gautam & Paul 2006)
Py. segnitium	None known	
Py. sylvaticum	Very broad	(Spencer 2004d)
Py. torulosum	Very broad	
Py. volutum	Poaceae	(Sprague 1950; Kerns & Tredway 2008)

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Figure S II.1: Twenty-five most frequently isolated oomycete species recovered from *P. australis*-invaded and non-invaded soils at different sampling dates. Isolation frequency reflects the number of isolates of a given species obtained at a particular sampling time relative to the total number of isolates recovered at that sampling time.

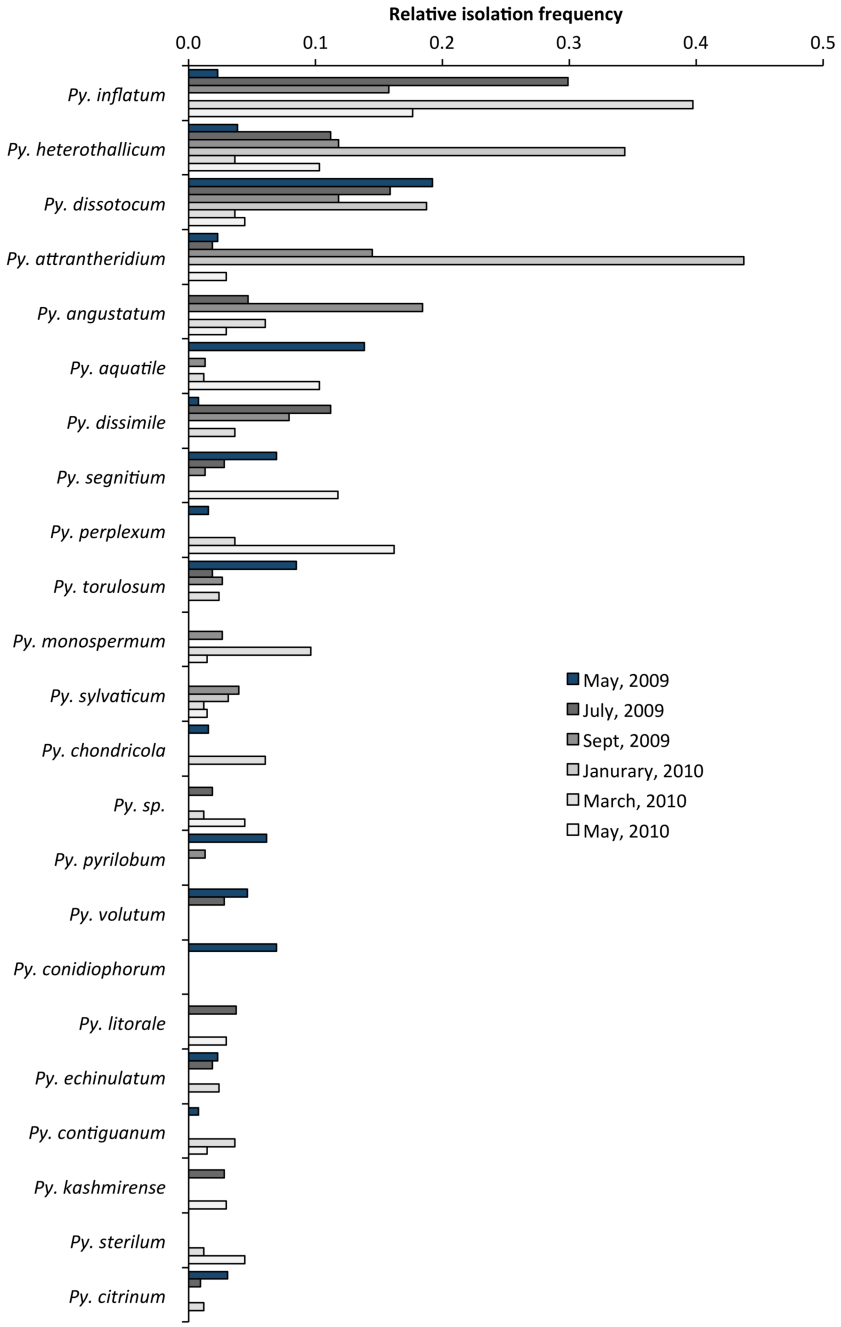


Figure S II.2: Distribution of oomycete species across sampling sites.

Isolation frequency reflects the number of isolates of a given species obtained at a particular site relative to the total number of isolates recovered from that site.

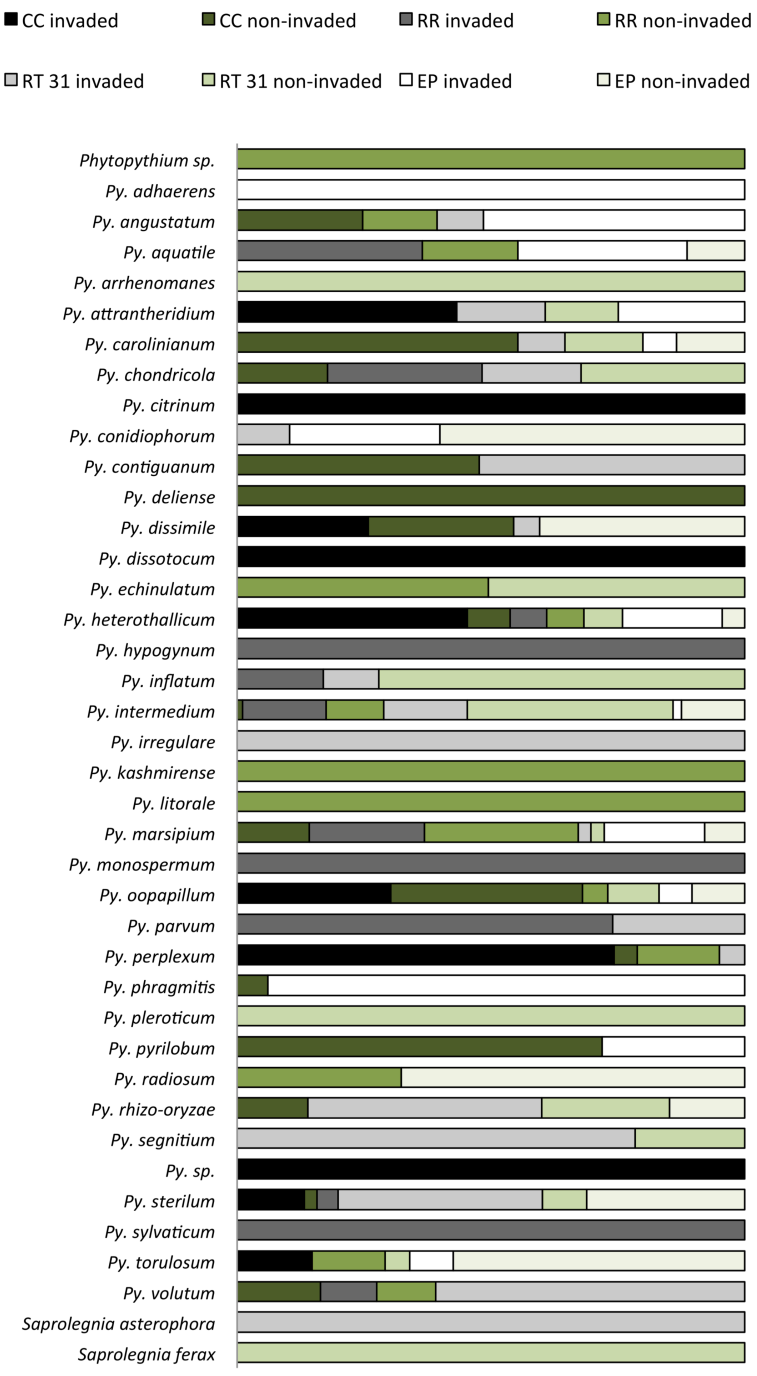


Figure S II.3: Overall seedling survival following inoculation by a single isolate of a given *Pythium* species. Error bars indicate standard error of seedling survival.

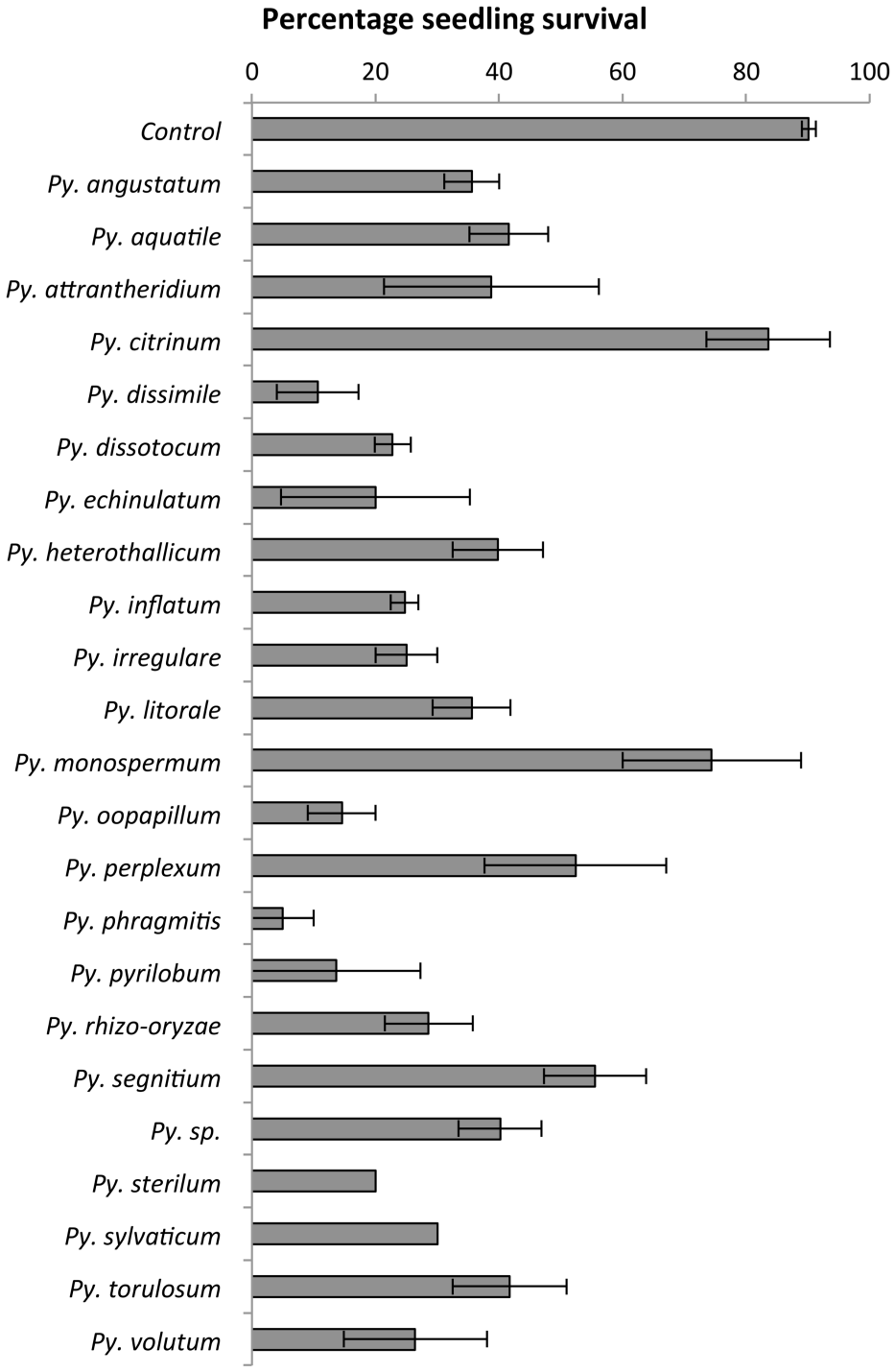
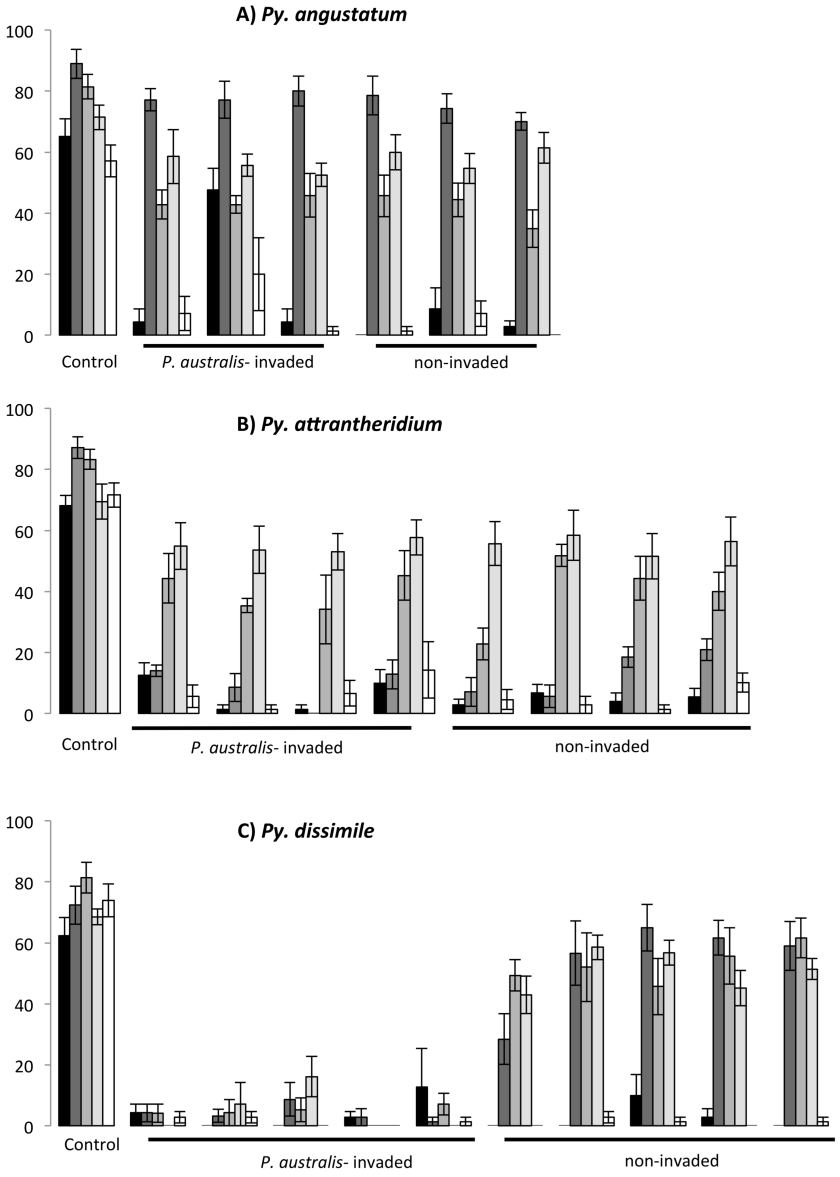
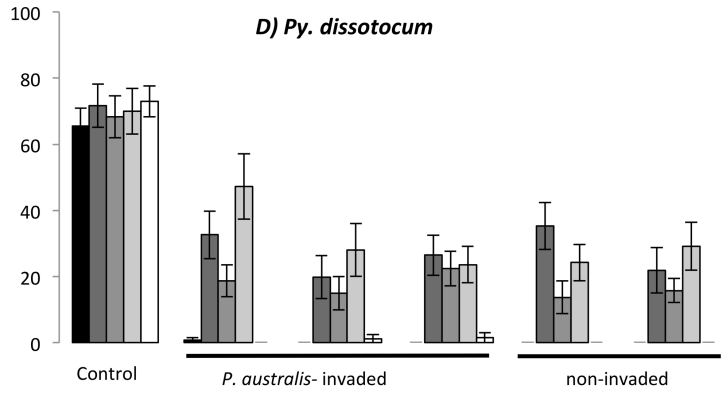
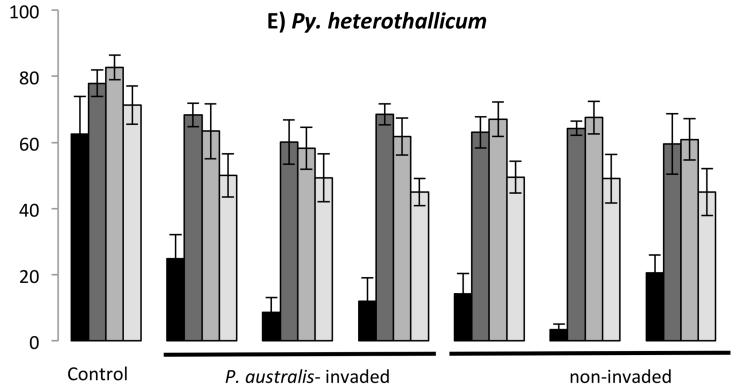
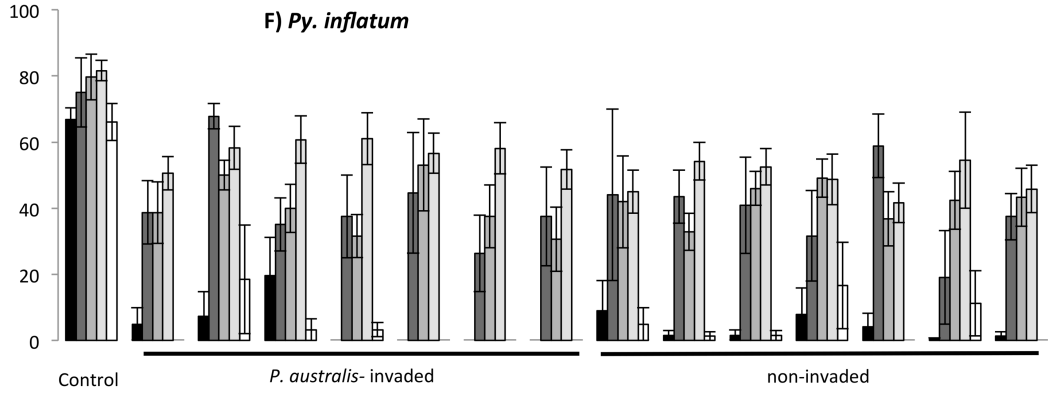


Figure S II. 4: Seedling survival of *E. glandulosum* (black bars), *P. australis* (dark grey bars), *P. a. americanus* (medium grey bars), *M. glomerata* (light grey bars) and *L. salicaria* (white bars) following inoculation with isolates recovered from *P. australis*-invaded and non-invaded soils. *Py. dissimile* (A), *Py. attrantheridium* (B), *Py. angustatum* (C), *Py. heterothallicum* (D), *Py. dissotocum* (E), *Py. inflatum* (F). Error bars indicate standard errors from the mean of seedling survival.




D) Py. dissotocum
100
80
60
40
20
0
Control
P. australis- invaded
non-invaded


E) Py. heterothallicum
100
80
60
40
20
0
Control
P. australis- invaded
non-invaded


F) Py. inflatum
100
80
60
40
20
0
Control
P. australis- invaded
non-invaded

Supporting Information Chapter 3

Table S III.1. Seed burial sites.

Site	***Abbreviation (#)***	***Longitude***	***Latitude***
Resource Ecology and Management Centre, Cornell University, Ithaca NY	1	76°28'5.40"W	42°26'30.31"N
Wildflower Garden, Cornell Plantations, Cornell University, Ithaca NY	2	76°28'9.13"W	42°27'1.92"N
Malone Unit, Department of Environmental Conservation, Savannah NY	3	76°44'43.55"W	43° 4'27.34"N
Martin's Marsh 1, Department of Environmental Conservation, Savannah NY	4	76°42'33.33"W	43° 5'7.11"N
Martin's Marsh 2, Department of Environmental Conservation, Savannah NY	5	76°42'27.60"W	43° 5'2.03"N
Martin's Marsh 3, Department of Environmental Conservation, Savannah NY	6	76°42'36.16"W	43° 5'2.05"N
Teal Pond 1, Department of Environmental Conservation, Savannah NY	7	76°42'20.62"W	43° 5'7.98"N
Teal Pond 2, Department of Environmental Conservation, Savannah NY	8	76°42'17.83"W	43° 5'9.51"N
Teal Pond 3, Department of Environmental Conservation, Savannah NY	9	76°42'18.03"W	43° 5'5.41"N
Private Residence, Cayuga View Rd., Trumansburg NY	10	76°37'39.43"W	42°32'42.44"N

Table S III.2. Model results for effect of soil overwintering on seed germination of the 10 different plant species tested. Data were analyzed using a generalized linear mixed model (GLMM) with binomial distribution. Models included fungicide and stramenicide treatments as a fixed effects and collection location as a random effect‡.

Species	**Intercept ± SE**	**Soil treatment ± SE**
Asclepias incarnata	*1.79 0.29***	
Calamagrostis canadensis	*-0.34 0.13***	
Carex comosa	*-4.19 0.37****	
Carex frankii	*-0.20 0.25*	
Epilobium glandulosum	*1.14 0.21****	-0.67 0.23 (None)**
		-0.6411 0.24 (stramenicide)**
Muhlenbergia glomerata	*1.28 0.22****	
Americanus	*0.27 0.13**	
Lythrum salicaria	*2.33 0.31***	0.75 0.38 (None)*
		0.80 0.38 (stramenicide)*
Australis	*1.26 0.16****	
Phalaris arundinacea	*1.03 0.21****	0.31 0.24(None)
		0.98 0.27 (stramenicide)

‡ Empty cells denote parameters that were not part of the best model, as determined by ANOVA comparisons of models with and without each term.

*, **, *** Asterisks indicate P-values from log-likelihood tests between a model without the term and a model with all terms included (* $P < .05$; ** $P < .01$, *** $P < .001$).

Table S III.3. Fungal species isolated from different (A) plant species and (B) soil treatments**.**

A

Fungal species	Plant species										Total
	A. inc	*C. can*	*C. com*	*C. fra*	*E. gla*	*L. sal*	*M. glo*	*P. a. amer*	*P. aust*	*Ph. aru*	
Alternaria alternata	12	4	12	24	3	6	7	5	2	1	76
Alternaria infectoria	1		1		1	1					4
Aureobasidium sp.			1								1
Cadophora luteo-olivacea		1	3			1	2				7
Curvularia inaequalis								1			1
Cylindrocarpon sp.	1										1
Diatype stigma						1					1
Diplodia seriata								1			1
Epicoccum nigrum	4	1	2	2	5	1	1	1		6	23
Epicoccum sorghinum								1			1
Fusarium proliferatum								1			1
Fusarium solani	1		1	1							3
Fusarium sporotrichioides			1		4						5
Fusarium tricinctum	1		1	1	1						4
Leptosphaeria sp.										1	1
Leptosphaerulina trifolii					1						1
Mucor circinelloides					2						2
Mucor hiemalis									1		1
Nectria nigrescens						1					1
Nigrograna mackinnonii				1							1
Paraconiothyrium sp.	1										1
Paraphaeosphaeria neglecta						1					1
Paraphaeosphaeria sp.	1										1
Paraphaeosphaeria sporulosa										1	1
Penicillium aculeatum	1										1
Penicillium glabrum						1					1
Penicillium sp.										1	1
Peyronellaea glomerata			3	2	1	1		38	9	1	55
Phaeosphaeria poae	1		1				1			1	4
Phoma sp.								1	1		2
Pilidium concavum			1				1				2
Pythium heterothallicum									1		1
Pythium logandrum	1								1		2
Saccharicola bicolor		1									1
Sarocladium strictum							1				1
Sphaeropsis sapinea										1	1
Stagnospora trichophoricola							1				1
Trametes gibbosa					1						1
Trichoderma koningiopsis	1										1
Uncultured fungal endophyte				1							1
Total	26	7	27	32	19	14	14	49	15	13	216

B

Fungal species	Seed treatment			Total
	Fungicide	None	Stramenicide	
Alternaria alternata	2	38	36	76
Alternaria infectoria	1	1	2	4
Aureobasidium sp.			1	1
Cadophora luteo-olivacea		6	1	7
Curvularia inaequalis			1	1
Cylindrocarpon sp.	1			1
Diatrype stigma			1	1
Diplodia seriata	1			1
Epicoccum nigrum		10	13	23
Epicoccum sorghinum			1	1
Fusarium proliferatum			1	1
Fusarium solani			3	3
Fusarium sporotrichioides		4	1	5
Fusarium tricinctum		1	3	4
Leptosphaeria sp.			1	1
Leptosphaerulina trifolii		1		1
Mucor circinelloides			2	2
Mucor hiemalis		1		1
Nectria nigrescens	1			1
Nigrograna mackinnonii			1	1
Paraconiothyrium sp.			1	1
Paraphaeosphaeria neglecta			1	1
Paraphaeosphaeria sp.		1		1
Paraphaeosphaeria sporulosa			1	1
Penicillium aculeatum			1	1
Penicillium glabrum	1			1
Penicillium sp.			1	1
Peyronellaea glomerata	1	27	26	54
Phaeosphaeria poae		1	3	4
Phoma sp.		1	1	2
Pilidium concavum		2		2
Pythium heterothallicum	1			1
Pythium logandrum	1	1		2
Saccharicola bicolor		1		1
Sarocladium strictum	1	1		2
Sphaeropsis sapinea		1		1
Stagonospora trichophoricola				0
Trametes gibbosa			1	1
Trichoderma koningiopsis			1	1
Uncultured fungal endophyte			1	1
Total	11	98	106	216

Figure S III.1. Seed burial container set-up.

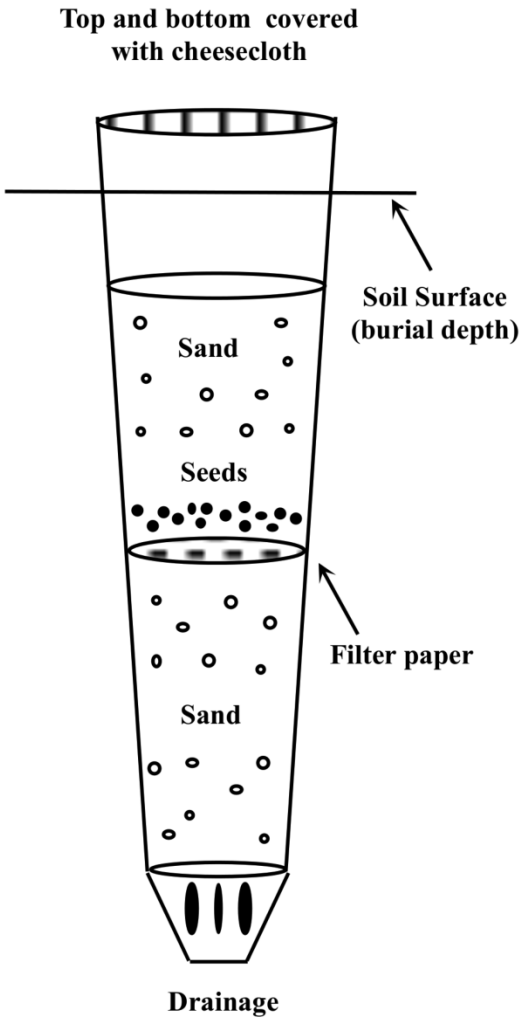
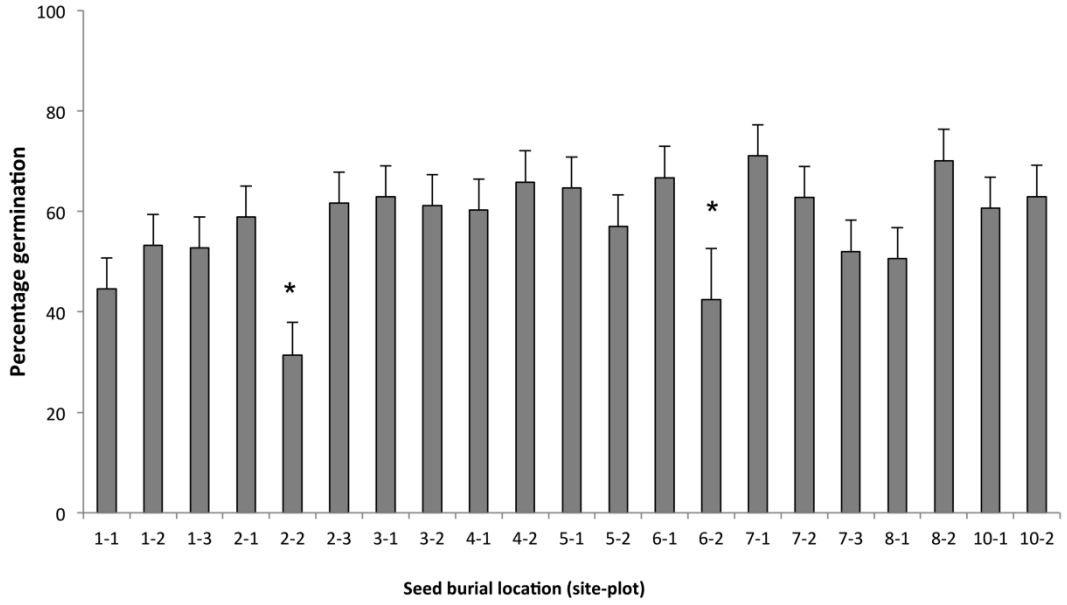


Figure S III.2. Percentage germination of all seeds (non-treated and biocide-treated) after overwintering at 10 different sites. Field sites are designated as a "x-y" value where x=site number and y=the plot number at that site. Data are means (+1SE) with significant differences ($p < 0.05$) represented by an asterisk (*).



Supporting Information Chapter 4

Table S IV.1: Experimental plant species.

Congeneric pair	Species	Origin	Stratification
Apocynaceae	*Asclepias incarnata*	Native (North American)	30 days, cold
	Cynanchum louiseae	Non- native (European)	30 days, cold
Iridaceae	*Iris versicolor*	Native (North American)	120 days, cold
	Iris pseudacorus	Non-native (Eurasian)	120 days, cold
Onagraceae	*Epilobium glandulosum*	Native (North American)	
	Epilobium hirsutum	Non-native (Eurasian)	
Poaceae 1	*Phragmites australis (americanus)*	Native (North American)	
	Phragmites australis (australis)	Non-native (Eurasian)	
Poaceae 2	*Calamagrostis canadensis*	Native (North American)	
	Phalaris arundinacea	Non-native (Eurasian)	

Figure S IV.1: Effect of soil conditioned by each plant species. Each chart reflects a different seedling species (a-j) and the x-axis shows the plant species conditioning the soil in which it grew. Along the x-axis, plant species are ordered by their genetic distance to the seedling species. Bars reflect mean dry biomass and standard errors from the mean.

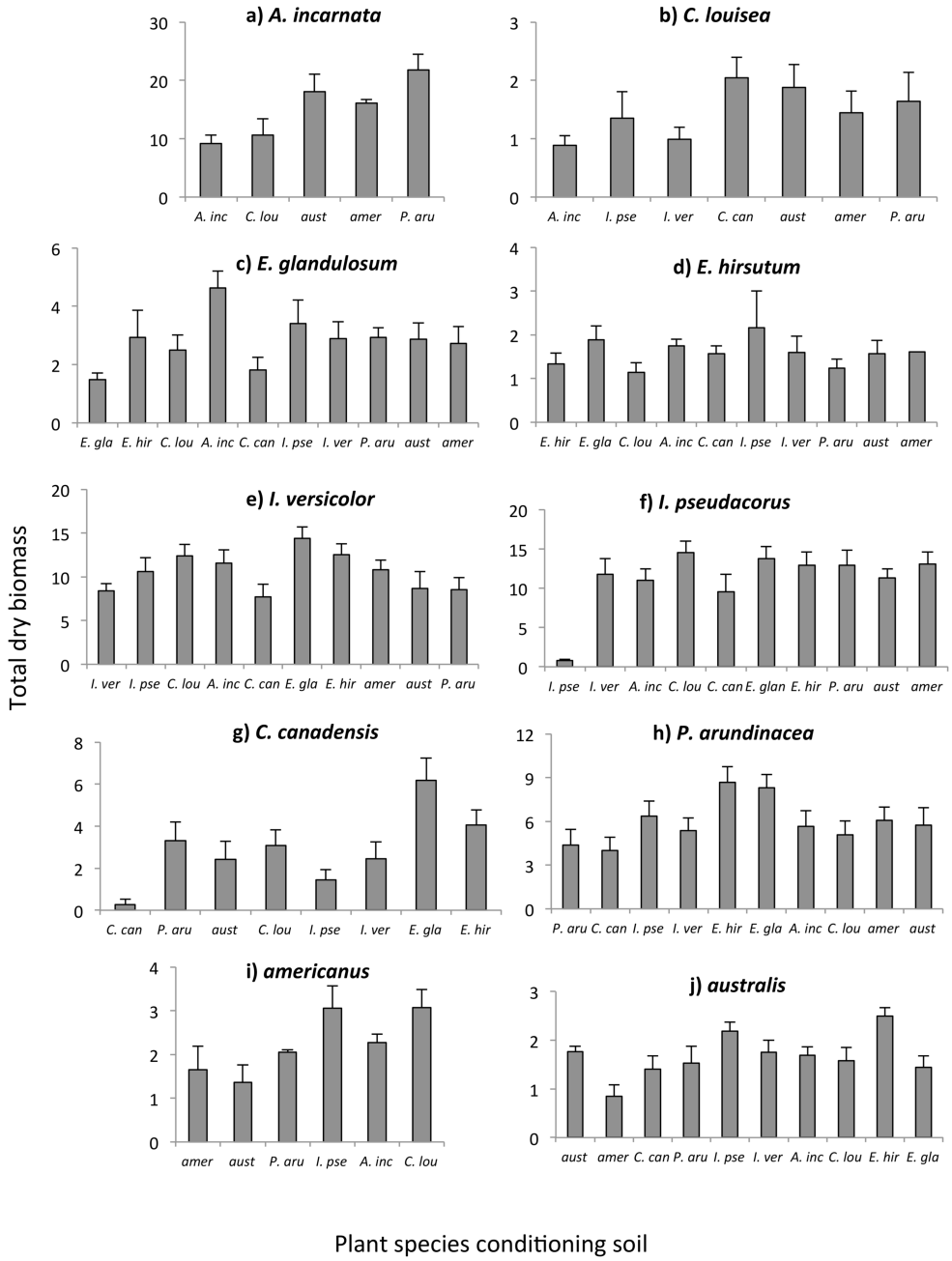


Figure S IV.2: Feedback by phylogenetic distance. Feedback measured as log [(dry biomass when grown in a particular plant species)/ dry biomass in potting mix]. Phylogenetic distance reflects the dissimilarity of *rbcL* sequences from the seedling species to the plant species that conditioned the soil. Plant families (and p-values) are listed and asterisks indicate statistically significant fit lines.

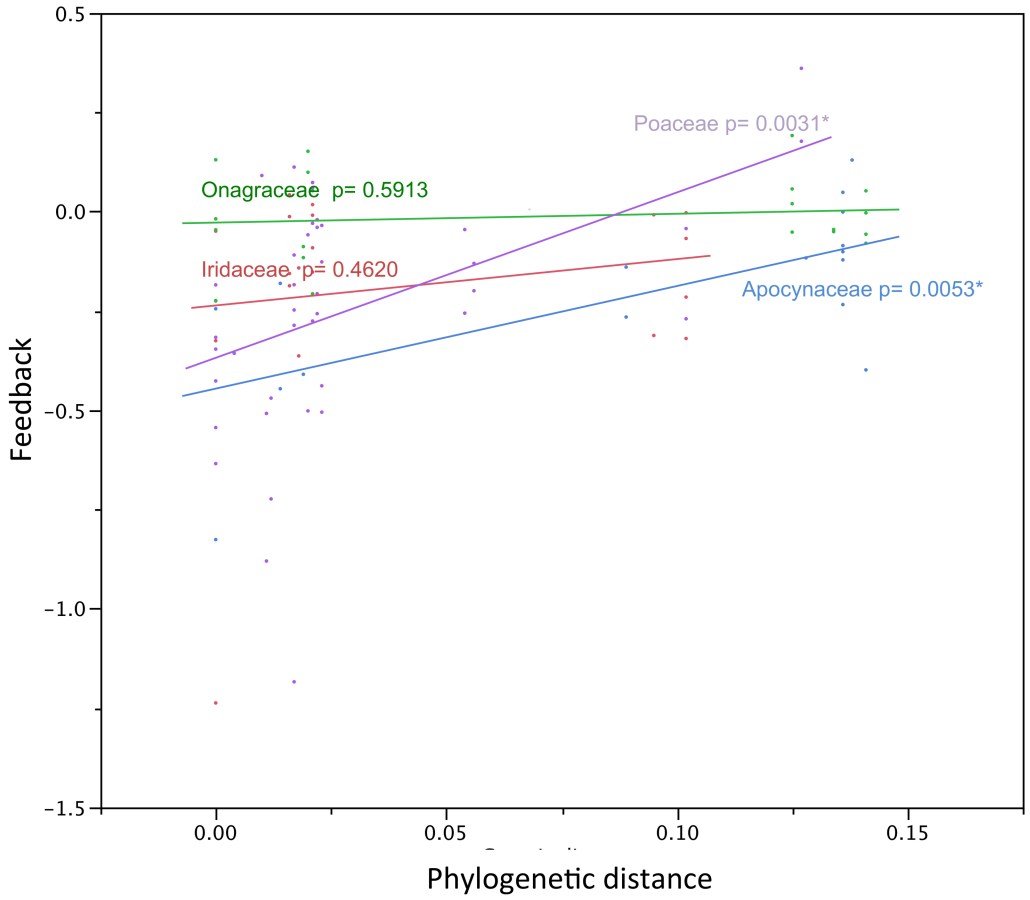


Table S IV.2: Illumina sequencing read results, a) before and b) after mapping to reference database.

a) Total reads per sample after QC and demultiplexing.

Plant species conditioning soil	Number of reads
A. incarnata	1034495
C. louisea	749087
E. glandulosum	864098
E. hirsutum	1004095
I. versicolor	880513
I. pseudacorus	658662
C. canadensis	788498
P. arundinacea	795567
americanus	628656
australis	533781

b) Number of sequences mapped to reference database.

Plant species conditioning soil	Number of reads	Number of OTUs	Proportion of OTUs unique to soil type
A. incarnata	13844	30	0.17
C. louisea	29540	24	0.04
E. glandulosum	12292	33	0.24
E. hirsutum	16912	29	0.17
I. versicolor	26794	23	0.04
I. pseudacorus	54450	13	0.00
C. canadensis	28233	13	0.00
P. arundinacea	22650	21	0.10
americanus	41624	19	0.16
australis	34883	12	0.00

Figure S IV.3: Principal coordinates analysis of fungal communities by plant species. Based on Illumina sequences of soil conditioned by each plant species, with those plant species that experienced negative PSFs caused by soil biota marked by black circles and all other plant species marked by grey squares.

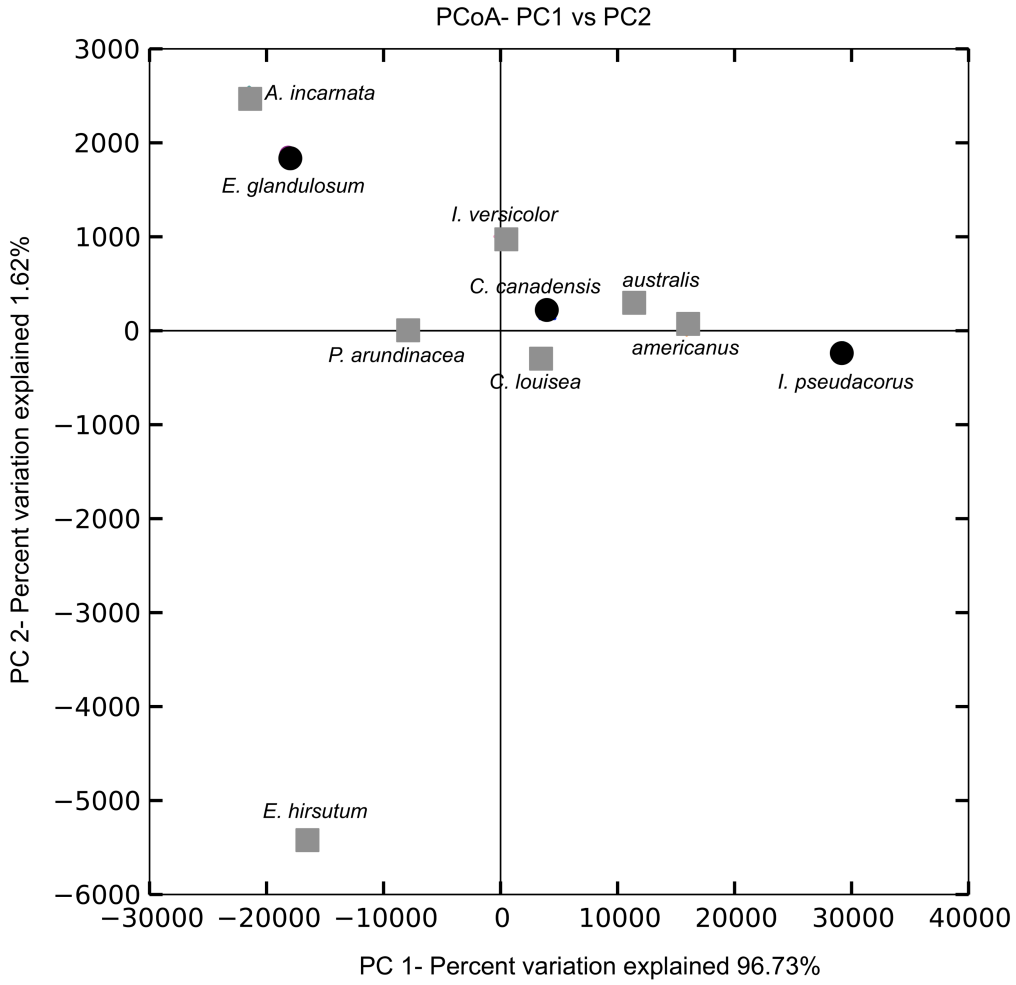


Table S IV.3: Number of reads for each fungal OTU detected. List includes all OTUs with > 10 reads from the soil of any plant species. Fungal OTUs are sorted by Phylum (Ascomycota, Basidiomycota…) and listed by best genus match or the closest fungal group match if of an unidentified genus.

	Number of reads per OTU for each plant species										
Fungal OTU	*I. ver.*	*P. arund.*	*austra lis*	*A. inc.*	*E. gla.*	*E. hir.*	*I. pse.*	*ameri canus*	*C. can.*	*C. lou.*	**Total # reads**
Ascomycota											
Phialosimplex	21810	13749	33243	318	3577	5293	51020	37545	25636	24932	**217123**
Aspergillus	80	4009	70	844	1180	1285	490	333	1186	798	**10275**
Pseudogymnoascus	1527	23	384	914	4929	135	54	125	0	207	**8298**
unidentified, phylum Ascomycota	54	23	0	42	12	6663	0	0	0	798	**7591**
unidentified, family Trichocomaceae	54	2492	105	734	246	1556	599	416	621	473	**7294**
unidentified, class Leotiomycetes	322	91	279	291	172	51	817	291	169	30	**2512**
unidentified, phylum Ascomyctoa	80	204	209	152	541	186	163	208	56	236	**2037**
Penicillium	482	91	105	138	221	68	54	166	141	473	**1940**
Tetracladium	188	0	0	720	356	237	54	0	0	59	**1614**
Talaromyces	80	0	0	14	74	0	0	1207	0	30	**1405**
unidentified, class Dothideomycetes	0	815	0	0	0	0	0	0	0	0	**815**
unidentified, order Helotiales	27	23	35	14	0	0	218	208	0	30	**554**
Devriseia	0	385	0	42	0	17	0	42	0	0	**485**
Aureobasidium	0	0	0	69	37	51	0	0	0	207	**364**
unidentified, class Dothideomycetes	54	0	0	14	12	17	0	0	0	0	**97**
Cochliobolus	27	0	35	0	0	0	0	0	28	0	**90**
Scutellinia	0	0	0	0	0	0	0	83	0	0	**83**
Phoma	0	0	0	0	37	17	0	0	0	0	**54**
unidentified, order Chaetothyriales	0	0	0	0	0	51	0	0	0	0	**51**
Lalaria	27	23	0	0	0	0	0	0	0	0	**49**
unidentified, order Pleosporales	0	0	0	0	0	17	0	0	0	30	**46**
unidentified, family Pezizaaceae	0	0	0	0	0	0	0	42	0	0	**42**
Microdochium	0	0	0	0	0	0	0	42	0	0	**42**
unidentified, family Pseudeurotiaceae	0	0	0	42	0	0	0	0	0	0	**42**
unidentified, family Taphrinaceae	0	0	0	28	12	0	0	0	0	0	**40**
Oidiodendron	0	0	0	0	37	0	0	0	0	0	**37**
unidentified, family Sclerotiniaceae	0	0	0	0	37	0	0	0	0	0	**37**
unidentified, family Trichocomaceae	0	23	0	0	12	0	0	0	0	0	**35**
unidentified, order Capnodiales	0	0	0	0	0	34	0	0	0	0	**34**
unidentified, family Mycosphaerellaceae	0	0	0	28	0	0	0	0	0	0	**28**
Rhizoscyphus	27	0	0	0	0	0	0	0	0	0	**27**
unidentified, order Saccharomycetales	27	0	0	0	0	0	0	0	0	0	**27**
Cyphellophora	0	23	0	0	0	0	0	0	0	0	**23**
unidentified, order	0	0	0	0	0	17	0	0	0	0	**17**

Pleosporales unidentified, family Herpotrichiellaceae	0	0	0	14	0	0	0	0	0	0	**14**
unidentified, phylum Ascomycota	0	0	0	0	12	0	0	0	0	0	**12**
Schwanniomyces	0	0	0	0	12	0	0	0	0	0	**12**
Basidiomycota											
unidentified, family Ceratobasidiaceae	938	0	0	5967	0	186	436	0	0	325	**7851**
unidentified, family Ganodermataceae	295	181	174	512	356	304	272	291	113	443	**2943**
Minimedusa	0	0	0	2603	0	0	0	0	0	0	**2603**
Athelia	54	45	35	55	61	34	0	42	28	30	**384**
Ganoderma	0	0	0	14	25	0	0	0	28	59	**126**
unidentified, family Thelephoraceae	0	23	0	14	0	17	0	0	28	30	**111**
Mrakia	0	0	0	0	0	17	54	0	0	0	**71**
Tomentella	0	0	0	0	0	0	0	42	0	30	**71**
Fuscoporia	0	0	0	0	0	0	0	0	0	59	**59**
Pleurotus	0	45	0	0	12	0	0	0	0	0	**58**
Entoloma	0	0	0	0	0	17	0	0	0	30	**46**
Cortinarius	0	0	0	0	12	0	0	0	28	0	**41**
unidentified, order Agaricales	0	0	0	14	12	0	0	0	0	0	**26**
unidentified, family Polyporaceae	0	0	0	0	25	0	0	0	0	0	**25**
Pluteus	0	0	0	0	0	17	0	0	0	0	**17**
Leucosporidium	0	0	0	0	0	17	0	0	0	0	**17**
unidentified, order Polyporales	0	0	0	14	0	0	0	0	0	0	**14**
Stereum	0	0	0	0	12	0	0	0	0	0	**12**
Sistotremastrum	0	0	0	0	12	0	0	0	0	0	**12**
unidentified, class Tremellomycetes	0	0	0	0	12	0	0	0	0	0	**12**
Zygomycota											
Mortierella	375	249	35	138	61	220	54	416	28	59	**1637**
Mucor	27	0	0	28	86	203	0	83	0	30	**456**
Umbelopsis	27	23	0	14	37	0	0	0	0	30	**130**
Rhizopus	0	0	0	0	0	68	0	0	0	0	**68**
Other fungi (Chytridiomycota etc.)	27	23	0	14	12	17	0	42	0	0	**134**
Total number of reads	**26606**	**22559**	**34709**	**13802**	**12255**	**16811**	**54287**	**41624**	**28092**	**29422**	**280167**

Fig. S IV.4. Common and unique fungal taxa. Best fungal genus matches to common OTUs present in all conditioned soils (circle) and those OTUs unique to the soil of particular plant species (boxes). Plant species whose soils contained no unique OTUs are listed in their own box.