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Soil ecology of the exotic dune grass Leymus arenarius.

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SOIL ECOLOGY OF THE EXOTIC DUNE GRASS *LEYMUS ARENARIUS*

By

Matthew L. Reid

B.A. Hendrix College, 2009

M.S. University of Louisiana at Monroe, 2013

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A Dissertation Approved on

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ABSTRACT

SOIL ECOLOGY OF THE EXOTIC DUNE GRASS *LEYMUS ARENARIUS*

Matthew L. Reid

April 9, 2018

Exotic plants can alter the structure and function of native communities both above- and belowground, which is particularly important in primary successional sand dune ecosystems where plant-soil interactions are critical drivers of successional dynamics. In Great Lakes sand dunes, the native foundation grass *Ammophila breviligulata* dominates early in succession until an accumulation of detrimental soil organisms causes its die-back of the grasses. Die-back of this native foundation species can be slowed by mutualistic interactions between plants and mycorrhizal fungi. Changes to the plant community resulting from invasion, and subsequent effects on the soil community have the potential to disrupt the successional dynamics of dune systems.

Leymus arenarius is a dune-building grass native to northern European sand dunes, and is currently invading Great Lakes dunes. This dissertation examines the effects of *L. arenarius* invasion on soil community structure, multi-trophic interactions within the soil, and plant community development. In a greenhouse study manipulating the presence/absence of soil organisms, I found evidence of altered multi-trophic interactions. Specifically, arbuscular mycorrhizal fungi provided greater protection from plant-parasitic nematodes to *L. arenarius*, resulting in weaker biomass reductions relative to *A. breviligulata*. In a field survey, I found that *L. arenarius* had little effect on the

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structure of soil nematode communities and soil bacterial functional diversity relative to uninvaded areas. In a growth chamber experiment, I found that direct competitive effects from *L. arenarius* were generally similar to effects from *A. breviligulata*, for later successional native species. However, *L. arenarius* more strongly suppressed growth of secondary invaders, relative to suppression by *A. breviligulata*. Soil conditioning effects from *L. arenarius* were present, but were generally weak suggesting that indirect effects of invasion on soil community composition are less important than direct competitive effects.

Experimentally-derived differences in multi-trophic interactions between *L. arenarius* and *A. breviligulata* suggested that the invasion of *L. arenarius* into communities dominated by *A. breviligulata* would result in altered plant-soil interactions. However, the invasion of *L. arenarius* did not result in large impacts on plant and soil communities. Overall, these results suggest that *L. arenarius* has relatively high functional similarity to its native counterpart, *A. breviligulata*.

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CHAPTER I

INTRODUCTION

Invasions by exotic plant species are an increasing problem worldwide and pose serious economic and environmental threats due to their potential to alter natural communities and ecosystem processes (Mack et al. 2000; Pimentel et al. 2005). This is especially true when exotic species replace native foundation species, or are themselves foundation species, capable of creating and modifying habitat (Cuddington and Hastings 2004). While most studies on exotic plants have focused on the impacts to native vegetation, a more predictive approach will require consideration of the linkages between plants and soil biota (Kardol and Wardle 2010) and how exotic plant invasion could alter soil communities, in turn altering native biodiversity and ecosystem functioning. These considerations become especially important in successional systems, where soil legacy effects from exotic plants may have the potential to impact longer term successional dynamics and ecosystem development (Fierer et al. 2010; van de Voorde et al. 2011).

Several recent efforts have improved understanding about the belowground impacts of exotic plant invasions. For example, exotic plant invasions alter soil communities through changes to bacteria (Kourtev et al. 2002), arbuscular mycorrhizal fungi (Lankau et al. 2014; Mummey and Rillig 2006), and invertebrates (Chen et al. 2007; Renčo and Baležentiené 2015; Tanner et al. 2013; Xiao et al. 2013). Even after removal of the exotic plants, negative soil legacy effects created by the exotic plant may

impede full recovery of the community to its pre-invasion state (D'antonio and Meyerson 2002). For example, Lankau et al. (2014) found that arbuscular mycorrhizal fungi (AMF) communities were still depauperate six years after removal of garlic mustard (*Alliaria petiolata*).

Many studies to date have been limited to exotic plant effects on single groups of belowground organisms. However, there is a complex soil food web involving plant roots, fungi, nematodes, mites, springtails, bacteria, and protists that is critical to ecosystem functioning (Bardgett and van der Putten 2014). Future studies of mechanisms regulating impacts of invasive species will need to consider multi-species interactions belowground, especially interactions among plants, soil nematodes, and arbuscular mycorrhizal fungi. Nematodes occupy nearly every trophic level in the soil food web (Bongers and Ferris 1999), including plant-parasites/root herbivores, bacteria-feeders, fungal-feeders, and predators. This makes nematode communities a great metric for assessing soil food webs. Arbuscular mycorrhizal fungi (AMF) are root symbionts that benefit a wide variety of plants by helping with nutrient or water uptake (Smith and Read 2010). AMF are particularly beneficial to plants by aiding in the uptake of phosphorus, a limiting nutrient for plants (Smith and Read 2010). Additionally, AMF can protect roots from the negative impacts of belowground plant-parasitic nematodes (de la Pena et al. 2006; Little and Maun 1997).

Biotic interactions, such as with AMF and nematodes, can affect the success of exotic plant invasion. One broad hypothesis proposed to explain invasion success is the enemy release hypothesis (Heger and Jeschke 2014; Keane and Crawley 2002), whereby exotic species are released from the negative effects of specialized enemies from their

native range. However, application of the enemy release hypothesis belowground has been contentious (Colautti et al. 2004), but there is some supporting evidence for various aspects of this hypothesis (Heger and Jeschke 2014). Additionally, many exotic plants may exhibit reduced mycorrhizal dependence (Seifert et al. 2009), where exotic plants rely less on AMF mutualists than native plants. This can allow exotic species to colonize areas with lower AMF abundance or diversity (Reeves et al. 1979), or potentially disrupt these mutualistic associations to the detriment of native plants (Callaway et al. 2008; Stinson et al. 2006). However, other exotic species may utilize native mycorrhizal networks for to their benefit (Carey et al. 2004; Marler et al. 1999)

STUDY SYSTEM

In primary successional sand dune ecosystems, interactions among plants, soil nematodes, and mycorrhizal fungi are critical drivers of plant community dynamics. The Great Lakes sand dunes are the classic example of primary succession (Cowles 1899), in which the pioneer plant species *Ammophila breviligulata* colonizes bare sand and initiates the dune-building process. Early in succession, *Ammophila breviligulata* spreads vegetatively through underground rhizomes, and becomes dominant on the foredunes. Here, it can outcompete other plant species in this system (Cheplick 2005). *Ammophila breviligulata* forms mutualistic associations with AMF that enhance its growth, while an accumulation of plant-parasitic nematodes and pathogenic fungi eventually cause *Ammophila* to die back (Seliskar and Huettel 1993), allowing for colonization by later successional species.

Great Lakes sand dunes are currently facing invasion from numerous exotic plant species. Of particular interest is the exotic plant *Leymus arenarius*, a dune-building grass

native to northern European sand dunes. In its native range, *Leymus arenarius* forms mutualistic relationships with AMF that enhance its growth (Greipsson and El-Mayas 2000), while an accumulation of detrimental soil organisms, including plant-parasitic nematodes can reduce its vigor (Greipsson and El-Mayas 2002). Thus, *Leymus arenarius* appears to be functionally similar to the native grass *Ammophila breviligulata* in its new invasive range. This dissertation is an examination of the invasion biology of *Leymus arenarius*, regarding its interactions with soil organisms, impacts of invasion on soil communities, and potential impacts on plant community dynamics.

ORGANIZATION OF DISSERTATION

In this dissertation, I examine how *Leymus arenarius* interacts with native soil organisms and both native plants and secondary invasive plant species in a primary successional sand dune ecosystem. My broad questions of interest are: 1) Is *Leymus* invasion associated with altered structure of soil communities and interactions within the soil community? and 2) Does *Leymus* invasion have the potential to alter plant successional dynamics via changes belowground or via direct competition? I used a combination of field surveys and manipulative greenhouse and growth chamber experiments to answer these questions regarding *Leymus* invasion.

In chapter two of the dissertation, I address the question: Do the exotic *Leymus arenarius* and the native *Ammophila breviligulata* have different interactions with two groups of soil organisms with important roles in dune succession – arbuscular mycorrhizal fungi (AMF) and plant-parasitic nematodes (PPN)? I manipulated presence/absence of two generalist belowground species that occur in Great Lakes dunes, *Rhizophagus intraradices* (AMF) and *Pratylenchus penetrans* (PPN) in a factorial

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greenhouse experiment, and assessed the biomass production and root architectural traits of the plants. Inoculation with the AMF *Rhizophagus* increased above- and belowground biomass production by ~32% for both plant species. Inoculation with the PPN *Pratylenchus* decreased aboveground biomass production by ~36% for both plant species. However belowground, the exotic *Leymus* was significantly more resistant to PPN than the native *Ammophila*, and colonization by AMF provided greater protection to *Leymus* in belowground tri-trophic interactions with PPN, relative to *Ammophila*. This suggests that differences in belowground interactions with AMF and PPN may have the potential to promote *Leymus* invasion.

In chapter three, I addressed the question: Do soil communities associated with *Ammophila* and *Leymus* differ in composition and function? Specifically, I examined soil nematode composition, and in collaboration with Andrea Howes, examined soil bacteria functional diversity. Overall, we found that soil bacterial functional diversity and soil nematode community composition were not strongly associated with factors relating to *Leymus* invasion. Rather, the structure of these soil communities was associated primarily by abiotic climate factors and factors relating to dune successional status and soil edaphic properties.

In the fourth chapter of the dissertation, I address the question: Do *Leymus* and *Ammophila* differ in their effects on plant successional dynamics via direct competitive effects and indirect soil conditioning effects. In a growth chamber experiment, I used a plant-soil feedback design to assess the effect of soil conditioning by the native *Ammophila breviligulata* and the exotic *Leymus arenarius* on other commonly co-occurring plant species. Specifically, seedlings of eight co-occurring native and exotic

plant species were grown in one of four treatments: 1) *Ammophila*-conditioned soil with

an *Ammophila* competitor, 2) *Ammophila*-conditioned soil without a competitor, 3)

Leymus-conditioned soil with a *Leymus* competitor, and 4) *Leymus*-conditioned soil

without a competitor. Overall, I found only a weak negative effect of *Leymus*-conditioned

soil on the growth of other native and exotic plants. There were strong effects of direct

competition from both *Ammophila* and *Leymus* competitors on both native and exotic

species. However, competition from *Leymus* was greater than competition from

Ammophila for the secondary invasive species. This suggests that *Leymus* invasion does

not promote, but rather has the potential to suppress, secondary invasion in sand dunes.

The fifth and final chapter summarizes the main conclusions of the dissertation

and presents ongoing and future research directions examining the causes and

consequences of *Leymus* invasion.

CHAPTER II

NATIVE AND EXOTIC FOUNDATION GRASSES DIFFER IN TRAITS AND

RESPONSES TO BELOWGROUND TRI-TROPHIC INTERACTIONS

SUMMARY

A plant's growth and fitness are influenced by species interactions, including those belowground. In primary successional systems, belowground organisms are known to have particularly important control over plant growth. Exotic plant invasions in these and other habitats may in part be explained by altered associations with belowground organisms compared to native plants. We investigated the growth responses of two foundation grasses on Great Lakes sand dunes, the native grass *Ammophila breviligulata* and the exotic grass *Leymus arenarius*, to two groups of soil organisms with important roles in dune succession: arbuscular mycorrhizal fungi (AMF) and plant-parasitic nematodes (PPN). We manipulated presence/absence of two generalist belowground species known to occur in Great Lakes dunes, *Rhizophagus intraradices* (AMF) and *Pratylenchus penetrans* (PPN) in a factorial greenhouse experiment, and assessed the biomass production and root architectural traits of the plants. There were clear differences in growth and above- and belowground architecture between *Ammophila* and *Leymus,* with *Leymus* plants being bigger, taller, and having longer roots than *Ammophila*. Inoculation with *Rhizophagus* increased above- and belowground biomass production by ~32% for both plant species. Inoculation with *Pratylenchus* decreased

aboveground biomass production by ~36% for both plant species. However belowground, the exotic *Leymus* was significantly more resistant to PPN than the native *Ammophila*, and gained more benefits from AMF in belowground tri-trophic interactions than *Ammophila*. Overall, our results indicate that differences in plant architecture coupled with altered belowground interactions with AMF and PPN have the potential to promote exotic plant invasion.

INTRODUCTION

Invasions by exotic plant species are an increasing problem worldwide and pose serious economic and environmental threats due to their potential to alter natural communities and ecosystem processes (Mack et al. 2000; Pimentel et al. 2005). Ecologists have long sought to understand why certain exotic plants become invasive. Biotic interactions, including those belowground have the potential to affect an exotic plant's growth and fitness (Van der Putten et al. 2001). Herbivores, mutualists, and parasites are all present in soils, forming a complex food web based on plant roots. For example, root herbivores such as plant-parasitic nematodes (PPN), can cause extensive damage to plants both above- and belowground (Neher 2010) and can be critical drivers of plant community dynamics (De Deyn et al. 2003). Invasive plant interactions with herbivores, including PPN, are often viewed in the context of the enemy release hypothesis, a broad hypothesis best viewed as a hierarchy of hypotheses (Heger and Jeschke 2014). Within this hierarchy of hypotheses, many studies have shown that exotic invasive plants are less susceptible to and less infested by root herbivores and parasites than native plant species (Maron et al. 2014; Van der Putten et al. 2005), bringing belowground support to certain sub-hypotheses of enemy release (Colautti et al. 2004;

Heger and Jeschke 2014). For example, the European dune grass *Ammophila arenaria* has had multiple introductions to parts of North America and South Africa, where it has spread and become invasive (David et al. 2015; Knevel et al. 2004). Biogeographic release from root herbivores has been implicated as a contributing factor in the invasiveness of this species (Knevel et al. 2004; Van der Putten et al. 2005).

Belowground mutualists can also play important roles in plant invasions. Arbuscular mycorrhizal fungi (AMF) are root symbionts that benefit a wide variety of plants by helping with nutrient and water uptake. AMF are particularly beneficial to plants by aiding in the uptake of phosphorus, a limiting nutrient for plants (Smith and Read 2010). Many exotic invasive plants are less dependent on AMF for their growth (Seifert et al. 2009; Vogelsang and Bever 2009), but see (Bunn et al. 2015). However, other exotic invasive plants are known to exploit the native AMF community to their advantage, via increased nutrient acquisition (Lee et al. 2013) or parasitism of the common mycorrhizal network among plants (Callaway et al. 2001).

These soil organisms interact not only with plant roots but with other soil organisms as well. For example, AMF can protect roots from the negative impacts of belowground PPN (Azcón-Aguilar and Barea 1997; de la Pena et al. 2006). This network of interactions among plant roots, soil nematodes, fungi, and other soil organisms is complex, and can affect plant performance. Plant species are expected to vary in their dependence on and susceptibility to belowground organisms (Reinhart and Callaway 2006), and reduced susceptibility to belowground organisms such as AMF and PPN may play a role in the invasiveness of some exotic plant species (Mitchell et al. 2006). Multitrophic interactions among plants, microbes, and herbivores add another layer of

complexity, making it difficult to predict how soil organisms generally contribute to exotic plant invasion (Kempel et al. 2013).

Plant interactions with belowground organisms are believed to be especially important in early successional ecosystems (Reynolds et al. 2003; van de Voorde et al. 2011). In many cases, early colonizing plant species control succession by inhibiting or facilitating colonization by other plant species (Connell and Slatyer 1977). For example, in the primary successional Great Lakes sand dunes, the dominant native foundation grass, *Ammophila breviligulata* (hereafter, *Ammophila*), inhibits colonization of later-successional plants (Cheplick 2005). Dieback of *Ammophila* eventually occurs after an accumulation of soil pathogens, including fungal pathogens and PPN (Seliskar and Huettel 1993). Typically, only after *Ammophila* dieback has occurred are other plants able to establish. Mutualistic associations of *Ammophila* with AMF can slow dieback, and thus slow succession (Little and Maun 1997). Similar patterns of *Ammophila* colonization, growth, and dieback are seen in the European dune grass *Ammophila arenaria* (Van der Putten et al. 1993). Thus, plant-soil interactions play a critical role in plant successional dynamics in coastal dune systems.

An exotic early successional dune-building grass native to northern Europe, *Leymus arenarius* (hereafter, *Leymus*), has established on dunes throughout the Great Lakes (Martinus 2009; Reid, unpublished data). *Leymus* appears to be functionally similar to *Ammophila*, since both are C3 foundational dune-building grasses. However, even small physical differences between foundation species can contribute to significant ecological impacts (Hacker et al. 2012). In sand dunes, subtle differences in tiller density, height, and biomass of foundation grasses can alter dune geomorphology through

biophysical feedbacks (Hacker et al. 2012; Zarnetske et al. 2012). While no direct comparisons between *Ammophila* and *Leymus* have been made, evidence suggests that mature *Leymus* plants may reach heights of up to 1.5 meters (Borland et al. 2009), while mature *Ammophila* plants typically attain heights of less than 1 meter (Emery and Rudgers 2014). Large physical differences such as this may contribute to the ecological impact of *Leymus* where it invades.

To our knowledge, no studies have examined the ecology of *Leymus* in its invasive range, despite its invasion dating back until at least the 1960s (Larkin 2011). However in its native range, *Leymus* is known to form associations with AMF which benefit its growth and survival (Greipsson and El-Mayas 2000), while PPN and fungal pathogens can suppress its growth. Similar to *Ammophila*, AMF provide some protection from native fungal pathogens and PPN as well (Greipsson and El-Mayas 2002). It remains unclear what belowground interactions *Leymus* forms in its exotic range, and whether any shifts in these belowground interactions could influence the invasion success of this species.

The objective of this study was to assess the above- and belowground growth responses of the native foundation grass *Ammophila breviligulata* and the exotic foundation grass *Leymus arenarius* to soil organisms naturally occurring in Great Lakes sand dunes. We hypothesized that (1) despite both being classified as foundational dune-building species, *Leymus* would be larger and exhibit different above- and belowground traits when compared with *Ammophila*; (2) as a non-native species, *Leymus* would be less susceptible to plant-parasitic nematodes relative to *Ammophila*; (3) as a non-native species, *Leymus* would be less dependent on arbuscular mycorrhizal fungi relative to

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Ammophila; and (4) as a non-native species, *Leymus* will have altered tri-trophic interactions with these native soil organisms, resulting in less variation in growth responses than the native *Ammophila.* Alternatively, *Leymus* and *Ammophila* could have a functionally similar dependence on AMF and susceptibility to PPN. In a greenhouse experiment, we tested these hypotheses by measuring above- and belowground plant traits in response to AMF and PPN. Results from this experiment have the potential to yield insights into the role of belowground multi-species interactions during exotic plant invasion.

MATERIALS AND METHODS

Seed collection and germination

We collected seeds of *Ammophila* and *Leymus* from western Michigan sand dunes in September 2014 and stored them at -20°C until initiation of the experiment. We removed the palea and lemma from seeds of each species, surfaced sterilized them for 15 minutes in 10% bleach, washed in distilled water for 15 minutes, and placed in moist sealed petri plates. Seeds were stratified for two months at 4°C. Following cold stratification, petri plates were placed under a florescent light at room temperature until germination occurred.

Experiment set-up

In the greenhouse, 80 Tall One treepots (10 cm wide × 36 cm deep; Stuewe and Sons, Corvallis, Oregon), were filled with screened and washed play sand (Quikrete Inc., Atlanta, GA), with similar texture to Great Lakes dune sand. Treatments were applied in a 2 x 2 x 2 factorial design, with plants species (native *Ammophila* and exotic *Leymus*), AMF (presence/absence), and PPN (presence/absence) as the factors.

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We used the AMF species *Rhizophagus intraradices* (formerly *Glomus intraradices*), a generalist AMF known to associate with dune plants (Gemma and Koske 1997) for the experiment. Our AMF inoculum was obtained from pure cultures of *R. intraradices* grown at the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM) at West Virginia University. To half of all pots, ten grams of inoculum, consisting of INVAM potting media containing spores and root fragments with hyphae, were mixed with an equal volume of play sand and spread on top of filled pots. INVAM potting media without *R. intraradices* was used in the other half of pots as a control. One seedling of either grass species was planted into the pot, and a thin layer of play sand was applied to cover the inoculum and reduce potential contamination. All pots were kept well-watered and grown in ambient greenhouse conditions (daytime temp. range 23-32°C) with no supplemental light.

After two weeks, PPN were added to half of the pots in each AMF treatment. Two weeks has been shown to provide enough time for AMF to colonize plant roots and provide protection from nematode damage (de la Pena et al. 2006). The PPN species added to the experiment was *Pratylenchus penetrans*, a common migratory endoparasitic nematode known to occur on sand dunes and to infect the roots of dune plants (Seliskar and Huettel 1993). This species has also been shown to be suppressed by *R. intraradices* in other systems (Veresoglou and Rillig 2012). PPN inoculum was prepared from pure *P. penetrans* cultures growing in-vivo on sterile agar plates with alfalfa roots (*Medicago sativa*). Nematodes were extracted from alfalfa roots by cutting roots into 1-2 cm length fragments and keeping moist for two days, allowing for nematode migration out of the roots, following the methods of Young (1954). Nematodes were washed into a 50 mL

centrifuge tube, and stored at 4°C overnight. To inoculate the pots with nematodes, we pipetted a total of 2 mL of the aqueous solution containing nematodes into four small depressions in the rooting zone of the soil for each pot in the PPN+ treatment. This resulted in an average of 160 nematodes per pot, similar to field densities reported from sand dune systems (de la Peña et al. 2008). For pots not receiving the nematode treatment, we added an equal volume of sterile water as a control. Each treatment combination (Plant species (2) x AMF (2) x PPN (2)) was replicated 10 times, for a total of 80 pots. Pots were rotated weekly to reduce any effect of location within the greenhouse.

Plant and soil harvesting

After three months, we assessed above- and belowground plant traits. For aboveground traits, we counted the number of leaves produced, total leaf length, and tiller height. Aboveground biomass was clipped, dried at 65°C for 5 days, and weighed. Root systems were carefully removed from the pots, gently rinsed with water to remove sand grains, and placed in a large tray for processing. Individual whole root systems were scanned using WinRhizo root-scanning software (Regent Instruments, Canada). Root scans measured average root diameter, root length, root surface area, number of root tips, and number of forks. Upon completion of root scanning, five 3 cm subsamples of root were clipped for assessment of AMF root colonization and an additional five 3 cm subsamples were clipped to determine nematode abundance in the root tissue. AMF root colonization was assessed following methods in Vierheilig et al. (1998). Briefly, root samples were cleared in a hot solution of 10% KOH for 4 min, rinsed with running tap water for five minutes, and placed in acidified water for 15 min to remove any residual

KOH. Root samples were then stained in a warm 0.05% Trypan Blue stain for 6 min.

After staining, roots were rinsed with running tap water for 2 min and were allowed to

destain overnight in water to maximize contrast between stained hyphal structures and

unstained root tissue. Ten 1 cm length root fragments from each plant were mounted on

microscope slides. Using a compound microscope (Leica Microsystems, Wetzlar,

Germany), AMF colonization was scored as a percentage of fields of view at 200X

magnification with mycorrhizal hyphae, vesicles, or arbuscules following methods

described in McGonigle et al. (1990). Plants inoculated with the AMF *R. intraradices* had

root colonization rates of ~ 35%, while plants receiving the control inoculum without *R.*

intraradices showed AMF root colonization rates of less than 1%, indicating little to no

contamination in our experiment ($F_{1,76} = 112.75$, $P < 0.0001$). Nematodes were extracted

from roots using the incubation method of Young (1954), as described earlier, and were

counted under a microscope (Nikon SMZ1500 stereoscope). Plants receiving inoculation

with the PPN *P. penetrans* contained on average 3.9 nematodes from the 15 cm

subsample of root tissue, while plants receiving the control inoculum without *P.*

penetrans had zero nematodes recovered from the root subsamples, indicating no

contamination in our experimental plants ($F_{1,76} = 10.40$, $P = 0.0019$). The remaining roots

were dried for 5 days at 65°C and weighed to obtain biomass values.

Data analyses

Plant biomass response variables (above- and belowground) were analyzed using

three-factor ANOVAs, with plant species (*Ammophila/Leymus*), AMF

(presence/absence), and PPN (presence/absence) as the factors, with all interaction terms

included. Any significant statistical interaction between plant species and AMF or PPN

would indicate that *Ammophila* and *Leymus* differ in their relationships with these soil organisms. Tri-trophic effects for only the plants inoculated with AMF were further explored using ANCOVA, with plant species (*Ammophila/Leymus*) and PPN (presence/absence) as categorical variables and percent root colonization by AMF as a continuous factor, with all interaction terms included. To further explore tri-trophic interactions in the dual AMF and PPN inoculations, we conducted correlations between percent root colonization by AMF and PPN abundance for both plant species. ANOVA, ANCOVA, and correlation analyses were conducted using SAS/STAT version 9.4 (SAS/STAT Software 2013).

Aboveground plant architecture data including height, number of leaves, and total leaf length were analyzed using MANOVA with species (*Ammophila/Leymus*), AMF (presence/absence), and PPN (presence/absence) as the factors, with all interaction terms included. The MANOVA analysis was conducted using SAS/STAT version 9.4 (SAS/STAT Software 2013).

Among the root architecture data, root length, surface area, number of tips, and number of forks were highly correlated ($r > 0.88$ for all pairwise comparisons). Therefore, we choose to analyze only total root length and the uncorrelated trait of average root diameter. Root length and root diameter data were analyzed using ANOVA with plant species (*Ammophila/Leymus*), AMF (presence/absence), and PPN (presence/absence) as the factors, with all interaction terms included. Root length and root diameter data were ln-transformed to meet assumptions of the ANOVA model. ANOVA analyses were conducted in SAS/STAT version 9.4 (SAS/STAT Software 2013).

RESULTS

Plant growth and vegetative traits

Across all treatments, *Leymus* produced ~135% more aboveground biomass and ~192% more belowground biomass than *Ammophila* (Table 1). *Leymus* plants were 83% taller and had approximately 50% greater total leaf length than *Ammophila* (Table 2; Appendix I). *Leymus* root systems had 151% greater root length than *Ammophila*. Average root diameter was 7% smaller in *Leymus* than in the native *Ammophila* (Appendix I).

Responses to PPN

There was no significant difference in the abundance of nematodes recovered from the root subsamples between *Ammophila* and *Leymus* ($F_{1,76} = 1.37$, $P = 0.2453$) and there was no Plant Species x PPN interaction ($F_{1,76} = 1.37$, $P = 0.2453$). Inoculation with the PPN *P. penetrans* resulted in a reduction in both above- and belowground biomass for both plant species, though effect sizes differed. Both *Ammophila* and *Leymus* had similar decreases in aboveground biomass of approximately 36% (Table 1; Figure 1A). Belowground, *Leymus* had a 50% reduction in root biomass when inoculated with PPN, while *Ammophila* had a 62% reduction in root biomass when inoculated with PPN (Table 1, Figure 1B).

Across both plant species, PPN inoculation reduced plant height by 25%, the number of leaves by 18%, and total leaf length by 35% (Appendix I). PPN inoculation reduced root length by 52% and increased average root diameter by 14% across both plant species (Appendix I).

Responses to AMF

There was no significant difference in AMF colonization levels between *Ammophila* and *Leymus* ($F_{1,76} = 0.01$, $P = 0.9039$), and there was no Plant Species x AMF interaction ($F_{1,76} = 0.09$, $P = 0.7704$). Inoculation with the AMF *R. intraradices* increased both above- and belowground biomass for both species by ~32%. There was no significant AMF x Plant Species interaction for either above- or belowground biomass (Table 1), indicating that both species had similar responses to AMF (Figure 2A and B).

For both plant species, inoculation with AMF increased plant height by 15%, the number of leaves by 22%, and total leaf length by 47% (Appendix I). For belowground architecture, root length was 17% greater for plants inoculated with AMF (Appendix I).

Tri-trophic interactions

There was no significant three-way Plant Species x AMF x PPN interaction for either above- or belowground biomass (Table 1). When examining just the plants inoculated with the AMF *R. intraradices*, there was a significant three-way interaction among Plant Species x PPN x AMF % root colonization for belowground biomass (Table 3). This interaction indicated that the native *Ammophila* and the exotic *Leymus* had different responses to PPN along a gradient of AMF root colonization (Figure 3). In the presence of PPN, *Ammophila* had reduced belowground biomass, and variation in AMF root colonization (ranging from ~10% to 60%) did not alter *Ammophila* responses (Figure 3). AMF and PPN showed a more complex interaction in *Leymus* plants. In the absence of PPN, *Leymus* belowground biomass was negatively correlated with AMF percent root colonization. However, in the presence of PPN, this effect was reversed and *Leymus* attained greater belowground biomass with higher levels of AMF root colonization (Figure 3). For plants in the dual AMF and PPN inoculation treatment, there were weak,

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non-significant negative correlations between percent AMF root colonization and PPN abundance for both *Ammophila* ($r = -0.36$, $P = 0.3011$) and *Leymus* ($r = -0.37$, $P = 0.2886$).

Above- and belowground architectural traits also varied as a function of the AMF x PPN interaction (Table 2; Appendix I). PPN reduced plant height by 38% when AMF were absent. However, when AMF were present, the reduction caused by PPN was significantly lower at 13% (Appendix I). Similar effects were observed for number of leaves and total leaf length. Average root diameter varied as a function of the AMF x PPN interaction (Table 1). As mentioned above, root diameter was increased by 14% with PPN inoculation across both plant species, but this effect was modified by AMF inoculation (Appendix I). In the absence of AMF, PPN inoculation resulted in a 22% increase in average root diameter. But in the presence of AMF, this increase in root diameter with PPN inoculation was substantially weaker at 6%.

DISCUSSION

In this study, the exotic *Leymus* plants were larger in nearly all architectural and biomass measurements. Previous work has shown that plant architectural traits such as height, tiller density, and biomass can alter the architecture of developing sand dunes (Hacker et al. 2012; Zarnetske et al. 2012), indicating the potential for dune structure to be affected by continued *Leymus* invasion. Belowground, *Leymus* root systems were finer and much longer than *Ammophila*, which may provide an advantage in nutrient absorption from the soil (Hodge et al. 2009), though interactions with mycorrhizae may mitigate potential differences in nutrient absorption based on root traits (Cheng et al. 2016). Similarly, studies by Keser et al. (2014) and Vaness et al. (2014) have found that

invasive plants generally produce greater root biomass and have greater root length than co-occurring native species. These belowground differences between *Leymus* and *Ammophila* were unaltered by interactions with AMF or PPN. Inherent differences in root architecture, and the associated differences in response to root herbivores, including PPN, may provide a possible explanation for the invasion success of many exotic invasive plant species (Dawson 2015).

The exotic *Leymus* was less susceptible to the negative effects of the plant-parasitic nematode *Pratylenchus penetrans*, providing support for our second hypothesis and more generally for enhanced performance of invasive species over natives, a sub-hypothesis of enemy release (Heger and Jeschke 2014). This result is consistent with other studies of invasive species in which native plants often receive greater damage from root herbivores compared to exotic invasive species (Maron et al. 2014; Van der Putten et al. 2005). However, we found no support for our third hypothesis that the exotic *Leymus* was less dependent on arbuscular mycorrhizal fungi than the native *Ammophila*. Previous studies of exotic invasive plants have found evidence for reduced mycorrhizal dependence of exotic invasive plants (Seifert et al. 2009; Vogelsang and Bever 2009), but that mechanism does not appear to play an important role in invasion by *Leymus*. Rather, our results are consistent with a recent meta-analysis by Bunn et al. (2015) which found that native and exotic plants have similar responses to AMF. Similarly, our results are consistent with Menzel et al. (2017) showing that for exotic plants, being mycorrhizal likely contributes to invasion success. Levels of AMF root colonization from this experiment are comparable to previous field studies on *Leymus arenarius* (Greipsson and El-Mayas 2000) and *Ammophila breviligulata* (Emery and Rudgers 2014). In this

experiment, both plant species were C3 grasses. It is possible that different functional groups of plants would exhibit different responses to AMF (Hoeksema et al. 2010).

Among all plants, there was no significant Plant Species x AMF x PPN interaction. However, when analyzing just the plants inoculated with AMF we did find a significant three-way interaction (Plant Species x PPN x AMF % root colonization) on belowground biomass, providing some support for our fourth hypothesis, though not in the direction we anticipated. *Leymus* showed a negative response to higher levels of AMF root colonization in the absence of PPN, but this response was reversed when PPN were present, indicating that AMF may enhance enemy release from PPN. *Ammophila* showed no effect of percent root colonization levels on belowground biomass, regardless of the presence/absence of PPN. At high levels of root colonization, AMF appear to become an energy burden for *Leymus* when PPN are absent. However, AMF are known to provide some protection from PPN damage (Azcón-Aguilar and Barea 1997; de la Pena et al. 2006), and thus higher levels of AMF root colonization may provide greater protection for the plant when PPN are present, though inhibition of PPN levels in root tissue was weak. Alternatively, the observed differences in belowground biomass could be the result of phenotypic plasticity of the invasive *Leymus*. Keser et al. (2014) found that invasive clonal plants (such as *Leymus*) have greater plasticity in root-foraging and root production. In this study, belowground biomass of the native species showed no variation along an AMF colonization gradient, indicating a potential lack of plasticity. The differing responses of the native and exotic plants to tri-trophic interactions indicate the potential for belowground interactions among PPN and AMF to contribute to the success of exotic plants.

For this experiment, we chose to use a single species of plant-parasitic nematode and a single species of AMF to control for potential confounding interactions among other species of AMF and nematodes. In general, these two organisms had similar influence on belowground biomass for both plants, though the PPN had an effect size that was almost double the AMF effect on aboveground biomass. In a field setting, coastal sand dunes will have multiple species of AMF present (da Silva et al. 2015; Gemma and Koske 1997; Koske and Gemma 1997), with potentially different affinities for and benefits to plant species (Klironomos 2003), but see Sikes et al. (2012). Plant responses to AMF inoculation may have been affected by the common generalist AMF species used in the experiment. Use of a more specialized or endemic AMF species may show a weaker association with non-native plants. Likewise, plant responses to PPN were likely affected by the migratory endoparasitic nematode used in this experiment. Within the enemy release framework (Heger and Jeschke 2014), more generalist plant-parasitic nematode species would likely affect both native and exotic plants similarly, while more specialized sedentary endoparasites would likely have stronger negative effects on native species with reduced effects on exotic species. Additionally, plants on coastal sand dunes will harbor multiple species of plant-parasitic nematodes, as well as other feeding groups such as predators and bacteria-feeders (Wall et al. 2002). These nematodes interact with each other, with additional effects on the plant (Brinkman et al. 2015). These diverse soil community interactions have the potential to influence interactions with plants, and future studies should address these interactions that occur in natural settings and address their impacts on native and exotic plant performance and competitive dynamics.

Dune successional dynamics are driven by belowground interactions, and thus changes in these interactions with exotic plant species indicate the potential for altered successional trajectories or timelines. Since *Leymus* is seemingly less susceptible to PPN, it could remain dominant longer than the native *Ammophila*, limiting colonization by later successional species. As a foundation species, *Leymus* has the potential to alter the physical structure of its environment though sand capture. Our results suggest the potential for altered biotic interactions belowground as well. Plant species with the ability to alter both the physical and biotic structure of their environment pose a significant threat where they invade. Overall, this study yields some support for a subset of enemy release during exotic plant invasion. Further, exotic plant interactions with mutualists may enhance release from belowground herbivore enemies. Belowground tri-trophic interactions among plants, mutualists, and herbivores have the potential to contribute to exotic plant invasion and should be considered when addressing invasive plant species.

Table 1: Results of 3-way ANOVAs testing for significant differences in above- and belowground biomass and the ln-transformed root length and average root diameter resulting from effects of plant species, inoculation with arbuscular mycorrhizae (AMF), inoculation with plant-parasitic nematodes (PPN), and all possible interactions. Degrees of freedom = 1, 72 for all terms. Significant effects are in boldface.

Factor	Aboveground Biomass		Belowground Biomass		Root Length		Average Root Diameter	
	F	P	F	P	F	P	F	P
Plant Species	53.92	**<0.0001**	57.71	**<0.0001**	125.78	**<0.0001**	5.27	**0.0246**
AMF	6.40	**0.0136**	4.47	**0.0379**	3.67	0.0595	0.59	0.4449
PPN	16.15	**0.0001**	32.17	**<0.0001**	77.49	**<0.0001**	18.86	**<0.0001**
Plant Species x AMF	0.50	0.4827	0.59	0.4437	0.01	0.9040	0.63	0.4315
Plant Species x PPN	1.79	0.1851	4.44	**0.0387**	1.33	0.2527	0.92	0.3398
AMF x PPN	0.42	0.5202	0.12	0.7294	0.18	0.6702	4.72	**0.0332**
Plant Species x AMF x PPN	0.60	0.4395	0.60	0.4406	0.44	0.5083	3.09	0.0831

Table 2: Results of a 3-way MANOVA testing for significant differences in aboveground

plant architecture traits (height, number of leaves, total leaf length) resulting from

effects of plant species, inoculation with arbuscular mycorrhizal fungi (AMF),

inoculation with plant-parasitic nematodes (PPN), and all possible interactions.

Degrees of freedom = 3, 70 for all terms. Significant effects are in boldface.

Factor	Wilk's λ	F	P
Plant Species	0.4411	29.57	**<0.0001**
AMF	0.7401	8.19	**<0.0001**
PPN	0.7151	9.30	**<0.0001**
Plant Species x AMF	0.9561	1.07	0.3672
Plant Species x PPN	0.9348	1.63	0.1910
AMF x PPN	0.8762	3.30	**0.0254**
Plant Species x AMF x PPN	0.9703	0.72	0.5463

Table 3: Results of a 3-way ANCOVA testing for significant differences in above- and

belowground biomass resulting from effects of plant species, inoculation with

plant-parasitic nematodes (PPN), percent root colonization by AMF, and all

possible interactions. Degrees of freedom = 1, 32 for all terms. Significant effects

are in boldface.

Factor	Aboveground Biomass		Belowground Biomass	
	F	P	F	P
Plant Species	2.63	0.1148	8.20	**0.0073**
PPN	4.90	**0.0341**	12.00	**0.0015**
Root colonization	2.26	0.1422	0.02	0.8955
Plant Species x PPN	0.58	0.4511	5.30	**0.0280**
Root colonization x Plant Species	0.44	0.5099	0.14	0.7148
Root colonization x PPN	1.87	0.1808	4.16	**0.0496**
Plant Species x PPN x Root colonization	0.52	0.4748	5.14	**0.0303**

Figure Legends

Figure 1: Aboveground (A) and belowground (B) biomass production of the native grass *Ammophila breviligulata* and the exotic grass *Leymus arenarius* in response to PPN manipulation. Bars show means ± 1 SE

Figure 2: Aboveground (A) and belowground (B) biomass production of the native grass *Ammophila breviligulata* and the exotic grass *Leymus arenarius* in response to AMF manipulation. Bars show means ± 1 SE

Figure 3: Belowground biomass production along an AMF percent root colonization gradient for the native grass *Ammophila breviligulata* with (A) PPN absent and (B) PPN present and the exotic grass *Leymus arenarius* with (C) PPN absent and (D) PPN present

Figure 1
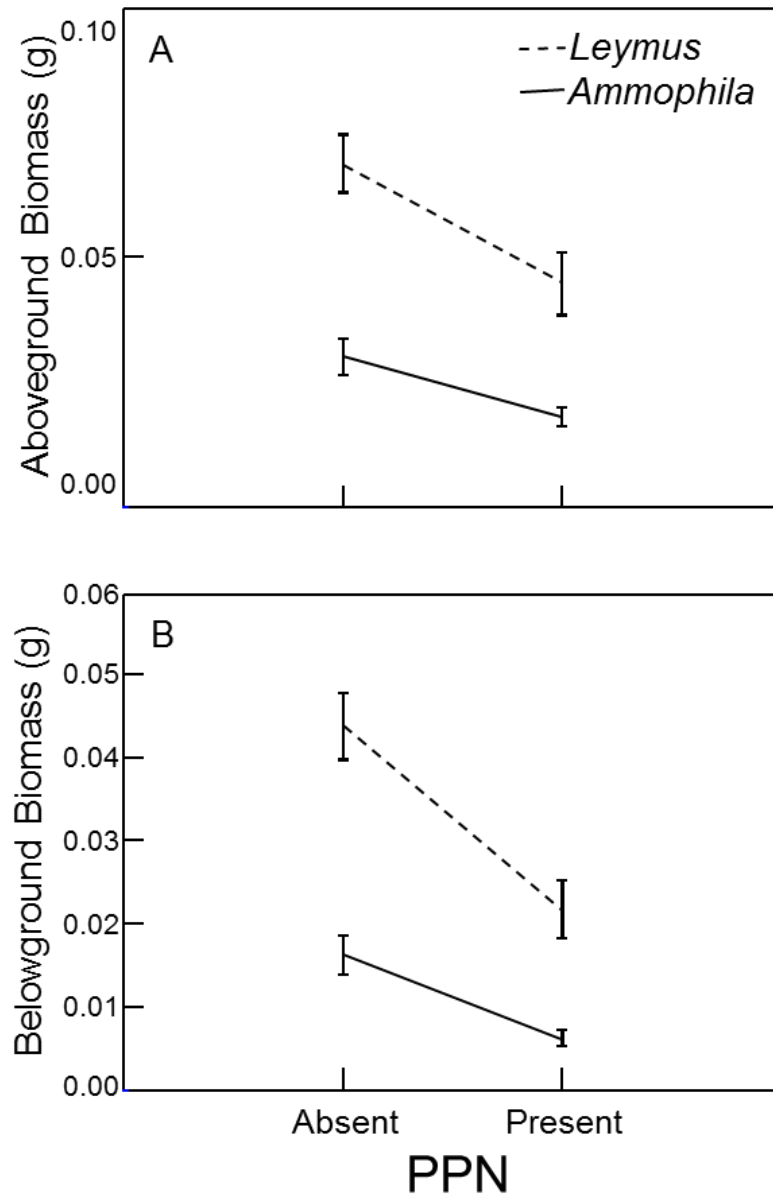
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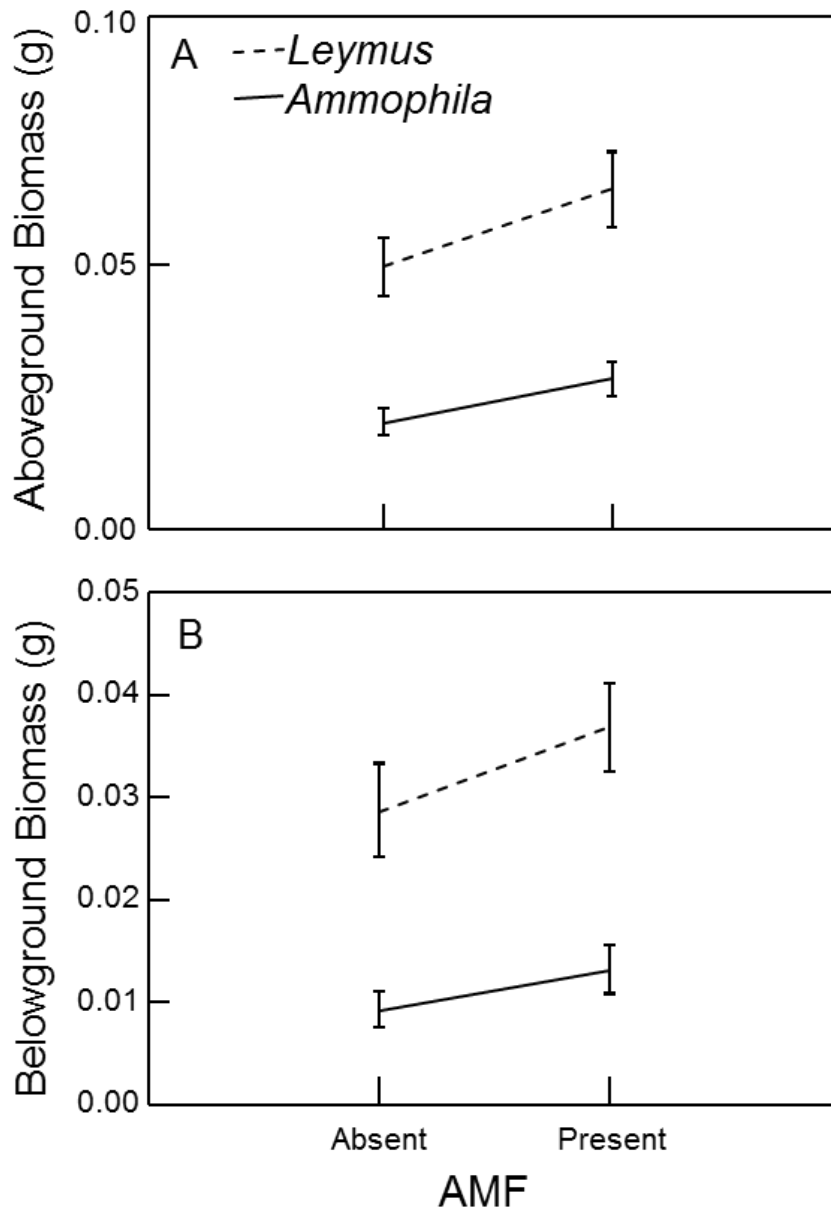
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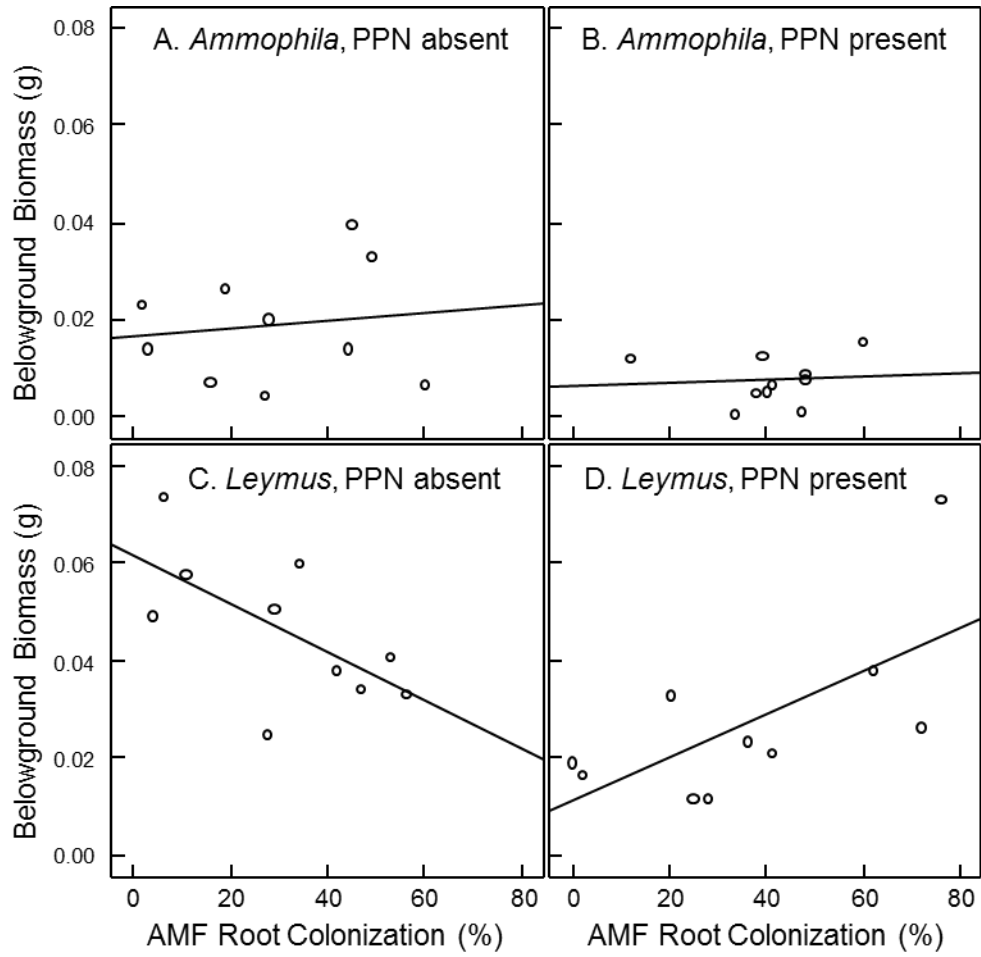
CHAPTER III

DUNE SOIL COMMUNITIES ASSOCIATED WITH CLIMATIC AND EDAPHIC

FACTORS, NOT EXOTIC PLANT PRESENCE

SUMMARY

Soil communities are shaped by multiple biotic and environmental components. We assessed the role of multiple factors in shaping soil bacterial and nematode communities in an ongoing plant invasion in western Michigan sand dunes. We aimed to determine which factors were most important for structuring soil communities. We surveyed seven sites containing the exotic plant, *Leymus arenarius*, in western Michigan, USA sand dunes. We sampled the plant community and collected soil cores to assess bacterial and nematode communities. Bacterial functional diversity was assessed using Biolog Ecoplates. Nematode communities were assessed based on morphological identification.

Bacterial functional community composition was most strongly associated with the native plant community, precipitation, soil organic matter, and latitude. Nematode diversity was most strongly associated with soil organic matter and successional position on the foredune, and less associated with native plant cover, precipitation, and latitude. Invasive plant cover and density had no strong association with bacterial or nematode communities. Soil communities of bacteria and nematodes in this early successional sand

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dune system were associated primarily with changes in climatic and edaphic factors, rather than exotic plant cover.

INTRODUCTION

Belowground communities are complex systems with multiple interacting organisms including animals, bacteria, fungi, and plant roots. Numerous recent studies have shown that soil communities at both local and regional scales are shaped by a combination of climatic factors such as temperature (Deslippe et al. 2012; Luo et al. 2014; Rinnan et al. 2009; Thakur et al. 2014; Wu et al. 2015) and precipitation (Barnard et al. 2015; Fuchslueger et al. 2014; Zeglin et al. 2013), and edaphic factors such as soil pH (Baker et al. 2009; Bartram et al. 2014; Fierer and Jackson 2006; Murphy and Foster 2014) and soil organic matter content (Arroyo et al. 2015). However, at a local scale, plants can also have strong influence on soil communities (Johnson et al. 2003; Marschner et al. 2004). In particular, changes in dominant plant identity, for example due to exotic plant invasions or habitat restoration efforts, have been shown to alter soil community structure and function of bacteria (Batten et al. 2006; Hawkes et al. 2005; Kourtev et al. 2002; Li et al. 2007; Urbanová et al. 2015), fungi (Lankau et al. 2014; Mummey and Rillig 2006), and invertebrates (Chen et al. 2007; Renčo and Baležentiené 2015; Tanner et al. 2013; Xiao et al. 2013), often through changes in carbon inputs via litter or root exudation. Exotic plants may also alter soil communities via suppression of belowground herbivores and pathogens, the "enemy release hypothesis" (Heger and Jeschke 2014), though application of the enemy release hypothesis belowground has been debated (Colautti et al. 2004). Such changes in soil communities may consequently affect

nutrient cycling and other ecosystem functioning (Hawkes et al. 2005; Nielsen et al. 2011; Wagg et al. 2014).

Previous studies have found that soil communities vary widely in their response to changes in abiotic and biotic factors (Wardle et al. 2001). For example, Papatheodorou et al. (2004) found that soil bacterial community function and bacterivorous nematodes had different responses to experimental warming and soil moisture manipulations. Thus, despite the trophic interactions between these two groups, they did not show synchronized changes to altered abiotic conditions. Even within a taxonomic group, organisms can occupy different trophic levels and may respond differently to local climatic or edaphic changes (Yeates et al. 1999), potentially due to variation in life history strategy (Bongers 1999). Additionally, soil food webs are driven by both top-down and bottom-up forces that can play strong roles in regulating soil community responses (Sylvain and Wall 2011). Soil nematodes offer a unique opportunity to evaluate the relative effects of biotic and abiotic factors on soil communities, as nematodes occupy nearly every trophic level in the soil food web (Bongers and Ferris 1999), including plant-parasites/root herbivores, bacteria-feeders, fungal-feeders, and predators.

In this study, we utilize an ongoing plant invasion in Michigan, USA sand dunes to address questions regarding the roles of plant invasion, edaphic, and regional climatic factors in shaping soil communities. In the Great Lakes region, dunes are dominated by the native ecosystem-engineering grass *Ammophila breviligulata*, whose growth has been shown to be inhibited by soil nematodes as dunes age (Seliskar and Huettel 1993). Recently, *Leymus arenarius* (blue lyme grass, hereafter "*Leymus*"), a foundation species

native to northern European sand dunes where it colonizes bare sand and initiates the dune building process (Greipsson and Davy 1997), has invaded Great Lakes sand dunes (Martinus 2009) and poses a potential threat to the structure and function of these ecosystems. Much like the other dune-building species *A. breviligulata* and *A. arenaria*, an accumulation of plant-parasitic nematodes and pathogenic fungi can reduce growth of *Leymus* in its native range (Greipsson and El-Mayas 2002). This invasion currently spans a 2° latitudinal gradient, representing July average 3.4°C and 6.3 cm monthly precipitation gradients in western Michigan (PRISM Climate Group, Oregon State University). In addition, local site conditions vary in terms of edaphic factors that may shape soil communities. In sand dunes, soil development follows a successional gradient, with soils closest to the water being most recently exposed and lower in organic matter content than more inland soils which have increased organic matter and plant diversity (Lichter 1998). Plant-soil interactions are often tightly coupled in these successional dune systems (Roy-Bolduc et al. 2016; Williams et al. 2013); thus this system is ideal for examining the relative importance of biotic and abiotic factors in shaping soil communities.

The objective of this study was to assess the role of multiple biotic and abiotic factors in structuring soil communities. Specifically, we ask 1) Is soil bacterial functional diversity associated with changes in native or exotic plant abundance, regional climate, or local edaphic factors? and 2) Are soil nematode communities associated with changes in native or exotic plant abundance, regional climate, or local edaphic factors? We hypothesized that all three factors would shape soil bacterial community functional diversity and composition and soil nematode community diversity and composition.

Specifically, we hypothesized that soil bacterial functional diversity would be most closely associated with local edaphic conditions, especially soil organic matter, as bacterial functional diversity is a measure of carbon usage and dunes are young soils with low organic matter (Lichter 1998), with influences from plant identity and climatic factors as well. Because dunes in this system are dominated by a single species, *Ammophila breviligulata*, we hypothesized changes in plant identity resulting from invasion would have a strong effect on soil nematode communities. Additionally, we anticipated local edaphic factors related to succession would be important for nematode communities, as shown in other systems (Matlack 2001; Pen-Mouratov et al. 2010). We tested these hypotheses in a survey of *Leymus* populations in western Michigan.

MATERIALS AND METHODS

Sampling sites

In July 2015, we located *Leymus* populations from seven state, county, and city parks along Lake Michigan dunes. Survey sites included Meinert Park, Ludington State Park, Green Point Dunes Nature Preserve, Saugatuck Dunes State Park, PJ Hoffmaster State Park, Nordhouse Dunes Wilderness Area, and the C.S. Mott Nature Preserve at Arcadia Dunes (Figure 1). *Leymus* population density varied in size from 5-50 tillers m^{-2}, and populations were surrounded by a matrix of *Ammophila* dominated dune grasslands at each site.

Field sampling methods

Through each "invaded" *Leymus* population, we ran a 20 m transect parallel to the shoreline and placed five 1 m x 1 m plots evenly spaced every 4 m along the transect. In each plot, we documented percent cover and tiller density of *Leymus* and *Ammophila* as

well as percent cover of plant litter. We considered a plot with any *Leymus* tillers as 'invaded.' If plots along the 'invaded' transect had no *Leymus* tillers, we excluded those plots. Within each plot along a transect, we collected and composited 10 soil cores (2 cm x 15 cm) from around *Leymus* plant roots. Soil cores were placed into labeled ziptop bags and stored on ice until transport to the lab, where they were stored at 4°C until processing. We paired each 'invaded' sampling transect with a similar 20 m 'uninvaded' transect through monoculture stands of *Ammophila* located ~20 m north or south of each 'invaded' transect. Soil and plant sampling were conducted as described above. Each pair of transects were equidistant from the shoreline to control for dune successional stage.

Edaphic variables

To quantify soil organic matter content, we first composited an equal volume of soil from each plot along a transect to generate a transect level soil sample for analysis. We used the ash-free massing method (Ryan et al. 1990) to determine percent organic matter in the soil. Briefly, 20 g of air dried soil from each transect was placed in a ceramic crucible and burned in a muffle furnace at 475°C for 4 hours. The muffle furnace was then turned off and the soil allowed to cool. Each soil sample was reweighed and the new weight was subtracted from the beginning weight and the quotient multiplied by 100 to obtain the percent of organic matter in the soil.

We used two dune location measurements to calculate a metric of dune successional stage, assuming transects closer to the dune edge represented younger dunes than those closer to the forest edge (Lichter 1998). ArcMap (ESRI 2011) was used to plot the coordinates of all transects at the sampling sites. A 2014 aerial image base-map (ESRI 2011) was then overlaid. The distances were then measured between each transect

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location, the forest edge and dune edge (where the dune vegetation and beach meet; Appendix II). Using these two variables, we calculated a composite 'succession' variable defined as the dune edge distance divided by the forest edge distance. Smaller values indicate a position closer to the dune edge (early dune succession), while larger values indicate a position closer to the forest edge (later dune succession).

Climatic variables

Latitude and longitude for each sample transect were used to gather average daily temperature high and average daily precipitation for the month of July 2015 using PRISM: Data Explorer (http://prism.oregonstate.edu). The PRISM data used are an estimated average for the month based on gridded 4 km^2 cells. PRISM uses reporting stations across the United States and assigns them weight for a particular location based on similarity to the particular grid cell in question. These similarities include location and coastal proximity (Daly et al. 2008). PRISM then uses regression algorithms to estimate averages for the specific grid cell (PRISM Climate Group, Oregon State University, http://prism.oregonstate.edu). We chose to use a single month average rather than yearly averages as microbial communities can respond quickly to seasonal variation (Cregger et al. 2012; Wasserstrom et al. 2017).

Soil bacterial functional diversity

We characterized soil bacterial functional diversity using Biolog Ecoplates™, which contain 31 carbon substrates to profile bacterial carbon consumption. The extraction and pipetting of soil bacteria onto Ecoplates followed the protocol described by Chen et al. (2013). Briefly, 5 g of composited soil from each transect was placed in a sterile 50 ml centrifuge tube. Next, 45 ml of sterile 0.85% NaCl was added using a sterile

pipet. The centrifuge tube was then shaken at 300 rpm for 30 minutes and allowed to settle for 5 minutes. 5 ml of the solution, extracted from the middle of the centrifuge tube, was combined with another 45 ml sterile 0.85% NaCl. This second solution was vortexed for 10-30 seconds to mix the two liquids. 150 μl of this 1:100 solution was pipetted into each well of a room temperature Ecoplate (Biolog Inc, Hayward, CA, USA). The Ecoplate then sat for an hour before running through the plate reader at 595 μm wavelength for baseline consumption data (time = 0.) Plates were then read at day 7 at 595 μm wavelength (BioTek, ELx800) after popping any bubbles that have formed on the surface of the wells with a sterile plastic toothpick (Howes 2017).

Nematode identification

In the lab, nematodes were extracted from 100 ml of composited sand per plot using the centrifugal flotation method (Jenkins 1964). The extracted nematodes were fixed in hot 3% formalin and were mounted on microscope slides and identified to genus under a microscope (Nikon SMZ1500 stereoscope). To generate data on specific feeding groups, plot-level nematode data were uploaded into the Nematode INdicator Joint Analysis program (NINJA; (Sieriebriennikov et al. 2014), which assigns feeding groups based on Yeates et al. (1993) and calculates the Maturity Index and Plant-Parasitic Index. The Maturity Index classifies nematodes into groups of colonizers and persisters, based on life history strategies and responses to disturbance (Bongers 1990). The Plant-Parasitic Index is a similar measure, but only for plant-parasitic nematodes.

Data analyses

We conducted a principal components analysis on all 10 abiotic and biotic variables to reduce dimensionality of data and to control for potential collinearity

(Graham 2003). Each factor was standardized by its standard deviation to account for differences in units of measurement. Based on the results of a randomization test and the stopping rules outlined in Peres-Neto et al. (2005), the first two principal components adequately described the structure of the data. We also chose to include the third principal component, because it was correlated with factors related to dune succession (Table 1).

We characterized composite soil community *diversity* by calculating average transect Shannon diversity (H') for bacterial function and nematodes, and we also calculated average transect richness for nematodes. Bacteria samples were already aggregated at the transect level for functional diversity analysis (as described above). Nematode community data were measured at the plot level. Hence, we calculated plot level genera richness and Shannon diversity (H'), then averaged across plots within a transect to generate transect level data. This accounted for differences in the number of plots along a transect, since not all invaded transects had five plots. We used general linear models with the first three PC axis scores as predictor variables to evaluate the relative contribution of biotic and abiotic variables to soil community diversity for bacterial function and nematodes separately. Models were run in SAS (SAS/STAT 2013). Correlations were used to visualize pairwise relationships between certain variables.

To characterize community *composition* of bacterial function and nematodes, we used non-metric multidimensional scaling (NMDS) (McCune et al. 2002). Data were plotted in two dimensions, and significant individual predictor variables were overlaid as vectors (biplots) on the NMDS graph. NMDS and associated axis scores were generated using PRIMER 6 and PERMANOVA+ statistical software (Anderson et al. 2008). We

then extracted the axis values for each transect and used general linear models with all three PC scores as predictor variables to evaluate the relative contribution of biotic and abiotic variables to soil community composition for bacterial function and nematodes separately. Models were run in SAS (SAS/STAT 2013).

RESULTS

Principal components analysis

The first three principle components (PC) used in the multiple regression model explained 71.0% of the variation in the measured variables (Table 1). The first PC axis explained 33.8% of variation and had strong correlation with primarily regional climate and native biotic components. Higher values of PC1 were associated with lower latitude and greater precipitation, and the important biotic components were those related to higher levels of native plant (*Ammophila*) percent cover and tiller density. The second PC axis explained 21.8% of variation and was correlated with biotic components related to higher levels of the invasive plant (*Leymus*) percent cover and tiller density. The third PC axis explained 15.3% of variation and was correlated primarily with factors related to later dune successional status and greater percent soil organic matter, as well as higher temperature (Table 1).

Bacterial community responses

Bacterial functional diversity (H'), as measured by our carbon substrate utilization on Biolog Ecoplates, was not associated with any of the three PC axes (Table 2), indicating no strong effects of regional climate and native biotic components (PC1), *Leymus* invasion (PC2), or dune successional variables (PC3) on functional diversity. However, composition of the bacterial community showed a significant association with

measured variables. For bacterial NDMS axis 1, PC1 was a significant predictor (p = 0.0229). For NMDS axis 2 both PC1 and PC3 were significant predictor variables, indicating that factors such as precipitation, native *Ammophila* cover and density, dune successional stage, and soil organic matter are important factoring structuring dune bacterial functional composition. Similarly, the NMDS overlays indicated bacterial functional composition was associated with latitude, *Ammophila* density, temperature, percent soil organic matter, and dune successional position (Figure 2A).

Nematode community responses

Nematode genera richness was correlated with PC3, associated with dune successional status and percent soil organic matter (Table 3). We found that soils with higher organic matter had lower richness of nematode genera (Figure 3A). Nematode diversity (H') was also affected by PC2 and PC3 (Table 3), indicating that both *Leymus* invasion and dune successional factors influence soil nematode diversity. However, the relationship between *Leymus* invasion and nematode diversity (H') is potentially driven by one high density invasion site (Figure 3B). None of the PC axes explained variation in soil nematode abundance (Table 3).

Nematode community composition was characterized using both NMDS and composite nematode indices: the Maturity Index and Plant-Parasitic Index. Neither NMDS axis had any relationship with any of the PC axes (Table 3), indicating community composition was unaffected by the regional climate and native biotic components (PC1), *Leymus* invasion (PC2), and dune successional status and soil organic matter (PC3). However, we did find differences in the nematode Maturity Index that were explained primarily by PC1, indicating regional climate and native *Ammophila* dynamics

are important in structuring these communities (Table 3). We found that the nematode communities associated with greater percent cover by the native *Ammophila* were more mature (Figure 3C). The overall model for the Plant-Parasitic Index was not significant, though again PC1 was the strongest predictor for this variable (Table 3).

Bacterivores were the dominant feeding group in all the nematode communities (Appendix II), making up 53% of the community on average. *Plectus, Acrobeles,* and *Heterocephalobus* were the most abundant bacterivores. The omnivorous genus *Eudorylaimus* was also highly abundant. *Leptonchus, Aphelenchoides,* and *Filenchus* were the most abundant fungivores. Predators and herbivores were noticeably less abundant. The most abundant predatory nematodes included *Mylonchulus, Nygolaimus,* and *Tripyla*, and the most abundant herbivores included *Trichodorus, Pratylenchus,* and *Tylenchorhynchus* (Appendix II).

DISCUSSION

In this study, we found that regional climate, native biotic factors such as *Ammophila* cover and tiller density, and factors relating to dune succession explained the majority of variation in soil bacterial and nematode community diversity and composition than *Leymus* invasion. Often, soil bacterial functional diversity and soil nematodes responded in similar manners. Climatic and edaphic factors have been shown to be important in structuring soil communities, particularly for soil microbes (Arroyo et al. 2015; Thakur et al. 2014; Zeglin et al. 2013), and the results of our survey support those findings.

Climatic variables of precipitation and temperature were important in structuring soil communities, and highlight the potential for climate change to alter soil communities

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in dune ecosystems. Previous work has shown that altered precipitation can alter bacterial community structure and function (Barnard et al. 2015; Fuchslueger et al. 2014; Papatheodorou et al. 2004; Zeglin et al. 2013). These changes in the bacterial community can vary over relatively short time frames (Cregger et al. 2012; Papatheodorou et al. 2004; Wasserstrom et al. 2017). In an old-field system, manipulations of precipitation to mimic patterns of climate change reveal strong shifts in community composition, primarily among Proteobacteria and Acidobacteria (Castro et al. 2010). We found that precipitation, a component of PC1, was a significant predictor of the bacterial NMDS axis 1 and axis 2, highlighting its importance in structuring bacterial functional composition. Soil nematodes are also likely to respond to changes in precipitation, since they rely on water films around soil particles for their survival and motility. Kardol et al. (2010) found that nematode communities were sensitive to experimental manipulation of climate change factors, including precipitation. The various functional groups of nematodes responded differently to altered precipitation, with bacterial-feeding nematodes showing stronger reductions in abundance than fungal-feeding nematodes (Kardol et al. 2010). Since bacterial-feeding nematodes were the most abundant feeding group in our samples (Appendix II), this indicates that potential reductions in precipitation could have strong impacts on the soil nematode communities in these sand dune ecosystems.

In other systems, increased temperature has been associated with changes in bacterial respiration rate (Zogg et al. 1997), abundance (Castro et al. 2010), and composition (Wu et al. 2015; Xiong et al. 2014). However, other studies have found that temperature played a relatively minor role in structuring soil bacterial communities (Bell

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et al. 2008; Kuffner et al. 2012). It is likely that multiple climate factors interact to shape soil bacterial communities (Castro et al. 2010). Increases in temperature are also likely to affect nematode communities. Stevnbak et al. (2012) found that temperature increases of 1°C reduced nematode abundance and shifted community composition to dominance by root herbivores with longer generation times.

Dune successional position and the correlated factor of soil organic matter were also important factors structuring bacterial and nematode communities in this survey. For soil nematodes, the relationship between genera richness and soil organic matter was in the opposite direction of what we hypothesized. While this was unexpected, Wall et al. (2002) also found decreases in nematode diversity moving from the beach inland. Our sampling was constrained to locations invaded by *Leymus* and nearby uninvaded areas, representing only a portion of the dune landscape. *Leymus* populations were generally near the first dune ridge, but even within this constrained sampling, we found dune successional position to be important. A recent study by Wasserstrom et al. (2017) also found landscape position to be important for structuring soil bacterial communities in dune systems, with lowest bacterial diversity on the youngest dunes, closest to the water. Diversity gradients in successional dune systems are well established for plants (Cowles 1899; Lichter 1998), and more recent work has highlighted similar patterns in dune soil communities as well (Emery et al. 2015; Wall et al. 2002; Wasserstrom et al. 2017).

Surprisingly, we found that the abundance of the exotic *Leymus* had little effect on soil community composition and diversity. While PC2, corresponding to *Leymus* invasion variables, was a significant predictor for nematode diversity (H'), this relationship was driven by one particularly dense *Leymus* population. Surveys of

additional *Leymus* populations of more moderate density would likely reveal whether there is a true relationship of invasion density and nematode diversity. Regardless, this overall lack of invasion effects was surprising given that studies have indicated that invasion status can play an important role in structuring soil microbial (Batten et al. 2006; Li et al. 2007) and nematode communities (Kostenko et al. 2015). Exotic plants in particular have been shown to alter communities of soil bacteria (Kourtev et al. 2002), soil nematodes (Liang et al. 2007; Xiao et al. 2013), and other soil organisms (Mummey and Rillig 2006; Zubek et al. 2016). However, different invasive plants have different impacts on native communities, and some invasive plants may exhibit minimal effects on soil communities (Stefanowicz et al. 2016).

The lack of an effect of exotic plant invasion in this study may be due to a couple factors. First, *Leymus arenarius* and *Ammophila breviligulata* are functionally similar species in many regards. Both are C-3 grasses that naturally colonize nutrient-poor sand dune ecosystems. They are both mycorrhizal and their growth can be inhibited by plant-parasitic nematodes (Greipsson and El-Mayas 2002; Greipsson and El-Mayas 2000; Reid and Emery 2017; Seliskar and Huettel 1993). However, we expected interspecific differences would lead to differences in their associated soil communities. Specifically, *Leymus* is a larger plant than *Ammophila* both above- and belowground (Reid and Emery 2017). We would expect greater inputs of detritus from root turnover and aboveground tissues to contribute to increased organic matter in the soil, which should alter soil communities. It is currently unknown whether these plant species differ in patterns of root exudation, which could also contribute to shaping soil communities. Previous work on other invasive plants in sand dunes has shown that invasive plants can alter both the

physical environment (David et al. 2015; Zarnetske et al. 2012) and heterogeneity of soil communities (Reid and Emery, in review). Such changes would be expected to alter soil community composition and diversity as well. Second, in this survey, many of the invasions we sampled were relatively young. It is possible that there had been insufficient time for impacts of invasion on the soil community to manifest. Longer duration studies would be necessary to determine if time since invasion is important.

Overall, we found that exotic plant density played a minor role in structuring communities of soil bacteria and nematodes. The high functional similarity of *Leymus* and *Ammophila* may explain this result, since previous studies often involved exotic species that were functionally different that their native counterparts. Rather, regional climatic variables of precipitation and temperature and native *Ammophila* density, along with successional position on the dune were the most important factors structuring soil communities. Our finding reveal that changes in climate, specifically patterns of precipitation and temperature have the potential to alter soil communities on sand dunes. Additionally, this study has also shown that invasion by a functionally similar exotic grass in sand dunes has minimal effects on soil bacterial function and nematode diversity, at least in the short term.

Table 1: Variable loadings on the first three principal components of the PCA, with the variables with the highest absolute loadings are indicated in bold. Percentage variation explained for each axis is indicated in parentheses.

Variable	PC1 (33.8%)	PC2 (21.8%)	PC3 (15.3%)
Latitude	**-0.438**	-0.301	0.112
% Litter cover	0.282	-0.296	0.035
% Soil organic matter	0.319	0.059	**0.457**
Precipitation	**0.383**	0.281	-0.300
Temperature	0.289	0.094	**0.340**
Succession	0.085	-0.217	**0.666**
Leymus tiller density	-0.217	**0.573**	0.215
Leymus % cover	-0.212	**0.578**	0.203
Ammophila tiller density	**0.411**	0.142	-0.192
Ammophila % cover	**0.359**	0.013	0.061

Table 2: Results of general linear models predicting soil bacterial functional diversity

(H') and composition based on NMDS axes. Significant effects are indicated in bold.

	df	Shannon Diversity (H')		NMDS Axis 1		NMDS Axis 2	
		F	P	F	P	F	P
Overall Model	3	0.69	0.5661	2.99	0.0531	4.65	**0.0116**
PC1	1	1.68	0.2090	5.99	**0.0229**	6.28	**0.0201**
PC2	1	0.21	0.6492	2.64	0.1186	3.02	0.0960
PC3	1	0.19	0.6666	0.33	0.5692	4.64	**0.0425**

Table 3: Results of general linear models examining differences in diversity and
composition of soil nematode communities. Significant effects are indicated in bold.

	df	Shannon Diversity (H`)		Richness		Abundance	
		F	P	F	P	F	P
Overall Model	3	3.38	**0.0363**	3.24	**0.0416**	2.23	0.1135
PC1	1	0.07	0.7999	0.04	0.8475	1.23	0.2789
PC2	1	5.50	**0.0284**	4.27	0.0508	3.86	0.0621
PC3	1	4.58	**0.0437**	5.41	**0.0296**	1.58	0.2213

	df	Plant-Parasitic Index		Maturity Index	
		F	P	F	P
Overall Model	3	1.49	0.2473	3.36	**0.0373**
PC1	1	4.37	**0.0496**	8.83	**0.0071**
PC2	1	0.00	0.9858	1.15	0.2952
PC3	1	0.48	0.4975	0.09	0.7623

	df	NMDS Axis 1		NMDS Axis 2	
		F	P	F	P
Overall Model	3	2.12	0.1268	0.93	0.4414
PC1	1	1.14	0.2981	0.74	0.3997
PC2	1	1.54	0.2277	2.06	0.1657
PC3	1	3.68	0.0681	0.01	0.9380

Figures

Figure 1: Map of western Michigan, USA showing sampling locations with *Leymus arenarius* invasion.

Figure 2: (A) Non-metric multidimensional scaling of bacterial functional diversity measured by carbon substrate utilization as a function of plant identity. Stress $= 0.16$. Overlays show predictor variables with $r > 0.5$. (B) Non-metric multidimensional scaling of nematode community composition as a function of plant identity. Stress $= 0.15$. Overlays show predictor variables with $r > 0.3$. No variables had $r > 0.5$. *Ammophila* plots are indicated by the open circles, and *Leymus* are indicated by the filled triangles.

Figure 3: (A) Simple linear relationship between nematode genera richness and percent soil organic matter. (B) Simple linear relationship between nematode diversity (H') and *Leymus* tiller density. (C) Simple linear relationship between nematode Maturity Index and percent cover by *Ammophila*.

Figure 1

Green Point Dunes
Nature Preserve

Arcadia Dunes

Nordhouse Dunes
Wilderness Area

Ludington State Park

Lake
Michigan

Meinert Park

PJ Hoffmaster
State Park

Saugatuck Dunes
State Park

N

0   15   30   60   90   120
                                    Kilometers
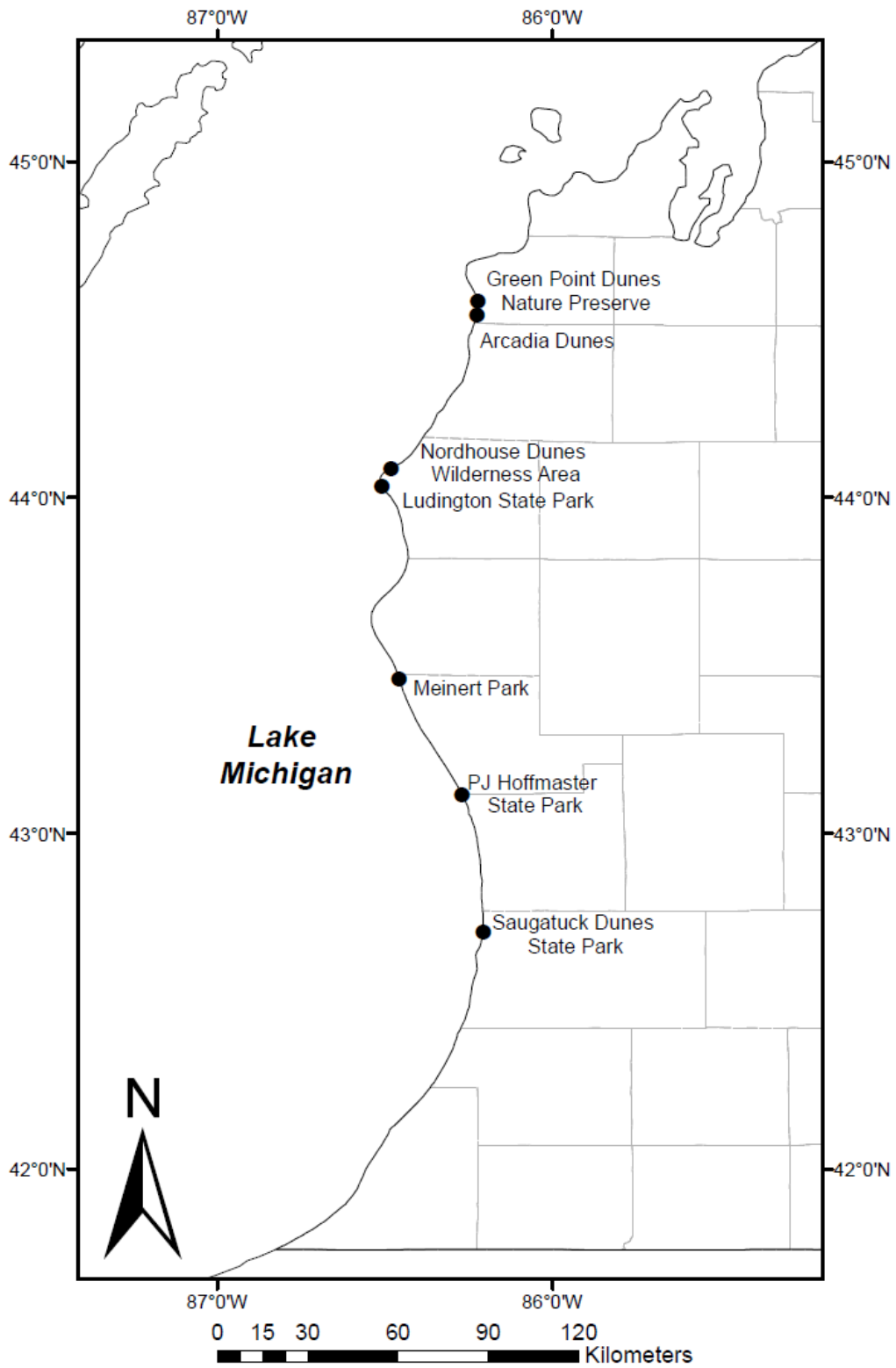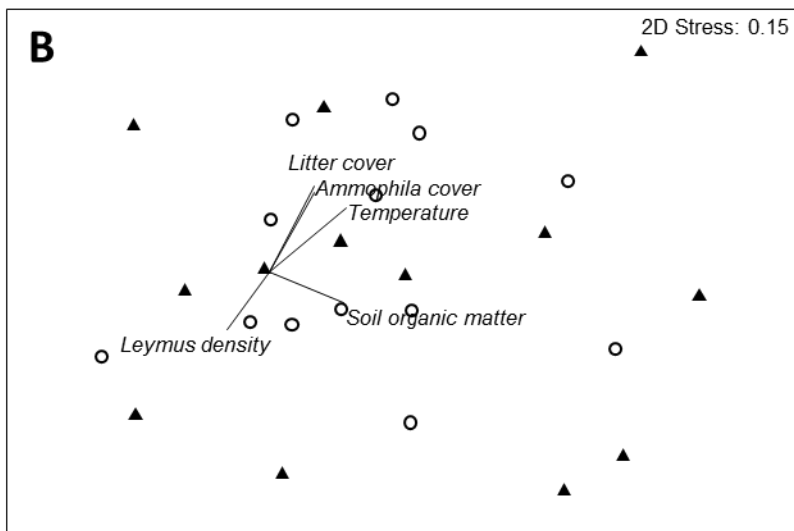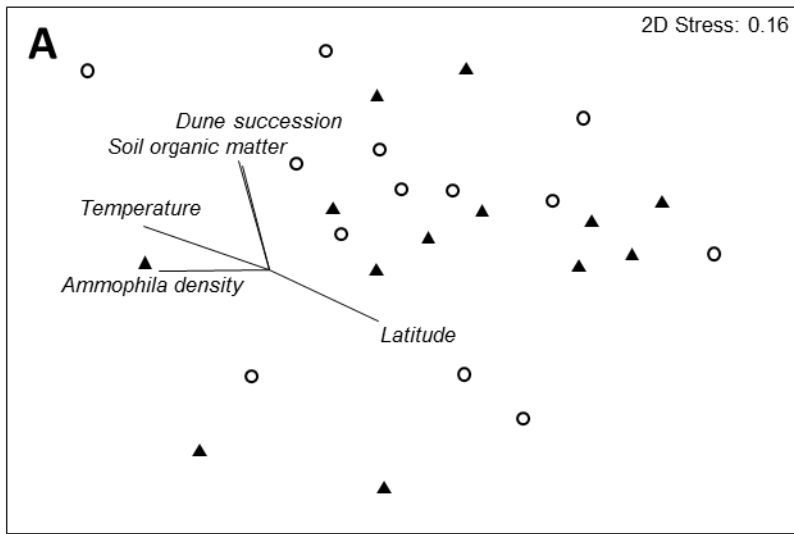
Figure 2

Figure 3
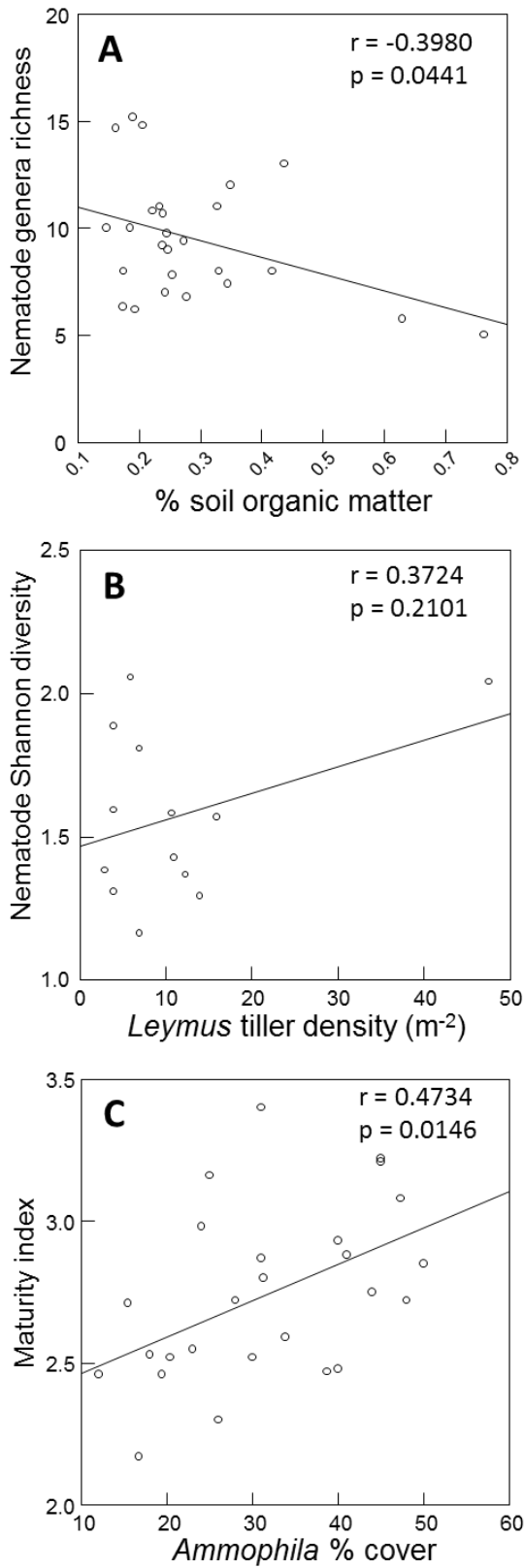
CHAPTER IV

NATIVE AND EXOTIC PIONEER GRASSES HAVE SIMILAR EFFECTS ON

SECONDARY SUCCESSION ON MICHIGAN DUNES

SUMMARY

Exotic plant invasions threaten to disrupt native plant communities. Changes to native plant community structure often occur via direct competitive effects from exotic plants. However, exotic plants also modify the soil microbial community, potentially causing indirect effects on native plant communities. We assessed the potential for *Leymus arenarius*, an exotic plant on Great Lakes sand dunes, to alter native plant performance via direct competitive effects and indirect soil conditioning effects. In a growth chamber experiment, we assessed the growth responses of eight dune plant species, representing both native and exotic taxa, across a dune succession gradient. Species were grown in soil conditioned by either the exotic *Leymus arenarius* or the native *Ammophila breviligulata*. Within the two soil conditioning treatments, we added a nested factor of competition from either *Ammophila* or *Leymus*. We found that direct competition from *Ammophila* and *Leymus* had stronger effects on plant growth than indirect effects of soil conditioning, although *Leymus*-conditioned soils suppressed plant growth by ~9%. The direct effects of competition from *Leymus* most strongly suppressed secondary invasive species (*Centaurea stoebe* and *Silene latifolia*). These results suggest

that *Leymus* has only a weak indirect effect via soil conditioning on native species, while direct effects of competition are stronger. Competitive effects from *Ammophila* and *Leymus* are similar, except on secondary invaders, suggesting that *Leymus* invasion may not hinder succession, but has the potential to suppress additional invaders.

INTRODUCTION

Invasions by exotic plant species have the potential to alter the development of natural communities and ecosystem processes (Mack et al. 2000; Pimentel et al. 2005). This is especially pertinent when exotic species replace native foundation species, or are themselves foundation species, capable of creating and modifying habitat (Cuddington and Hastings 2004). Native and exotic plants may have different effects on plant community structure and development. For example, Dickson et al. (2012) found that priority effects from early colonizing exotic plant species were greater than those of native plants. Additionally, there is evidence that exotic plant species may facilitate the establishment and growth of other exotic species, known as 'invasional meltdown' (Simberloff and Von Holle 1999). In such cases, exotic plants alter biotic and/or abiotic conditions to favor secondary invaders (Flory and Bauer 2014).

Exotic plants can reduce the success of neighboring native species via multiple mechanisms, including direct competition (Bakker and Wilson 2001; Broadbent et al. 2018; Vilà and Weiner 2004). Differences in functional traits between exotic and native plants can give exotic species a competitive advantage (van Kleunen et al. 2010). For example belowground, exotic plants often produce larger root systems, giving them an advantage in nutrient acquisition over co-occurring plant species (Keser et al. 2014; Vaness et al. 2014). Direct resource competition such as this is likely an important

interaction shaping the development of plant communities in invaded areas, particularly in nutrient-poor systems. Allelopathy is another direct mechanism by which exotic plants can affect native communities (Hierro and Callaway 2003). For example, the exotic forb *Centaurea diffusa* produces novel root exudates that inhibit germination and growth of native plant species (Vivanco et al. 2004).

Exotic plants also have the potential to alter native plant success by modifying the soil biotic community (Batten et al. 2008; Bennett et al. 2011), which has been shown for soil communities of bacteria (Kourtev et al. 2002; Li et al. 2007), mycorrhizal fungi (Lankau et al. 2014; Mummey and Rillig 2006), and nematodes (Chen et al. 2007; Xiao et al. 2013). Additionally, exotic plants can alter the abiotic conditions of the soil, including changes in pH (Kourtev et al. 2003) and nitrogen availability (Hawkes et al. 2005) contributing to potential indirect effects on co-occurring plants (Elgersma et al. 2011; Heneghan et al. 2006). Alterations to soil communities can persist even after removal of the exotic plant (e.g., legacy effects, Corbin and D'Antonio 2012), hindering natural community recovery and active restoration efforts.

During succession, such direct and indirect effects among native plants contribute to plant community dynamics in complex ways; some species are inhibited by early colonizers, while others are facilitated (Connell and Slatyer 1977). For example, van de Voorde et al. (2011) found that an early successional species conditioned soil that inhibited its continued growth, while simultaneously generating positive soil conditioning effects on some later colonizing species. Additionally, the strength and direction of these effects may change over succession. For example, Kardol et al. (2006) found that early successional species generated negative plant-soil feedbacks, mid successional species

generated neutral feedbacks, and later successional plant species generated positive feedbacks.

In uninvaded primary successional sand dunes systems, a native pioneer species will colonize bare sand, initiating the dune-building process. Plant-soil interactions are critical drivers of plant community change in sand dunes, since an accumulation of detrimental soil organisms will ultimately cause die-back of the native pioneer species (Van der Putten et al. 1993), which allows other species to colonize, changing patterns of plant dominance (Cowles 1899). Exotic species, particularly exotic perennial species, threaten the development of native dune plant communities (Buell et al. 1995; David et al. 2015; Hayes and Kirkpatrick 2012; Hilton et al. 2005). In Great Lakes sand dunes, the dominant native dune-building grass is *Ammophila breviligulata* (hereafter, '*Ammophila*'). Early in succession, *Ammophila* exhibits priority effects by inhibiting colonization by later successional plant species and may outcompete them for scarce resources (Cheplick 2005). However, *Ammophila* ultimately dies back following an accumulation of detrimental soil pathogens, including plant-parasitic nematodes and fungal pathogens (Seliskar and Huettel 1993). This dieback of *Ammophila* allows other plant species to establish. In this system, *Leymus arenarius* (blue lyme grass, hereafter '*Leymus*'), a dune-building grass native to northern European sand dunes, has recently begun establishing, posing a potential threat to the native dune community structure and function. *Leymus* shares many similarities with *Ammophila*—both are perennial dune-building C3 grasses that form mutualistic associations with arbuscular mycorrhizal fungi (AMF), and their growth can be inhibited by plant-parasitic nematodes (Greipsson and El-Mayas 2002; Seliskar and Huettel 1993). One conspicuous difference is in size, with

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Leymus being a larger plant, producing more biomass than *Ammophila*, both above- and belowground (Reid and Emery 2017), potentially making it a stronger competitor than *Ammophila*.

The objective of this study was to assess the effects of *Leymus* invasion on co-occurring plant species via direct competitive effects and indirect effects of soil conditioning. We hypothesized that *Leymus* would alter dune plant succession via the direct effects of competition and indirect effects of soil conditioning. Specifically, we predicted that soils conditioned by *Leymus* would indirectly reduce native plant performance, relative to soil conditioned by the native *Ammophila*. Consistent with 'invasional meltdown' (Simberloff and Von Holle 1999), we also predicted other secondary invasive species to perform better in *Leymus* conditioned soils, since plant invasion can enhance the success of secondary invaders (Flory and Bauer 2014). Additionally, we expected direct competition from *Ammophila* and *Leymus* plants to reduce the performance of co-occurring plants via direct competitive effects. We expected that the direct competitive effects would be greater from *Leymus* since it produces greater above- and belowground biomass than *Ammophila* (Reid and Emery 2017). We tested these predictions in a multi-factor growth chamber experiment.

MATERIALS AND METHODS

Seed collection and germination

We collected seeds of native *Ammophila breviligulata,* exotic *Leymus arenarius,* and six co-occurring common dune plant species—native species *Artemisia campestris, Lathyrus japonicus, Calamovilfa longifolia, Schizachyrium scoparium,* and exotic species *Centaurea stoebe,* and *Silene latifolia* from sand dunes in western Michigan in September

2015 and stored them at -20°C until initiation of the experiment. To stratify seeds of

Ammophila, Leymus, Artemisia, Calamovilfa, Schizachyrium, Centaurea, and *Silene*, we

surface sterilized them in bleach (3% sodium hypochlorite) for 15 min, rinsed with

running distilled water for 15 min, and placed in moist sealed petri plates, where they

were stored for two months at 4°C. Following cold stratification, petri plates were placed

under a florescent light at room temperature until germination occurred. *Lathyrus* seeds

were scarified in 96% sulfuric acid for 45 min, rinsed for 15 min in running distilled

water, surfaced sterilized in bleach (3% sodium hypochlorite) for 10 min, and rinsed for

15 min in running distilled water. Seeds were placed on moist sealed petri plates until

germination occurred.

Experimental set-up – Conditioning phase

We filled 224 Deepots (7 cm wide x 36 cm deep; Stewe and Sons, Corvallis, OR)

with screened and washed play sand (Quikrete Inc., Atlanta, GA), which has similar

texture to Great Lakes sand dunes. We also added 100 ml of live dune sand collected

from the field, which contained nematodes, AMF, and bacteria. In this phase of the

experiment, each pot was conditioned by one of the two pioneer species – *Ammophila*

breviligulata or *Leymus arenarius*. One seedling of either of these species was

transplanted into the pot and was allowed to grow and condition the soil for 90 days. All

pots were kept well-watered and grown in climate-controlled growth chambers (14 h

21°C days, 10 h 14°C nights – mimicking average summer temperatures in Michigan).

Once a month, 50 ml of 10% Hoagland's solution was added to each pot to minimize

nutrient stress. Each week, pots were rotated within the growth chamber to reduce any

effect of location within the chamber. After 90 days, plants were removed from all pots.

Half of the plants of each species were harvested for above- and belowground biomass. The other half of the plants were used in the second phase of the experiment (described below). We mixed soil within each conditioning group (*Ammophila*-conditioned and *Leymus*-conditioned) for use in the second phase of the experiment.

Experimental set-up – Response Phase

We redistributed the mixed *Ammophila*-conditioned sand among 112 pots, and transplanted a mature *Ammophila* tiller from the first phase of the experiment to half of the pots. Similarly, we redistributed the mixed *Leymus*-conditioned sand among 112 pots, and transplanted a mature *Leymus* tiller transplant from the conditioning phase of the experiment to half of the pots. This resulted in a total of four treatments: 1) *Ammophila*-conditioned soil with an *Ammophila* competitor, 2) *Ammophila*-conditioned soil without a competitor, 3) *Leymus*-conditioned soil with a *Leymus* competitor, and 4) *Leymus*-conditioned soil without a competitor. Because an *Ammophila* competitor only occurred in *Ammophila* conditioned soil (and similarly for *Leymus*), competition was treated as a nested variable for analyses (see *Data Analyses*). There were differences in biomass between *Ammophila* (aboveground biomass = 0.2390 g; belowground biomass = 0.1420 g) and *Leymus* tillers (aboveground biomass = 0.4010 g; belowground biomass = 0.3648 g) harvested from the first round of the experiment. There were no differences in the number of leaves between *Ammophila* and *Leymus* competitors transplanted into phase two of the experiment.

Our response species of interest were grouped into four successional categories, 1) pioneer species (*Ammophila breviligulata, Leymus arenarius*), 2) early succession species (*Artemisia campestris, Lathyrus japonicus*), 3) mid-succession species

(*Calamovilfa longifolia, Schizachyrium scoparium*), and 4) secondary invaders

(*Centaurea stoebe, Silene latifolia*). Each pot received one seedling transplant from one

of the eight response species. Because the competitors were tillers from the first phase of

the experiment, they were much larger than the response species seedlings. This

asymmetric competition between larger established grass competitors and smaller

seedlings mimics the interaction that new seedlings would experience under field

conditions. Each treatment combination (four treatments, eight response species) was

replicated seven times for a total of 224 pots. Plants grew for 90 days before being

harvested. All pots were kept well-watered and grown in climate-controlled growth

chambers (14 h 21°C days, 10 h 14°C nights). Once a month, 50 ml of 10% Hoagland's

solution was added to each pot to minimize nutrient stress. Each week, pots were rotated

within the growth chamber to reduce any effect of location within the chamber.

Plant harvesting and root staining

After 90 days, we harvested all plants from the experiment. For pots with a

competitor, we separated the competitor and response plants. For all plants, aboveground

biomass was clipped, dried at 65°C for 5 days, and weighed. Root systems were carefully

removed from the pots, rinsed to remove sand grains, dried at 65°C for 5 days, and

weighed.

Data analyses

We used two series of general linear models to assess biomass responses. For the

first series of general linear models, biomass data were analyzed as a function of

successional group (i.e. pioneer, early, mid, or secondary invaders), soil conditioning,

competition (nested within soil conditioning), and the two-way interactions of

successional group with soil conditioning and competition. Within successional group differences were assessed using Tukey post hoc tests. Separate general linear models were run for aboveground biomass and belowground biomass. The second series of general linear models was similar to the first series, except that species were not grouped together. Thus, biomass data were analyzed as a function of response species, soil conditioning, competition (nested within soil conditioning), and the two-way interactions of response species with soil conditioning and competition. Within response group differences were assessed using Tukey post hoc tests. Separate general linear models were run for aboveground biomass and belowground biomass. All biomass data were square root transformed to better meet model assumptions. All general linear models were performed in SAS (SAS/STAT 2013).

RESULTS

Variation among response groups and species

There were strong differences in above- and belowground biomass among the successional plant groups (Table 1). Across all treatments, the pioneer species group produced the most aboveground biomass, with the early succession, mid succession, and secondary invaders groups producing on average only 64.3%, 17.9%, and 39.2% of the biomass as the pioneer species group respectively (Figure 1). Across all treatments, the secondary invaders group produced the most belowground biomass with the pioneer species, early succession, and mid succession groups producing on average only 79.7%, 63.9%, and 15.8% of the belowground biomass as the secondary invaders group, respectively (Appendix III Table 1).

When species were analyzed individually, these differences in above- and belowground biomass were also present (Table 2). Across all treatments, the exotic pioneer grass, *Leymus arenarius* produced the most aboveground biomass, followed by the native early succession legume *Lathyrus japonicus*, and the native pioneer grass *Ammophila breviligulata*. Across all treatments, *Silene latifolia*, a forb in the secondary invaders group, produced the most belowground biomass, followed by the exotic pioneer grass *Leymus arenarius*, the native early succession legume *Lathyrus japonicus*, and *Centaurea stoebe*, a forb in the secondary invaders group (Appendix III Table 1).

Responses to soil conditioning

There were significant effects of soil conditioning on aboveground biomass when analyzed by successional group (p = 0.0387; Table 1) and when analyzed by response species (p = 0.0076; Table 2). Across all species, plants from *Leymus* conditioned soils produced 9% less aboveground biomass than *Ammophila* conditioned soils (biomass from *Ammophila* conditioned soil = 99.2 mg; biomass from *Leymus* conditioned soil = 90.2 mg). Effects of soil conditioning on belowground biomass were not statistically significant when analyzed by successional group (p = 0.1496; Table 1, Appendix III Figure 1) and were marginally significant when analyzed by response species (p = 0.0539; Table 2, Appendix III Figure 2), with plants producing 9.6% less belowground biomass in *Leymus* conditioned soils. For both above- and belowground biomass, there were no significant interactions between soil conditioning and either successional group (Table 1) or response species (Table 2).

Responses to direct competition

There was no significant difference in aboveground biomass between the mature competitor species at the second round harvest (df = 1, F = 2.58, p = 0.1111). However, there was a significant difference in belowground biomass between the two mature competitor species (df = 1, F = 36.97, p < 0.0001) with *Leymus* competitors producing 59.3% more belowground biomass than *Ammophila* competitors, independent of the identity of response seedlings. Presence of a competitor consistently had a strong effect reducing both above- and belowground biomass in the response plants. Across all species in *Ammophila* conditioned soil, presence of an *Ammophila* competitor reduced above- and belowground biomass by 52.1% and 57.9%, respectively. Across all species in *Leymus* conditioned soil, presence of a *Leymus* competitor reduced above- and belowground biomass by 67.4% and 75.6%.

There were significant interactions between competition (nested within conditioning) and both successional group (p < 0.0001; Table 1) and response species (p < 0.0001; Table 2). Presence of the competitor always reduced aboveground biomass within each conditioning treatment, however the two competitor species differed in their magnitude of effect. Within the secondary invaders group, presence of an *Ammophila* competitor reduced aboveground biomass by 33.7% relative to *Ammophila* conditioned soils without a competitor, while presence of a *Leymus* competitor had a larger impact, reducing aboveground biomass by 64.2% relative to *Leymus* conditioned soils without a competitor (Figure 1). Similar patterns were observed with the mid succession group, with an *Ammophila* competitor reducing aboveground biomass by 27.1%, while a *Leymus* competitor reducing biomass by 70.4% (Figure 1). Within the other groups, differences between *Ammophila* and *Leymus* competition were smaller—for the pioneer group,

Ammophila competition reduced aboveground biomass by 65.6%, while *Leymus* competition reduced aboveground biomass by 72.3% (Figure 1). Similarly for the early succession group, *Ammophila* competition reduced aboveground biomass by 46.1%, while *Leymus* competition reduced aboveground biomass by 58.0% (Figure 1). Similar patterns were observed for belowground biomass as well (Table 1, Appendix III Figure 1).

For the species-level analyses, many species did not show differences in biomass between *Ammophila* and *Leymus* competition (Figure 2). However, there were notable differences for the two secondary invaders. *Centaurea stoebe* showed no difference in growth in the two soil conditioning treatments, but aboveground biomass production was 64.9% lower with a *Leymus* competitor compared to an *Ammophila* competitor. Similarly, *Silene latifolia* showed no significant response to soil conditioning, but aboveground biomass production was 43.6% lower under *Leymus* competition than *Ammophila* competition. Similar trends were observed for belowground biomass as well (Appendix III Figure 2).

DISCUSSION

Direct and indirect effects of exotic plants can alter successional dynamics of native plant communities (Corbin and D'Antonio 2012; Titus and Tsuyuzaki 2002). Here we show that the ecological consequences of one type of indirect effect – soil conditioning – are not especially strong in a sand dune system. We found only minor effects of soil conditioning from *Leymus* on the growth of native plant species, where growth was reduced 9% compared to plants grown in soil conditioned by the native *Ammophila*. This effect persisted across plant successional status, from the pioneer

species *Ammophila* and *Leymus* to the mid successional species *Calmovilfa* and *Schizachryium*. These results generally support our hypothesis based on the many exotic plants that have been shown to alter soil communities, often to the detriment of native species (Batten et al. 2008; Bennett et al. 2011; Grman and Suding 2010; Jordan et al. 2011). However, the magnitude of soil conditioning effects were not very large, indicating that *Leymus* may not be substantially altering soil communities or conditions in a way to strongly affect native plants.

We did not find any positive effects of soil conditioning by *Leymus* on the secondary invaders, *Centaurea stoebe* and *Silene latifolia*. We expected to find positive effects of soil conditioning on secondary invaders, since exotic plant legacies may promote the invasion of secondary invaders (Flory and Bauer 2014), known as invasional meltdown (Simberloff and Von Holle 1999). However, much like with the native response species, there were only minor effects of soil conditioning on the growth of secondary invaders. It should be noted that we mixed soils across replicates for both soil conditioning treatments for the second phase of the experiment. Recent advances in plant-soil feedback study design by Reinhart and Rinella (2016) and Rinella and Reinhart (2018) have shown that the soil handling technique used in this study can generate false positive effects of soil conditioning. Since we found only a weak effect of soil conditioning, we can reasonably accept these results with caution.

More work is needed to tease apart the biotic or abiotic mechanisms responsible for even the small differences in soil conditioning found in this study. In a recent field survey of *Leymus* invasion, Reid et al. (Chapter III) found no difference in soil bacterial functional diversity and soil nematode community composition in invaded and uninvaded

areas. However, soil bacterial composition was not addressed in that survey, making it difficult to eliminate bacterial communities as a potential effect of soil conditioning. Other potential *Leymus* conditioning effects could be due to communities of arbuscular mycorrhizal fungi, non-mycorrhizal fungal communities (Kourtev et al. 2002) and residual allelopathic or other soil chemical properties (Lepinay et al. 2018). These were not quantified as part of this study, but our results suggest that any differences in these soil conditioning parameters are likely to be minimal.

We found that direct competition from the pioneer dune grasses *Ammophila* and *Leymus* had strong negative effects on the growth of most response species. This study did not examine potential mechanisms of competition, but one possible mechanism involves competition for soil nutrients. Sand dunes are nutrient-limited systems, particularly regarding soil nitrogen (Gilbert et al. 2008; Olff et al. 1993). Competition for limited soil nitrogen could be driving the competitive dynamics since the legume *Lathyrus japonicus* showed no effect of competition from either *Ammophila* or *Leymus* on aboveground biomass production (Figure 2C), while most other grasses and forbs did show negative growth responses to competition. *Ammophila* is known to be an important competitor on early successional sand dunes and acts to suppress colonizers (Cheplick 2005), and the results of this study suggest that *Leymus* would likely function in a similar manner, with one notable exception. The observation that only secondary invaders were conspicuously suppressed by *Leymus* at a greater level than *Ammophila* was unexpected. Since previous work has shown that *Leymus* is larger than *Ammophila* in terms of both above- and belowground biomass (Reid and Emery 2017), we had anticipated that effects of competition with *Leymus* would be stronger than *Ammophila* competition across all

species. Our mature *Leymus* competitors produced more belowground biomass than the mature *Ammophila* competitors, but this did not lead to significant differences in response plant biomass outside of the secondary invaders. Why competition from the exotic *Leymus* would be stronger only for the secondary invaders is not immediately clear and warrants further investigation.

The effects of *Leymus* via direct competition and indirect soil conditioning do not appear to change over the course of dune succession. This suggests that *Leymus* is unlikely to alter dune successional dynamics. Rather, we expect that it performs similarly to *Ammophila* where it outcompetes native species (Cheplick 2005) until its potential decline due to an accumulation of detrimental soil organisms (Seliskar and Huettel 1993). Such interactions between *Leymus* as an early colonizer and subsequent later colonizing species would be consistent with the inhibition model of succession (Connell and Slatyer 1977). Additionally, the results of this study suggest that suppression of secondary invaders by *Leymus* could be beneficial, as many secondary invaders in this system represent different functional groups with known negative effects on native plants (e.g., *Centaurea stoebe* effects on the endangered native dune plant *Cirsium pitcheri*; Rand et al. 2015). Land managers currently take an aggressive management approach with *Leymus*, actively treating known populations with herbicides. The results of this study suggest that impacts of *Leymus* on native plant communities are minimal, thus aggressive management approaches likely are unnecessary. However, manipulative field experiments are needed to fully assess the effects of *Leymus* on dune plant communities.

Previous studies have shown that exotic plants can alter successional dynamics by negatively affecting native species. However, the results of this study indicate that the

exotic pioneer grass *Leymus arenarius* and the native pioneer grass *Ammophila breviligulata* have relatively similar effects on native species from across a dune succession gradient. Thus, despite differences in size of these two pioneer species, they appear to have high functional similarity with regards to their direct and indirect effects on native plants, indicating that successional impacts from *Leymus* invasion are likely minimal.

Tables

Table 1: Above- and belowground biomass responses as a function of soil conditioning, competition nested within soil conditioning, plant successional group, and all possible interactions. Significant effects are indicated in bold.

Source	df	Aboveground Biomass		Belowground Biomass	
		F	P	F	P
Condition	1	4.33	**0.0387**	2.09	0.1496
Competition(condition)	2	75.99	**<0.0001**	60.28	**<0.0001**
Successional group	3	85.83	**<0.0001**	45.76	**<0.0001**
Successional group*condition	3	1.21	0.3061	2.00	0.114
Successional group*comp(condition)	6	6.85	**<0.0001**	5.35	**<0.0001**

Table 2: Above- and belowground biomass responses as a function of soil conditioning, competition nested within soil conditioning, response plant species, and all possible interactions. Significant effects are indicated in bold.

Source	df	Aboveground Biomass		Belowground Biomass	
		F	P	F	P
Condition	1	7.28	**0.0076**	3.76	0.0539
Competition(condition)	2	119.62	**<0.0001**	102.42	**<0.0001**
Response species	7	69.95	**<0.0001**	51.28	**<0.0001**
Response species*condition	7	1.22	0.2955	1.89	0.0735
Response species*comp(condition)	14	7.16	**<0.0001**	5.72	**<0.0001**

Figure Legends

Figure 1: Aboveground biomass of the A) pioneer group, B) early succession group, C) mid succession group, and D) secondary invaders group as a function of soil conditioning and competition, nested within soil conditioning. Bars represent ±1 SE. Note different x-axis scales. Different letters indicate significant differences among treatments within each group based on Tukey post-hoc tests.

Figure 2: Aboveground biomass of A) *Ammophila breviligulata*, B), *Leymus arenarius*, C) *Lathyrus japonicus,* D) *Artemisia campestris*, E) *Calamovilfa longifolia*, F) *Schizachyrium scoparium*, G) *Centaurea stoebe*, and H) *Silene latifolia* as a function of soil conditioning or competition (nested within soil conditioning). Bars represent ±1 SE. Note different x-axis scales. Different letters indicate significant differences among treatments within each species based on Tukey post-hoc tests.

Figures

Figure 1

A) Pioneer group

B) Early succession group

C) Mid succession group

D) Secondary invaders group

Figure 2

A) *Ammophila breviligulata*
B) *Leymus arenarius*
C) *Lathyrus japonicus*
D) *Artemesia campestris*
E) *Calamovilfa longifolia*
F) *Schizachyrium scoparium*
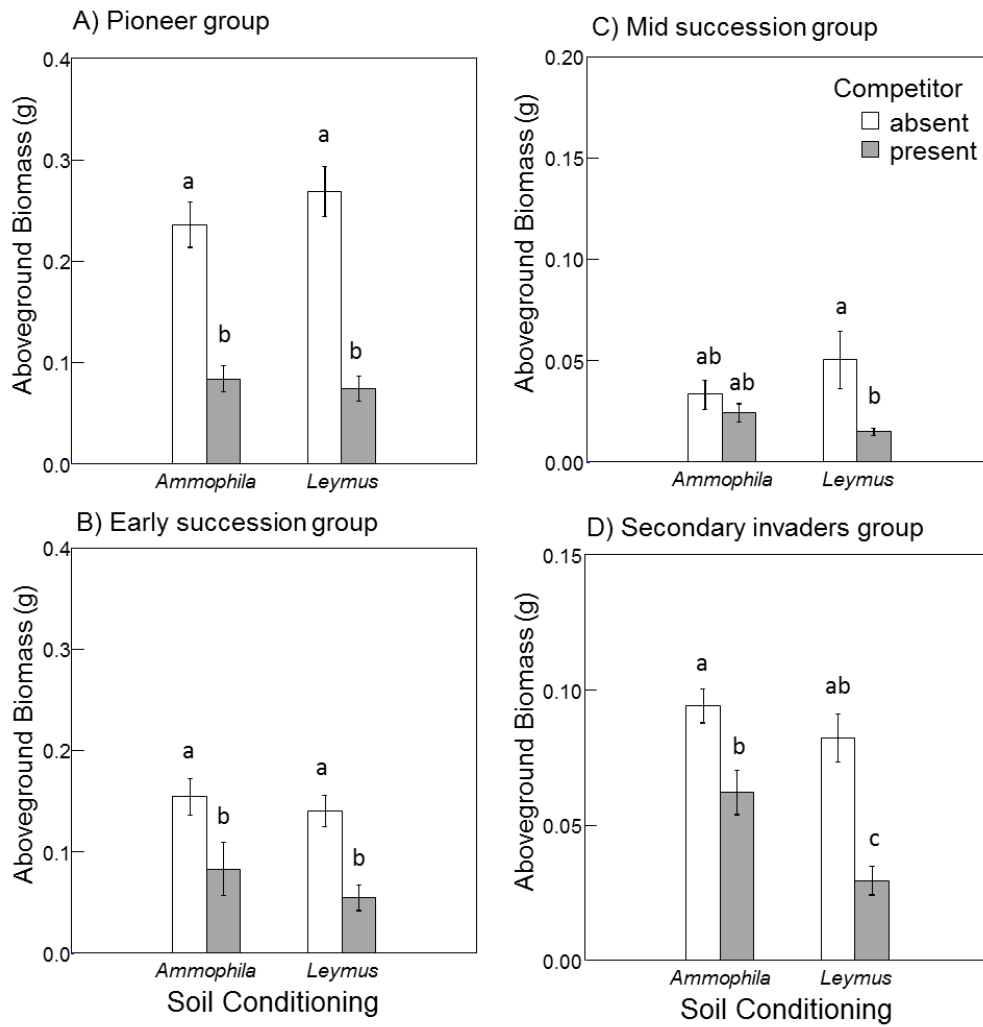G) *Centaurea stoebe*
H) *Silene latifolia*
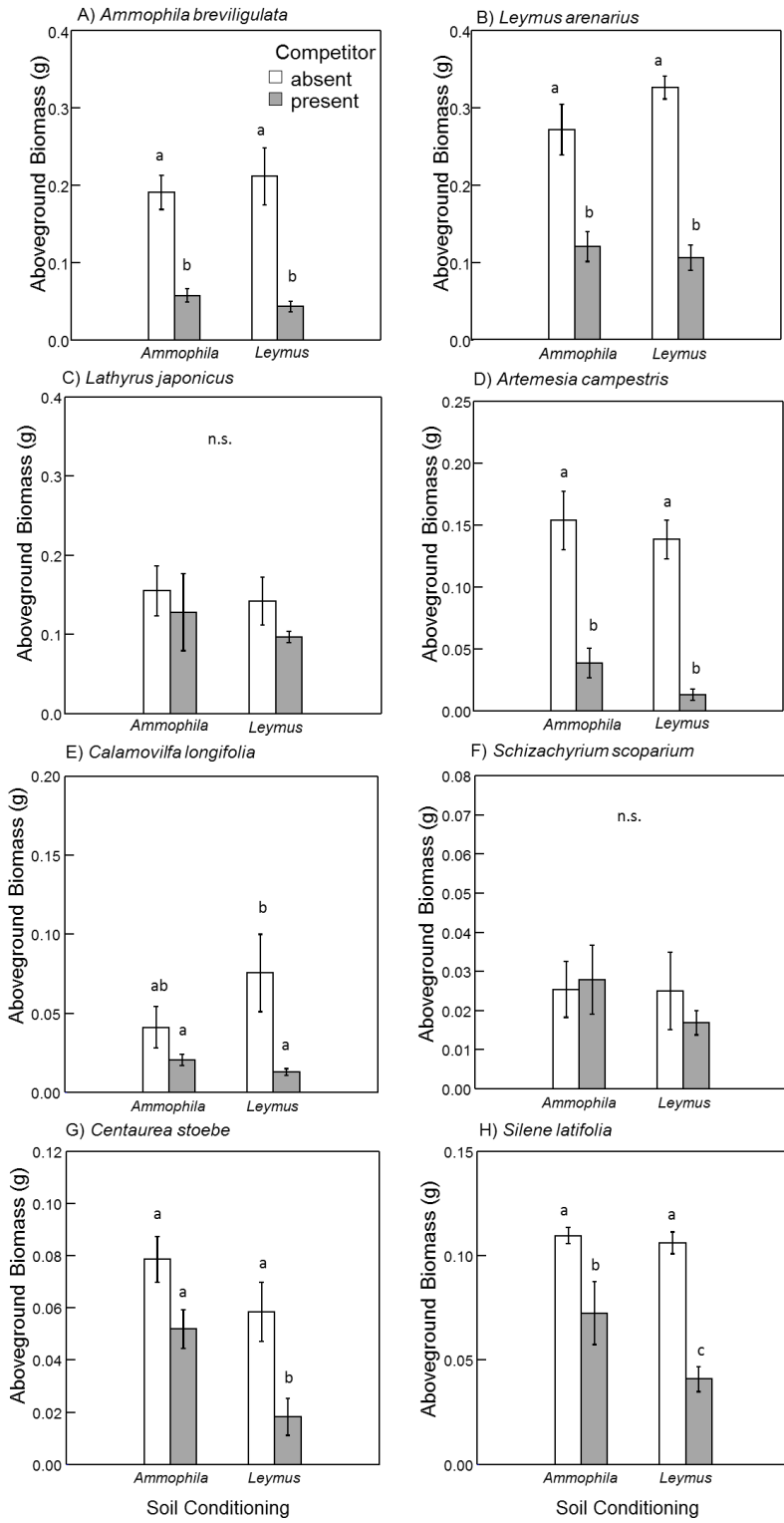
CHAPTER V

SUMMARY AND FUTURE DIRECTIONS

SUMMARY

This dissertation investigated the consequences of *Leymus arenarius* invasion on the structure of soil communities and interactions within the soil community, and whether *Leymus* invasion could potentially alter plant successional dynamics via direct competition or changes belowground. The greenhouse experiment from Chapter II showed that there were architectural differences in the exotic *Leymus* and the native *Ammophila*. Additionally, both *Leymus* and *Ammophila* benefitted from interactions with arbuscular mycorrhizal fungi (AMF) and experienced negative effects from plant-parasitic nematodes (PPN). However, in tri-trophic interactions between plant roots, AMF, and PPN, *Leymus* had a stronger net benefit from AMF in the presence of PPN than *Ammophila*, indicating that belowground multi-trophic interactions could be contributing to its invasion. In Chapter III, the *Leymus* field survey revealed that *Leymus* invasion was not associated with changes in the soil nematode community or the functional diversity of the bacterial community. Rather, climatic factors such as precipitation and temperature, and dune successional factors including soil organic matter, were the most important factors structuring these communities. In Chapter IV, the growth chamber experiment showed that both *Leymus* and *Ammophila* had similar strong direct competitive effects on the growth of later successional species. The notable

exception to this involved the effects on the secondary invaders *Centaurea stoebe* and *Silene latifolia*, which experienced stronger direct negative effects from *Leymus*, indicating the potential for *Leymus* to suppress other important secondary invaders in sand dunes. Overall, *Leymus* soil conditioning had only a weak effect reducing the growth of later successional plant species. Thus, direct competition is likely playing a larger role in any *Leymus* effects on the plant community. Overall, *Leymus* appears to have minimal impact on the structure of soil communities, but interactions within the soil community may be altered. These interactions benefit *Leymus*, which can have direct negative effects on co-occurring plant species. Combined, the results from this dissertation yield new questions regarding differences between *Ammophila* and *Leymus* in their interactions belowground, the role those belowground interactions play in invasion, and species traits that contribute to population growth on the dunes.

FUTURE DIRECTIONS

Much of my dissertation work focused on the consequences of *Leymus* invasion— primarily effects on soil and plant communities. However, there is still much work that could be done examining the causes of *Leymus* invasion, particularly regarding the enemy release hypothesis (Heger and Jeschke 2014). One facet of enemy release involves enemy preference for native versus exotic plants. Previous studies have examined preferences for native insect herbivores between native and exotic plant species (Bezemer et al. 2014). While feeding preference tests for root herbivores have been relatively rare, Brinkman et al. (2008) found plant-parasitic nematodes had differential attraction among dune plant species. Since native and exotic plants often differ in root tissue quality, exudation, and architecture, these differences could drive nematode preference (Demarta

et al. 2014; Hiltpold et al. 2014). I would hypothesize that enemy release would be demonstrated by nematode feeding preferences where native and exotic plants co-occur. In free-choice experiments, I would expect nematodes will exhibit greater preference for native plant roots over exotic plant roots. Feeding trials where different plant-parasitic nematode species are given a choice between *Leymus arenarius* and *Ammophila breviligulata* would reveal if herbivore feeding preferences could be an important factor of enemy release in *Leymus* invasion. Feeding trials could be established in simple agar plates (Brinkman et al. 2008) or in sand-filled Y-tubes (Boff et al. 2002), which would more closely resemble field conditions. Results from free-choice feeding trials would indicate whether native root herbivores can detect differences in plant roots, and if so exhibit plant species preferences.

Another facet of the enemy release hypothesis (Heger and Jeschke 2014) states that exotic species are often released from their specialized herbivores that suppress their growth in their native range (Keane and Crawley 2002). Studies that examine a species in both its native and exotic ranges offer the clearest test of this hypothesis (Hierro et al. 2005). Surveys of *Leymus* populations from its native European range and its introduced range in the Great Lakes would provide a clear test of potential differences in soil community composition, and its role in contributing to *Leymus* success in its introduced range. If enemy release is a factor in *Leymus* success, then *Leymus* populations in the Great Lakes region should have fewer specialized root herbivores and parasites relative to *Leymus* populations in its northern European range.

Finally, my dissertation and the future directions presented thus far have focused on community-level interactions. However, there is still much we do not know about the

population dynamics of *Leymus arenarius*, particularly as it compares to the native *Ammophila breviligulata*. Both species can undergo vegetative spread by sending up new tillers from underground rhizomes, and both species can reproduce sexually. My observations in the field suggest that *Leymus* has higher rates of sexual reproduction and seed production than *Ammophila*. Additionally, the seeds of *Leymus* are larger than *Ammophila*, providing a potential benefit to developing seedlings in this nutrient-poor environment. I would propose field surveys to monitor rates of flowering and seed production in unmanaged *Leymus* populations and nearby *Ammophila* populations. Seed germination and establishment trials could also be conducted in carefully controlled field experiments, to minimize the risk of *Leymus* seeds escaping. Simple experiments such as this could reveal if *Leymus* seeds germinate earlier than *Ammophila* seeds under field conditions and determine if they have greater survivability than *Ammophila*. Results from such experiments could reveal the likelihood of potential *Leymus* spread from established populations. Additionally, establishing permanent plots in the field would allow for annual tiller counts for defined populations of *Leymus* and *Ammophila* to assess differences in vegetative reproduction. Land managers currently take an aggressive approach to managing *Leymus* using chemical herbicides and seed head clipping to reduce reproduction. Results from the proposed experiments would reveal potential rates of *Leymus* reproduction, providing land managers with more information to assess the relative merit of *Leymus* management, given limited resources and other more common invasive species on sand dunes, such as *Gypsophila paniculata* and *Centaurea stoebe*.

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APPENDIX I

SUPPLEMENTAL DATA FROM CHAPTER II

Appendix I Table 1: Aboveground and back-transformed belowground plant architecture

traits of *Ammophila* and *Leymus*

	Ammophila		*Leymus*	
	Mean	95% CI	Mean	95% CI
Height (cm)	9.86	8.88-10.84	18.03	15.97-20.10
Number of leaves	2.58	2.40-2.75	2.48	2.23-2.72
Leaf length (cm)	20.31	17.16-23.47	30.54	25.91-35.16
Root length (cm)	223.84	193.05-259.60	563.85	469.79-676.74
Average root diameter (mm)	0.27378	0.257028-0.291627	0.25505	0.24692-0.26345

Appendix I Table 2: Aboveground and back-transformed belowground plant architecture traits as a function of PPN for *Ammophila* and *Leymus*

	Ammophila, PPN-		*Ammophila,* PPN+		*Leymus,* PPN-		*Leymus,* PPN+	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Height (cm)	10.86	9.18-12.53	8.87	7.88-9.85	21.10	18.66-23.53	14.97	12.05-17.89
Number of leaves	2.80	2.56-3.04	2.35	2.12-2.58	2.75	2.45-3.05	2.20	1.84-2.56
Leaf length (cm)	23.87	19.01-28.73	16.76	12.96-20.55	37.91	32.99-42.82	23.17	16.48-29.86
Root length (cm)	306.75	258.32-364.25	163.34	141.31-188.82	849.68	739.82-975.85	374.17	299.40-467.62
Average root diameter (mm)	0.25226	0.23494-0.27086	0.29713	0.26970-0.32734	0.24208	0.232340-0.25217	0.26872	0.25790-0.27999

Appendix I Table 3: Aboveground and back-transformed belowground plant architecture

traits as a function of AMF for *Ammophila* and *Leymus*

	Ammophila, AMF-		*Ammophila,* AMF+		*Leymus,* AMF-		*Leymus,* AMF+	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Height (cm)	9.53	8.59-10.47	10.20	8.37-12.01	16.15	12.95-19.35	19.915	17.29-22.54
Number of leaves	2.35	2.12-2.58	2.80	2.56-3.04	2.20	1.81-2.59	2.75	2.49-3.01
Leaf length (cm)	16.13	13.07-19.18	24.50	19.36-29.64	25.10	18.25-31.95	35.98	30.20-41.75
Root length	207.90	170.90-252.91	241.01	190.47-304.95	518.49	387.70-693.41	613.17	481.29-781.21
Average root diameter	0.28044	0.25167	0.26727	0.24810-0.28793	0.25496	0.24498-0.26535	0.25514	0.24151-0.26955
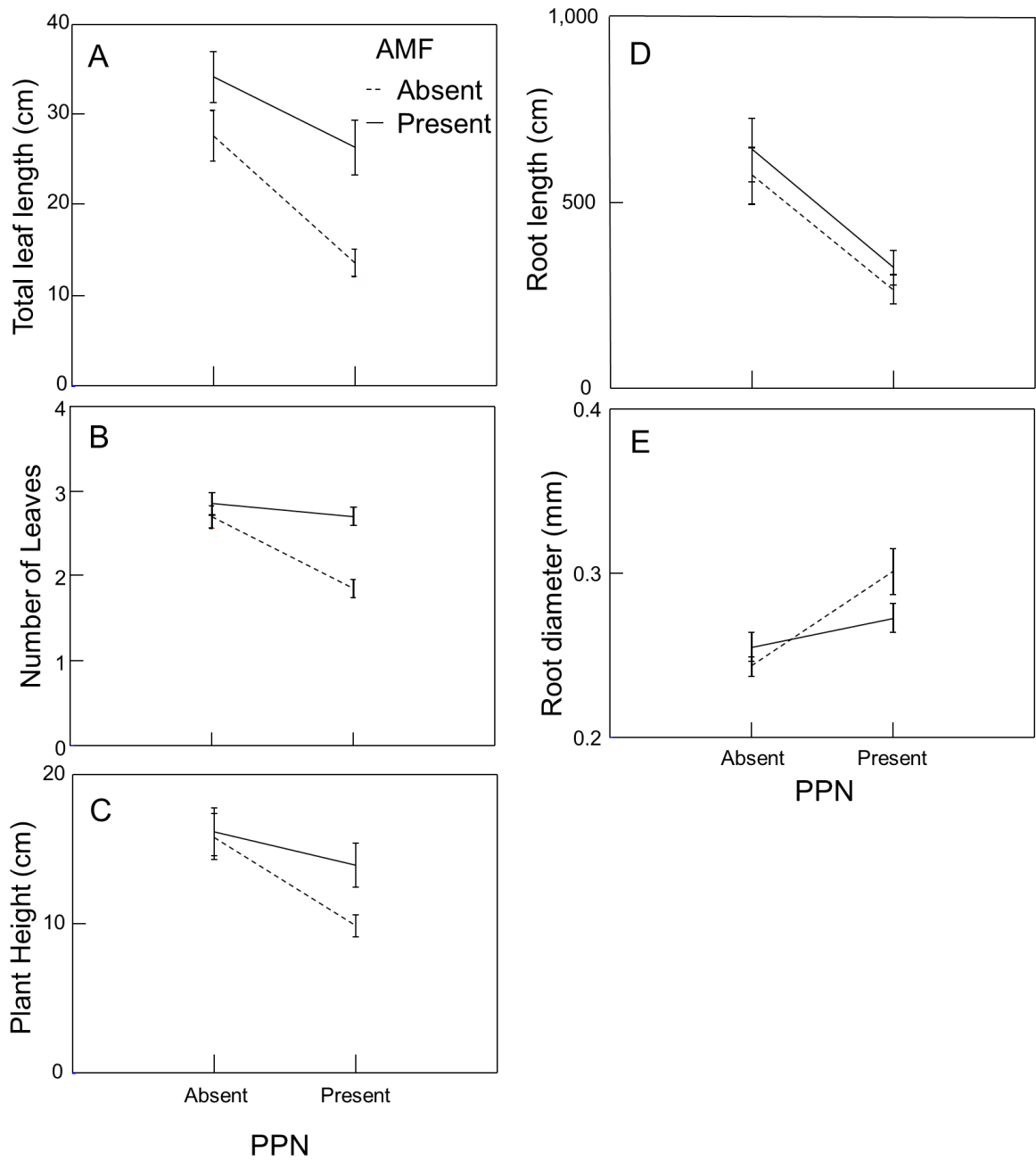
Appendix I Figure 1: Effects of AMF x PPN interaction on A) leaf length, B) number of leaves, C) plant height, D) root length, and E) root diameter.

SUPPLEMENTAL DATA FROM CHAPTER III

Appendix II Figure 1: Example of landscape analysis based on ArcGIS data. GPS coordinates were overlaid on aerial images of the site, allowing for measurements of distance to dune edge and forest edge, indicated by the arrows, in order to calculate our succession variable. Modified from Howes (2017).

Appendix II Table 1: List of the 30 most abundant nematode taxa identified in the field survey, with corresponding feeding group, as assigned by the Nematode Indicator Joint Analysis (Sieriebriennikov et al. 2014), based on Yeates et al. (1993). Values indicate absolute abundance for each taxon, ranked from most to least abundant.

Taxon	Feeding Group	Abundance
Plectus	Bacterivore	2617
Eudorylaimus	Omnivore	1952
Acrobeles	Bacterivore	744
Heterocephalobus	Bacterivore	419
Rhomborhabditis	Bacterivore	197
dauer	Bacterivore	135
Leptonchus	Fungivore	125
Trichodorus	Herbivore - ectoparasite	125
Mylonchulus	Predator	124
Rhabditis	Bacterivore	114
Aphelenchoides	Fungivore	89
Filenchus	Fungivore	84
Nygolaimus	Predator	84
Pratylenchus	Herbivore - migratory endoparasite	84
Tripyla	Predator	83
Tylenchorhynchus	Herbivore - ectoparasite	69
Cephalobus	Bacterivore	66
Coomansus	Predator	63
Leptolaimus	Bacterivore	49
Eucephalobus	Bacterivore	36
Acrobeloides	Bacterivore	25
Criconematidae	Herbivore - ectoparasite	16
Dorylaimoides	Fungivore	16
Longidorus	Herbivore - ectoparasite	14
Mesodorylaimus	Omnivore	14
Longidorella	Herbivore - ectoparasite	11
Eumonhystera	Bacterivore	9
Diphtherophora	Fungivore	8
Alaimus	Bacterivore	7
Ditylenchus	Fungivore	6

APPENDIX III

SUPPLEMENTAL DATA FROM CHAPTER IV

Appendix III Table 1: Average above- and belowground biomass for each species across all treatments.

	Aboveground Biomass (g)		Belowground Biomass (g)	
Species	Mean	SD	Mean	SD
Ammophila breviligulata	0.1259	0.0925	0.0961	0.0966
Leymus arenarius	0.2166	0.1113	0.2274	0.1592
Artemisia campestris	0.0890	0.0708	0.0864	0.0912
Lathyrus japonicus	0.1305	0.0783	0.1717	0.1164
Calamovilfa longifolia	0.0376	0.0404	0.0410	0.0617
Schizachryium scoparium	0.0238	0.0181	0.0230	0.0176
Centaurea stoebe	0.0518	0.0300	0.1215	0.0814
Silene latifolia	0.0823	0.0348	0.2846	0.1871

Appendix Figure Legends

Appendix III Figure 1: Belowground biomass of the A) pioneer group, B) early

succession group, C) mid succession group, and D) secondary invaders group as a

function of soil conditioning and competition, nested within soil conditioning. Note

different x-axis scales. Different letters indicate significant differences among treatments

within each group based on Tukey post-hoc tests.

Appendix III Figure 2: Belowground biomass of A) *Ammophila breviligulata*, B),

Leymus arenarius, C) *Lathyrus japonicus,* D) *Artemisia campestris*, E) *Calamovilfa*

longifolia, F) *Schizachyrium scoparium*, G) *Centaurea stoebe*, and H) *Silene latifolia* as a

function of soil conditioning or competition (nested within soil conditioning). Bars

represent ±1 SE. Note different x-axis scales. Different letters indicate significant

differences among treatments within each species based on Tukey post-hoc tests.
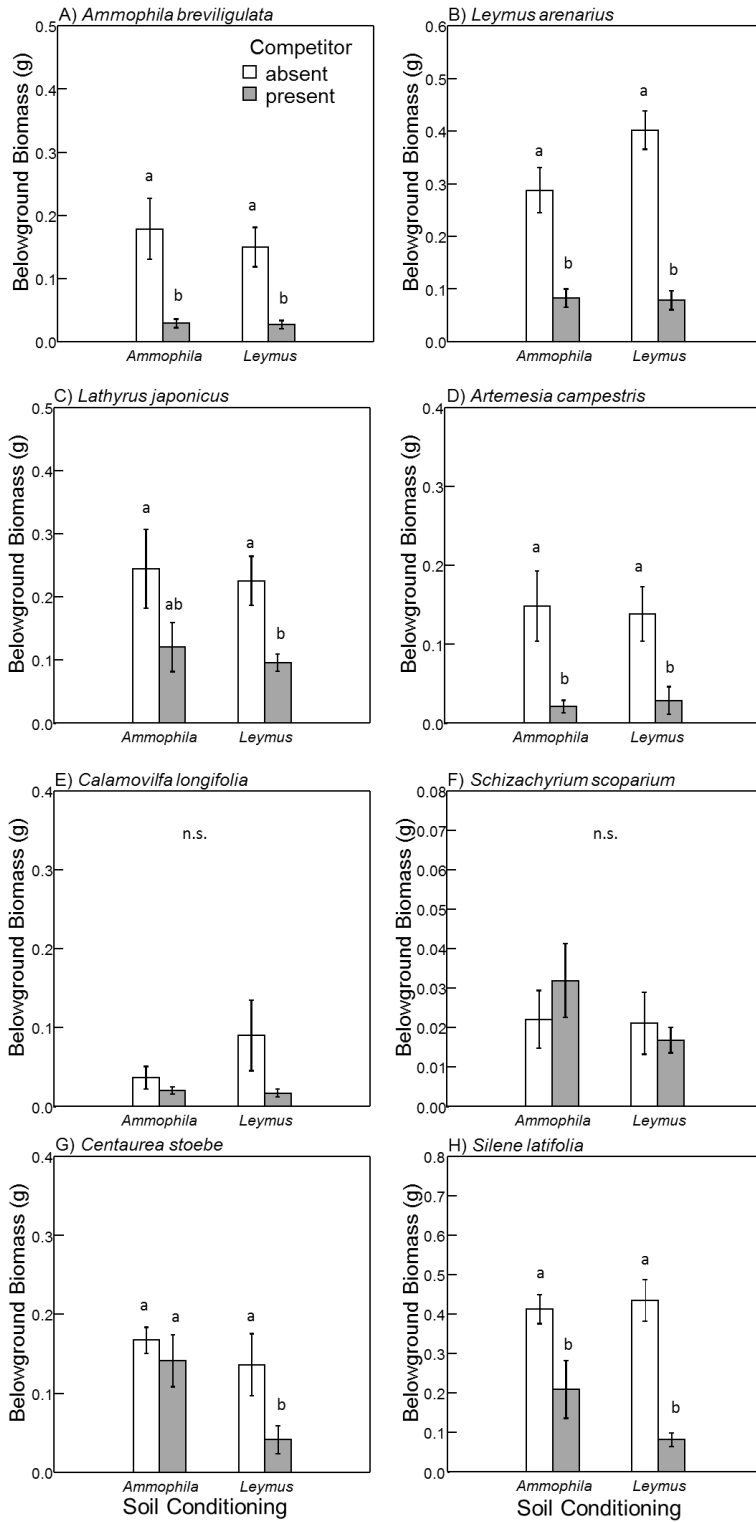
Appendix III Figure 1

A) Pioneer group

B) Early succession group

C) Mid succession group

D) Secondary invaders group

Appendix III Figure 2

A) *Ammophila breviligulata*

B) *Leymus arenarius*

C) *Lathyrus japonicus*

D) *Artemesia campestris*

E) *Calamovilfa longifolia*

F) *Schizachyrium scoparium*

G) *Centaurea stoebe*

H) *Silene latifolia*
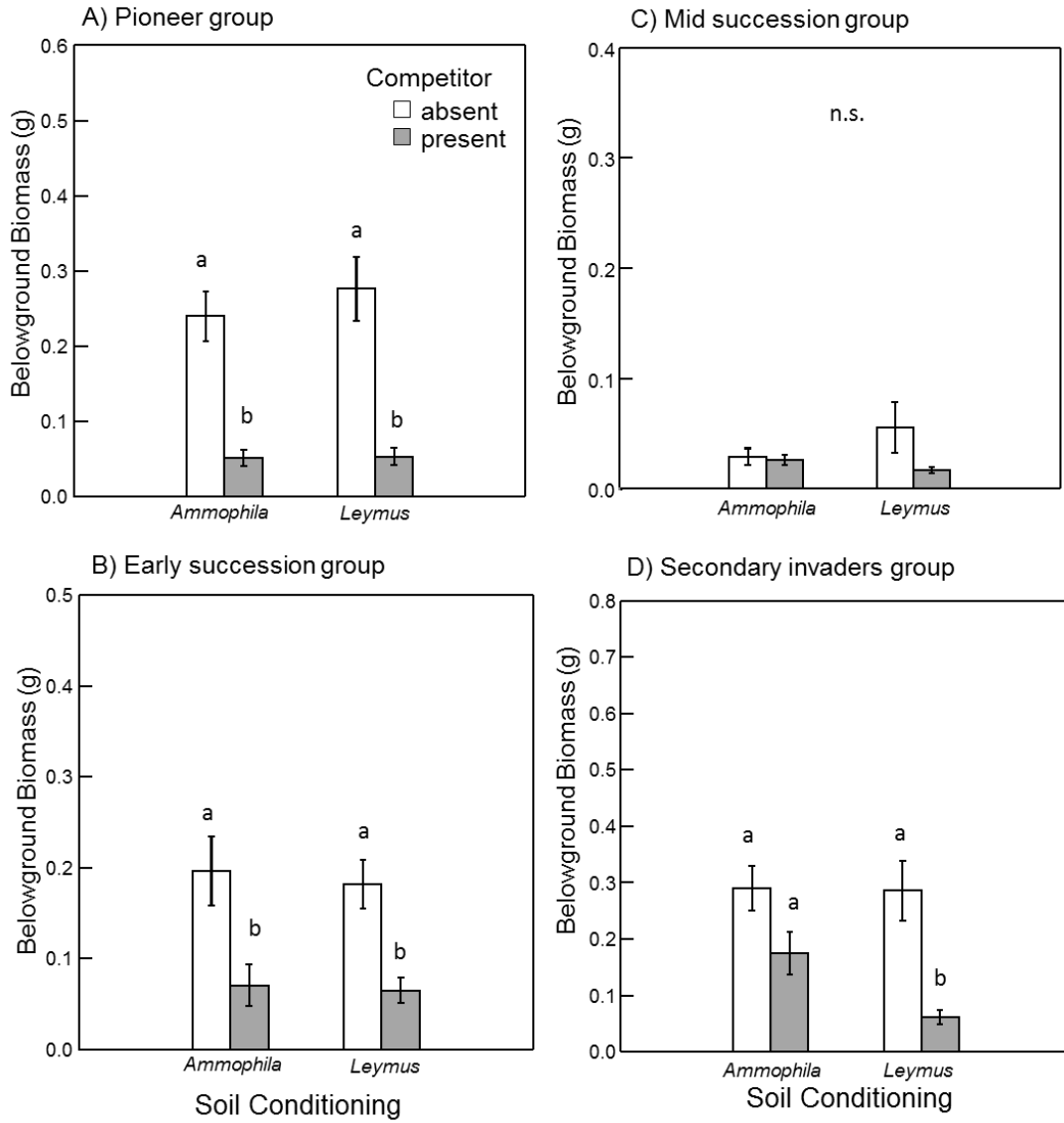
CURRICULUM VITAE

Matthew L. Reid
Department of Biology, University of Louisville
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EDUCATION:

Ph.D. Biology, University of Louisville, Louisville, KY   2013 – 2018
    Dissertation: Soil ecology of the exotic dune grass *Leymus arenarius*.
    Advisor: Dr. Sarah Emery.
M.S. Biology, University of Louisiana at Monroe, Monroe, LA   2010 – 2013
    Thesis: A quarter century of plant succession in a bottomland hardwood forest in
    northeastern Louisiana. Advisor: Dr. Joydeep Bhattacharjee.
B.A. Biology, Hendrix College, Conway, AR   2005 – 2009
    Cum Laude, Distinction in Biology

RESEARCH INTERESTS:

My research interests broadly focus on plant community and soil ecology. My dissertation work focuses on understanding the consequences of exotic plant invasion on belowground community interactions. Specifically, I examined the effects of exotic plant invasion on nematode community structure, tri-trophic interactions among plant roots, nematodes, and mycorrhizal fungi, and ultimately community development in a primary successional sand dune ecosystem. Work from my M.S. thesis focused on patterns of secondary succession in bottomland hardwood forests in Louisiana.

EXTERNAL RESEARCH FUNDING:

-NSF Doctoral Dissertation Improvement Grant, DEB #1701084 [$18,547]   2017
-Garden Club of America Fellowship in Ecological Restoration [$8,000]   2015
-Kentucky Academy of Sciences Botany Fund [$1,858]   2015
-Sigma Xi Grant-in-Aid of Research [$600]   2014

FELLOWSHIPS AND AWARDS:

-UofL Doctoral Dissertation Completion Award [$11,664]   2018
-Soil Ecology Society Parkinson Travel Award to SES Meeting [$300]   2017
-Biology Graduate Student Association Travel Award to SES Meeting [$100]   2017
-Soil Ecology Section Graduate Student Travel Award to ESA Meeting [$500]   2016
-Biology Graduate Student Association Travel Award to ESA Meeting [$150]   2016
-UofL Arts & Sciences Graduate Student Union Research Fund [$100]   2015
-Beechmont Garden Club Award, University of Louisville [$1,000]   2014

-UofL Intramural Research Initiation Grant [$4,976]   2014
-UofL Graduate Student Research and Creative Activities Fund [$500]   2014
-UofL Biology Graduate Student Association Research Fund [$235]   2014
-UofL Graduate Student Council Research Fund [$300]   2014
-University Fellowship, University of Louisville [$89,274]   2013 – 2015
-Society of Wetland Scientists Student Travel Award to ASB Meeting [$200]   2012
-Association of Southeastern Biologists Graduate Student Travel Award [$385]   2012
-Southwestern Association of Naturalists Student Travel Award [$860]   2012
-Charles E. DePoe Memorial Scholarship in Biology, ULM   2012
-Association of Southeastern Biologists Graduate Student Travel Award [$350]   2011
-Tom Kee Memorial Scholarship in Biology, ULM   2011

PUBLICATIONS: (***** denotes undergraduate co-author)

7. Emery S.M., **M.L. Reid**, L. Bell-Dereske, and K.L. Gross. 2017. Soil mycorrhizal and nematode activity and diversity vary in response to bioenergy crop identity and fertilization. GCB Bioenergy 9:1644-1656.

6. **Reid M.L.** and S.M. Emery. 2017. Native and exotic foundation grasses differ in traits and responses to belowground tri-trophic interactions. Plant Ecology 218:172-183.

5. Battaglia C.D., C.R. Faidley, A.N. Hudson, M.D Brown, T.M. Pardue, **M.L. Reid**, A.A. Bass, C.L. Townsend, and J.L. Carr. 2015. Distribution records for Louisiana amphibians and reptiles. Herpetological Review 46:579-581.

4. **Reid M.L.**, S.R. Allen*, and J. Bhattacharjee. 2014. Patterns of spatial distribution and seed dispersal among bottomland hardwood tree species. Castanea 79:255-265.

3. **Reid M.L.** and J. Bhattacharjee. 2014. Episodic flooding of the Ouachita River: Levee-mediated mortality of trees and saplings in a bottomland hardwood restoration area. Southeastern Naturalist 13:493-505.

2. **Reid M.L.**, C.D. Battaglia, C.L. Rice, and J.L. Carr. 2014. Colonization of northern Louisiana by the Mediterranean Gecko, *Hemidactylus turcicus*. Journal of North American Herpetology 1:93-97.

1. Bhattacharjee J. and **M.L. Reid**. 2011. Revolution of Colors: Impact on our fragile environment. National Workshop and Seminar on Vegetable dye and its application on textiles. 2nd - 4th December, Department of Silpa-Sadhana, Visva-Bharati University, Sriniketan. Birbhum West Bengal, India, p. 117-121.

MANUSCRIPTS IN REVIEW:

Reid M.L. and S.M. Emery. Scale-dependent effects of *Gypsophila paniculata* invasion and management on above- and belowground diversity and heterogeneity. Biological Conservation.

INVITED PRESENTATIONS:

Reid M.L. 2016. Ecological impacts of baby's breath (*Gypsophila paniculata*) invasion and management in sand dunes. Research Rendezvous, Sleeping Bear Dunes National Lakeshore. Empire, MI.

CONFERENCE PRESENTATIONS – PAPERS:

Emery S.M., **M.L. Reid**, L. Bell-Dereske, and K.L. Gross. 2017. Soil mycorrhizal and nematode activity and diversity vary in response to bioenergy crop identity and management. Ecological Society of America. Portland, OR.

Reid M.L. and S.M. Emery. 2017. *Leymus arenarius* invasion alters belowground interactions with mycorrhizae and nematodes without altering nematode community structure. Soil Ecology Society. Fort Collins, CO.

Reid M.L. 2017. Exotic plant invasion alters above- and belowground heterogeneity. UofL Biology Department Awards Day. [Best Graduate Student Presentation Award]

Howes A, S. Emery, and **M. Reid**. 2016. Abiotic factors contribute to functional diversity of soil bacteria in dunes of Lake Michigan. Kentucky Academy of Sciences. Louisville, KY.

Reid M.L. and S.M. Emery. 2016. Shifts in soil nematode communities associated with plant invasion in primary succession sand dunes. Ecological Society of America. Fort Lauderdale, FL.

Reid M.L. and S.M. Emery. 2015. Do soil communities differ between native and invasive dune grasses on Great Lakes sand dunes? Midwest Invasive Plant Network Symposium. Indianapolis, IN.

Reid M.L. and S.M. Emery. 2015. Can exotic plant invasion be facilitated by belowground tri-trophic interactions? Kentucky Academy of Science. Highland Heights, KY. [1st Place in Ecology & Environmental Sciences Graduate Student Paper Presentations]

Reid M.L. and J. Bhattacharjee. 2013. Floods and levees: Implications for bottomland hardwood restoration/Inundaciones y diques: Implicaciones para restauración de bosque de madera dura de tierras bajas. Southwestern Association of Naturalists. Lake Charles, LA.

Reid M.L., D.W. Pritchett, and J. Bhattacharjee. 2013. Twenty-eight years of understory vegetation dynamics during bottomland hardwood forest succession. Louisiana Academy of Sciences. Grambling, LA.

Reid M.L. and J. Bhattacharjee. 2013. Ecological analysis of a bottomland hardwood forest community undergoing secondary succession. ULM Student Research Symposium. Monroe, LA. [1st Place in Biology Graduate Student Paper Presentations]

Reid M.L. and J. Bhattacharjee. 2012. Twenty-seven years of secondary succession in a bottomland hardwood forest. ULM Student Research Symposium. Monroe, LA. [2nd Place in Biology Graduate Student Paper Presentations]

Reid M.L., D.W. Pritchett, and J. Bhattacharjee. 2012. A quarter century of plant succession in a bottomland hardwood forest/Un cuarto siglo de sucesión de plantas en un bosque de madera dura de tierras bajas. Southwestern Association of Naturalists. Valle de Bravo, Mexico.

Reid M.L., D.W. Pritchett, and J. Bhattacharjee. 2012. Twenty-seven years of bottomland hardwood forest succession. Association of Southeastern Biologists. Athens, GA.

Reid M.L., D.W. Pritchett, and J. Bhattacharjee. 2012. Succession in bottomlands: Twenty-seven years of change. Louisiana Academy of Sciences. Alexandria, LA.

CONFERENCE PRESENTATIONS – POSTERS:
Allen S.R.*, **M.L. Reid**, and J. Bhattacharjee. 2013. Effects of seed dispersal dynamics on distribution patterns of six dominant tree species in a bottomland hardwood forest. Louisiana Academy of Sciences. Grambling, LA.
Allen S.R.*, **M.L. Reid**, and J. Bhattacharjee. 2013. Dispersal and distribution patterns of trees in a bottomland hardwood forest. ULM Student Research Symposium. Monroe, LA.
Allen S.R.*, C.N. Ho*, **M.L. Reid**, and J. Bhattacharjee. 2012. Distribution patterns of tree species during secondary succession in bottomlands. Louisiana Academy of Sciences. Alexandria, LA. [Best Undergraduate Poster in Botany, Environmental Sciences, and Zoology Division]
Kuethe B.M.*, C.N. Ho*, **M.L. Reid**, and J. Bhattacharjee. 2012. Vegetation and other factors affecting tree seedlings during old-field succession. Louisiana Academy of Sciences. Alexandria, LA.
Reid M.L. and J. Bhattacharjee. 2011. Effects of levee on flooding-induced mortality of trees and saplings in a bottomland hardwood restoration area. Association of Southeastern Biologists. Huntsville, AL.
Reid M.L. and J. Bhattacharjee. 2011. Flooding-induced mortality of trees and saplings in a bottomland hardwood restoration area. Louisiana Academy of Sciences. Monroe, LA.
* denotes mentored undergraduate project

TEACHING EXPERIENCE:
-Guest Lecturer, BIOL 563/663: Population and Community Ecology   2017
-Graduate Teaching Assistant, BIOL 244: Principles of Quantitative
        Biology (UofL)   2015, 2017
-Graduate Teaching Assistant, BIOL 1023: Principles of Biology II
        Laboratory (ULM)   2010 – 2013
        Aided development of new lab on fungi and revamped two labs on plant diversity.
-Lab Assistant, BIOL 365: Ecology and Evolution Lab (Hendrix College)   2009
-Lab Assistant, BIOL 210: Botany Lab (Hendrix College)   2007 – 2008

MENTORING EXPERIENCE:
University of Louisville
To date, I have mentored 4 undergraduates (Peter Clenny, Sarah Fosnight, Jeffrey Fox, Matthew Harper) and 1 M.S. student (Andrea Howes) resulting in 1 presentation to date.
University of Louisiana at Monroe
I mentored 3 undergraduate students (Stephanie Allen, Cathy Ho, Brittany Kuethe) resulting in 4 poster presentations and 1 publication.

PROFESSIONAL DEVELOPMENT:
STEM GTA Teaching Mini-Academy. School of Interdisciplinary and Graduate Studies, University of Louisville, 25-29 July 2016.

Identification of Freshwater and Terrestrial Nematodes Course. Netherlands Institute of Ecology, Wageningen, Netherlands. 15-26 June 2015.

PROFESSIONAL SERVICES & PUBLIC OUTREACH:
-Organizer – Soil Ecology Lab for the Louisville Youth Science Summit   2017
-Assisted in planting and maintenance of native plant garden at UofL   2016 – 2017
-Graduate Student Coordinator, UofL BioBlitz – Lincoln Boyhood National
        Memorial   2016
-Assisted in set-up of KAS Annual Meeting at University of Louisville   2016
-Representative to Graduate Committee, UofL Biology Graduate Student
        Association   2015 – 2018
-Volunteer – Breckinridge County 4-H Science Day at UofL   2015
-Volunteer – Louisville Youth Science Summit   2014
-Planning Committee – ULM BioBlitz – Charles Allen Nature Preserve   2013
-Volunteer – 73rd Annual Meeting of the Association of Southeastern Biologists   2012
-Appraiser – Louisiana Destination ImagiNation 2012 State Tournament   2012
-Volunteer – Wetlands Exploration Day, Chemin-a-Haut State Park
        ([Bastrop Daily Enterprise](#))   2011
-Volunteer – Fall Celebration, Black Bayou Lake National Wildlife Refuge   2011
-Volunteer – 72nd Annual Meeting of the Association of Southeastern Biologists   2011
-Volunteer – Museum Guide, ULM Museum of Natural History   2010 – 2012

MANUSCRIPT REVIEWER:
Canadian Journal of Forest Research (1), Ecology and Evolution (1), Plant Ecology (1)

PROFESSIONAL SOCIETY MEMBERSHIPS:
Ecological Society of America, Soil Ecology Society, Sigma Xi, Kentucky Academy of Sciences, Tri-Beta