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Shifts in the relative importance of competition and mutualism for communities and ecosystems

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SHIFTS IN THE RELATIVE IMPORTANCE OF COMPETITION AND MUTUALISM FOR COMMUNITIES AND ECOSYSTEMS

by

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DISSERTATION

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DEDICATION

I would to dedicate this dissertation to the late Waldo Dereske, who always pushed me to be a better person and to always take advantage of your opportunities to enjoy this moment. He was a creature of intelligence and independence. I wish you were here today to ram you head into my elbow as I lace up my running shoes.

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ABSTRACT

Plant species interact with at least one, likely many, microbial mutualist throughout their life cycles. These microbial mutualists can have strong effects on plant communities and ecosystem processes. Fungal endophytes within the genus *Epichloë* associate with ~20%–30% of grass species and have been shown to have strong effects on plant communities. Here I described the effect of *Epichloë amarillans* associated with the dominant grass species, *Ammophila breviligulata*, on nutrient cycling, below-ground microbial community, and compare the strength of its effects on plant communities to plant-plant competition.

In chapters one and two, I examine the effects of *Epichloë* on litter decomposition and below-ground microbial communities in the Great Lake dunes within the context of altered precipitation and soil moisture. In chapter 1, using litterbags, I found that the endophyte presence in litter increased initial rates of decomposition, though the effect disappeared after one growing season. Later litter decomposition was slowed by endophyte presence in *A. breviligulata* conditioning the soil microenvironment. In

chapter 2, using microscopy and 454 pyrosequencing, I found that the endophyte reduced the abundance of soil fungi and the diversity of an important fungal group, arbuscular mycorrhizal fungi, though this effect on diversity disappeared with altered precipitation. The presence of the endophyte also shifted the positive relationship between root associated bacteria and soil moisture to a negative relationship where diversity decreased with increasing soil moisture.

In chapter three, I tested the relative effects of *Epichloë* and competition on plant community dynamics by jointly manipulating plant-plant interactions and the presence of the endophyte within the context of altered timing of precipitation events. I found that plant-plant interactions were the strongest driver of plant community composition and diversity. However, the endophyte altered the effects of plant-plant interactions on the plant community by increasing the negative effects of competition on *A. breviligulata* growth while increasing facilitative effects of its host on the dune plant community. Increased precipitation did not alter the effects of the endophyte but did reduced the strength of plant-plant interactions. Microbial mutualisms are drivers of ecosystem and community processes playing as important a role as antagonistic interactions.

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Chapter 1

Plant-fungal symbiosis affects litter decomposition during primary succession

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ABSTRACT

Microbial symbionts of plants can affect decomposition by altering the quality or quantity of host plant tissue (substrate) or the micro-environment where decomposition occurs (conditioning). In C_3 grasses, foliar fungal endophytes (Clavicipitaceae) can increase plant resistance to drought and/or produce alkaloids that reduce herbivory --- effects that may also influence host litter composition and subsequent litter decomposition. We studied the effect of the endophyte *Epichloë* sp. on litter decomposition in the Great Lakes dunes (USA) using a reciprocal design altering endophyte presence/absence in both *Ammophila breviligulata* (American beachgrass) substrate (litter bags) and its conditioning of the decomposition microenvironment. Symbiont treatments were crossed with rain-out shelters that altered growing season precipitation. The first year of decomposition, senesced leaf substrate from *A. breviligulata* with *Epichloë* decomposed 21% *faster* than endophyte-free substrate. By the third year, conditioning by live symbiotic plants *reduced* cumulative decomposition by 33% compared to plots planted

with endophyte-free plants. Of the traits we examined – litter quantity, C:N ratio, mineral composition, fungal colonization, and carbon chemistry – increased litter quantity via greater tiller production was the primary trait shift associated with endophyte symbiosis. *Epichloë* in *A. breviligulata* litter also altered litter nitrogen decomposition dynamics, as evidenced by lower nitrogen and protein content in decomposed tissue from plants that hosted the endophyte. Differences in initial litter quality and subsequent colonization by saprotrophic fungi were ruled out as key drivers. Altered precipitation had negligible effects on decomposing processes in the dunes. Grass-*Epichloë* symbiosis altered nutrient cycling through increasing the rate of litter decomposition when present in the litter and through reducing litter decomposition by conditioning the decomposition microenvironment. *Epichloë* are widespread symbionts of grasses. Thus, their effects on decomposition could be an important, but often overlooked, driver of nutrient cycling in grass-dominated ecosystems.

INTRODUCTION

Interactions between above- and below-ground communities can be major drivers of the rate of decomposition in terrestrial ecosystems (Wardle et al. 2004, Kardol and Wardle 2010). However, most focus on above-ground interactors has been on herbivores (Wardle et al. 2012), with much less attention to the potential roles of above-ground microbes that live in plants. Separately, both above- and below-ground microbes can influence decomposition (Osono 2006, Purahong and Hyde 2011, Omacini et al. 2012, Nuccio et al. 2013, Yuan and Chen 2014). For example, fungal and bacterial endophytes of leaves can affect decomposition by altering the quantity and composition of host litter (Raghavendra and Newcombe 2013, Rogers et al. 2012, Saikkonen et al. 2015). A

predictive framework for understanding the influence of aboveground microbes on litter decomposition requires studies that test for such above–below-ground interactive effects over a broad range of species and ecosystems.

One group of aboveground microbial symbionts, *Epichloë* spp. (Clavicipitaceae, Ascomycota) (Schardl 2010) have been shown to have strong effects on decomposition of host litter, but only has been tested in two non-native host-endophyte systems (Omacini et al. 2012). These fungal endophytes are obligate symbionts of grasses that cannot survive in senesced plant tissue. *Epichloë* can provide a range of benefits to plants, including herbivore deterrence (Crawford et al. 2010), drought tolerance (Oberhofer et al. 2014), and resistance to pathogens (Wäli et al. 2006), in exchange for carbon and shelter within host tissue (Thrower and Lewis 1973, Clay 1990). These benefits, particularly, the production of fungal alkaloids and increases in plant biomass, have potential for cascading effects on decomposition processes. Three prior studies have examined how epichloid endophytes affect decomposition. In all cases, endophyte presence slowed host litter decomposition (reviewed by Omacini et al. (2012)). However, the host plants and endophytes in these studies: *Lolium arundinaceum–Epichloë coenophiala* (Lemon et al. 2005, Siegrist et al 2010) and *Lolium multiflorum– Epichloë occultans* (Omancini et al. 2004) were non-native to the study ecosytem and occurred in rich agricultural/grassland soils. Thus, prior work may not be broadly representative of *Epichloë* effects in diverse ecosystems, and importantly the effects of native *Epichloë* symbiosis on decomposition remains unknown.

Further work in a greater diversity of systems is also needed in order to uncover the mechanisms through which above-ground endophytes affect decomposition (Fig. 1.1).

Within the two systems tested to date, the authors hypothesized that fungal-produced alkaloids, which can persist in the litter, reduced the rate of host litter decomposition by suppressing microbial activity (Omacini et al. 2004, Lemons et al. 2005, Casas et al. 2011). However, Siegrist et al. (2010) showed that fungal alkaloids were mostly lost from host litter 60 d after leaf senescence, suggesting limited long-term effects of this mechanism. On the other hand, alkaloids could shift the initial saprotrophic community, which may affect long-term decomposition rates (Kivlin and Treseder 2014). Alternative mechanisms have also been proposed. For example, *Epichloë* increase host biomass (Omacini et al. 2006), potentially altering litter production and the microenvironment for decomposition (Omacini et al. 2004). *Epichloë* can increase root exudate production (Omacini et al. 2012), which could also shift the composition of rhizosphere microbial assemblages (Jenkins et al. 2006) (Fig. 1.1). For example, in *L. arundinaceum* pastures, soil microbial biomass and respiration were 14% lower in fields with *Epichloë* symbiosis than in endophyte-free fields (Franzluebbers et al. 1999). *Epichloë* can also inhibit AMF root colonization on host roots (Chu-Chou et al. 1992) and on the roots of competing plant species (Antunes et al. 2008).

Additionally, climate may shift the effects of plant symbionts on decomposition (Cheng et al. 2012). This shift in the effects of plant symbionts represents a context dependency in the mutualism which may require altering the abiotic environment to fully understand the effects of the symbiont on the quality and quantity of host litter. However, to our knowledge, no studies have yet examined whether *Epichloë* may alter decomposition under altered climates. Recent research has suggested that altered precipitation may play a larger role than increased temperatures in driving the long term

rates of litter decomposition (Suseela et al. 2013). Experiments in temperate grasslands that directly manipulated precipitation showed that drought and reduced total rainfall slowed decomposition (Suseela et al. 2013, Walter et al. 2013), possibly due to reduced microbial activity. Importantly, microbial symbionts can alter host responses to altered environmental conditions (Worchel et al. 2012, Kivlin et al. 2013), which may subsequently affect decomposition rates.

The Great Lakes dune ecosystem may be particularly responsive to climate shifts (Pendleton et al. 2005, Pendleton et al. 2010). Under the highest CO_2 emission scenarios, general circulation models project that this region will experience a 5 °C increase in mean annual temperature by 2070-2099 (Hayhoe et al. 2010). Furthermore, downscaled predictions from the IPCC Fourth Assessment (IPCC 2007) vary from +19% to -31% change in growing season precipitation (Rudgers et al. 2015) while IPCC Fifth Assesement ensemble model predicts a 10-25% increase in annual preciptiation for the region (IPCC 2014).

We examined *Epichloë* effects on decomposition in the Great Lakes dunes of the USA. *Ammophila breviligulata* is the main dune builder in both the Great Lakes and Atlantic coastal dune ecosystems (Cowles 1899, Lichter 1998a). It hosts an undescribed species of *Epichloë* (Emery et al. 2010). The most commonly used nursery stocks for dune restoration material have 100% endophyte prevalence, whereas the prevalence of *Epichloë* in Great Lakes *A. breviligulata* populations is more variable [~22% of Great Lakes populations were symbiotic (Emery et al. 2010, Emery and Rudgers 2014)]. To assess the interactive effects of above-ground plant symbionts and climate on decomposition in a native ecosystem, we manipulated *Epichloë* sp. in *Ammophila*

breviligulata under alternative precipitation regimes. Specifically, we asked 1) Does *Epichloë* affect decomposition directly by altering litter composition (substrate) or indirectly by altering the microenvironment for decomposition (conditioning)? 2) Does the precipitation regime directly affect decomposition or modify how the endophyte affects decomposition? 3) Do the traits that underlie changes in decomposition include shifts in litter substrate quantity, substrate quality, or colonization by saprotrophic fungi?

METHODS

STUDY SITE

The experiment was located in Leelanau State Park, Leelanau Co., Michigan, USA (45.183°, -85.576°) within a large blowout on the leading edge of a second foredune ~200 m from the Lake Michigan shoreline. In the Great Lakes dunes, the accumulation of an organic layer, soil carbon, and soil nitrogen is slow and only stabilizes ~450 years after succession begins (Lichter 1998b). Thus, small changes in short term litter input and decomposition can have strong effects on long term nutrient accumulation.

EXPERIMENTAL DESIGN

In May 2010, we established a 2 × 3 factorial experiment to alter the presence or absence of *Epichloë* sp. symbiosis in *A. breviligulata* populations in the context of a climate manipulation (reduced, ambient, or augmented precipitation). Replication consisted of 15 plots (2 m × 2 m) per treatment (90 plots total), each with 25 transplanted *A. breviligulata* individuals, and each randomly assigned to a treatment combination (Emery et al. 2015).

Precipitation manipulation: We constructed modified Sala rain-out shelters to manipulate growing season precipitation (Yahdjian and Sala 2002). Clear, plastic gutters removed ~30% of ambient precipitation from the reduced precipitation plots. We added the

collected rain to the augmented water plots to increase the precipitation by ~30%. Both augmented and ambient precipitation plots had mock shelters with gutters oriented upside-down to control for effects on light levels without altering ambient precipitation. A detailed description of the experimental design was presented by Emery et al. (2015). Though *A. breviligulata* is a rhizomatous grass, lateral transfer of water between plots through rhizomes is likely not a problem because each plot is surrounded by 1 m of bare sand and no tillers are growing between plots. Additionally, our precipitation manipulation led to an average of 9% higher moisture in augmented plots compared to reduced plots at both 20 and 40 cm depth [Supplementary material Appendix 1.1 Table A1.1 and Fig. A1.1 (Rudgers et al. 2015)].

Conditioning treatment: To manipulate endophyte presence in *A. breviligulata*, we used endophyte-free seeds collected at a nearby site in Sleeping Bear Dunes National Lakeshore (44.858°, -86.063°) during fall 2006. We used a sterile needle to insert hyphae from *Epichloë* sp. isolates cultured from *A. breviligulata* into the meristem of each seedling (E+ treatment) and sham-inoculated other seedlings (E- treatment) (Leuchtmann and Clay 1988). This inoculation method had an 8% success rate (Emery et al. 2015; Rudgers et al. 2015) similar to *Epichloë* inoculation of other grass species (Chung et al. 1997). Only successfully inoculated genotypes were used for our E+ treatment. Plant responses from the field experiment for 2010–2013 were presented by Emery et al. (2015) and Rudgers et al. (2015). Here, we counted the number of *A. breviligulata* tillers per plot on 27 May 2015 to estimate above-ground plant biomass using allometric equations.

Substrate decomposition bags: Senesced leaves (hereafter, substrate) were collected during April and May 2011 from random individuals of 32 E- genotypes and 21 E+ genotypes of greenhouse-grown *A. breviligulata* planted into a 50:50 mix of sterile sand and Metro Mix 220 (Rice University greenhouse, Houston, TX average daily temp 24 °C, no supplemental lighting). The same stock plant genotypes used to establish the field experiment were included in the litter bags. Material was air dried at 25 °C and thoroughly mixed within each endophyte status. A total of 540 (270 E- and 270 E+) litter bags (10×10 cm) were constructed from fiberglass window screening (3 mm mesh). Each bag contained 4 g of plant material. On 26 May 2011, six randomly chosen litter bags (three E- and three E+) were buried near the center of each 2 × 2 m plot. E+ and E- bags were alternated spatially, placed ~10 cm apart in a circle, and buried ~15 cm deep. We have found that sand accumulation greater than 18 cm can occur over the winter and spring months (*unpubl. data)*, so a litter burial depth of 15 cm likely happens regularly in the dune ecosystem. Each year, for three years, two bags (one E+ and one E-) were collected from each plot at the end of the growing season (September), allowing assessments of decomposition over ~3 mo, ~15 mo, and ~27 mo of continuous field burial (Table 1.1).

Data collection: Bags from 2011 and 2012 were air dried at 25 °C and weighed to determine mass loss. The bags collected in 2013 were stored at 4 °C for ~1 mo, during which time bags were weighed wet and subsampled for microscopy, ^{13}C nuclear magnetic resonance (NMR) spectroscopy, mineral content, and elemental composition (Table 1.1). The remaining material was air dried to calculate moisture content and mass loss. Due to the limited amount of available substrate and the lack of a strong effect of the

precipitation treatment, microscopic and chemical analyses were conducted on subsets of treatment combinations as described in Table 1.1.

Traits: Aboveground biomass and standing dead litter: Standing dead litter in combination with live biomass links the results from the litter bag experiment to the amount of above-ground decomposition occurring naturally in plots. Biomass of standing dead litter reflects the amount of litter produced by plants minus the loss of litter to decomposition, burial by sand, and mechanical removal by wind. To estimate the effects of *Epichloë* and altered precipitation on birth and death of tillers and litter quantity, we measured the amount of live *A. breviligulata* biomass, tiller turnover rate, and standing dead litter biomass in each plot (Table 1.1). Near the northeastern corner of each plot we established a permanent 0.25 × 0.25 m subplot. In 2013, we measured tiller turnover (tiller birth and death rates) in the plots by tagging all tillers the subplot in each plot in May, and then censusing all new and dead tillers in each subsequent month until the end of the season in September. Daily birth (and death) rates of tillers were calculated as the number of new (or dead) tillers at a census divided by tiller number at the previous census and the number of days since the last census. Rates were averaged across all months to give daily birth and death rates for the entire season. In the beginning of June 2014, we removed all of the standing dead litter from each subplot. On 28 June 2015, we surveyed each subplot by counting live tillers and collecting all of the standing dead litter. This litter thus represented ~1 y of accumulation. Litter was oven dried at 80 °C for 72 h, then weighed.

We counted the number of live tillers at the whole plot scale on 27 May 2015 to estimate the effects of *Epichloë* on biomass production. At our site, tiller number is

related to live biomass by the allometric equation: y=1.3519 * Tiller number, r^2 =0.92, p <0.00001 (Bell-Dereske and Rudgers *unpubl. data*).

Traits: Substrate carbon and nitrogen content: To assess endophyte effects (substrate and conditioning) on litter chemistry, samples from litter bags were oven dried at 60 °C for 72 h then ground with a Ball Mill MM301 (Retsch, Haan, Germany). Ground samples were dried at 60 °C for 72 h then sent to Oklahoma State University Soil, Water, and Forage Laboratory (Stillwater, OK) for percentage carbon (C) and nitrogen (N) analysis on a LECO Tru-Spec C:N analyzer (Leco Corp., St. Joseph, MI, USA).

Traits: NMR spectroscopy and litter mineral composition: To examine effects of *Epichloë* on litter substrate carbon chemistry, we subjected both fresh litter [greenhouse-grown litter harvested 24 January 2014 from 15 genotypes of E+ and 15 genotypes of E- (five genotypes per sample)] and decomposed litter (Table 1.1) to ^{13}C-NMR analysis. To assess the average effect of the endophyte's presence in substrate across our other treatments, we combined subsamples of litter among the conditioning endophyte treatment and plot location for a total of three composite samples of each endophyte substrate treatment. Litter was air dried and ground with liquid nitrogen using a mortar and pestle.

Solid state ^{13}C cross polarization magic angle spinning (CP MAS) NMR spectra were collected on each sample using a Bruker Avance 200 MHz solid-state NMR spectrometer (Bruker Corp., MA). The spectrometer was equipped with 4 mm magic angle spinning (MAS) probe and operated at rotor spinning frequency of 7 kHz. Cross polarization (CP) spectra were acquired by applying a 90 degree ^{1}H pulse, a 1.0 ms ^{13}C contact pulse, composite pulse proton decoupling, and a 5 s recycle delay. The ^{13}C NMR

spectra were divided into chemical shift regions corresponding to different functionalities: alkyl C (0-45 ppm), N-alkyl/methoxyl (45-60 ppm), O-alkyl (60-95 ppm), di-O-alkyl (95-110 ppm), aromatic C (110-145 ppm), phenolic (145-165 ppm), and amide/carboxyl (165-215 ppm). The relative allocation of signal was assigned to the seven individual spectral regions. We used a molecular mixing model (MMM) (Baldock et al. 2004) to determine the major biochemical components (e.g., carbohydrate, protein, lignin, and lipid) in each sample. The C:N ratio and signal distribution across the seven predefined ^{13}C NMR spectral regions were input into the MMM to model the concentration of each biochemical component. Spinning sideband (SSB) was integrated and corrected during MMM calculation. The MMM additionally provides information on the degree of decomposition of the sample as reported in the alkyl/O-alkyl peak ratios (Baldock et al. 1997). The same fresh and decomposed litter samples described above were analyzed for litter mineral composition (Ca, P, Na, Mg, K, S, Mn, Cu, Fe, and Zn) using wet digestion and inductively coupled plasma spectroscopy (Oklahoma State University Soil, Water, and Forage Laboratory, Stillwater, OK).

Response: Colonization by saprotrophic fungi: To determine how substrate and conditioning treatments affected the colonization of litter by non-*Epichloë* fungi, we determined the percentage of litter area colonized by fungi using acid fuchsin stain following methods in Brundrett et al. (1996). We used the gridline intersect method to count the presence of distinct fungal structures and measure hyphal length within 30 fields of view at 200X magnification (Brundrett et al. 1996); each field of view was 1 mm^2 of litter containing 100 ocular grid squares.

Statistical Methods: We used a negative exponential model to calculate the litter loss rate: $\ln(x_t/x_0) = - kt$ where x_0 is the initial mass of the litter, x_t is litter mass at year t, and k is the decay constant per year (Olson 1963). Values that showed small increases in mass were treated as a loss of 0g (9 / 539 observations). Values with mass loss >100% were treated as a loss of 4 g (6 / 539 observations). Results did not significantly change when the actual values were analyzed.

We analyzed (k) and C N litter composition using a general linear mixed effects model containing conditioning endophyte (endophyte presence in the plot), precipitation, and substrate endophyte (endophyte presence in litter) treatments, including all interactions as well as plot as a random, nested factor. As spatial blocking factors, we included categorical variables of plot spatial position indicating column (north – south gradient) and row (east –west gradient) in the analyses of (k). To meet assumptions of Gaussian distributions of errors and homogeneity of variances, we log-transformed Year 2 data (2012) and used cube root for Year 3 (2013). Years were analyzed separately due to differences in litter mass loss calculation for Year 3 litter (detailed above) increasing the variance of the measurement compared to litter from Year 1 and 2. Tiller birth and death rates, standing dead litter (g/tiller), plot level standing dead biomass (y=Dead litter per tiller x Tiller number), and plot level live biomass were analyzed with general linear models with endophyte treatment, precipitation treatment, spatial position of plots, and all interactions as described above. NMR spectra and mineral composition were statistically analyzed using Welch's two sample t-test in R version 3.0.2 (R Core Team 2014). We analyzed the percentage of litter surface area colonized by fungi with a general linear mixed model including the fixed effects of conditioning endophyte, substrate endophyte,

and their interaction, with plot as a random factor. All a general linear mixed effects model and linear models were analyzed using PROC GLMMIX, SAS v. 9.3 (SAS Institute, Cary, NC).

RESULTS

1) Does Epichloë *affect decomposition directly by altering litter composition (substrate) or indirectly by altering the microenvironment for decomposition (conditioning)?*

Endophyte substrate

The effects of *Epichloë* presence on the rate of litter decomposition varied temporally during the experiment. During the first growing season (May – Sept 2011), substrate produced by E+ plants decomposed 21% faster than E- substrate (Fig. 1.2a). Percentage mass loss was 20% ± 0.8% SE for E+ substrate and 17% ± 0.8% SE for E- substrate. However, the influence of the endophyte substrate treatment disappeared after the first growing season and had no effect in the second or third year of decomposition (Fig. 1.2a, Supplementary material Appendix 1.1 Table A1.2).

Endophyte conditioning

In contrast, by the third year of ongoing decomposition, litter had decomposed 33% more slowly in E+ *A. breviligulata* conditioned plots compared to endophyte-free (E-) conditioning plots (Fig. 1.2b). Cumulative litter mass loss over three years was 43% ± 3.2% SE for litter in E+ conditioned plots and 51% ± 3.2% SE for litter in E- conditioned plots. This effect was not observed during the first two years (Fig. 1.2b), and

there was no significant interaction between the endophyte conditioning and endophyte substrate treatments (Supplementary material Appendix 1.1 Table A1.2).

2) Does the precipitation regime directly affect decomposition or modify how the endophyte affects decomposition?

For both the litter bags and standing dead litter, precipitation treatments had no significant influence on decomposition. In addition, precipitation did not interact with endophyte substrate or endophyte conditioning treatments to affect either decomposition rates or the amount of standing litter (Supplementary material Appendix 1.1 Table A1.2 – A1.3).

3) Do the traits that underlie changes in decomposition include shifts in litter substrate quantity, substrate quality, or colonization by saprotrophic fungi?

Epichloë *effects on litter quantity*

In a natural field setting, the endophyte could affect the quantity of substrate as well substrate quality and the micro-environmental conditions during decomposition. Endophyte presence in live plants increased the above-ground biomass of *A. breviligulata* by 31% (Fig. 1.3a). Yet, there was no net change in standing dead litter *per* plot (Fig. 1.3a) because *Epichloë* presence in live plants reduced the amount standing dead litter *per tiller* by 26% compared to endophyte-free plots (Fig. 1.3b). Furthermore, when we estimated the amount of above-ground litter lost during decomposition as [estimated live biomass - estimated standing dead litter] (Fig. 1.3 a), we found that E+ plots lost, on average, 48.4 g (~20% of live litter biomass) of litter while E- plots showed an insignificant increase in litter of 2.2 g (~1% of live litter biomass). Thus, the presence of *Epichloë* increased the loss of litter by 21% compared to E- plots, exactly the same effect

size we reported for *Epichloë* presence in substrate during the first season of decomposition in our litter bags (Fig. 1.2a). Though, the presence of *Epichloë* increased cumulative sand accumulation in plots during 2010–2013 (Emery et al. 2015), sand accumulation during the period of standing dead tiller accumulation (2014-2015) was generally low (average: 2.08 ± 0.23 SE cm) and did not strongly correlate with the mass of standing dead litter per tiller (r=0.17 p>0.1). Additionally, endophyte presence increased the rate of tiller senescence 84% and had no effect on tiller birth rate (Supplementary material Appendix 1.1 Fig. A1.2, Table A1.3).

Epichloë *effects on substrate litter quality*

The presence of *Epichloë* affected the downstream composition of litter substrate over the course of decomposition. *Epichloë* did not affect the structural carbon (NMR, Fig. 1.4 a, Supplementary material Appendix 1.1 Fig. A1.3), nitrogen composition (Fig 1.5a), litter mineral composition (all elements and minerals: Supplementary material Appendix 1.1 Table A1.4), or carbon:nitrogen (C:N) ratio (Fig. 1.5c) of freshly senesced litter. However, after three years of decomposition, E+ substrate had 28% lower amide content than E- substrate (Fig. 1.4b) and 22% lower N content (Fig. 1.5b). Despite these shifts in chemistry, there was no significant effect of the substrate *Epichloë* treatment on the percentage of leaf tissue colonized by fungi, over all fungal morphotypes combined (Supplementary material Appendix 1.1 Fig. A2) or for any individual fungal morphotype (all p>0.1, *data not shown*)). It is unclear if these endophyte-mediated shifts in substrate also affect the C:N ratio because our different methods showed divergent results. *Epichloë* in the substrate increased the C:N ratio of bulked samples of decomposed litter (by 39% compared to endophyte-free litter, n = 3, Fig. 1.5d). However, during the same

year of decomposition, the endophyte had no effect on the C:N ratio of samples taken from each plot, where the sample size was larger (5% decrease compare to E-, $n = 15$, Supplementary material Appendix 1.1 Table A1.2).

Epichloë *conditioning of the environment for decomposition*

After three years of decomposition, endophyte conditioning due to the presence of live plants in field plots had no effect on the C:N ratio of decomposed litter (Supplementary material Appendix 1.1 Table A1.2). *Epichloë* conditioning also did not alter the percentage of leaf tissue colonized by fungi for any fungal morphotype (all fungi Supplementary material Appendix 1.1 Fig. A1.2; each morphotype: all $p>0.2$, *data not shown*).

DISCUSSION

Substrate produced by symbiotic plants had faster initial rates of decomposition than substrate from symbiont-free plants. This increase in initial decomposition rate may have reduced the amount of standing dead litter per tiller in plots where the endophyte was present in live plants. However, the endophyte substrate treatment was not important after the first year of decomposition. Instead, the decomposition microenvironment (i.e., endophyte conditioning) became increasingly important, with endophyte presence in live plants in field plots reducing the rate of decomposition of both E+ and E- substrate types by the third year of decomposition. Mechanisms that may underlie these results include shifts in local nutrient availability, microbial activity, and microbial community composition, but are unlikely to be caused by initial differences in litter quality. These temporally dependent shifts in the importance of *Epichloë* in host plant litter

decomposition could have large effects on the nutrient cycling in the nearly sterile dune soil.

Epichloë effects on substrate increased rates of early decomposition

Endophyte symbiosis in *A. breviligulata* substrate sped up initial rates of litter decomposition. In contrast, all three previous studies found slower decomposition when an *Epichloë* species was present in the substrate (Omacini et al. 2012). One hypothesis for these divergent results is that all prior work examined grass-*Epichloë* symbioses that were non-native introductions to the ecosystem studied and where the interactions between *Epichloë* and below-ground microbes maybe novel. A second hypothesis is that prior studies focused on *Epichloë* species that produce high levels of toxic alkaloids, while the *A. breviligulata* endophyte lacks the genes for alkaloid production, with the exception of the pyrrolopyrazine, peramine (J.A. Rudgers, N. Charlton, C. A. Young, *unpubl. data*). Some possible differences among studies can be ruled out: The length of decomposition in our experiment (98–847 d) overlapped the range of the previous experiments [83 d (Omacini et al. 2004), 170 d (Siegrist et al. 2010), and 256 d (Lemons et al. 2005)]. Additionally, the percentage of litter lost in sandy soils of the Great Lakes dunes during the first growing season (mean 19% ± 0.6% SE) was comparable to, but on the low end of, litter loss reported in the previous experiments (~15% to 72% (Omacini et al. 2004, Siegrist et al. 2010)). After the first growing season, however, the endophyte substrate treatment had no effect on the rate of litter decomposition, suggesting these symbiont effects were either ephemeral during early succession or show interannual variability. This initial increased decomposition due the endophyte will likely lead to a

pulse of nutrients that may lead to ephemeral increases in productivity of the plant community and associated microbial community.

Epichloë altered host leaf traits and litter quantity

Epichloë presence increased aboveground biomass production by 31%, which was comparable to *Epichloë*-driven increases previously reported in this system [3-19% increase in tiller production (Emery et al. 2015)]. However, the amount of standing dead litter per live tiller was reduced by 26% when *Epichloë* was present. Although *Epichloë* increased the amount of live biomass, the endophyte-driven reduction in dead litter per tiller produced no difference in the amount of standing dead litter at the plot-scale. It is possible that the rapid decomposition of substrate with *Epichloë* could lead to pulses of nutrients and a positive feedback with the host leading to the increased live biomass of *A. breviligulata* we have seen in this system (Emery et al. 2015). Factors such as sand accumulation, tiller turnover, and wind can be ruled out as drivers of the reduction in standing dead litter, as these factors were either minor (e.g., sand accumulation and wind; Emery et al. 2015) or positively related to litter biomass (e.g., tiller turnover). Ruling out sand burial, tiller turnover, and wind removal suggests a direct link between our litter bag experiment and the above-ground decomposition processes: higher initial rates of decomposition of *Epichloë* substrate may cause faster initial leaf litter loss from tillers when *Epichloë* is present in live plants.

The lack of initial differences in substrate chemistry due to *Epichloë* presence suggests that the endophyte's influence on the initial rate of decomposition is not driven by aspects of substrate quality measured in our study. Previous research on *Lolium perenne* and *L. arundinaceum* has found that the presence of *Epichloë* alters the

metabolic composition of host plant tissue though many of these effects were dependent on soil nitrogen and CO_2 concentration (Brosi et al. 2011; Hunt et al. 2005; Newman et al. 2003; Rasmussen et al. 2008). *Epichloë* presence reduced the carbon:nitrogen (C:N) ratio of *L. arundinaceum* tissue though this effect was lost during decomposition (Siegrist et al. 2010). It is possible that *Epichloë* alters the surface physical structure or internal architecture of litter in ways that increased access for saprotrophs (particularly microarthropods and bacteria, since fungal colonization was not affected). Dupont et al. (2015) found that *Epichloë* presence increased expression of genes regulating host cell walls and reduced the thickness of the cell walls. Alternatively, the legacy effects of the endophyte could alter utilization/conversion of nutrients from inorganic to organic forms by the soil microbial community (Cornwell et al. 2008, García-Palacios et al. 2016). Other aspects of litter chemistry, such as levels of the endophyte-produced, insect-deterrent alkaloid, peramine (Panaccione et al. 2014), which were not measured here, may also contribute to the effect of the endophyte on substrate decomposition rate.

After three years of decomposition, substrate produced by plants with the endophyte was depleted in protein and nitrogen compared to substrate made by endophyte-free plants. Since we cannot decouple the contributions of plant-derived versus microbe-derived protein, it is difficult to identify specific mechanisms through which the endophyte may be altering nitrogen dynamics in this system. In general, however, higher lignin content slows rates of decomposition (Cornwell et al. 2008). Because we found no effects of *Epichloë* presence on lignin content in fresh litter, effects of *Epichloë* on decomposition rates are not likely occurring through shifts in initial structural carbons, but instead accrue through other (as yet unmeasured) traits, such as

leaf toughness and surface structure (García-Palacios et al. 2016), that influence how nutrients are utilized by saprotrophs.

Soil conditioning by *Epichloë* reduced rates of later decomposition

Our results show temporal shifts in the importance of endophyte conditioning on the decomposition microenvironment that may be missed by short-term studies. Notably, plots conditioned by *A. breviligulata-Epichloë* symbiosis significantly reduced the rate of decomposition of both substrate types by the third year of decomposition, with no detectable effects in earlier years. To date, our study is the longest decomposition experiment examining the effects of *Epichloë* symbiosis (847 d; the next longest was 256 d (Lemons et al. 2005)). Previous studies have found ephemeral effects of *Epichloë* on short time scales. For example, Siegrist et al. (2010) showed that endophyte conditioning of plots reduced the decomposition of litter only during the earliest stage of decomposition (21 d). In contrast with this result and ours, Lemons et al. (2005) reported that *Epichloë* conditioning *increased* the decomposition rate compared to endophyte-free plots, but this effect only occurred when a larger mesh (169 mm^2 pore size) was used for litter bag construction, suggesting that effects were driven by invertebrates. Lemons et al. (2005) reported no significant effect of plot endophyte status when a smaller mesh size (0.1 mm^2 pore size, more similar to our mesh) was used. Slower decomposition rates when endophyte-symbiotic plants dominate conditioning suggest that endophyte-mediated alterations in the soil biotic or abiotic microenvironment (e.g., root exudation, carbon priming, nutrient competition) influence later stages of decomposition or ecological succession. Effects of *Epichloë* on the soil micro-environment may become stronger with the length of soil conditioning; however, our experiment fell within the

time range of soil conditioning in prior experiments: ~40 mo compared to ~10 mo (Omacini et al. 2004) to ~75 mo (Siegrist et al. 2010). This reduced decomposition over time could lead to increased carbon sequestration similar to what has been recorded in the *Lolium arundinaceum* dominate systems (Franzluebbers et al. 1999).

Mechanisms of *Epichloë* conditioning on the decomposition microenvironment

The mechanism underlying *Epichloë*-altered decomposition that has received the greatest attention has been endophyte-produced alkaloids. Previous screening of four alkaloid gene clusters showed that the *Epichloë* in our *A. breviligulata* has only the genes to produce the pyrrolopyrazine, peramine (J.A. Rudgers, N. Charlton, and C.A. Young, *unpubl, data*), an anti-insect defensive chemical (reviewed by Schardl 1996). Peramine is unique among the *Epichloë* alkaloids in being found throughout host plant tissues (Koulman et al. 2007, Panaccione et al. 2014). Thus, it could affect rhizosphere soils through root exudates or root decomposition. To our knowledge, no previous studies have examined the direct effects of peramine on decomposition or on saprotrophs. Additionally, few studies have examined direct effects of peramine on herbivores; however, it does effectively deter the Argentine stem weevil, a common grass pest (Gerard 2000). We did not measure alkaloid levels in the litter or roots in this experiment, but *Epichloë* has been shown to alter the root exudate profile in *L. arundinaceum*, which also produces peramine (Guo et al. 2015). If endophyte-produced peramine was a major mechanism underlying reduced rates of decomposition in plots with the endophyte, we would expect to see inhibition of saprotrophs. Although we found no effect of the endophyte on the percentage of litter colonized by fungi, endophyte conditioning altered

the soil microbial community [i.e. arbuscular mycorrhizal fungi and bacteria (Bell-Dereske et al. *unpubl. data*)].

Altered precipitation regime had no direct or indirect effect on the rate of decomposition

Rates of litter decomposition can be accelerated by pulses of precipitation (e.g., Chang et al. 2007), but this effect may be most important in ecosystems with high annual precipitation (Manzoni et al. 2010). On the other hand, decomposition rates are typically reduced by drought events (Walter et al. 2013). Surprisingly, we found no effects of either increased or decreased precipitation on rates of litter decomposition in Great Lakes dunes. Given soil moisture levels that are typical for dunes (Baldwin and Maun 1983), our precipitation treatments likely did not reduce the precipitation to drought levels, although they did alter soil moisture in our plots (Emery et al. 2015). Additionally, because dune soils are composed of medium fine sand with no organic layer, water percolates through the soil rapidly (Lichter 1998b). Overall, our data suggest the decomposition processes in Great Lakes dunes are resistant to precipitation variation that falls within 30% of ambient levels, and that symbiotic endophytes trump the effects of precipitation on decomposition rate. Therefore, climate change driven changes in the average precipitation during the growing season may not have strong effects on the nutrient cycling through decomposition, but other aspects of climate change, such as increased temperature (Creamer et al. 2015) and the interactive effect of temperature and precipitation (Suseela et al. 2013), may lead to altered nutrient cycling processes.

CONCLUSION

This study is the first to report a temporal shift in the relative importance of the pathways through which above-ground fungal symbionts can alter litter decomposition. Initial decomposition of *A. breviligulata* litter was faster if this substrate came from plants hosting the endophyte. This early increase in decomposition rate did not decrease the amount of standing dead biomass per m^2 because the endophyte also increased aboveground plant biomass. Later in the decomposition process, endophyte symbiosis in living host plants slowed the rate of decomposition, perhaps by altering the soil microenvironment. Since previous research has shown that the endophyte is found ~22% of *A. breviligulata* populations and its occurrence is spatially heterogeneous (Emery et al. 2010), both the effects of endophyte in the substrate and conditioning of the soil will likely lead to endophyte-driven spatial heterogeneity in nutrient cycling within the dunes. This spatial heterogeneity may have important implications in successional processes as soil nutrients play an important role in plant succession (Lichter 1998a). Given the widespread occurrence of *Epichloë* within grasses and more generally, of fungal symbionts in plants, temporal shifts in their influence on decomposition processes may have strong effects on nutrient cycling and carbon sequestration during succession in many ecosystems.

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Conflict of Interest

L.B.D, X.G., C.A.M., R.L.S., S.M.E., and J.A.R have no conflicts of interest to declare.

Data Accessibility

Data will be archived in Dryad (http://datadryad.org/) and will be made available to the public upon acceptance of manuscript.

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Tables

Table 1.1. Response variables measured. Material is the substrate analyzed for each response: Fresh litter was collected from greenhouse grown stock plants, Year 1 litter was collected on 1 September 2011 (98 d of decomposition), Year 2 was collected on 22 September 2012 (485 d), and Year 3 was collected on 19 September 2013 (847 d).

Material	Response	Treatments Examined	*n*	Method
Year 1-3 litter	Litter decomposition	Substrate*Conditioning*Precipitation*Year	15	Litter loss rate (Olson 1963)
Standing dead	Litter mass	Conditioning*Precipitation	15	Mass balance
Tillers	Aboveground biomass	Conditioning*Precipitation	15	Field survey
Tillers	Tiller turnover	Conditioning*Precipitation	15	Monthly census of tiller birth and death
Fresh litter	C:N	Substrate	3	Elemental analyzer
Year 3 litter	C:N	Substrate*Conditioning*Precipitation	5	Elemental analyzer
Fresh litter	^{13}C NMR	Substrate	3	Solid state ^{13}C CP MAS NMR
Year 3 litter	^{13}C NMR	Substrate	3	Solid state ^{13}C CP MAS NMR
Fresh litter	Mineral composition	Substrate	3	Inductively coupled plasma spectroscopy
Year 3 litter	Mineral composition	Substrate	3	Inductively coupled plasma spectroscopy
Year 3 litter	Fungal colonization	Substrate*Conditioning	5	Gridline intersect 200X Microscopy

Figures

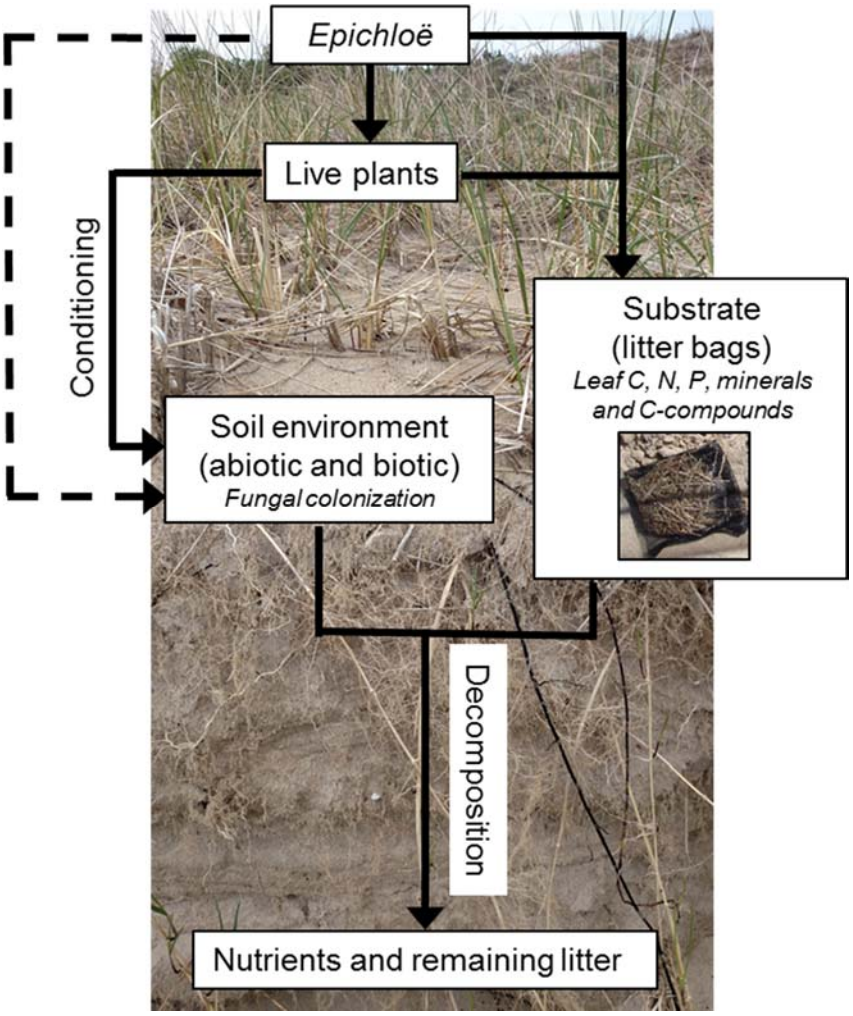

Figure 1.1. Possible pathways for the effects of *Epichloë* on decomposition of host litter. The fungal endophyte may alter decomposition directly (solid lines) through the amount of litter production (substrate quantity) or the composition of the litter (substrate quality). The endophyte may also alter decomposition indirectly (dashed lines) by shifting the microenvironment in which decomposition occurs (conditioning). At the bottom of each box is the list of mechanisms that we examined for each pathway (in italics).

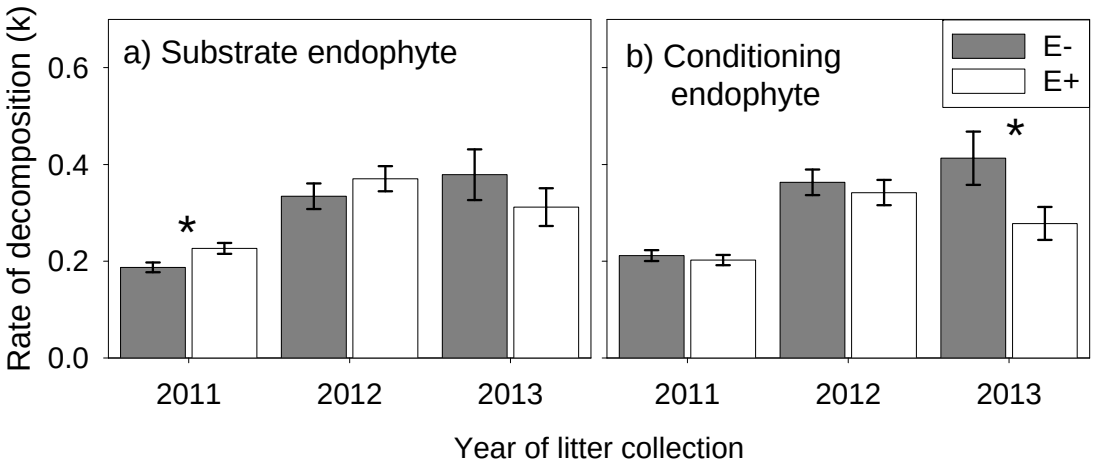

Figure 1.2. Rates of decomposition of *Ammophila breviligulata* substrate from litter bags deployed in May 2011 and collected in September 2011 (n=15), 2012 (n=15), or 2013 (n=14). a) *Epichloë* (white bars (E+)) substrate decomposed faster than endophyte free substrate (filled bars (E-)) only during the first growing season (2011). b) Litter bags (of either endophyte status) placed in plots conditioned by *A. breviligulata* with *Epichloë* (white bars (E+)) decomposed more slowly than litter bags placed in endophyte free plots (filled bars (E-)), after three growing seasons (2013). There was no interaction between substrate endophyte status and conditioning endophyte treatment. Error bars are +/- standard error. "*" significant pairwise difference at $p \leq 0.05$.

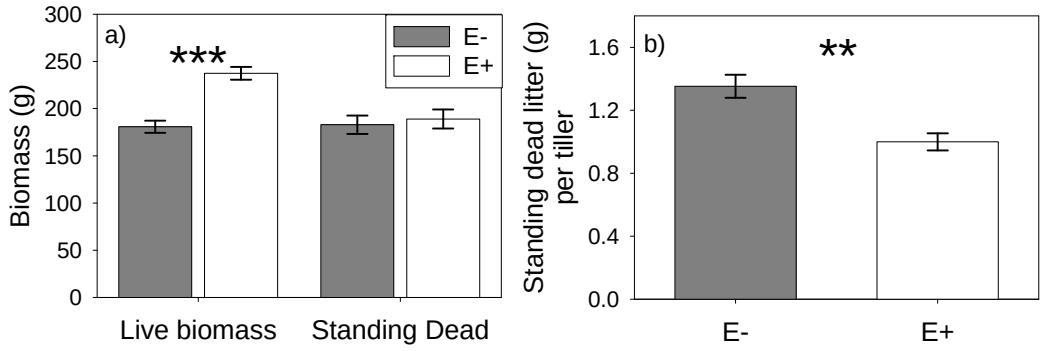

Figure 1.3. *Ammophila breviligulata* live biomass and standing dead litter per plot and per tiller (n=15). a) *Epichloë* (white bars (E+)) increased the live biomass per plot for host plant *A. breviligulata* compared to endophyte free plots (filled bars (E-)); however, this increased biomass did not increase the amount of standing dead litter per plot. This was due b) standing dead litter mass (g) per live tiller of *A. breviligulata* being lower in *Epichloë* plots than in endophyte free plots. Error bars are +/- standard error. "**" significant at $p \leq 0.001$ and "***" $p \leq 0.0001$.

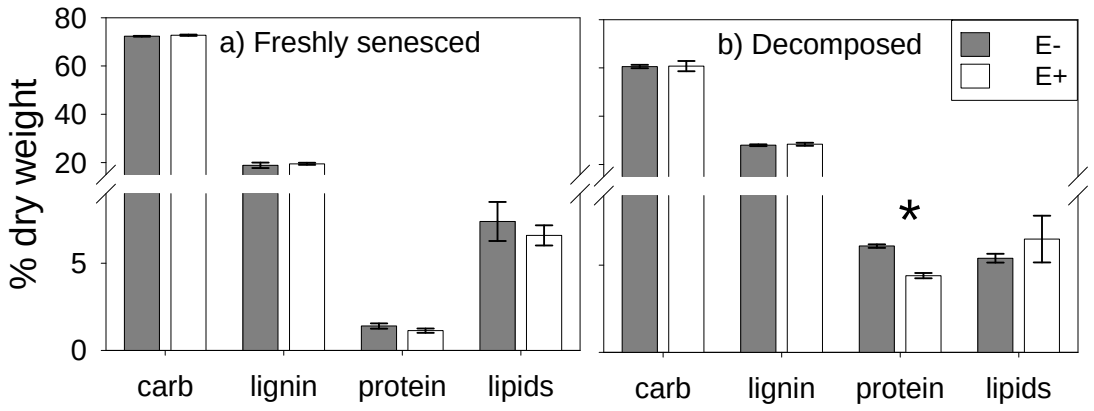

Figure 1.4. Carbon chemistry of *Ammophila breviligulata* litter, produced by plants with (white bars (E+)) or without (filled bars (E-)) *Epichloë* (n=3). The presence of the endophyte did not affected percentage litter composed of carbohydrates (carb), lignin, protein, and lipids modeled from ^{13}C NMR spectroscopy in a) freshly senesced litter. However for b) decomposed litter collected after three years, E+ substrate was depleted in protein compared to E- substrate. Bars are means +/- s.e. "*" significant at $p \leq 0.05$.

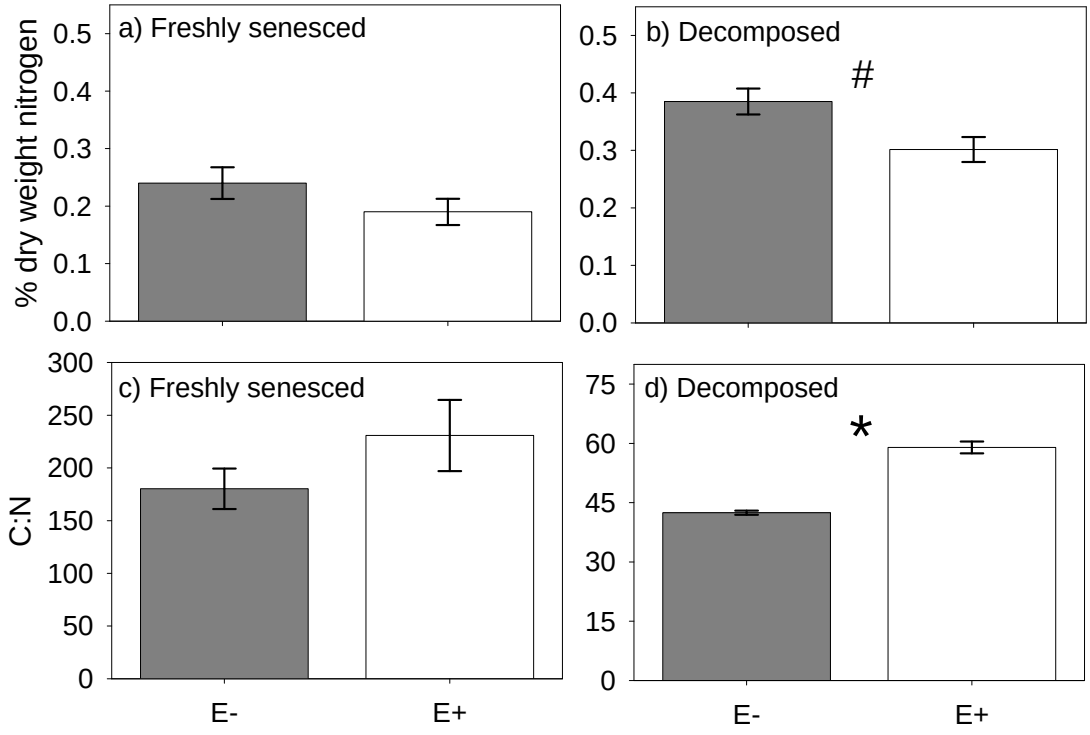

Figure 1.5. Percentage nitrogen and carbon:nitrogen (C:N) ratio of *Ammophila breviligulata* litter (n=3). Both a) fresh and b) decomposed substrate from *A. breviligulata* with the endophyte (white bars) tended to have lower nitrogen content than substrate from plants without the endophyte (filled bars). c) There was no significant difference in C:N ratio between freshly senesced *A. breviligulata* substrate with *Epichloë* (E+) and endophyte free (E-) substrate. d) The carbon:nitrogen ratio was significantly higher in bulked, decomposed substrate from plants with *Epichloë* than without *Epichloë*. "*" signify significant differences between means $p \leq 0.05$. "#" signifies a nearly significant difference between means at $p \leq 0.06$. Bars show means ± s.e.

Supplementary Tables

Bell-Dereske, L., Gao, X., Masiello, C. A., Sinsabaugh, R. L., Emery, S. M., and Rudgers, J. A. *in review*.
Plant-fungal symbiosis affects litter decomposition during primary succession. Oikos

APPENDIX 1.1

Table A1.1. Statistical results from repeated measures mixed models examining the effects of conditioning endophyte and precipitation manipulations on soil volumetric water content (VWC, %) at two soil depths, 20 cm and 40 cm. Significant effects ($p \leq 0.05$) are shown in bold. Table is reproduced with permission from Rudgers et al. (2015).

		VWC 20 cm		VWC 40 cm	
Effect	*df*	X^2	*P*	X^2	*P*
Conditioning endophyte	1,84	2.36	0.1244	0.40	0.5293
Precipitation	2,84	19.39	**<0.0001**	13.04	**0.0015**
Conditioning endo x Precipitation	2,84	3.16	0.2064	2.31	0.3147
Date	16,1291	4642.65	**<0.0001**	4086.98	**<0.0001**
Conditioning endo × Date	16,1291	24.42	0.0807	15.97	0.4549
Precipitation × Date	32,1291	144.04	**<0.0001**	72.36	**<0.0001**
Conditioning endo × Precipitation × Date	32,1291	51.09	**0.0174**	36.82	0.2556

Table A1.2. Statistical results for general linear mixed models examining the effect of *Epichloë* symbiosis in live *Ammophila breviligulata* (Conditioning endophyte: E-/E+), rainfall manipulation (Precipitation: reduced, ambient, or augmented), and *Epichloë* presence in the *A. breviligulata* that produced the litter (Substrate endophyte: E-/E+) on litter loss rates across three litter collection years (analyzed separately) as well as on CN ratios of decomposed litter from Year 3. Spatial coordinates within the dune blowout (column and row) were included as categorical covariates. Significant effects ($p \leq 0.05$) are shown in bold.

		Year 1 Rate of decomposition (*k*)		Year 2 Rate of decomposition (*k*)		Year 3 Rate of decomposition (*k*)			Year 3 Litter C:N ratio		
Effect	*df*	X^2	*P*	X^2	*P*	*df*	X^2	*P*	*df*	X^2	*P*
Conditioning endophyte	1,64	1.13	0.2886	0.25	0.6165	1,64	5.55	**0.0185**	1,42	0.37	0.5449
Precipitation	2,64	1.25	0.5366	2.33	0.3119	2,64	0.92	0.6305	2, 42	1.49	0.4740
Substrate endophyte	1,84	8.39	**0.0038**	3.00	0.0834	1,82	0.01	0.9361	1,6	1.27	0.2604
Row	6,84	9.53	0.1461	3.01	0.8079	6,82	6.92	0.3288			
Column	14,84	15.79	0.3265	17.11	0.2505	14,82	6.21	0.9609			
Cond endo × Subs endophyte	1,84	0.12	0.7303	1.00	0.3162	1,82	0.10	0.7531	1,6	0.64	0.4219
Litter endophyte × Precipitation	2,84	0.81	0.6685	2.12	0.3470	2,82	1.45	0.4851	2,6	2.59	0.2743
Cond endo × Precipitation	2,64	0.19	0.9100	1.26	0.5339	2,64	3.28	0.1936	2,42	2.34	0.3098
Cond endo × Subs endo × Precipitation	2,84	0.97	0.6144	3.31	0.1908	2,82	0.13	0.9393	2,6	1.89	0.3881

Table A1.3. Statistical results for the general linear models examining the effect of *Epichloë* symbiosis in *Ammophila breviligulata* (Conditioning endophyte: E-/E+), and rainfall manipulation (Precipitation: reduced, ambient, or augmented) on *A. breviligulata* live biomass, the amount of standing dead litter per plot (g) extrapolated from standing dead litter (g) per live tiller, the amount of standing dead litter (g) per live tiller, and tiller birth and death rates. Spatial coordinates within the dune blowout (column and row) were included as categorical covariates. Significant effects ($p \leq 0.05$) are shown in bold.

		Live biomass (g) per plot		Standing dead litter (g) per plot		Standing dead litter (g) per tiller		Tiller birth rates		Tiller death rates	
Effect	*df*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Conditioning endophyte	1,64	35.65	**<0.0001**	0.18	0.67	14.48	**0.0003**	0.31	0.58	**7.55**	**0.008**
Precipitation	2,64	1.96	0.145	0.29	0.75	0.18	0.83	1.11	0.33	0.50	0.61
Row	6,64	11.13	**<0.0001**	7.67	**<0.0001**	2.88	**0.015**	**8.59**	**<0.0001**	**2.67**	**0.022**
Column	14,64	3.17	**0.0008**	2.20	0.017	2.23	**0.016**	1.43	0.17	0.95	0.52
Conditioning endophyte × Precipitation	2, 64	2.34	0.10	0.79	0.46	0.46	0.63	2.07	0.13	0.50	0.61

Table A1.4. Statistical results for t-tests examining the effect of *Epichloë* presence in the *Ammophila breviligulata* that produced the litter (Substrate endophyte: E-/E+) on the elemental and mineral composition of the freshly senesced (Fresh Litter) and decomposed litter (Year 3 Litter).

Percentage of dry litter mass (n=3)

Fresh Litter

	E-		E+			
Element	Mean	STE	Mean	STE	*t-statistic*	*P*
P	0.051	0.006	0.037	0.008	-1.41	0.24
Ca	0.716	0.074	0.626	0.065	-0.91	0.41
K	1.054	0.090	1.468	0.312	1.28	0.31
Mg	0.216	0.023	0.245	0.007	1.20	0.34
Na	0.232	0.026	0.199	0.017	-1.07	0.35

Year 3 Litter

	E-		E+			
Element	Mean	STE	Mean	STE	*t-statistic*	*P*
P	0.103	0.007	0.134	0.024	1.25	0.32
Ca	1.688	0.075	1.634	0.062	-0.56	0.61
K	0.063	0.004	0.064	0.008	0.11	0.92
Mg	0.238	0.013	0.249	0.005	0.74	0.52
Na	0.018	0.001	0.018	0.003	-0.17	0.88

Leaf composition PPM (n=3)

Fresh Litter

	E-		E+			
Element	Mean	STE	Mean	STE	*t-statistic*	*P*
Fe	246.631	33.123	313.494	36.662	1.35	0.25
Mn	117.855	43.516	56.455	6.125	-1.40	0.29
Zn	25.931	5.509	21.887	1.965	-0.69	0.55
Cu	9.236	1.997	2.575	1.798	-2.48	0.07
S	0.173	0.006	0.156	0.009	-1.63	0.18

Year 3 Litter

	E-		E+			
Element	Mean	STE	Mean	STE	*t-statistic*	*P*
Fe	3423.39	16.756	3425.43	1063	0.00	1.00
Mn	148.225	13.694	152.394	10.344	0.24	0.82
Zn	49.118	2.826	56.926	3.391	1.77	0.15
Cu	15.233	0.906	15.045	2.710	-0.07	0.95
S	0.103	0.011	0.115	0.015	0.62	0.57

Supplementary Figures

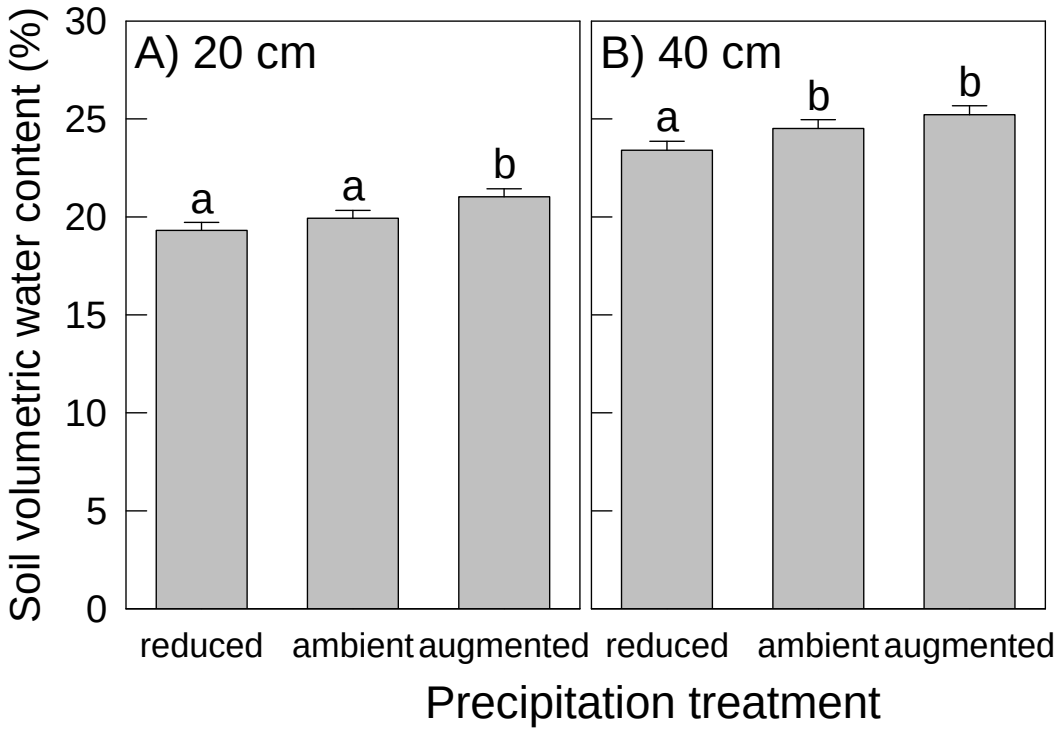

Fig. S1.1. Effects of the rainout shelters on volumetric water content (%) at two soil depths (n=15). **A**) Means ± s.e. VWC at 20 cm depth, **B**) Means ± s.e. VWC at 40 cm depth. Letters indicate differences ($p \leq 0.05$) among precipitation treatments within a soil depth. Figure is reproduced with permission from Rudgers et al. (2015).

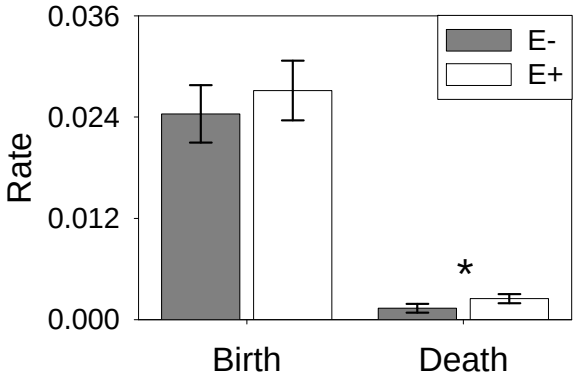

Fig. A1.2. Tiller turnover (daily birth and death rates) of *Ammophila breviligulata* under endophyte conditioning (E+) and without the endophyte (E-) (n=15). *Epichloë* increased the rate of tiller death compared to plots without the endophyte. Bars show means ± s.e. "*" signify significant differences between endophyte treatment means ($p \leq 0.05$).

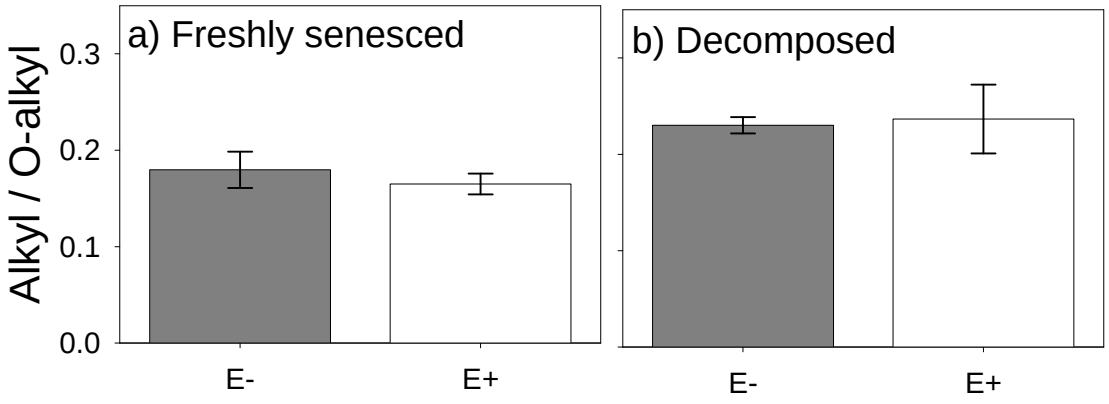

Fig. A1.3. Alkyl/O-alkyl peak ratio of *Ammophila breviligulata* litter from ^{13}C NMR spectroscopy (n=3). There was no significant effect of the presence of the endophyte in *A. breviligulata* substrate (white bars (E+)) on the alkyl/O-alkyl peak ratio compared to endophyte free substrate (filled bars (E-)) in neither a) freshly senesced litter or b) decomposed litter. Bars show means ± s.e.

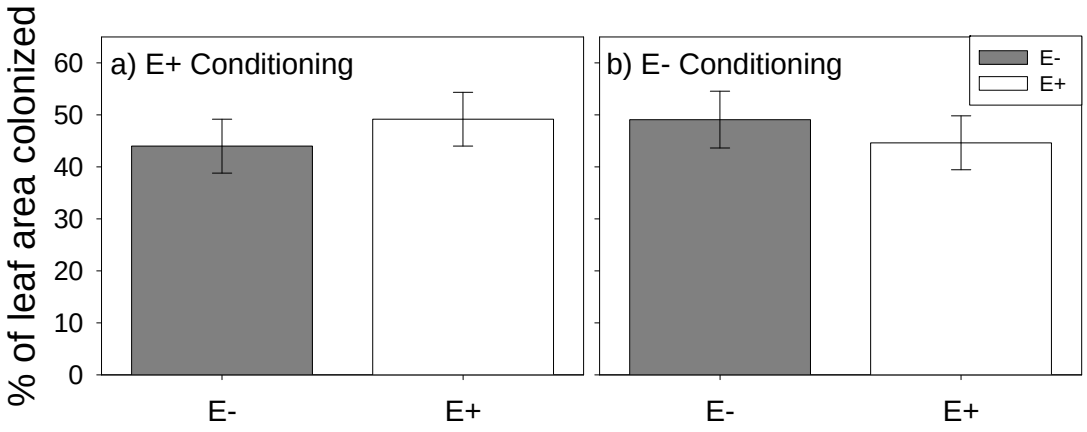

Fig. A1.4. Percentage *Ammophila breviligulata* litter area colonized by fungi (n=5). There was no difference in fungal colonization on substrate with *Epichloë* (white bars) versus endophyte free (filled bars) substrate taken from plots a) conditioned by *A. breviligulata-Epichloë* symbiosis or b) E- conditioning. Bars show means ± s.e.

References

Rudgers, J. A., et al. 2015. Fungal symbiont effects on dune plant diversity depend on precipitation. - Journal of Ecology 103: 219-230.

Chapter 2

Leaf endophyte interacts with precipitation to alter belowground microbial communities in primary successional dunes

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ABSTRACT

Understanding interactions between above- and belowground components of ecosystems is an important next step in community ecology. These interactions may be fundamental to predicting ecological responses to global change because indirect effects occurring through altered species interactions can outweigh or interact with the direct effects of altered environmental drivers. In a multi-year field experiment, we tested the effects of a mutualistic leaf endophyte (*Epichloë* sp.) associated with the dune-builder American beachgrass (*Ammophila breviligulata*) and altered precipitation regime on the belowground microbial community. We monitored belowground shifts in the abundance and composition of arbuscular mycorrhizal (AM) fungi and bacteria in response to the endophyte and level of precipitation (±30%), which affects soil moisture. Under ambient precipitation, presence of the leaf endophyte reduced diversity and abundance of AM fungi in *Ammophila* roots, but effects weakened under altered precipitation. *Epichloë* also

reduced extraradical hyphal length and increased AM fungal glomalin production under augmented precipitation. With *Epichloë* present, root-associated bacterial diversity declined with higher soil moisture, whereas in its absence, bacterial diversity increased with higher soil moisture. Thus, an aboveground fungal mutualist not only altered the abundance and composition of belowground microbial communities but also affected how belowground communities respond to climate.

Key Words: *Epichloë*, bacteria, *Ammophila breviligulata,* arbuscular mycorrhizal fungi, symbiosis, context-dependent

INTRODUCTION

Historically, terrestrial community ecology focused on interactions among organisms that occur aboveground and in plain sight. A critical frontier involves investigations of interactions between above- and belowground components of ecosystems, including microbial assemblages (Van der Putten, 2012, Philippot *et al.*, 2013). Understanding the direction and magnitude of above/belowground interactions may be fundamental to predicting ecological responses to global change because species interactions can create indirect effects that either exacerbate or ameliorate the direct effects of a changing climate, making net outcomes unpredictable (Tylianakis *et al.*, 2008, Kivlin *et al.*, 2013, Singer *et al.*, 2013).

Above/belowground interactions between foliar insects and soil microbes are well known to have strong effects on community and ecosystem processes (e.g., Kostenko *et al.*, 2012, Van der Putten, 2012, A'Bear *et al.*, 2014). However, a potentially important, yet little studied, dimension of integrating above/belowground systems involves interactions between above/belowground *microorganisms*. Belowground microbial

symbionts of plants are recognized to play critical roles in terrestrial ecosystems (Bardgett & van der Putten, 2014) such as decomposition (Rousk & Frey, 2015), soil stability (Fokom *et al.*, 2012), and nitrogen cycling (Phillips *et al.*, 2014). In contrast, with the exception of foliar pathogens, the significance of aboveground microbial communities in plants has received less attention (Bacon *et al.*, 1977, Arnold & Lutzoni, 2007, Omacini *et al.*, 2012). However, during the past 25 years, culturing, microscopic, and sequence analyses of asymptomatic leaves and stems has revealed a ubiquitous and diverse community of bacterial and fungal endophytes that can have diverse ecological effects (Bacon & White, 2000, Andrews & Hirano, 2012).

One group of aboveground plant microorganisms may have particularly strong belowground effects. The epichloid fungi (family Clavicipitaceae, genus *Epichloë*) (Leuchtmann *et al.*, 2014) occur systemically in aboveground plant tissues, often conferring protection against abiotic (drought, heat) or biotic stressors (herbivores, foliar pathogens) (Rodriguez *et al.*, 2009). Endophyte benefits to host plants can be exceptionally large, resulting in several-fold increases in plant survival, biomass, or reproduction (Rudgers *et al.*, 2005, Cheplick & Faeth, 2009). Strong belowground effects of the *Epichloë* have been well-documented in one system thus far. In tall fescue grass (*Schedonorus arundinaceus*), *Epichloë coenophiala* suppressed spore abundances of arbuscular mycorrhizal (AM) fungi in field soil (Chu-Chou *et al.*, 1992) and reduced their root colonization in both host plants (Mack & Rudgers, 2008) and neighboring plant species (Antunes *et al.*, 2008). In tall fescue pastures, endophyte presence also reduced soil microbial biomass and soil respiration (Franzluebbers *et al.*, 1999, Franzluebbers & Stuedemann, 2005, but see, Van Hecke *et al.*, 2005). Mesocosm experiments showed

endophyte-mediated suppression of soil archaea, high G+C gram-positive bacteria, delta-proteobacteria, and Planctomycetes in the tall fescue rhizosphere (Jenkins *et al.*, 2006), as well as reduced microbial utilization of several substrates (Buyer *et al.*, 2011). A recent long-term study, however, reported increased relative abundance of AM fungi and decreased Ascomycota (Rojas *et al.*, 2016), suggesting the possibility that interactions shift during community succession. *Epichloë*-mediated shifts in soil microbial composition may cause higher soil carbon sequestration (Iqbal *et al.*, 2012) and alter nitrogen dynamics in pastures (Franzluebbers & Stuedemann, 2005, Bowatte *et al.*, 2011). Belowground responses to endophyte presence may be caused in part by endophyte-altered composition of plant root exudates (Novas *et al.*, 2011, Guo *et al.*, 2015) and root volatile organic compounds (Rostas *et al.*, 2015). In addition, endophyte presence can reduce rates of litter decomposition (Lemons *et al.*, 2005, Omacini *et al.*, 2012).

These studies have focused on tall fescue-*Epichloë* interactions due to its economic and agricultural importance; however, in native ecosystems, such interactions remain largely unresolved (Cheplick & Faeth, 2009, Omacini *et al.*, 2012). Interestingly, the few reported effects in native ecosystems thus far show opposite patterns to those in agronomic ecosystems. Surveys of populations with naturally high *Epichloë* prevalence showed higher abundance and colonization rates of AM fungi for two native host species (Novas *et al.*, 2005, Novas *et al.*, 2009). In addition, experimental work in a third native grass species showed that *Epichloë* increased the abundance of mutualistic AM fungal species and reduced parasitic AM fungal taxa, with net benefits to plant performance (Larimer *et al.*, 2012). Finally, in contrast to managed agronomic ecosystems, the effects

of aboveground endophytes in native ecosystems may show higher context dependency in their ecological outcomes, dependent on exogenous, environmental conditions, such as climate, due to a longer history of above/belowground interactions within the native ecosystem (Cheplick & Faeth, 2009).

Understanding the degree of context-dependency could improve our ability to predict outcomes of above/belowground interactions under future climates. Here, we investigated the influence of an aboveground fungal endophyte symbiosis on belowground microbes in a native dune ecosystem to add to our understanding of the importance and prevalence of above/belowground interactions in natural ecosystems. To evaluate the degree of context-dependency, we altered precipitation (± 30% ambient) to replicate projected climate changes for Great Lakes dune ecosystems (Emery *et al.,* 2015). Coastal and lacustrine ecosystems are expected to be amongst the most vulnerable to climate change due to their already fragile nature and predicted increases in the intensity of severe weather events, such as storms and droughts, which will accelerate erosion and reduce dune stability (Schlacher *et al.*, 2008).

Dune ecosystems include diverse microbial taxa with a variety of functional roles. Dunes are extremely nitrogen and water limited, and so diazotrophic groups such as Rhizobiales and Burkholderiales, along with drought-tolerant groups such as Acidobacteria, may play particularly important roles in these systems (Dalton *et al.*, 2004, Evans & Wallenstein, 2014). AM fungi are common plant associates in dunes (Koske & Gemma, 1997, Perumal & Maun, 1999) as well and may influence plant species composition and soil formation (van der Heijden *et al.*, 1998, Bever *et al.*, 2010). Additionally, in Great Lakes dunes, *Epichloë* endophytes are present in aboveground

tissues of the dominant, dune-building grass, *Ammophila breviligulata*, and are especially common in plant material available for dune restoration (Emery *et al.*, 2010). Our previous work in this system has shown that *Epichloë* presence increased host growth and reduced the diversity of plant species colonizing the dunes (Emery *et al.*, 2010, Emery & Rudgers, 2013, Emery & Rudgers, 2014, Emery *et al.*, 2015, Rudgers *et al.*, 2015). In addition, *Epichloë* presence in live host plants reduced decomposition rates of litter placed near live plants (Bell-Dereske *et al., in press*).

For this study, we asked: (1) Does aboveground endophyte symbiosis in *A. breviligulata* affect belowground biomass and the diversity or composition of root- or soil-associated microbes in Great Lakes dunes? (2) Does the amount of growing season precipitation cause context-dependency in the effect of aboveground symbiosis on belowground microbes, or directly affect the diversity or composition of root- or soil-associated microbes? To provide new insight into dune soil microbial ecology, we also explored the question, (3) Does the diversity and composition of bacteria differ between *A. breviligulata* roots and the surrounding dune soil matrix?

METHODS

STUDY SYSTEM

Sand dunes cover much of the Great Lakes shoreline, forming the most extensive freshwater dunes in the world and covering >1,000 km^2 in Michigan alone (Albert, 2000). Great Lakes sand dunes are dominated by *A. breviligulata*, which stabilizes moving sand during the early stages of dune succession and contributes to early soil carbon enrichment (Olson, 1958, Nuñez *et al.*, 2011). Additionally, *A. breviligulata* contributes to biotic

engineering of dunes which can be rapid, altering dune geomorphology within months to years (Godfrey, 1977, Lichter, 1998). After dunes are stabilized, other plant species colonize and out-compete *A. breviligulata*, succeeding ultimately to a mixed deciduous-pine forest (Lichter, 1998, Lichter, 2000).

Drought may be a particularly important element of climate change for dune plants and microbes in the Great Lakes region. The survival of native dune plants has been shown to be water limited along Lake Michigan (Lichter, 2000, Ensign *et al.*, 2006), and water was more limiting to plant survival than nutrients in a study of Canadian dunes (Houle, 1997). Climate models project increases in evapotranspiration rates and drops in lake levels in the Great Lakes (reviewed in Gronewold *et al.* (2013), potentially increasing water stress for plants. Across ten general circulation models from the IPCC Fourth Assessment Report (IPCC (2007), www.cccsn.ec.gc.ca/?page=dd-gcm), predicted changes in precipitation for the region ranged from 31% decrease to 19% increase by 2071 – 2100 compared to baseline data back projected by each GCM for 1971 – 2000 (Emery *et al.*, 2015, Rudgers *et al.*, 2015) while the IPCC Fifth Assesement ensemble model predicts a 10-25% increase in annual preciptiation for the region (IPCC 2014).

STUDY SITE

The experimental site is located in Leelanau State Park, Leelanau Co., Michigan, USA (45°10.964', -85° 34.578'). We established the experiment on a large blowout on the leading edge of the second foredune, approximately 200 m from the shoreline of Lake Michigan. The blowout was largely devoid of vegetation and showed ongoing sand

movement at the time of establishment. The habitat between the first and second dunes was a gravel bed with little vegetation.

EXPERIMENTAL DESIGN

During late May 2010, we established a 2 × 3 factorial field experiment to alter the presence/ absence of endophyte symbiosis in *A. breviligulata* populations in the context of a growing season climate manipulation (reduced, ambient, or augmented precipitation). A full description of the experimental design is reported in Emery et al. (2015).

Precipitation manipulation. We constructed modified Sala rain-out shelters to manipulate growing season precipitation (Yahdjian & Sala, 2002). Clear plastic shingles removed ~30% of ambient rainfall from the reduced rainfall plots. We then added collected rain to the augmented water plots after each rain event with watering cans. Both augmented and ambient rainfall plots had mock shelters with shingles oriented upside-down to control for any effects on light levels or temperature, without altering the amount of ambient rainfall. Each year, shelter roofs were re-installed at the beginning of the growing season (late May) and removed as plants began to senesce (mid-Sept).

Endophyte manipulation. To manipulate endophyte presence, we used endophyte-free seeds collected at a nearby site in Sleeping Bear Dunes National Lakeshore (44°51.472', -86°3.834') during fall 2006. *Epichloë* occurred in ~22% of Great Lakes populations (Emery *et al.,* 2010, Emery & Rudgers 2014). To manipulate endophyte presence, we germinated seedlings on 1% water agar and inoculated half with endophyte isolates of the

Epichloë sp. grown on potato dextrose agar. We used a sterile needle to either wound (sham-inoculate, E- treatment) or insert hyphae into the meristem of each seedling (E+ treatment) (Leuchtmann & Clay, 1988). Following inoculation, seedlings were grown in the greenhouse in a 50:50 mix of sterile play sand and Metro-Mix 220 (Sun Gro Horticulture, Agawam, MA). As plants matured, we cloned genotypes by gently separating tillers from the original stock plants. Thus, we were able to plant the same set of 12 *A. breviligulata* genotypes into every E+ plot, and a second set of 12 genotypes into every E- plot, thereby homogenizing plant genotypic variation within each endophyte treatment. We matched plant genetic variation (3 genotypes m^{-2}) to naturally occurring levels (Fant *et al.*, 2008).

RESPONSE VARIABLES

Plot level measurements. To examine plot level abiotic conditions, we measured volumetric water content (VWC) at a depth of 40 cm monthly (May - July) in three random locations per plot. We used an M300 soil moisture meter (Aquaterr Instruments & Automation, Costa Mesa, CA). Soil moisture was averaged across the growing season for each year.

To examine how treatments affected plant performance, we counted *A. breviligulata* tillers per plot each September from 2011-2015. Aboveground effects of the precipitation and endophyte treatments on *A. breviligulata* were reported in Emery *et al.,* (2015). In the current study, we sampled root biomass during September 2014 using a bulb auger (volume ~ 695cm^3) to collect the tillers and roots from clumps of ~1-5 tillers. Roots were oven dried and weighed, to calculate per tiller root biomass for each plot. We

then estimated plot-level root biomass for each year using September tiller counts × per tiller root biomass.

AM Fungal Root colonization. Fungal abundance in *A. breviligulata* roots was quantified from composited root samples collected in July from each plot during 2011-2014. Roots were rinsed and placed into 50 ml centrifuge tubes and then soaked in hot 10% KOH for 30 m and stained using the ink (Sheaffer Pen, Shelton, CT) and vinegar method (Vierheilig *et al.*, 1998). From each plot, ten 1 cm root sections were mounted on a microscope slide. Using a compound microscope (Leica Microsystems, Wetzlar, Germany) at 200× magnification, the percentage of roots colonized by AM fungal hyphae was recorded using the gridline intercept method (McGonigle *et al.*, 1990) with 100 views per slide [(number of views with structures visibly present in roots/total number of views) × 100]. We separately counted coarse AM hyphae, fine AM hyphae (both of which appear blue-black and non-septate). Fine AM fungi have been found to more tolerant of extreme environmental conditions than coarse AM fungi (Orchard *et al.*, 2016) though their taxonomy is still under debate (Schüßler & Walker, 2010)

Extra-radical hyphal length. We quantified the length of extraradical AM fungal hyphae in 20 g soil subsamples from each plot collected during 2011-2014. Each subsample was mixed with 500 ml DI water in a 100 ml beaker and stirred at 80% speed for 2 min with a magnetic stir bar. Before solid material settled, the solution was poured through 500 µm and 212 µm sieves to separate sand and large organic material from the hyphal suspension. Residue from the 212 µm filter was rinsed back into a 50 ml beaker using 10

ml of DI water. Twenty drops of 4% Trypan Blue stain was added and left to sit for 45 min. This solution was then filtered through a 38 µm sieve and rinsed with DI water until water ran clear from the sieve. The residue on the 38 µm sieve was rinsed back into a 400 ml beaker using 200 ml of DI water and agitated for 2 min with the stir bar. A 20 ml sample was removed from ~1 cm below the water surface and drained through a 25 mm glass microanalysis vacuum filter holder fitted with a 0.45 µm mesh nylon membrane. The membrane was then rinsed and dried under vacuum and mounted onto a slide. Hyphal length was estimated using the gridline-intercept method based on 50 fields of view per sample (McGonigle et al. 1990) under a stereomicroscope (Nikon SMZ1500 at 70X). Hyphal lengths were standardized to mm hyphae/g soil based on soil sample mass.

Soil glomalin content. AM fungal spores and extra-radical hyphal cell walls contain the recalcitrant soil protein glomalin (Wright & Upadhyaya, 1996). Glomalin may represent 4-8% of soil organic carbon in natural ecosystems (Rillig *et al.*, 2001), and thus is one measure of ecosystem function (carbon sequestration) provided by mycorrhizal communities. Total soil glomalin was estimated by extracting from 1 g soil subsamples per plot during 2010-2014 using the 50 mM sodium citrate buffer and autoclaving method described in Janos et al. (2008). We quantified the Bradford reactive fraction (Bio Rad, Hercules, CA, USA) using bovine serum as a standard. Total soil glomalin has several extractible fractions and Bradford reactive soil protein (BRSP) has been shown to consistently represent the largest fraction of total soil glomalin (approximately ~90% by volume (Koide & Peoples, 2013). Therefore, we used BRSP to operationally define glomalin.

Root and soil collection for microbial composition. Root and soil samples were collected from each plot in September 2012 for microbial characterization. Roots were collected from three randomly chosen *A. breviligulata* individuals per plot. Soils were collected from near 3 plants per plot and homogenized. Roots and soils collected for bacterial extracts were preserved with sucrose lysis buffer (Giovannoni *et al.*, 1990) added to saturation. All samples were shipped on dry ice within 24 h of collection. AM fungi root samples to be used in pyrosequencing (details below) were stored at -80°C and samples for bacterial extraction were stored at -20°C until processing.

454 Pyrosequencing: AM fungi. Freeze-dried root samples were washed with DI water and sterilized with 10% bleach. Samples were disrupted with 0.2 cm^3 of 0.1 mm diameter Zirconia Silica beads (BioSpec Products) in a Mixer Mill 300 (Retsch, Haan, Germany). Samples (100 mg) were then extracted using the DNeasy Plant kit (Qiagen, Hilden, Germany) following the manufacturer protocol. DNA concentration was quantified using a NanoDrop 1000 (Thermo Fisher Scientific, Wilmington, DE) and standardized to 20 ng/µL. Extracted samples were then amplified and sequenced by Mr. DNA (Shallowater, TX). The 28S region of the rDNA was targeted using AM fungal specific primers. Briefly, PCRs were performed in triplicate 25 µL reactions containing 0.25 mM forward and reverse fusion primer, 0.25 mM dNTP (each), 1x Platinum PCR buffer (Lifetech, Carlsbad, CA), 1.5 mM MgCl2, 1 U Platinum Taq Polymerase (Lifetech, Carlsbad, CA) and 2 µL (~40ng) of DNA template. Fusion primers were designed so that the forward primer consisted of the Roche adapter A, followed by a 10 base error-correcting barcode

for multiplexing (Hamady *et al.*, 2008), and using FLR3 (5'-TTG AAA GGG AAA CGA TTG AAG T-3'). The reverse primers included the Roche adapter B, followed by the reverse PCR primer FLR4 (5'-TAC GTC AAC ATC CTT AAC GAA-3') (Gollotte *et al.*, 2004). The thermal cycler program included an initial 5 min denaturation at 95 °C, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 30 s. A final 7 min extension completed the PCR. PCR amplicons were purified using the Mo-bio Gel Purification Kit (Carlsbad, CA) following the manufacturer's instructions, quantified spectrophotometrically, and combined in equimolar concentrations for multiplexed pyrosequencing. Sequencing template was quantitated fluorometrically using a picogreen dye kit, assayed for quality and fragment length on an Agilent Bioanalyzer DNA 1000 chip before library preparation using Roche titanium reagents and titanium procedures. Samples were then sequenced on a Roche 454 FLX titanium instrument (Basel, Switzerland) following manufacturer's protocols.

454 Pyrosequencing: Bacteria. DNA from each of the 90 root associated (endophytic and surface of the root) and soil samples (0.3 g) were extracted following the cetyltrimethylammonium bromide (CTAB) method described in Mitchell & Takacs-Vesbach (2008), modified to include a bead beating step. Briefly, 0.2 cm^3 of 0.1 mm diameter Zirconia Silica beads (BioSpec Products, Bartlesville, OK), 300 µL of 1% CTAB, and 100 µg and 1 mg each of proteinase K and lysozyme, respectively, were added to preserved sample. Samples were incubated with continuous vertical rotation (~35 rpm) at 37 °C for 0.5 h. Sodium dodecyl sulfate was added (final concentration 2%), and samples were returned to the laboratory rotator for 0.5 hour at 60 °C. Samples were

then bead-beaten on a vortexor for 5 min at the medium setting. Nucleic acids were extracted with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1), followed by an extraction with chloroform and precipitated in 95% ethanol after the addition of 0.1 volume 3 M sodium acetate. Nucleic acids were washed once in 70% ethanol, air dried, and re-suspended in 40 µL 10 mM Tris, pH 8.0.

DNA extractions served as template to survey bacterial diversity with barcoded amplicon pyrosequencing of 16S rDNA genes in each of the 180 samples. The 16S rDNA gene pyrosequencing was performed as described previously (Schwartz *et al.*, 2014). Briefly, PCRs were performed in triplicate 25 µL reactions containing 0.25 mM forward and reverse fusion primer, 0.25 mM of each dNTP, 1x Platinum PCR buffer (Lifetech, Carlsbad, CA), 1.5 mM MgCl2, 1 U Platinum Taq Polymerase (Lifetech, Carlsbad, CA) and 2 µL of DNA template. Fusion primers were designed so that the forward primer consisted of the Roche adapter A, followed by a 10 base error-correcting barcode for multiplexing (Hamady *et al.*, 2008), and the universal bacterial primer 939F 5' TTG ACG GGG GCC CGC ACA AG-3'. The reverse primers included the Roche adapter B, followed by the reverse PCR primer 1492R 5'-GTT TAC CTT GTT ACG ACT T-3'. The thermal cycler program included an initial 5 min denaturation at 95 °C, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 30 s. A final 7 min extension completed the PCR. Sample amplicons were purified, quantified, and aggregated as described above. All samples from this study were run on one half region of a sequencing plate, with no more than 96 samples total per region. Pyrosequencing was performed on a Roche 454 FLX instrument (Basel,

Switzerland) following manufacturer's protocols at the Molecular Biology Facility in the UNM Biology Department.

BIOINFORMATICS

AM fungal 28S rRNA sequencing resulted in 611,624 raw sequences which then were quality filtered and trimmed to 300bp using fastq_filter in USEARCH8 with default settings (http://drive5.com/usearch/). Sequences were chimera checked, filtered de novo, and clustered at 97% similarity into unique operational taxonomic units (OTUs, i.e., DNA sequences or amplicon types) using UPARSE implemented in UEARCH8 (Edgar, 2013). USEARCH has been successfully used for the processing and OTU clustering of AM fungal amplicons (Van Geel *et al.*, 2014, De Beenhouwer *et al.*, 2015, Johansen *et al.*, 2015, van Geel *et al.*, 2015, Van Geel *et al.*, 2016). USEARCH8 quality filtering, chimera checking using UCHIME, and OTU clustering lead to 44 OTUs and 277,799 reads. Taxonomic affiliation was assigned to OTUs by comparing the representative set of DNA sequences to the MaarjAM data base using megablast (Öpik *et al.*, 2010). Representative sequences were aligned and a tree was built in PASTA (Mirarab *et al.*, 2015) using RAXML and all other default settings with reference sequences from the online database schuessler.userweb.mwn.de/amphylo/ constructed from (Redecker *et al.*, 2013) and (Schüßler & Walker, 2010). The tree was rooted using *Batrachochytrium dendrobatidis* as the outgroup. Sequences that did not blast to species in the MaarjAM database (<95% Query coverage and <95% Max identity) but were monophyletic with references sequences in the AM fungal phylogeny were additionally blasted to the NCBI database; OTUs that did not hit AM fungal entries were removed from analysis because

they were highly likely to be non-AM fungi. We also removed OTUs with < 5 reads total to avoid over splitting (Thiéry *et al.*, 2012) and sequencing errors (Dickie, 2010). Taxonomic filtering resulted in 34 OTUs and 276957 reads (Table S2.1). We transformed OTU tables using variance stabilizing transformation (VST) in the DeSeq2 package (Love *et al.*, 2014) in R (R Core Team 2015) to control for biases in PCR amplification and to avoid biases due to rarefaction (McMurdie & Holmes, 2014). The inverse Simpson diversity index was calculated for each sample using the vegan package of R (Oksanen *et al.*, 2016). Results were qualitatively the same using a rarefied OTU table [reads = 500 (Table S2.3)]. A Bray-Curtis distance matrix was generated from the VST normalized community using Primer V6 (Clarke & Gorley, 2006). Weighted and unweighted Unifrac (Lozupone & Knight, 2005) distance matrices were generated from the VST normalized community in QIIME. Because results for weighted and unweighted matrices were similar, only results for weighted Unifrac are reported. Importantly, the resulting community matrix was not significantly different than the community matrix produced by quality filtering, chimera checking, and OTU clustering using the Quantitative Insights into Microbial Ecology (QIIME) pipeline (Caporaso *et al.*, 2010). Specifically, the Bray-Curtis matrices were strongly correlated (Mantel: Spearman correlation $r = 0.86$ $P < 0.01$), and the ordination structure was significantly correlated (Procrustes correlations $r = 0.73$ $P = 0.001$).

Bacterial 16S rRNA gene sequencing resulted in 826,382 raw sequences which were quality filtered, denoised, screened for PCR errors, and chimera checked using AmpliconNoise and Perseus to minimize potential artifacts (Quince *et al.*, 2011). The

QIIME pipeline was used to analyze alpha and beta diversity of the DNA sequence data (Caporaso et al. 2010). OTUs were identified by the 97% DNA identity criterion using the uclust OTU picker (Edgar, 2010) in QIIME. A set of representative DNA sequences was chosen for each unique OTU in QIIME and used for all subsequent analyses. Taxonomic affiliation was assigned to OTUs by comparing the representative DNA sequences to the Green Genes database (gg8.15.13). These DNA sequences were aligned using MUSCLE (Edgar, 2004), and a phylogenetic tree necessary for the beta diversity analysis was constructed using FastTree (Price *et al.*, 2009). We filtered the OTU table to remove samples with < 300 reads and OTUs with < 5 reads, resulting in 142 total samples (70 root; 72 soil), 8,180 OTUs (5,003 OTUs in the root; 2,120 in the soil), and 807,991 sequences remained. The filtered bacterial OTU table was then normalized using VST as described for the AM fungi data above. To examine the effects of the treatments on root versus soils, filtered OTU tables were separated into root vs. soil community, then re-filtered to remove samples with < 300 reads (removing one root sample and one sample) and OTUs with < 5 reads within each table (Table S2.1). The separated files of raw OTU reads were then normalized using VST as described above. Diversity was calculated on the VST normalized data using the inverse Simpson diversity index for each sample using the vegan package of R. Results using this diversity metric were qualitatively similar to a rarefied OTU table [reads = 1000 (Table S2.4)]. Bray-Curtis distance matrices were generated from the VST normalized communities using Primer V6 (Clarke & Gorley, 2006). Weighted and unweighted Unifrac (Lozupone & Knight, 2005) distance matrices were generated from the VST normalized communities in QIIME. Because results for weighted and unweighted matrices were similar, only

results for weighted Unifrac are reported. All raw sequence data from this study are available through the NCBI Sequence Read Archive under accession SAMN05354971.

STATISTICAL ANALYSES

Dunes are a highly spatially heterogeneous environment, ranging in depth to the groundwater table, nutrient content, aeolian sand deposition, and many other factors. To fully explore our response variables within context of the spatial heterogeneity of the dunes, we compared statistical models using our treatments as fixed factors (i.e. endophyte, precipitation, and sampling year when multiple years of data were collected) and spatial blocking within the experiment as a fixed factor (Precipitation Model) to statistical models with soil moisture (VWC) as a continuous variable instead of the precipitation factor (Soil moisture Model). In the **Precipitation Model**, because we were interested in (1): how the endophyte affects the belowground communities and (2): if these effects are dependent on precipitation, we examined the effects of our endophyte and precipitation treatments and their interaction on the abundance of fungi and community indices AM fungi and bacteria described above. We included as spatial blocking factors both the column (blocked into three groups based on north – south gradient (ColumnBlock)) and row (blocked into three groups based on east – west gradient (RowBlock)) position of each plot. In the **Soil moisture Model**, we replaced precipitation treatment with the VWC measured for each plot (40 cm depth), averaged over sampling points within the plot and over the growing season. On average over the growing season, our precipitation treatment decreased soil moisture by 3% in reduced compared to control plots and increased soil moisture by 9% in augmented compared to

reduced precipitation (Rudgers *et al.*, 2015). Since the position of the plot on the dune slope (i.e. RowBlock and ColumnBlock) strongly affected the soil moisture (Fig. S2.1; Table S2.1), spatial blocking factors were dropped from Soil moisture Models to avoid collinearity with VWC.

Root colonization and soil fungi analyses. Using the Precipitation Model, we analyzed responses of soil moisture (VWC at 40 cm), percentage of root colonization by fungi, extraradical hyphal length (ERH), glomalin, and plot-level estimated root biomass using mixed effect models with plot as a random factor using the lme4 package (Bates *et al.*, 2015) in R (R Core Team 2015). Because per tiller root biomass was only measured in 2014, we used a general linear model for this variable (one observation per plot). To meet assumptions of Gaussian distributions of errors and homogeneity of variances, we square-root transformed total AM fungal colonization of roots, log-transformed root biomass and fine AM fungi colonization, cube-root-transformed ERH, and inverse square-root transformed glomalin estimates.

Community composition analyses: Fungi. Using the Precipitation Model, we analyzed the response of the inverse Simpson diversity of the AM fungal community using a linear model in R. AM fungal community structure was analyzed using permutational analysis of variance (PERMANOVA) on the Bray-Curtis and Unifrac weighted (VST normalized matrix) distance matrices using Primer V6 (Clarke & Gorley, 2006).

Community composition analyses: Bacteria. Using the Precipitation Model with the addition of location of the bacterial community [to address (3) if the bacterial diversity and community differ between roots and soil], we examined responses of

inverse Simpson diversity of the bacterial community, along with the relative abundance (in percentage of VST normalized sequences per sample) of key soil functional groups Acidobacteria, Actinobacteria, Rhizobiales, and Burkholderiales, using mixed effect models with plot as a random factor using the lme4 package (Bates *et al.*, 2015) in R (R Core Team 2015). To meet assumptions of Gaussian distributions of errors and homogeneity of variances, we log-transformed Rhizobiales, Burkholderiales, Actinobacteria, and Acidobacteria relative abundance and inverse Simpson diversity. Precipitation Model effects on bacterial community structure were analyzed using PERMANOVA with factors described above plus the location of collection (root vs. soil), and all interaction terms with the addition of plot as a random factor. If RowBlock or ColumnBlock were not significant in the full Precipitation Model, they were dropped from the final models.

Community composition analyses: Soil moisture Model. Using the Soil moisture Model, we analyzed bacteria and AM fungi community structure using PERMANOVA on the Bray-Curtis and weighted Unifrac (VST normalized matrix) distance matrices using Primer V6. We examined the effects of the location of collection (root versus soil) and Soil moisture Model treatments on the relative abundance (in percentage of VST normalized sequences per sample) of Acidobacteria, Actinobacteria, Rhizobiales, and Burkholderiales using mixed effect models with plot as a random factor using the lme4 package (Bates *et al.*, 2015) in R (R Core Team, 2015). Since soil moisture had significant interactive effects with location of collection (root versus soil) in the bacterial community composition and our focus bacterial taxonomic groups, we analyzed the effects of the Soil moisture Model on bacterial Simpson diversity and proportion of

sequences composed of Acidobacteria, Actinobacteria, Rhizobiales and Burkholderiales on the separate root and soil communities. All mixed effects and linear models were implemented in R (R Core Team, 2015).

RESULTS

(1) Does aboveground endophyte symbiosis in A. breviligulata *affect belowground biomass and the diversity or composition of root- or soil-associated microbes in Great Lakes dunes?*

Roots. Estimated plot-level root biomass was 27% greater when *Epichloë* was present compared to endophyte free plots ($X^2 = 3.93$, $P = 0.047$; Fig. 2.1a Table S2.2), consistent with our previous findings of increased aboveground *A. breviligulata* biomass when *Epichloë* was present (Emery *et al.*, 2015).

Fungal abundance. Epichloë presence altered *A. breviligula* root colonization and the abundance of soil fungi, but had little effect on the overall composition of belowground communities associated with *A. breviligulata* roots and soils. Across all years of sampling, *Epichloë* reduced the length of soil ERH by 11% compared to endophyte free plots ($X^2 = 4.69$ $P = 0.030$; Table S2.2). The endophyte-driven reduction in ERH was strongest in 2014, where the presence of *Epichloë* reduced the ERH by 19% compared to the endophyte free plots (Fig. 2.2a). The effects of *Epichloë* on colonization of fine AM fungal hyphae in roots varied across years (endophyte x year $X^2 = 8.45$ $P = 0.038$), tending to reduce colonization in 2013 (by 35%) but causing an increase in colonization (50%) during 2012 (Fig. 2.3a). However, *Epichloë* did not alter hyphal colonization by the combined coarse and fine AM fungal morphotypes ($P > 0.10$, Table

S2.2). Glomalin abundance also showed no overall net response to *Epichloë* presence ($P > 0.65$, Table S2.2).

Microbial diversity and composition. Epichloë presence did not have a net main effect on belowground microbial diversity or community composition, despite evidence for context dependency (see question 2). *Epichloë* presence also did not alter the diversity of AM fungi ($P > 0.55$; Table S2.3) or inverse Simpson diversity of bacteria ($P > 0.80$; Table S2.4). Additionally, *Epichloë* presence did not shift community composition of the AM fungal community (Bray-Curtis $P > 0.95$; Weighted Unifrac $P > 0.90$; Table S2.5) or the bacterial community (Bray-Curtis $P > 0.55$; Weighted Unifrac $P > 0.50$; Table S2.6). There was no main endophyte effect on the focal diazotrophic bacteria (all $P > 0.70$; Table S2.7) or on the focal bacterial phyla (all $P > 0.45$; Table S2.7).

(2) Does growing season precipitation cause context-dependency in above/belowground interactions or directly affect the diversity or composition of root- or soil-associated microbes?

Direct effects of precipitation on belowground responses. Precipitation directly altered root colonization and soil fungi abundance, but did not affect bacterial or AM fungal diversity or composition. In 2012, *A. breviligulata* roots from the augmented precipitation treatment had > 2X higher colonization by the fine AM fungal morphotype than plots receiving ambient precipitation (precipitation x year $X^2 = 12.71$, $P = 0.048$; Fig 2.3b; Table S2.2). However, there was a trend for increased root colonization of combined AM fungal morphotypes under ambient precipitation (precipitation x year $X^2 = 12.30$, $P = 0.055$; Fig 2.3c; Table S2.2). Reduced precipitation increased soil ERH by

44% over ambient precipitation in 2014 but had very little effect in all other years (precipitation x year $X^2 = 19.71$ $P = 0.003$; Fig 2.2b; Table S2.2). Precipitation treatments had no main effect on the diversity or composition of bacterial or AM fungal communities (all $P > 0.09$; Table S2.4-S2.6).

Belowground context-dependency: Roots. Epichloë increased per tiller root biomass during 2014 by 50% compared to endophyte free plants under ambient precipitation, but had little to no effect on per tiller root biomass under altered precipitation (endophyte × precipitation $F_{2,84} = 2.68$ $P = 0.075$; Fig. 2.1b; Table S2.3). This suggests that the strongest endophyte effects should be found under ambient precipitation.

Belowground context-dependency: Fungi. The amount of precipitation modified how the endophyte affected AM fungal diversity and glomalin production. *Epichloë* reduced the diversity of AM fungal variance stabilizing transformation (VST) normalized OTUs by 25% under ambient precipitation, but did not strongly affect diversity under altered precipitation (endophyte × precipitation $F_{2,63} = 3.31$, $P = 0.043$; Fig. 2.4; Table S2.3). Consistent with the VST results, endophyte presence reduced AM fungal rarefied diversity overall endophyte $F_{1,61} = 5.31$, $P = 0.025$; Table S2.3) having the strongest negative effect under ambient precipitation (Fig. S2.3). The interactive effect on glomalin varied with year (endophyte × precipitation × year $X^2 = 18.13$, $P = 0.020$; Table S2.2). *Epichloë* increased glomalin only under augmented precipitation in year 2012 (when we sampled microbial composition), with little effect in other years (Fig. 2.5). Despite this

interactive effect of *Epichloë* and precipitation on diversity and glomalin, there was no interactive effect on root colonization by AM fungi across years ($P > 0.45$; Table S2.2).

Belowground context-dependency: Bacteria. Though our precipitation treatments did not alter the effects of *Epichloë* on the inverse Simpson diversity of the bacterial community in the Precipitation Model (all $P > 0.15$; Table S2.4), endophyte presence did interact with soil moisture in the Soil moisture Model to alter the response of the belowground bacterial community. Because the location of the bacterial association with *A. breviligulata* (roots versus soil) and spatial blocking both had strong effects on the diversity and composition of bacterial community (Fig. 2.9 and 10; Fig. S2.2; Table S2.4 and S2.6), we split the bacterial OTU matrix into root associated bacteria versus soil associated bacteria and ran our Soil moisture Model on the separate communities. *Epichloë* presence caused root associated bacterial diversity to decline with soil moisture at 40 cm depth (endophyte × soil VWC $F_{1,65} = 5.52$, $P = 0.022$; Table S2.9). However, in the absence of *Epichloë*, root-associated bacterial diversity increased with greater soil moisture (slope of E+ ($R^2 = 0.14$) was 252% less than slope of E- ($R^2 = 0.01$); Fig. 2.6a). In contrast to root bacteria, soil bacterial diversity was not affected by *Epichloë*, and increased with greater soil moisture regardless of *Epichloë* presence (soil VWC $F_{1,67}$=6.21, P=0.015, $R^2 = 0.11$; Fig. 2.6b; Table S2.9). Endophyte presence did not alter the response of root bacterial, soil bacterial, or AM fungal community structure to soil moisture (all endophyte × soil VWC P>0.10), but composition of the three belowground communities did shift with soil moisture (Table S2.10).

Endophyte presence altered the responses of diazotrophs and soil bacteria phyla to soil moisture. For putative diazotrophs, *Epichloë* presence caused the relative abundance

of root associated Rhizobiales to decline with soil moisture; however when the endophyte was absent, there was a weak increase in the abundance of root-associated Rhizobiales with higher soil moisture (endophyte × soil VWC $F_{1,65}$ =6.73, P = 0.012, slope of E+ (R^2 = 0.17) was 183% less than slope of E- (R^2 = 0.025); Fig. 2.7a). Endophyte presence did not affect soil Rhizobiales or Burkholderiales associated with either roots or soil (all: endophyte × soil VWC P > 0.15), although relative abundance decreased with increasing soil moisture across all three groups (soil Rhizobiales: soil VWC $F_{1,67}$ = 20.69, P < 0.001, R^2 = 0.22; root Burkholderiales soil VWC $F_{1,65}$ = 7.09, P = 0.010, R^2 = 0.07; and soil Burkholderiales soil VWC $F_{1,67}$ = 17.11, P < 0.001, R^2 = 0.19; Fig. 2.7; Table S2.10).

Interestingly, endophyte presence altered how soil Actinobacteria and Acidobacteria responded to soil moisture (Fig. 2.8), but did not affect the response of these phyla when they resided in roots (both: endophyte × soil VWC P > 0.15; Fig. 2.8a,c). Soil Actinobacteria tended to increase with higher soil moisture when the endophyte was present (E+ R^2 = 0.035), but tended to decrease when the endophyte was absent (E- R^2 = 0.031) (endophyte x soil VWC, $F_{1,67}$ = 4.14 P = 0.046; Fig. 2.8b; Table S2.10). Soil Acidobacteria increased in relative abundance with increasing soil moisture only when the endophyte was absent (endophyte x soil VWC, $F_{1,67}$ = 12.41, P = 0.001, slope of E+ (R^2 < 0.001) was 112% less than slope of E- (R^2 = 0.30); Fig. 2.8d; Table S2.10).

(3) Does the diversity and composition of bacteria differ between A. breviligulata *roots and the surrounding dune soil matrix?*

In the nutrient poor soil of dunes, proximity to *A. breviligulata* roots altered the composition and diversity of the bacterial community (Fig. 2.9 and 2.10). VST normalized root-associated bacteria were ~99% more diverse than the soil bacterial community ($X^2 = 52.64$, $P < 0.001$; Fig. 2.10a) and rarefied diversity was 9-fold higher in roots than in soil ($X^2 = 415.24$, $P < 0.001$; Fig. S2.4). Rhizobiales were more abundant in soils, where that clade made up 10% of the sequences, than in the roots of *A. breviligulata* where Rhizobiales constituted just 3% of total sequences ($X^2 = 57.25$, $P < 0.001$; Fig. 2.7c,d). Burkholderiales was also more abundant in the soils than root with Burkholderiales representing 5% of total squences in the soil versus 4% of total sequences in the roots ($X^2 = 21.48$, $P < 0.001$; Fig. 2.7a,b).

DISCUSSION

Leaf Epichloë *increased belowground biomass while reducing AM fungal diversity and extraradical hyphae*

Epichloë presence in the aboveground tissue of *A. breviligulata* increased root biomass nearly 30%, and reduced AM fungal diversity and extraradical hyphae (ERH) production. The tradeoff between root biomass and ERH production is found in other systems (De Deyn *et al.*, 2009), with plants investing more in roots having less need for ERH to acquire nutrients. To our knowledge, ours is the first study that has used AM-specific primers to examine whether *Epichloë* affects root fungal composition in the field. Here, *Epichloë* reduced the diversity of the AM fungal community. In contrast, Rojas et al. (2016) showed that the *Epichloë* presence increased the relative abundance of AM fungal sequences when they examined the *soil* fungal community via more general *ITS*

fungal primers, which do not yield good resolution of AM fungi. The reduction in AM fungal diversity reported here could influence plant succession through shared hyphal networks or altered access to symbiont partners (van der Heijden *et al.*, 1998, Enkhtuya *et al.*, 2005).

An endophyte-mediated shift in soil fungi could also influence dune ecosystem processes. Here, the presence of *Epichloë* reduced the hyphal length of extraradical hyphae in the soil consistently across precipitation treatments and years. Reduced ERH may contribute to slower decomposition in the dunes and help explain our prior observation that *Epichloë* presence in live *A. breviligulata* plants reduced the rate of decomposition of litter buried in our plots (Bell-Dereske *et al., in press*). Endophyte-reduced abundances of soil fungi may similarly cause the slower decomposition rates in tall fescue pastures (e.g., Siegrist *et al.*, 2010) and also explain their higher levels of carbon sequestration (Iqbal *et al.*, 2012). In contrast to our result for ERH, a long-term field study on the tall fescue – *E. coenophiala* symbiosis showed no endophyte effect on soil fungal biomass. However, Ascomycota strongly declined with endophyte-presence (Rojas *et al.*, 2016). Although we did not sequence other soil or root fungi, we found no significant shifts in root colonization by dark septate endophyte morphotypes. Also, in contrast to our results, a study of *Bromus auleticus – Epichloë pampeana* suggested that *Epichloë* presence increased the diversity of soil fungal cultures, specifically phosphorus-solubilizing, rhizospheric fungi (Arrieta *et al.*, 2015). Furthermore, a previous study on tall fescue found endophyte-mediated *increases* in the activity of the fungal community (Casas *et al.*, 2011). Thus, our results suggest that the belowground effects of *Epichloë* are not easily generalizable across host species or ecosystems.

Despite detecting an influence of *Epichloë* on AM fungi and ERH, we found that most microbial responses were insensitive to endophyte presence. That belowground fungi were more sensitive than bacteria to the net effect of *Epichloë* is in line with prior work showing higher sensitivity of fungi than bacteria to *Epichloë* presence in tall fescue pastures (Rojas *et al.*, 2016). Additionally, Great Lakes dunes are characterized by a spatially heterogeneous abiotic environment (Lichter, 1998, Ensign *et al.*, 2006). Our results suggest this heterogeneity is the primary driver of both root and soil microbial diversity and composition. For example, spatial blocking effects tended to outweigh the biotic influence of *Epichloë* presence in leaves for many microbial responses variables. Factors that could be structuring the microbial community are likely to be scale-dependent, with global trends driven by soil pH (Fierer & Jackson, 2006) and temperature (Zhou *et al.*, 2016), and local patterns reflective of variable soil moisture, nutrients, or salinity (Van Horn *et al.*, 2013, Okie *et al.*, 2015).

Limited context-dependency in fungal community responses

Most fungal responses did not depend on the precipitation treatment, with the exception of soil glomalin. Additionally, both glomalin and fine AM fungal hyphae showed year-to-year variability in responses to *Epichloë*, which may also indicate that the climate context can alter aboveground/belowground interactions. For example, *Epichloë* tended to increase colonization by fine AM fungal hyphae in 2012, but reduced the abundance of fine hyphae in 2013. The fine hyphae (previously categorized as *Glomus tenue* (Schüßler & Walker, 2010)) have been suggested to be drought-resistant (Staddon *et al.*, 2004). Our site experienced increased drought during August - September, 2012

(Fig. S2.5). Interestingly, our augmented precipitation treatment also increased fine AM fungi in 2012, which may indicate that *Epichloë* and precipitation addition helped to alleviate the effects of extreme drought on fine AM fungi. However, the effect of fine AM fungi on host plant responses to climate are poorly understood due to their low detection in environmental samples and difficulties in culturing compared to other AM fungal morphotypes (Orchard *et al.*, 2016). Perhaps by increasing colonization by fine AM fungal hyphae, *Epichloë* presence increased the AM fungi-produced protein glomalin in soils during the same year. However, this effect was present only under augmented precipitation, demonstrating context-dependency on water availability. In the tall fescue – *Epichloë coenophiala* system, endophyte presence reduced glomalin (Buyer *et al.*, 2011); however, unlike our system. *Epichloë* also reduced total root colonization and spores of AM fungi (Chu-Chou *et al.*, 1992, Mack & Rudgers, 2008). Inter-annual variability in climate could underlie differences among years, but a longer time series would be needed to resolve such an influence in our system.

Epichloë *causes context-dependent responses of bacteria to soil moisture*

To our knowledge, ours is the first study to report that a foliar endophyte alters how belowground bacterial diversity responds to an abiotic gradient. Resolving such relationships is important for refining predictions on how plant-microbe interactions will change under future environmental conditions. Specifically, root-associated bacterial diversity decreased in wetter soils only when *Epichloë* was present (Fig. 2.5a). The abundance of root-associated Rhizobiales showed the same negative relationship with soil moisture when the endophyte was present. However, with *Epichloë* absent, root

bacterial diversity and Rhizobiales abundance increased with soil moisture, similar to the overall positive effect of soil moisture on soil bacterial diversity. Since roots had vastly higher microbial diversity and richness than soils, this context dependent effect could have a strong influence on the total diversity of bacterial species in dune ecosystems. Specifically, even a relatively small reduction in diazotrophic bacteria (i.e. Rhizobiales) in response to soil moisture could affect plant succession because dune soils are so nitrogen poor (Lichter, 1998, Lichter, 2000). In contrast, we found no effect of *Epichloë* on the Burkholderiales, but both the root soil communities showed a negative relationship with soil moisture. Despite the responsiveness of bacterial diversity, and specifically of diazotrophs, in our system, we have not detected significant shifts in total N, nitrate or ammonium in dune soils, based on ion resin exchange membranes placed in plots during the 2013 growing season (*data not shown*). Future investigations of N process rates could be useful for resolving the N cycle in this system.

Previous research has found that Actinobacteria are likely copiotrophic and sensitive to changes in soil moisture, whereas Acidobacteria are more oligotrophic and resilient to changes in moisture (Fierer *et al.*, 2007, Evans & Wallenstein, 2014). Consistent with this past work, soil Actinobacteria tended to increase in relative abundance with soil moisture when the endophyte was present and showed greater sensitivity to soil moisture than soil Acidobacteria. The effect of *Epichloë* on bacterial abundance was similarly context dependent on soil type in tall fescue pastures, where endophyte presence reduced the abundance of more phyla of bacteria in clay loam soils than in loamy sand (Jenkins *et al.*, 2006). It remains unclear why *Epichloë* alters the responsiveness of bacterial diversity to soil moisture (or soil texture). Growth and feeding

strategies seem to be phylogenetically conserved in some of the dominant soil phyla (Fierer *et al.*, 2007) which leads to somewhat predictable shifts in the community composition in response to changes in soil moisture (Evans *et al.*, 2014). It is possible that by altering plant root characteristics, such as root exudates (Franzluebbers & Hill, 2005, Guo *et al.*, 2015) or root biomass, *Epichloë* shifts limitations on bacterial diversity from carbon-based resource availability to water limitation, increasing bacterial responsiveness to soil moisture. Adding carbon to E- plots could provide a direct test of this hypothesis. Alternatively, *Epichloë* presence also widened the range of soil moistures observed across plots (Fig. 2.5), possibly making it easier to detect an influence of soil moisture on bacterial diversity. Prior work has shown that *Epichloë* can promote host tolerance of drought (Malinowski & Belesky, 2000), including our past work on *A. breviligulata* (Emery *et al.*, 2010). Previous studies have additionally suggested that *Epichloë* can alter plant water relations in ways that retain soil moisture for longer periods of time (Elmi & West, 1995, Kannadan & Rudgers, 2008). Thus, plots with *Epichloë* could have an expanded range of soil moisture values. In support of this hypothesis, the coefficient of variation in soil moisture for E+ plots was 42% higher (CV = 16%) than in endophyte-free plots (CV = 11%), and differences in the range of soil moistures observed were not due to imbalance in the sample sizes among treatments.

Ammophila breviligulata *roots harbor islands of bacterial biodiversity*

In many plants, roots selectively filter microbial communities, constraining microbial diversity relative to that of the surrounding soil matrix (Wang *et al.*, 2016). In contrast to this general pattern, roots of *A. breviligulata* act more as islands of microbial

biodiversity than as a selective filter. Perhaps root exudates from *A. breviligulata* provide much needed resources for bacteria, explaining the elevated diversity of bacteria compared to that of the soil. On the other hand, resource inputs from root exudates could lead to antagonistic interactions among bacterial species, increasing the diversity of the root community (Czárán *et al.*, 2002, Schlatter *et al.*, 2015). In more productive ecosystems, the root and rhizosphere typically harbor lower bacterial diversity than surrounding soils (reviewed in Faure et al. (2008)), an effect that grows stronger with a longer time of interaction with plant roots (Shi *et al.*, 2015). Although most research on the selective effect of roots on bacterial communities has focused on few well studied plants, such as *Arabidopsis thaliana* (Bulgarelli *et al.*, 2013), barley, and rice (Bulgarelli *et al.*, 2015, Edwards *et al.*, 2015), studies of wild species have found a similar selective effect of the host root on bacterial communities (Dean *et al.*, 2015, Nuccio *et al.*, 2016).

Roots of *A. breviligulata* harbored higher relative abundances of Burkholderiales than of Rhizobiales, suggesting that roots may selectively favor this group of diazotrophic bacteria. In contrast to the diversity pattern of the whole bacterial community, soils actually had higher proportions of both diazotrophic clades than did roots. Prior work suggested that members of Burkholderiales inhabits the rhizosheathes of grasses, such as *Ammophila*, that grow in extremely nutrient poor soils (Wullstein *et al.*, 1979, Wullstein, 1991, Bergmann *et al.*, 2009). Diazotrophic bacteria in the root sheaths of *A. breviligulata* may be an important, but unresolved, part of the nitrogen cycle in nutrient-poor dune ecosystems. For example, the roots of the sister species *A. arenaria* hosted the diazotrophic bacterial species *Burkholderia tropicalis* in European dunes (Dalton *et al.*, 2004). Although we did not directly examine levels of nitrogen

fixation or *nif* gene expression, our detection of Burkholderiales suggests that root-associated taxa are present in North American dunes as well. In addition, Rhizobiales made up a significant fraction of the soil bacterial community (~10%), suggesting that free-living diazotrophs could make important contributions to the nitrogen cycle in dune soils.

CONCLUSION

An aboveground fungal endophyte reduced AM fungal diversity and abundance and also altered how root-associated bacterial diversity and glomalin production responded to soil moisture. Most belowground responses to aboveground fungi varied among years, demonstrating context-dependency that may be caused by interannual variation in evapotranspiration and drought. Within the spatially heterogeneous, low nutrient, and high disturbance ecosystem of Great Lakes dunes, plant roots acted as an important resource for belowground microbes, increasing microbial diversity relative to that in the soil. Our work highlights the importance of examining aboveground microbes as factors that influence belowground microbes and sheds new light on above/belowground *microbial* interactions.

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Conflict of Interest

None to declare.

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Figures

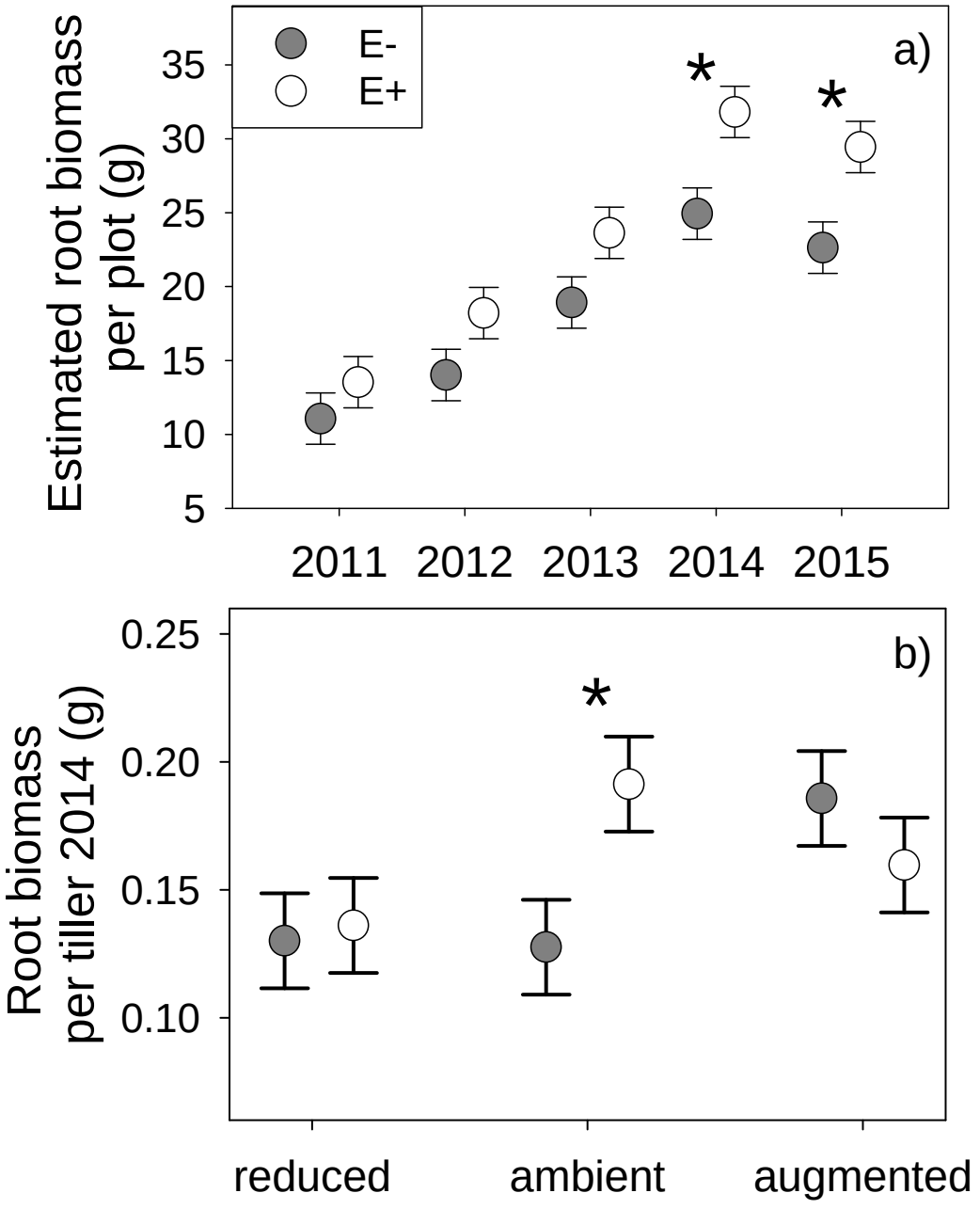

Figure 2.1. Root biomass a) estimated per plot (September tiller survey × per tiller root biomass in 2014) and b) per tiller in 2014 showing treatments with *Epichloë* (E+, open symbol) versus endophyte-free (E-, filled symbol). For b) precipitation treatments are 30% reduced (black circle symbol), ambient (grey triangle), or 30% augmented (dark square). "*" indicates $P < 0.05$ Tukey HSD test. Symbols show means ± s.e.

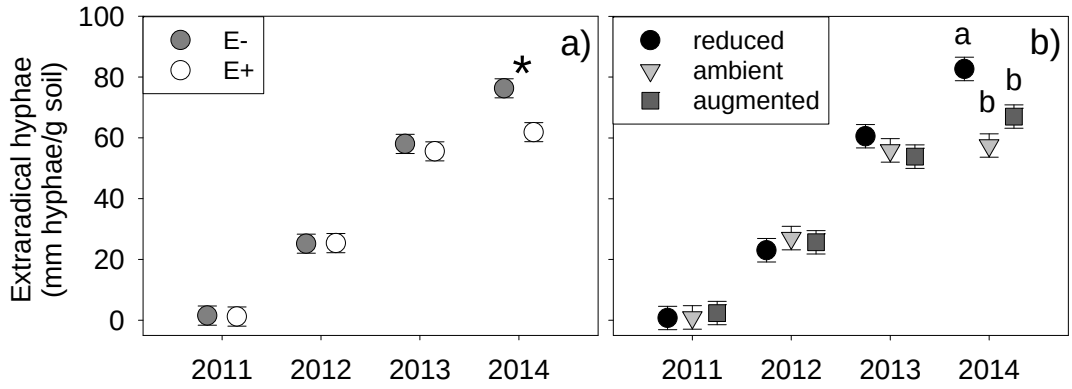

Figure 2.2. Extraradical hyphae (ERH) in soil (mm hyphae/g soil) from a) treatments with *Epichloë* (E+ open symbols) vs. endophyte free (E- filled symbols) and b) for precipitation treatments: 30% reduced (black circle symbol), ambient (grey triangle), or 30% augmented (dark square). "*" represents $P < 0.05$. Letters represent Tukey HSD significant differences between means ($P < 0.05$). Symbols show means ± s.e.

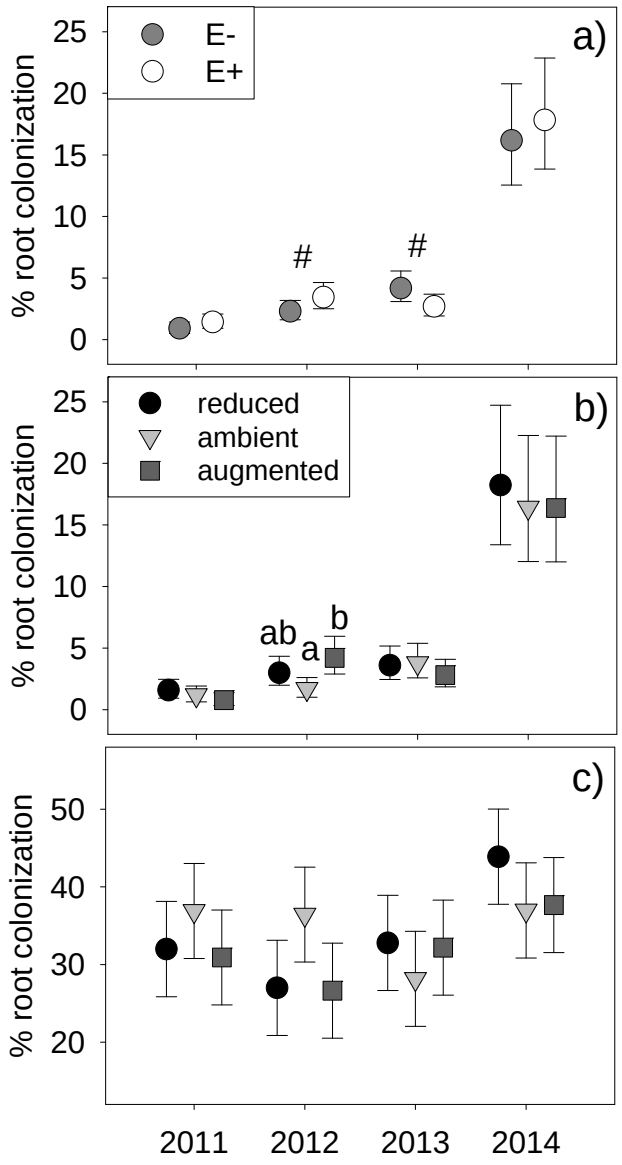

Figure 2.3. Perentage of root area colonized by hyphae ± 95% C.I. (not ± s.e.) of a) fine AM fungal morphotype in plot with *Epichloë* (E+ open symbols) and endophyte free (E- filled symbols), b) fine AM fungal morphotype under altered precipitation, c) and all AM fungal morphotypes under altered precipitation. For plots b)-c) precipitation treatments: 30% reduced (black circle symbol), ambient (grey triangle), or 30% augmented (dark square). Reported percentage area of roots colonized by the fine AM fungal morphotype are back transformed. Letters represent significant differences between means ($P < 0.05$, Tukey HSD). "#" shows $P < 0.10$ in Tukey HSD tests.

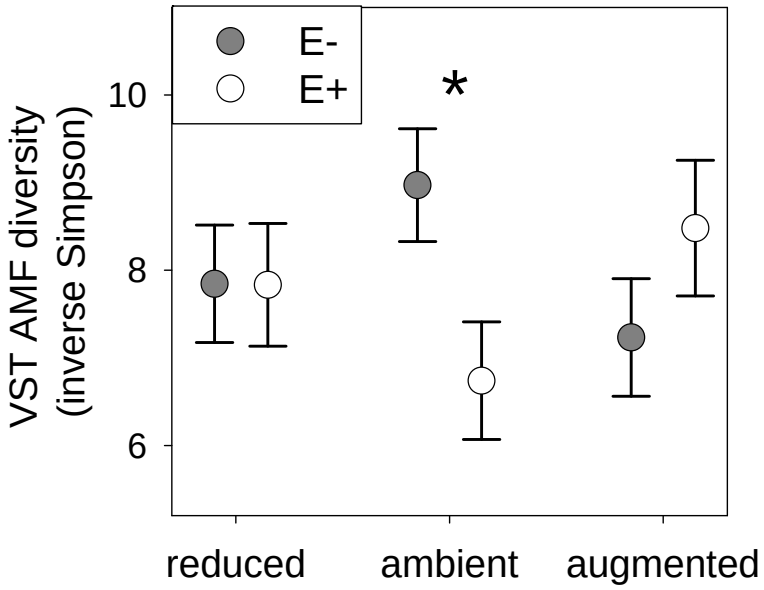

Figure 2.4. Inverse Simpson diversity of AM fungal sequences from variance stabilizing transformation (VST) normalized root communities from plots with 30% decreased (reduced), ambient, or 30% increased precipitation (augmented) with *Epichloë* (E+ open symbols) and endophyte free (E- filled symbols). "*" represents $P < 0.05$ Tukey HSD pairwise comparison. Bars show means ± s.e

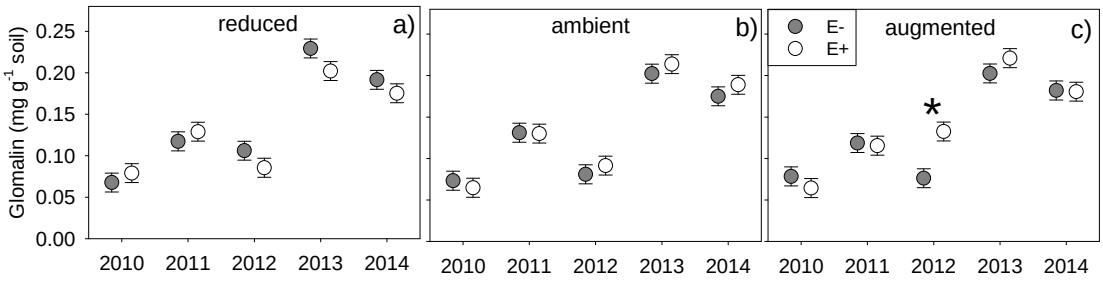

Figure 2.5. Glomalin in soils from plots with a) 30% decreased, b) ambient, or c) 30% increased precipitation with Epichloë (E+ open symbols) and endophyte free (E- filled symbols). "*" represents P < 0.05 Tukey HSD pairwise significance. Bars show means ± s.e.

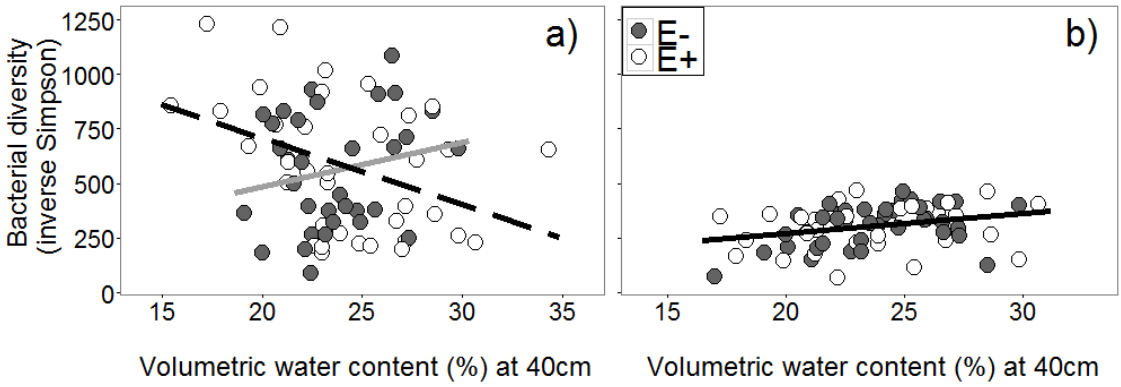

Figure 2.6. Linear regressions of soil moisture at 40cm versus a) root bacterial and b) soil bacterial inverse Simpson diversity from variance stabilizing transformation (VST) normalized communities. In plots with the *A. breviligulata-Epichloë* symbiosis, there was a negative correlation between soil moisture and bacterial OTU diversity (E+ open symbols and dashed line, $y = -30.68*x + 1321.01$, $R^2 = 0.14$). In plots without endophytes, there was a weak positive correlation between soil moisture and bacterial diversity (E- filled symbols and solid gray line $y = 20.18*x + 81.64$, $R^2 = 0.01$). Soil moisture was positively correlated with soil bacterial diversity (black line $y = 9.78*x + 70.83$, $R^2 = 0.11$).

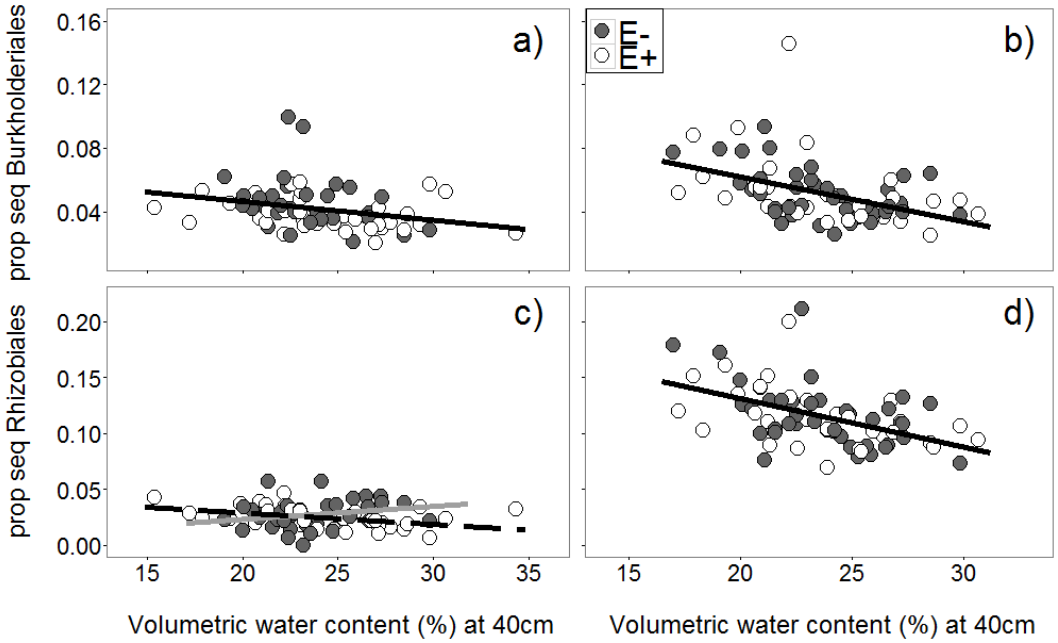

Figure 2.7. Linear regressions of soil moisture at 40cm versus relative abundance of variance stabilizing transformation (VST) normalized sequences composed of a) Burkholderiales in samples from roots, b) Burkholderiales from soils, c) Rhizobiales from roots, and d) Rhizobiales from soils associated with *A. breviligulata.* Endophyte presence (E+ open symbols and E- filled symbols) did not affect Burkholderiales abundance, but there was a positive correlation between soil moisture and the proportion of sequences composed of Burkholderiales (root: $y = -0.0012*x + 0.069$, $R^2 = 0.07$ and soil: $y = -0.0028*x + 0.12$, $R^2 = 0.19$). Soil moisture was negatively correlated with proportion of sequences composed of Rhizobiales (E+ white dots and dashed line $y = 0.0010*x + 0.049$, $R^2 = 0.17$), however, there was a positive correlation between soil moisture when the endophyte was absent (E- grey dots and solid gray line $y = 0.0012*x – 0.0016$, $R^2 = 0.025$). Soil Rhizobiales decreased with increasing soil moisture (black line: $y = -0.0043*x + 0.22$, $R^2 = 0.22$).

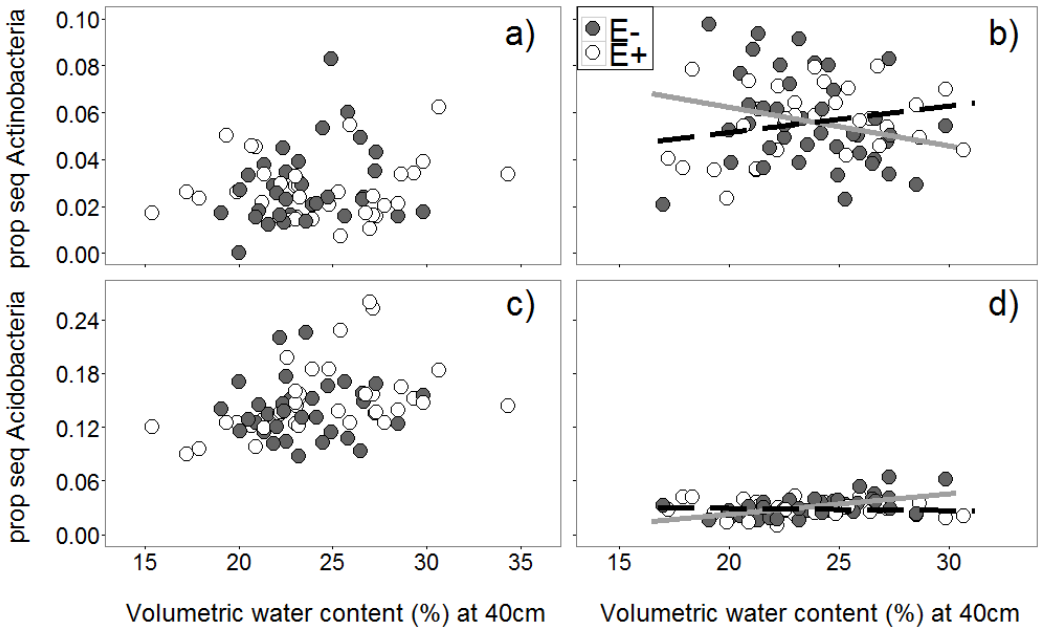

Figure 2.8. Linear regressions of soil moisture at 40cm versus relative abundance of variance stabilizing transformation (VST) normalized sequences composed of a) Actinobacteria in samples from roots, b) Actinobacteria in samples from soils, c) Acidobacteria from roots, and d) Acidobacteria from soils associated with *A. breviligulata.* In plots with the *Ammophila-Epichloë* symbiosis, there was a weak positive correlation between soil moisture and proportion of sequences composed of Actinobacteria (E+ open symbols and dashed line, $y = 0.0011*x + 0.029$, $R^2 = 0.035$). In plots without endophytes, there was a weak positive correlation between soil moisture and the abundance of Actinobacteria (E- filled symbols and solid gray line $y = -0.0017*x + 0.095$, $R^2 = 0.031$). There was no correlation between soil moisture and Acidobacterial abundance when the *Epichloë* symbiosis was present (E+ open symbols and dashed line, $y= 0.00027*x + 0.034$, $R^2 < 0.001$). When the endophyte is absent, there is a positive correlation between soil moisture and the portion of sequences composed of Acidobacteria (E- filled symbols and solid gray line $y= 0.0022*x - 0.022$, $R^2 = 0.30$).

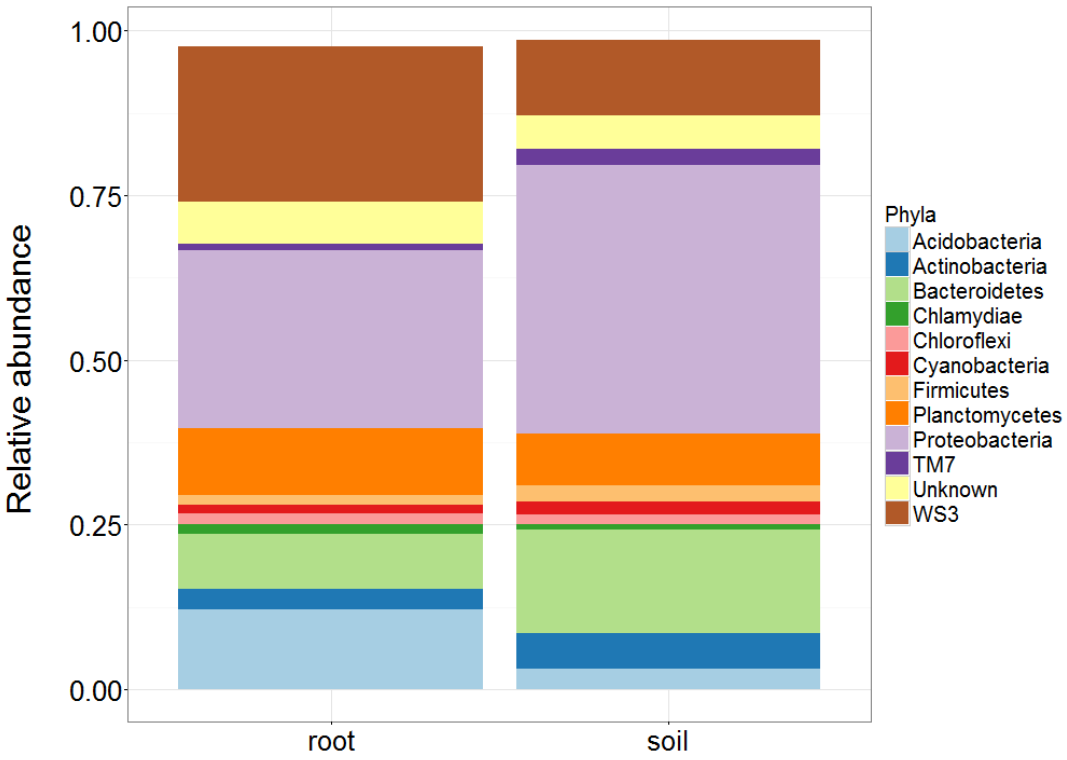

Figure 2.9. Relative abundance (variance stabilizing transformation (VST)) of reads in each of the dominant bacterial phyla for communities associated with *A. breviligulata* roots versus the surrounding soil matrix.

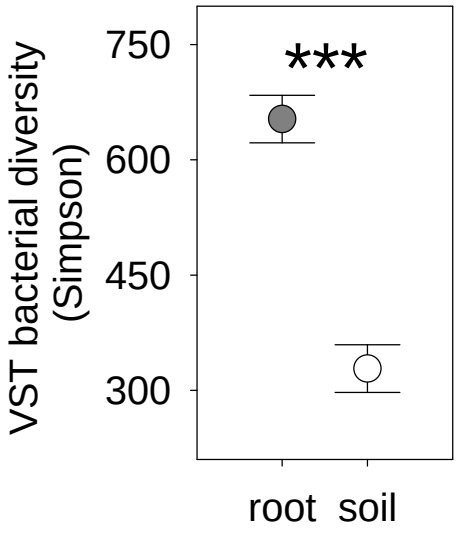

Figure 2.10. Bacterial inverse Simpson diversity in *A. breviligulata* from variance stabilizing transformation (VST) normalized communities in roots (filled symbols) and the surrounding soils (open symbols). "***" represents $P < 0.001$ significance. Bars show means ± s.e.

Supplemental Tables

Table S2.1. Numbers of samples and reads after sequencing, quality control, and filtering in arbuscular mycorrhizal (AM) fungal, root bacterial, and soil bacterial datasets.

		AM fungi	Root bacteria	Soil bacteria
		276,957 reads	287,684 reads	513,337 reads
		34 OTUs	4,998 OTUs	2,118 OTUs
Precipitation	Endophyte	Samples	Samples	Samples
Reduced	E+	11	12	9
	E-	12	12	14
Ambient	E+	12	12	12
	E-	13	9	12
Augmented	E+	9	12	9
	E-	12	12	15

Table S2.2. Models where spatial blocking factors did not significantly affect the response variable (Precipitation Models). Statistical results from mixed effects models examining the effects of endophyte presence (E+ or E-), precipitation (30% decreased, 30% increased or ambient) and year of collection, with plot as a random factor, on the estimate root biomass per plot, percentage area of roots colonized by all arbuscular mycorrhizal (AM) fungi hyphal morphotypes, fine AM fungal morphotype, soil glomalin concentration (mg per g of soil), soil extraradical hyphae [ERH mm hyphae/g soil)]. Significant factors are bolded.

	Root biomass per plot			AM fungal hyphae		Fine AM fungal hyphae	
Effect	*df*	X^2	*P*	X^2	*P*	X^2	*P*
Endophyte	1	**3.93**	**0.047**	2.36	0.124	0.82	0.366
Precipitation	2	0.31	0.146	1.32	0.517	1.66	0.437
Year	3	**99.49**	**<0.001**	**22.38**	**<0.001**	**335.30**	**<0.001**
Endo×Precip	2	0.28	0.112	0.63	0.730	1.57	0.455
Endo×Year	3	4.51	0.178	4.20	0.241	**8.45**	**0.038**
Precip×Year	6	14.91	0.909	12.3	0.055	**12.71**	**0.048**
Endo×Precip×Year	6	1.39	0.317	4.70	0.583	2.36	0.884

	Glomalin			ERH	
Effect	*df*	X^2	*P*	X^2	*P*
Endophyte	1	0.16	0.691	**4.69**	**0.030**
Precipitation	2	1.19	0.550	5.17	0.075
Year	3	**857.45**	**<0.001**	**1103.65**	**<0.001**
Endo×Precip	2	1.35	0.509	1.16	0.561
Endo×Year	3	2.78	0.595	7.42	0.060
Precip×Year	6	2.06	0.979	**19.74**	**0.003**
Endo×Precip×Year	6	**18.13**	**0.020**	7.88	0.247

Table S2.3. Models where spatial blocking factors did not significantly affect the response variable (Precipitation Models). Statistical results from general linear models testing endophyte (E+ or E-), precipitation (30% decreased, 30% increased or ambient) treatment effects on root biomass per tiller in 2014 and inverse Simpson diversity of the AM fungal community either variance stabilizing transformation (VST) or rarefied (reads = 500). Significant factors are bolded.

	Root biomass per tiller			AM fungi VST Simpson			AM fungi Rarefied Simpson		
Effect	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Endophyte	1,84	0.83	0.366	1,63	0.35	0.557	1,61	**5.31**	**0.025**
Precipitation	2,84	2.55	0.084	2,63	0.00	0.999	2,61	0.36	0.702
Endo×Precip	2,84	2.68	0.075	2,63	**3.31**	**0.043**	2,61	2.27	0.112

Table S2.4. Models where spatial blocking factors did not significantly affect the response variable (Precipitation Models). Statistical results from mixed effects models examining the effects of endophyte presence (E+ or E-), precipitation (30% decreased, 30% increased or ambient), and Location (root or soil), and plot as a random factor on inverse Simpson diversity of the bacterial community either variance stabilizing transformation (VST) or rarefied (reads = 1000), raw OTU richness, and proportion of sequences composed of Actinobacteria (see Methods). Significant factors are bolded.

		Bacterial VST Simpson		Bacterial Rarefied Simpson	
Effect	*df*	X^2	*P*	X^2	*P*
Endophyte	1	0.05	0.818	1.65	0.198
Precipitation	2	4.77	0.092	0.59	0.745
Location	1	**52.64**	**<0.001**	**415.24**	**<0.001**
Endo×Precip	2	0.16	0.923	3.58	0.167
Endo×Location	1	0.07	0.793	0.54	0.464
Precip×Location	2	3.74	0.154	2.44	0.295
Endo×Precip×Location	2	0.45	0.801	0.53	0.766

		OTU Richness		Actinobacteria	
Effect	*df*	X^2	*P*	X^2	*P*
Endophyte	1	0.07	0.794	0.02	0.899
Precipitation	2	4.76	0.093	1.76	0.415
Location	1	**38.11**	**<0.001**	**96.32**	**<0.001**
Endo×Precip	2	0.15	0.926	1.80	0.407
Endo×Location	1	0.06	0.799	0.02	0.889
Precip×Location	2	3.67	0.160	1.90	0.387
Endo×Precip×Location	2	0.52	0.772	0.36	0.834

Table S2.5. Models where spatial blocking factors had a significant effect on the response variable (Precipitation Models). Statistical results from PERMANOVA examining the effects of (E+ or E-), precipitation (30% decreased, 30% increased or ambient), as well as spatial position of plots in the dunes (RowBlock, ColumnBlock) on belowground community composition (arbuscular mycorrhizal (AM) fungi and root/soil bacteria) using Bray-Curtis (BC) and Weighted (WU) dissimilarity. Significant factors are bolded.

	BC AM Fungi			BC Root Bacteria			BC Soil Bacteria		
Effect	*df*	*Pseudo-F*	*P*	*df*	*Pseudo-F*	*P*	*df*	*Pseudo-F*	*P*
Endophyte	1,59	0.01	0.988	1,59	0.86	0.857	1,61	0.85	0.845
Precipitation	2,59	0.64	0.778	2,59	1.04	0.308	2,61	1.06	0.269
RowBlock	2,59	**3.34**	**0.003**	2,59	**2.07**	**<0.001**	2,61	**2.61**	**<0.001**
ColBlock	2,59	**2.19**	**0.030**	2,59	**1.47**	**0.006**	2,61	**1.40**	**0.009**
Endo×Precip	2,59	0.98	0.443	2,59	0.94	0.680	2,61	0.96	0.570

	WU AM Fungi			WU Root Bacteria			WU Soil Bacteria		
Effect	*df*	*Pseudo-F*	*P*	*df*	*Pseudo-F*	*P*	*df*	*Pseudo-F*	*P*
Endophyte	1,58	0.19	0.904	1,59	0.67	0.900	1,61	0.83	0.711
Precipitation	2,58	1.44	0.205	2,59	1.22	0.157	2,61	1.04	0.361
RowBlock	2,58	**3.16**	**0.011**	2,59	**2.38**	**<0.001**	2,61	**4.35**	**<0.001**
ColumnBlock	2,58	1.85	0.095	2,59	1.21	0.160	2,61	**1.48**	**0.019**
Endo×Precip	2,58	1.06	0.382	2,59	0.89	0.649	2,61	1.03	0.388

Table S2.6. Models where spatial blocking factors had a significant effect on the response variable (Precipitation Models). Statistical results from PERMANOVA examining the effects of endophyte presence (E+ or E-), precipitation (30% decreased, 30% increased or ambient), and Location (root or soil), plot as a random factor, and spatial position of plots in the dunes (RowBlock, ColumnBlock) on the bacterial community using Bray-Curtis (BC) and Weighted (WU) dissimilarity. Significant factors are bolded.

	Bacterial BC			Bacterial WU	
Effect	*df*	*Pseudo-F*	*P*	*Pseudo-F*	*P*
Endophyte	1,85	0.95	0.588	0.92	0.542
Precipitation	2,85	1.02	0.359	1.24	0.147
RowBlock	2,85	**2.24**	**<0.001**	**2.77**	**<0.001**
ColumnBlock	2,85	**1.47**	**0.001**	**1.59**	**0.019**
Location	1,85	**69.81**	**<0.001**	**89.08**	**<0.001**
Endo×Precip	2,85	1.00	0.474	0.93	0.584
Endo×Location	2,85	0.90	0.730	0.758	0.789
Precip×Location	2,85	1.16	0.091	1.02	0.407
Endo×Precip×Location	2,85	0.91	0.776	0.87	0.699
Plot	41,85	0.94	0.947	0.88	0.965

Table S2.7. Models where spatial blocking factors had a significant effect on the response variable (Precipitation Models). Statistical results from mixed effects models examining the effects of endophyte presence (E+ or E-), precipitation (30% decreased, 30% increased or ambient), and Location (root or soil), spatial position of plots in the dunes (RowBlock, ColumnBlock) and plot as a random factor on the proportion of sequences composed of Rhizobiales, Burkholderiales, Acidobacteria, and Actinobacteria (see Methods). Significant factors are bolded.

	Burkholderiales			Rhizobiales		Acidobacteria		Actinobacteria	
Effect	*df*	X^2	*P*	X^2	*P*	X^2	*P*	X^2	*P*
Endophyte	1	0.01	0.943	0.14	0.706	0.18	0.668	0.46	0.496
Precipitation	2	3.42	0.181	1.65	0.438	0.18	0.952	0.92	0.631
RowBlock	2	**31.86**	**<0.001**	**18.50**	**<0.001**	**16.22**	**<0.001**	2.43	0.297
ColumnBlock	2	0.85	0.654	3.83	0.147	1.17	0.557	4.57	0.102
Location	1	**31.86**	**<0.001**	**874.59**	**<0.001**	**1084.42**	**<0.001**	**91.82**	**<0.001**
Endo×Precip	2	1.29	0.524	4.84	0.089	1.49	0.474	2.20	0.333
Endo×Location	1	0.79	0.374	0.02	0.878	1.91	0.167	0.01	0.934
Precip×Location	2	4.18	0.124	1.30	0.521	5.56	0.062	180	0.407
Endo×Precip×Loc	2	3.01	0.222	0.16	0.924	1.33	0.514	0.54	0.762

Table S2.8. Models where spatial blocking factors had a significant effect on the response variable (Precipitation Models). Statistical results from mixed effects models examining the effects of endophyte presence (E+ or E-), precipitation (30% decreased, 30% increased or ambient), and year of collection, spatial position of plots in the dunes (RowBlock, ColumnBlock) and plot as a random factor on the volumetric water content (VWC) of soils at a depth of 40 cm. Significant factors are bolded.

	VWC40 cm		
Effect	*df*	X^2	*P*
Endophyte	1	0.42	0.518
Precipitation	2	**8.24**	**0.016**
RowBlock	2	**47.85**	**<0.001**
ColumnBlock	2	**7.87**	**0.018**
Year	3	**67.03**	**<0.001**
Endo×Precip	2	2.87	0.239
Endo×Year	3	1.61	0.447
Precip×Year	6	1.53	0.822
Endo×Precip×Year	6	3.81	0.432

Table S2.9. Models with volumetric water content as proxy for precipitation treatments and spatial blocking (Soil moisture Models). Statistical results from general linear models testing endophyte (Endo: E+ or E-) and volumetric water content of the soils at 40 cm (VWC 40) effects on inverse Simpson diversity calculated variance stabilizing transformation (VST) community matrices of root and soil bacteria, and proportion of root and soil community VST normalized sequences composed of Burkholderiales, Rhizobiales, Acidobacteria, and Actinobacteria. Significant factors are bolded.

	Root bacterial diversity VST			Root Burkholderiales		Root Rhizobiales		Root Acidobacteria		Root Actinobacteria	
Effect	*df*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Endo	1,65	**5.69**	**0.010**	2.54	0.116	**6.09**	**0.016**	1.63	0.207	1.00	0.321
VWC 40	1,65	0.24	0.629	**7.09**	**0.010**	2.78	0.100	3.21	0.078	2.73	0.103
Endo× VWC 40	1,65	**5.52**	**0.022**	1.86	0.177	**6.73**	**0.012**	1.98	0.164	1.13	0.291

	Soil bacterial diversity VST			Soil Burkholderiales		Soil Rhizobiales		Soil Acidobacteria		Soil Actinobacteria	
Effect	*df*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Endo	1,67	0.003	0.405	0.03	0.865	0.51	0.480	**10.97**	**0.001**	**4.11**	**0.047**
VWC 40	1,67	**6.21**	**0.015**	**17.11**	**<0.001**	**20.69**	**<0.001**	**7.63**	**0.007**	0.16	0.694
Endo× VWC 40	1,67	0.78	0.379	0.01	0.920	0.46	0.500	**12.41**	**0.001**	**4.14**	**0.046**

Table S2.10. Models with volumetric water content as proxy for precipitation treatments and spatial blocking (Soil moisture Models). Statistical results from PERMANOVA examining endophyte (E+ or E-) and volumetric water content of the soils at 40 cm (VWC 40) as well as spatial position of plots in the dunes (RowBlock, ColumnBlock) on belowground community (arbuscular mycorrhizal (AM) fungi and bacteria) composition using Bray-Curtis (BC). Significant factors are bolded.

	BC Root Bacteria			BC Soil Bacteria			BC AM Fungi		
Effect	*df*	*Pseudo-F*	*P*	*df*	*Pseudo-F*	*P*	*df*	*Pseudo-F*	*P*
Endophyte	1,65	1.20	0.124	1,67	0.84	0.849	1,65	0.61	0.670
VWC 40	1,65	**1.69**	**0.007**	1,67	**3.00**	**<0.001**	1,65	**3.85**	**0.010**
Endo×VWC40	1,65	1.23	0.107	1,67	0.86	0.791	1,65	0.57	0.698

Supplemental Figures

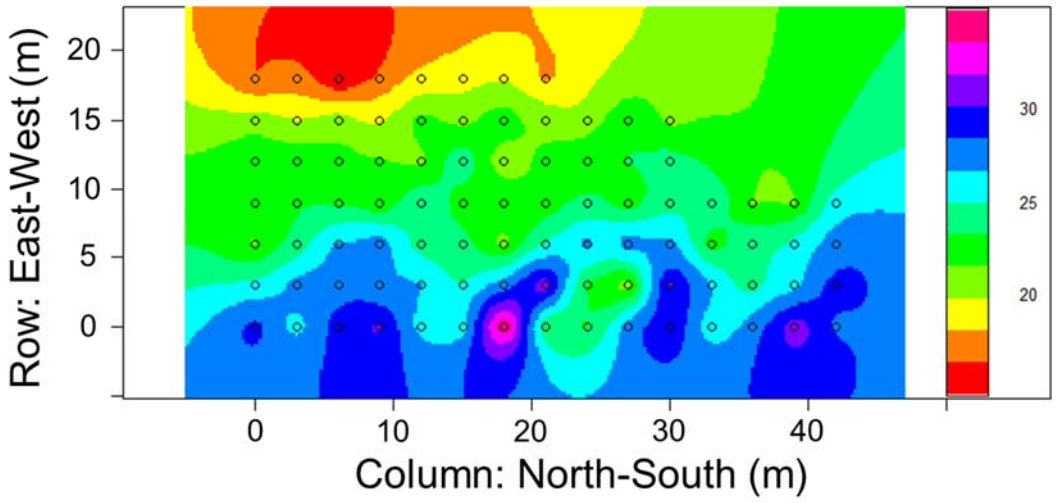

Figure S2.1. Kriging map of 2012 volumetric water content (%) of the soils at 40cm. Row runs vertically from lower interdune area (0m) to near the top of the dune (20m) and east to west. Column runs horizontally along the dune and north to south. Circles represent plots with the experiment.

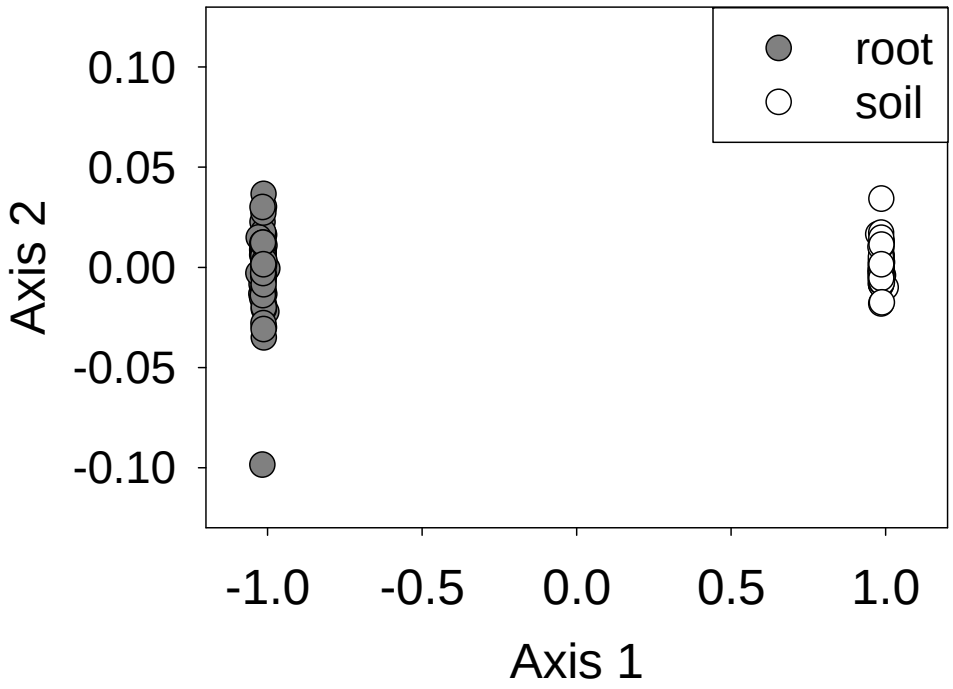

Figure S2.2. NMDS plot of bacterial communities in *A. breviligulata* roots (filled symbols) and the soil surrounding soils (open symbols).

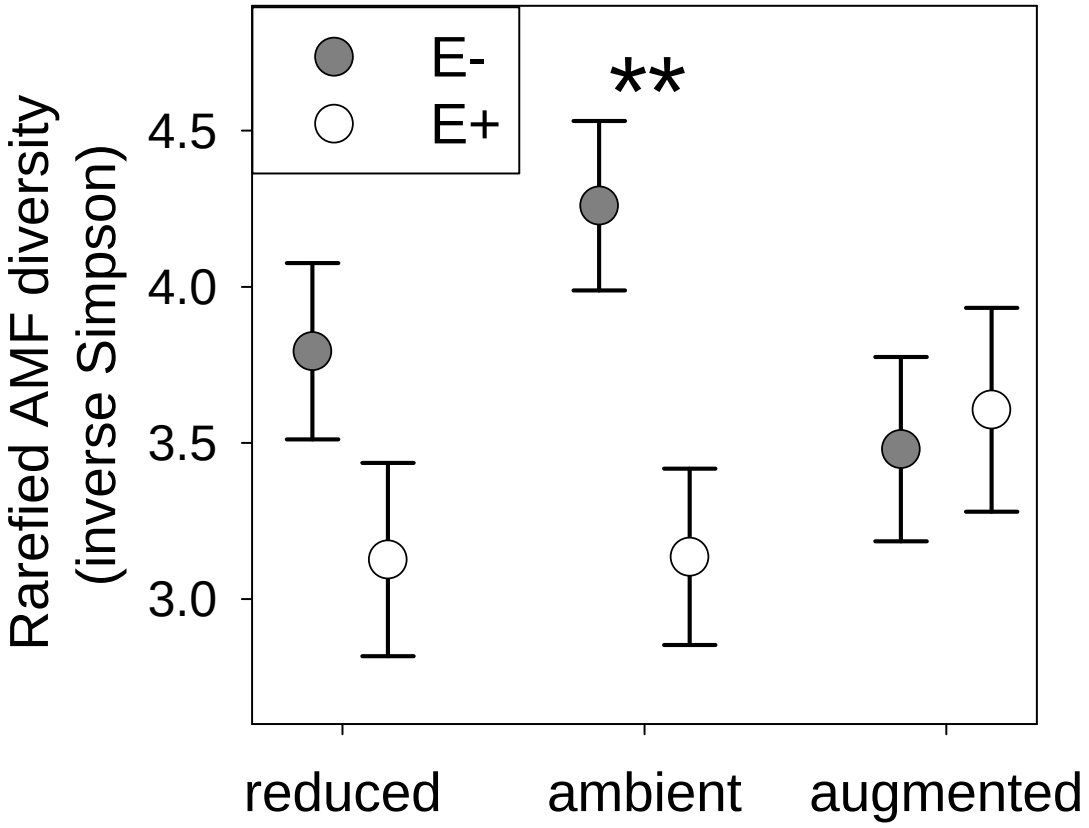

Figure S2.3. AM fungal inverse Simpson diversity of rarefied sequences (reads = 500) from roots samples from plots with 30% decreased (reduced), ambient, or 30% increased precipitation (augmented) with *Epichloë* (E+ open symbols) and endophyte free (E- filled symbols). "**" represents $P < 0.01$ Tukey HSD pairwise comparison. Bars show means ± s.e

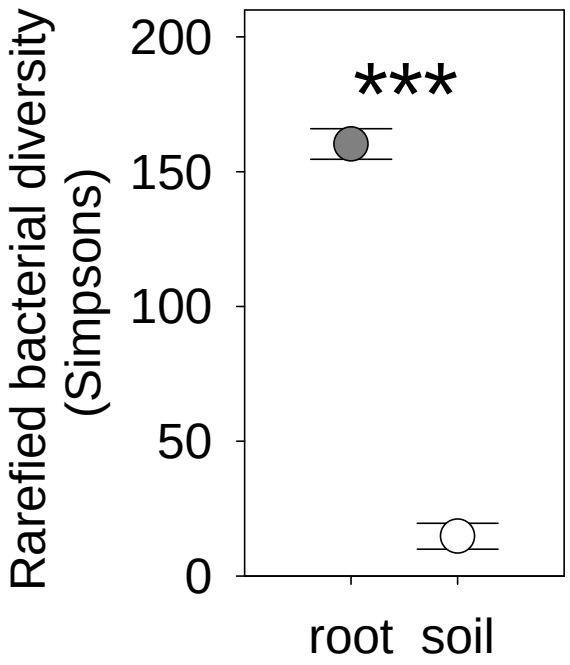

Figure S2.4. Bacterial rarefied (reads = 1000) inverse Simpson diversity in *A. breviligulata* roots (filled symbols) and the soil surrounding soils (open symbols). "***" represents $P < 0.001$ significance. Bars show means ± s.e.

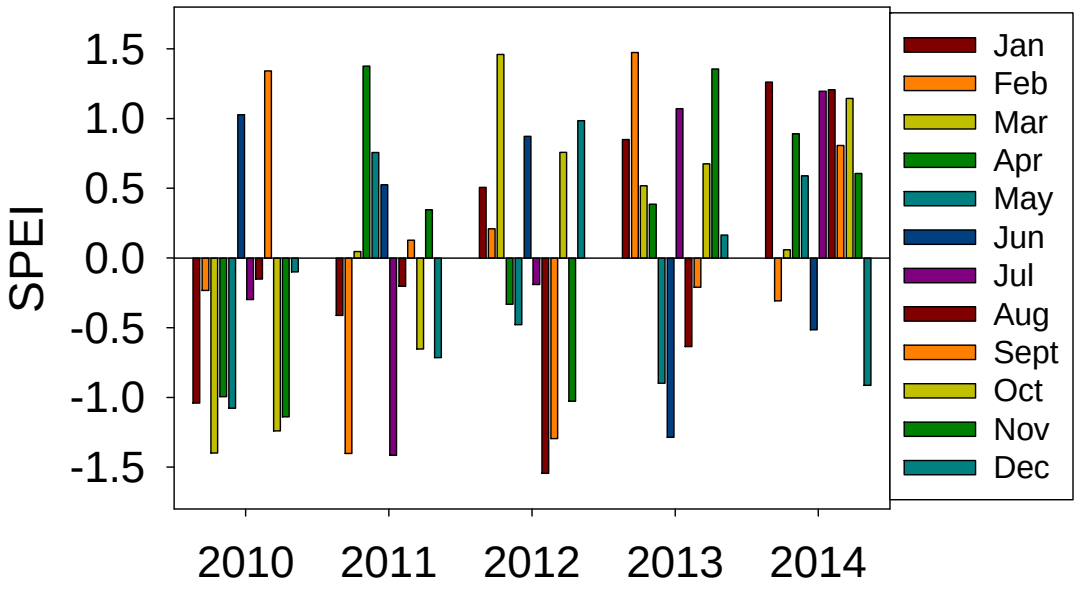

Figure S2.5. 2010-2014 Weather Data. Standardised Precipitation-Evapotranspiration Index (SPEI) calculated based on potential evapotranspiration estimated from the Thornthwaite (1948) equation for Northport, MI. Data from National Oceanic and Atmospheric Administration (NOAA)'s Global Historical Climatology Network-Monthly Summary for weather station 00206007 (http://www.ncdc.noaa.gov/cdo-web/search). SPEI was calculated using SPEI package (Vicente-Serrano *et al.*, 2010) in R.

Chapter 3

Interactions among plants are a stronger driver of plant community composition than microbial mutualism

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ABSTRACT

Many of the current theoretical underpinnings of plant community ecology are based on negative species interactions, mainly competition and herbivory. However, most plant species interact with mutualists at some stage of their life cycles, and these mutualistic interactions can shift competitive outcomes and alter community composition. If these interaction outcomes vary with climate, understanding the roles of species interactions in affecting community structure is fundamental to predicting ecological responses to climate change. Here we jointly manipulated mutualism with a fungal endophyte and plant-plant interactions to determine their relative importance in Great Lakes dune communities and test if outcomes shift under alternative precipitation regimes. Interspecific interactions had the strongest effects, increasing evenness of the overall plant community, by reducing the relative biomass of the dominant grass. Microbial mutualism decreased plant diversity overall, but increased subdominant community diversity in the final year of the experiment. Altered precipitation regimes had very little effect on the plant community, but increased precipitation overall reduced the strength of interspecific effects on the plant community. Consistent with previous studies of exploring the relative strengths of plant-plant interactions versus microbial

mutualisms, plant-plant interactions were the largest driver of community composition, though the microbial mutualism had strong effects on the subordinate community and modified the plant-plant interaction.

Key Words: *Epichloë*, interspecific competition, *Ammophila breviligulata,* symbiosis, context-dependent, intra-annual variation in precipitation

INTRODUCTION

Historically, plant community ecology has been dominated by the study of antagonistic species interactions, mainly competition and herbivory (Tilman 1993; Chesson 2000). Although mutualistic and positive species interactions can have similar, or even stronger, effects on plant communities (Clay & Holah 1999; Bastolla *et al.* 2009; Afkhami *et al.* 2013) than antagonisms, they are less well studied at the community scale (Bruno *et al.* 2003; Bulleri *et al.* 2016). Furthermore, ignoring the effects of mutualisms can lead to reduced ability to predict and model community composition and interaction.

Mutualism effects on community diversity can be negative or positive depending on the breadth of their association with members of the community. First, a mutualist can increase the competitive ability of its partner, leading to competitive exclusion of other species that compete for similar resources. For example, in temperate grasslands, nitrogen-fixation mutualisms with rhizobia increased the competitive supremacy of the dominant legume and depressed plant species diversity and evenness (Keller 2014). Alternatively, mutualisms can support foundation species that facilitate diversity, as in coral reefs (Bowen *et al.* 2016). For example, the loss of a keystone mistletoe species involved in a mutualistic interaction web caused cascading declines in diversity within

plant-animal seed-dispersal and pollination mutualisms (Rodriguez-Cabal *et al.* 2013). Understanding how mutualism affects community-scale patterns requires manipulating mutualism and competition jointly to determine the degree to which mutualism influences the outcome of competitive interactions, and indirectly alters species composition. Such factorial manipulations can also test the relative importance of competition versus mutualism in affecting community diversity and composition but, to our knowledge, have not been conducted in a field setting (but see Smith et al. 1999 for a manipulation of dominant species abundance × mutualism).

Most plant species interact with microbial mutualists during at least one life history stage (Bacon & White 2000; Smith & Read 2008). These interactions can have strong effects on pairwise plant-plant interactions – effects that are likely to scale-up to the community level (Van Der Heijden *et al.* 2003; Xiao *et al.* 2012). For example, ~80% of land plants associate with mycorrhizal fungi (Wang & Qiu 2006; Smith & Read 2008) which have received the most attention in studies of the effects of microbial mutualists on plant community structure (Scheublin *et al.* 2007; Wagg *et al.* 2011). Microbial mutualists, such as arbuscular mycorrhizal (AM) fungi, may reduce diversity if the dominant plants benefit more from the mutualism than subordinate species (e.g., Hartnett & Wilson 1999; Janoušková *et al.* 2011). Alternatively, microbial mutualists may promote plant diversity if mutualists promote niche differentiation among plant species or equalize fitness differences among plant species (van der Heijden *et al.* 1998; Collins & Foster 2009; Sabais *et al.* 2012).

Although understudied relative to AM fungi (Omacini *et al.* 2012), foliar fungal endophytes can also affect plant communities. Fungal endophytes in the family

Clavicipitaceae live within the above-ground tissues of ~20%–30% of grass species, as well as in morning glories, legumes, and sedges (Leuchtmann 1993; Schardl *et al.* 2004; Beaulieu *et al.* 2013). In the best studied example to date, plots sown with tall fescue grass symbiotic with an endophyte showed higher dominance of tall fescue, lower diversity of plants, and slowed succession compared with plots of endophyte-free tall fescue (Clay & Holah 1999; Rudgers & Clay 2007; Rudgers *et al.* 2007; Rudgers & Clay 2008). However, tall fescue is a non-native, agronomic grass, and the effects of fungal endophytes in native communities remain poorly understood (Cheplick & Faeth 2009). In one non-agronomic study, a native host-endophyte symbiosis increased plant diversity by inhibiting an invasive grass species (Afkhami & Strauss 2016). However, prior studies have not directly manipulated interspecific competition in combination with mutualism to compare their relative strengths or disentangle the indirect pathway of mutualism's effect.

A pressing issue in ecology is to understand how climate change influences species interactions and coexistence. Interactions can have indirect effects on species responses to climate change that either exacerbate or ameliorate the direct effects of a changing climate (Van der Putten 2012; He *et al.* 2013; Kivlin *et al.* 2013). For example, if climate warming increases the abundance of a competitor species, then another plant species may decline from the combined effects of increased competition plus the direct impacts of warming (Adler *et al.* 2011). Alternatively, beneficial species interactions could ameliorate the negative impacts of climate change (Bulleri *et al.* 2016). Relatively few studies have examined the roles of microbial mutualists in modifying plant species responses to climate change (Van der Putten 2012; Rudgers *et al.* 2015; Terrer *et al.* 2016). For example, while endophytic fungi can confer drought tolerance to host plants

(Kivlin *et al.* 2013), possibly improving performance in warmer, drier climates, little work has directly tested for shifting fungal endophyte benefits in the context of a future climate (but see Hunt *et al.* (2005) for CO_2 and Emery *et al.* (2015); Rudgers *et al.* (2015) for precipitation).

Here, we tested the relative importance and interactive effects of mutualism and interspecific interactions on plant community composition under climate change. We focused on a native ecosystem of conservation concern: freshwater dunes of the Great Lakes (Kost *et al.* 2007). The Great Lakes dunes offer a socioeconomically relevant and tractable system for studying the effects of climate change on drivers of plant community dynamics and plant succession. First, dunes provide the first line of defense against storm surge (Seabloom *et al.* 2013), important wildlife habitat for endemic species (Roche *et al.* 2010), and a locus for tourism and recreation. Second, the dune plant community has relatively low diversity (1–5 species/m^2, Cowles (1899)), and compositional changes are rapid (and thus, detectable) during the process of primary succession. Third, arid conditions characterize dune ecosystems (low soil water holding capacity) along with high aeolian disturbance regime (Cowles 1899; Lichter 1998, 2000). These conditions may make dune communities especially sensitive to the effects of climate change (Hellmann *et al.* 2010). Projected increases in the frequency of extreme rain events with future climates (reviewed in Easterling *et al.* 2000) may have particularly strong effects on the dune ecosystem and are relatively understudied in grasslands in general (Smith 2011; Knapp *et al.* 2015). We specifically asked: (1) What is the relative importance of microbial mutualism, interspecific plant-plant interactions, and precipitation regime as drivers of plant community diversity, composition, and productivity? (2) Does the

precipitation regime alter how microbial mutualism or interspecific interactions influence on plant community structure?

STUDY SYSTEM

In Great Lakes dunes, primary succession begins with colonization of bare sand by American beachgrass (*Ammophila breviligulata*, hereafter referred to as *Ammophila*). Following establishment of *Ammophila*, other grasses (e.g., *Calamovilfa longifolia*, *Schizachyrium scoparium*), forbs (*Asclepias syriaca*, *Cirsium pitcheri*), and woody shrubs (*Salix exigua*, *Artemisia campestris*) recruit within a few years. By the mid-successional stage (145 y), *Ammophila* has largely disappeared from the system, which eventually (beginning 225-440 y) becomes a hardwood forest. *Ammophila* individuals can host a seed-borne fungal endophyte [*Epichloë amarillans* (Belanger *et al. in review*) hereafter referred to as *Epichloë*], which grows systemically in leaves (Emery *et al.* 2010). Although natural *Ammophila* populations vary in the presence and prevalence of the endophyte, the symbiosis is very common in plant material used in restoration (Emery *et al.* 2010; Emery & Rudgers 2014). Effects of the endophyte on dune restoration success and dune succession remain unresolved because the community-scale consequences of unintentional endophyte introductions have not been determined. Based on this natural history, we made the following predictions for this system. 1) The endophyte will increase *Ammophila* biomass. 2) *Ammophila* will suffer more from interspecific competition than later successional plant species. 3) However, presence of the endophyte will lessen the negative influence of interspecific competition on *Ammophila*. Given these plant-plant dynamics, we predicted that 3) presence of the

endophyte in *Ammophila* would reduce the diversity and evenness of the remaining plant community, with 4) the strongest reduction occurring when plants had the strongest interspecific interactions.

METHODS

STUDY SITE

Our experiment was set-up in Sleeping Bear Dunes National Lakeshore Leelanau Co., Michigan, USA (44.941975°, -85.827510°) ~80 m from the Lake Michigan shoreline. Experimental plots were distributed between two former homesteads that were ~90 m apart and slated to be restored by the National Park Service (NPS). The houses were removed from the sites before 2001. The NPS removed vegetation and re-contoured sites Fall 2011; thus, sites had little to no vegetation prior to planting. Beginning summer 2012, we established a randomized 2×2×3 factorial design manipulating fungal endophyte presence in *Ammophila* (present = E+ or absent = E-), intra/interspecific plant interactions (Monoculture = only intraspecific interactions or Mixture = interspecific + intraspecific interactions), and precipitation regime (established July 2013: average, increased storm event frequency (High), or increased storm event size (Extreme)). Treatments were blocked by homestead site with 2/3 of the plots at the western homestead site and 1/3 at the eastern site. Each plot was 3m×3m and contained 36 *Ammophila* plants and 9 individuals each of five common dune plants spanning diverse plant functional types: *Asclepias syriaca* (Apocynaceae), *Calamovilfa longifolia*, *Elymus canadensis, Schizachyrium scoparium* (Poaceae), *Salix exigua* (Salicaceae), and (total of 81 plants per plot and 60 plots, Fig. 3.1).

TREATMENTS

Endophyte Treatment: To manipulate the endophyte, seeds of *Ammophila* were collected from endophyte-free parents at SBDNL (Emery et al. 2010). Endophytes were isolated from symbiotic *Ammophila*, grown on Petri plates, then inoculated into the meristem of half of the seedlings (*E*+) following (Leuchtmann & Clay 1988); the other half were sham-inoculated (*E*-). As plants matured, we cloned genotypes by gently separating tillers from the original stock plants. We planted 11 symbiotic *Ammophila* genotypes into every E+ plot, and 11 endophyte-free genotypes into every E- plot, closely matching plant genetic variation (3 genotypes m^{-2}) to naturally occurring levels (Fant *et al.* 2008).

Plant Interactions Treatment: This treatment is a modification of the experimental null model approach proposed by Goldberg (1994) and first inspired by Campbell and Grime (1992). This design separates the effects of interspecific versus intraspecific plant-plant interactions and allows measurement of the *community-level* response to plant-plant interactions (Goldberg 1994), which is lacking in the pairwise studies that constitute the bulk of existing work in this area. In the **Monoculture** treatment, all plant species were planted into a plot in conspecific patches (subplots) allowing for only intra-specific interactions (Fig. 3.1a). In the **Mixture** treatment, species were intermixed within each subplot at the same density as the Monocultures, allowing for both intra- and inter-specific interactions, but diluting intra-specific interactions relative to inter-specific (Fig. 3.1b). Experimental plants, with the exception of *Ammophila*, were propagated from seed (*A. syriaca*, *C. longifolia*, *E. canadensis, and S. scoparium*) and cuttings (*S. exigua*) by

Greystone Gardens (Honor, MI) during spring 2012. All species were represented by local genotypes collected from the community surrounding our plots. Plots were planted during June 2012 and watered evenly for one growing to season allow for establishment. Every other week during the growing season; we cut trenches between all subplots using a 42 cm drainage spade to eliminate belowground interactions between plant species in the Monocultures, and to control for the effects of disturbance in the Mixtures.

Precipitation Treatment: Our research focuses on the effects of precipitation on communities because water is one of the most limiting factors in the dunes (Lichter 2000). Furthermore, increased storm intensities could accelerate sand erosion and soil nutrient leaching (Jung *et al.* 2011; Klug *et al.* 2012) with detrimental effects on dune communities. Under the highest CO_2 emissions general circulation models, the Great Lakes region is expected to experience an increase in annual precipitation and an increase of 5 ^{o}C in annual temperature by 2070-2099 (Hayhoe *et al.* 2010). Regionally downscaled models from the Coupled Model Intercomparison Project Phase 5 (CMIP5) from IPCC Fifth Assessment Report (IPCC 2014) projections for precipitation range from decrease of 20% to an increase of 10% in summer precipitation and an increase of between 15% and 30% in spring precipitation to by the end of the century (RCP8.5, baseline from 1986-2005 http://www.cccsn.ec.gc.ca/?page=download-intro). Coarse spatial scale CMIP5 multimodel ensemble for the RCP8.5 scenario predict an increase of ~40% in days exceeding the 95 percentile of rain compared to a baseline of 1961-1990 (Sillmann *et al.* 2013). July 2013, we began precipitation treatments to mimic a projected 35% increase in precipitation during the growing season and the 49% increase in the

frequency of extreme rain events (>2 cm/day) based on regional climate models from the IPCC Fourth Assessment Report (IPCC 2007; Vavrus & Van Dorn 2010). The **High Precipitation** treatment received an additional 35% of the 30-year mean (1971–2000 Maple City, MI, http://www.ncdc.noaa.gov/cdo-web/search), ~11.46 cm total, applied in *weekly* increments of ~0.64 cm during the growing season (end of May– beginning of Sept) and testing the effects of an increase in the average precipitation over the growing season. The **Extreme Precipitation** treatment received a 35% precipitation increase in *monthly* increments of large (~2.87 cm) events testing the effects an increase in growing season precipitation occurring in large pulse rain events. The **Ambient Precipitation** treatment received natural rainfall. Water was pumped from Lake Michigan, stored in a 550 gallon tank, then amended via an in-line nutrient injector (D8R Dosatron, Clearwater, Florida) to match local rainfall chemistry by adjusting pH (4.8 to 5.3 monitored with Bluelab pH meter Tauranga, New Zeeland) using 99% citric acid powder (C□H□O□) and nitrogen content (1.4 ppm) using calcium nitrate (Soul Synthetics Grow-N (8-0-0) Aurora Innovations, Eugene, Oregon). Amended water was applied to each subplot using xeric sprayers (Rain Bird, Azusa, CA) on 30.5 cm risers (Fig 3.1c,d). Amendments were based on National Atmospheric Deposition Program's National Trends Network rainwater chemistry measurements from 2002-2009 from the nearest station (Peshawbestown, MI Site ID: MI29 http://nadp.sws.uiuc.edu/ntn/).

TREATMENT EFFECTIVENESS

To monitor the prevalence of in *Epichloë* in *Ammophila* plants in the plots, August 2016 we collected tillers from three *Ammophila* individuals from four replicates of each E+

plant interaction × precipitation treatment combination. We additionally monitored endophyte free plots by collecting tillers from three *Ammophila* individuals in four randomly chosen E- plots. Tillers were transported to the University of New Mexico on ice and stored at 4°C. Thin sections of the leaf sheath were removed from each tiller and stained with aniline blue (Clark *et al.* 1983). Leafs were scored at 100–400X on a light microscope (Leica Microsystems, Wetzlar, Germany) as described by Rudgers et al. (2009).

To monitor the effects of the our watering treatments on soil moisture, we installed soil moisture probes (10HS Decagon, Pullman, WA) at a depth of 20 cm in the center subplot of four replicate plots of each precipitation treatment split between the two sites. Readings were taken every 30 min throughout the growing season using a Hobo Micro station (H21, Onset, Bourne, MA). We tested for precipitation treatment effects on both the mean and coefficient of variation in soil moisture.

RESPONSE VARIABLES

Plant Community Responses: All plant communities were surveyed during peak biomass in early September 2013-2015. This project acts as high diversity restoration of formally degraded homesteads; therefore, the plots could not be destructively harvested based on permitting with SBDNL. Biomass was estimated using allometric equations derived from biomass harvests of extra plants that we grew outside of the plots for this purpose (Table S3.1). Diversity and evenness was calculated for each year using Pielou and Shannon-Weiner indices respectively on the estimated biomass of each experimental species per plot. Each indices was used to calculate the change from the community composition at

the beginning of the experiment (i.e. 2012). Since the loss of species from experimental plots was limited, *S. exigua* was lost from one Monoculture plot by 2014 and *C. longifolia* was lost from 12 Monoculture plots and two Mixture plots by 2015 (total 12 Monoculture and 2 Mixture plots lost a species), and changes in plant diversity responded similarly to changes in evenness, we focus on the effects of our treatments on changes in evenness.

Soil Nutrient Responses: A profile of 14 soil nutrients was assessed with plant root simulator (PRS, Western Ag Innovations, Saskatoon CA) ion exchange probes. Four sets PRS probes were installed during May 2013 and again during May 2014 in the same subset of plots each year. We focused on comparing soil nutrients between E+/E- and Ambient/Extreme precipitation treatments for Mixture plots only, with five replicates of each endophyte × precipitation treatment combination. Due to a difference in burial length (2013: 120 d and 2014: 85 d), nutrient measurements were standardized to rates per day. Copper, boron, and cadmium were below the detection thresholds and were not examined. Additionally, in 2013 probes from one plot were lost during transport.

Soil Moisture: To examine plot level soil moisture beyond our continuous measurement described above, we measured volumetric water content (VWC) at a depth of 20 cm and 40 cm June 2013, June 2014, and July 2014 in *Ammophila* subplots of each Monoculture treatment. We used an M300 soil moisture meter (Aquaterr Instruments & Automation, Costa Mesa, CA). Soil moisture was averaged across subplots within each plot and the growing season for each year.

STATISTICAL ANALYSES

We analyzed the change [each year's peak biomass – biomass at the beginning of the experiment (i.e. June 2012)] in plant community evenness (Pielou), and total productivity both with and without *Ammophila* in the calculation to give inference on the whole community response as well as the separate response of the subordinates, which were not directly engaged in the fungal endophyte mutualism. We also examined the change in biomass of each species individually. All analyses were general linear mixed effects model containing the fixed effects of our three treatments: plant interactions, endophyte presence, and precipitation, as well as the year of sampling. We also analyzed the effects of the endophyte and precipitation treatment on soil moisture at 20 cm and 40 cm in *Ammophila* Monoculture subplots. Homestead site (east or west) was included as a categorical blocking factor. Models included all interactions among treatments and year, as well as plot as a random grouping factor. Analyses were implemented in the lme4 package (Bates *et al.* 2015) in R (R Core Team 2016). To meet assumptions of Gaussian distributions of errors and homogeneity of variances, we square root-transformed change in plant community productivity, individual species biomass, and VWC at 40 cm. Since we were interested in the effect of the microbial mutualist and altered precipitation on the strength of plant-plant interactions, we conducted post-hoc independent contracts between Monoculture and Mixture treatments for each level of the endophyte and precipitation treatments.

Change in plant community structure, both with and without *Ammophila* included, was analyzed using permutational analysis of variance (PERMANOVA) on Euclidean distance matrices using a model containing plant interactions, endophyte, and precipitation treatments, the year of sampling, and plot as a random factor nested in endophyte × interaction × precipitation × site. PERMANOVA was implemented in Primer V6 (Clarke & Gorley 2006). In addition, within each year, we used metaMDS to calculate ordination coordinates for non-metric multidimensional scaling (NMDS) plots used for visualisation of the community composition using the vegan package (Oksanen *et al.* 2016) implemented in R (R Core Team 2016). To examine the effects of our treatments on beta diversity, we used PERMDISP to calculate the the average distances from the centroid on the Euclidean distance matrices with pariwise tests of significance in Primer V6. A Euclidean distance matrix was constructed for total nitrogen and ten other soil nutrients after each nutrient was separately z-scored to weight nutrient responses equally, controling for differences in the magnitude of nutrient measurements (i.e. calcium made 100X more the ions captured than the next most abundant nutrient). The nutrient profile was then analyzed using PERMANOVA to test the effects of the endophyte and precipitation treatments (only Ambient vs. Extreme), year of sampling (2013 or 2014), homestead site as a blocking factor, and plot as a random, nested in endophyte × precipitation × site, using Primer V6. SIMPER analysis was used to determine which nutrients contributed most to differences between treatments. In addition, within each year, ordination coordinates were calculated using metaMDS for constructing NMDS plots visualisation of the and *P*-values and R^2 for each factor were calculated using adonis in the vegan package implemented in R.

RESULTS

Treatment efficacy

In 2016, the average endophyte frequency per plot was 93% ± 4.7% SE in E+ plots and 4% ± 3.6% SE in E- plots. Across all three years, our precipitation treatments resulted in a 10% increase in daily soil moisture under weekly watering (High) and 5% increase in daily soil moisture under monthly watering (Extreme) compared to the ambient treatment. Additionally, the High treatment increased coefficient of variance (CV) in daily soil moisture by 9% and the Extreme precipitation treatment increased CV in daily soil moisture by 16% compared to ambient precipitation (Fig. S3.1).

(1) What is the relative importance of microbial mutualism, interspecific plant-plant interactions, and precipitation regime as drivers of plant community diversity, composition, and productivity?

Of the three treatments, interspecific interactions had the strongest effect on plant community evenness and composition. Over the course of the experiment, evenness generally declined over time. The presence of interspecific interactions reduced the loss of evenness in the community by 42% relative to communities with only intraspecific interactions (community $X^2 = 30.07$, $P < 0.001$) although effects were strongest in 2014 year (community × year $X^2 = 8.06$, $P = 0.018$; Fig. 3.2a). Consistent with the effects on evenness, interspecific interactions had a strong effect on community composition (community $PseudoF = 11.95$, $P < 0.001$) that increased with time (community × year $PseudoF = 8.34$, $P < 0.001$; Table S3.4; Fig. 3.3a,c,e). Contrary to the strong effects of

interspecific interactions on community evenness, there was no change in total productivity of the plant community ($P > 0.3$; Fig. 3.4a). As we expected for a primary successional species, *Ammophila* biomass decreased by 41% when in Mixtures than when competing with only conspecifics, and this effect of interspecific interactions became stronger each year (community × year $X^2 = 15.01$, $P < 0.001$; Table S3.2; Fig. 3.4b). This decreased *Ammophila* growth when competing with other species was the main driver of increased evenness in plots with interspecific interactions.

Endophyte presence had the second largest effect on the plant community by increasing the loss of plant evenness by 34% (endophyte $X^2 = 8.99$, $P = 0.003$; Fig. 3.2a) and increasing the dominance of *Ammophila*. Additionally, endophyte presence had a strong effect on community composition (endophyte *PseudoF* =8.58, $P = 0.003$) with the strongest effect in 2014 and weakest effect in 2013 (endophyte × year *PseudoF* = 4.03, $P = 0.011$, Fig. 3.3a,c,e). Endophyte presence had stronger effects on biomass of the dominant (*Ammophila*) than did altering interspecific interactions by increasing the change in biomass by 59% compared to endophyte free plots (endophyte $X^2 = 10.26$, $P = 0.001$). On the other hand, the endophyte increased the negative effects of competition on the growth of *Ammophila* (Fig. 3.4b). Since the endophyte is restricted to *Ammophila*, and the host plant was sown at the highest relative density (36 of the 81 individuals in each plot), endophyte effects on the community evenness were unsurprisingly driven by changes in host biomass. Specifically, the negative effect of endophyte presence on evenness was strongest in 2013 and 2014, when the endophyte increased the loss of plant evenness by 77% and 50% respectively (Fig. 3.2a), corresponding with the years where endophyte increased *Ammophila* change in biomass the most compared to endophyte free

plots (2013: 97% and 2014: 65%; Fig. 3.4b). The endophyte decreased the positive effects of interspecific interactions on plant community evenness compared to endophyte free communities (Fig. 3.2a). Additionally, endophyte presence tended to increase the plant community productivity (endophyte $X^2 = 3.16$, $P = 0.075$), though this was driven by the *Ammophila* biomass in plots with only intraspecific interactions (Fig. 3.4a). However, the endophyte-driven decline in plant evenness was outweighed by variability among plots and became non-significant by the final year of experiment (Fig. 3.2a), suggesting the possibility that endophyte effects shift during plant succession. In support of possible shifting effects of the endophyte on the plant community, endophyte presence increased beta diversity by 52% in 2015 ($t_{28} = 2.381$, $P = 0.029$) though the effect was strongest when only intraspecific interactions were present (Fig. 3.2b).

Interspecific interactions increased the loss of subordinate evenness by 27% and altered the composition of the subordinate community, with the effects becoming stronger with time (evenness: community × year $X^2 = 7.27$, $P = 0.026$, Fig. 3.2c; community composition: community × year $PseudoF = 5.23$, $P < 0.001$; Table S3.4; Fig. 3.3b,d,f). On the other hand, interspecific interactions increased the biomass of the subordinate community by 84% and this effect became stronger over the course of the experiment (community × year $X^2 = 14.78$, $P < 0.001$; Fig. 3.4c). Endophyte presence decreased the loss of evenness in the subordinate plant community, in contrast to the endophyte-driven decline in whole plant diversity, but this effect occurred only during the final year. In 2015, the loss of subordinate evenness was 20% lower in E+ than in E- plots (endophyte × year $X^2 = 6.72$, $P = 0.035$; Table S3.2). This positive effect of the endophyte on subordinate community reduced the negative effects of interspecific

interactions on subordinate evenness (Fig. 3.2c). However, endophyte presence tended to reduce the biomass of the subordinate community in 2014 (endophyte × year $X^2 = 4.65$, $P = 0.098$) by reducing the positive effects of interspecific interactions (Fig. 3.4c). This endophyte-driven increased subordinate evenness, with associated decrease in biomass, occurred primarily because *S. scoparium* biomass tended to increased more slowly when the endophyte was present (endophyte × year $X^2 = 8.30$, $P = 0.016$) with the effect being the strongest in 2014 (Fig. 3.4d), with corresponding slight increases in the biomass of other plant species (Fig. 3.5 and S3.2). When *Ammophila* was removed from the analysis, endophyte presence did not affect community composition ($P > 0.25$); though in 2014 endophyte presence reduced the beta evenness of the subordinate community by 30% compared to endophyte free plots ($t_{28} = 2.375$, $P = 0.046$; Table S3.3; Fig. 3.2d and 3.3d).

Precipitation had little to no effect on plant community evenness and composition ($P > 0.30$; Table S3.2 and S3.4; Fig. 3.6a and S3.3). On the other hand, the effects of precipitation on community productivity varied interannually, with productivity tending to increase with higher average growing season precipitation, but slightly decline with large pulses in precipitation (precipitation × year $X^2 = 12.11$, $P = 0.017$ Fig. 3.7a). This change in productivity was primarily driven by the effects of altered precipitation on *Ammophila* biomass; which, tended to be higher under higher average precipitation, but slightly declined under large pulsed rain events (precipitation × year $X^2 = 13.05$, $P = 0.011$; Table S3.2; Fig. 3.7b). Along these same lines, in 2013 large pulses of rain decreased the beta evenness of the plant community by 36% compared to plant receiving only ambient precipitation (Table S3.3; Fig 3.6b and S3.3a)

Both soil nutrients and soil moisture responded strongly to the endophyte and the precipitation regime. Under interspecific interactions (Mixtures), both large pulses of precipitation and endophyte presence altered the profile of plant-available nutrients in dune soils. Large rain had the strongest effect on the soil nutrient profile (PERMANOVA: precipitation $PseudoF = 3.27$, $P = 0.008$; Fig. S3.5c,d). Phosphorus and zinc were 35% and 25% lower while sulfur was 81% higher under extreme rain events compared to ambient precipitation, as indicated by SIMPER analysis, accounting for 30% of the cumulative difference between precipitation treatments (Table 3.S6). Endophyte presence also shifted the soil nutrient profile (PERMANOVA: endophyte $PseudoF = 2.52$, $P = 0.047$; Fig. S3.5a,b) with magnesium, calcium, and total nitrogen all declining by 25%, 21% and 27%, respectively, in the presence of the endophyte, as indicated by SIMPER analysis, accounting for 30% of the cumulative difference between precipitation treatments (Table S3.6). Endophyte presence increased soil moisture at 20 cm depth by 14% compared to endophyte free Monoculture plots ($X^2 = 4.75$, $P = 0.029$) though it had no effect on soil moisture at 40 cm (Table S3.7; Fig. S3.6).

(2) Does the precipitation regime alter how microbial mutualism or interspecific interactions influence on plant community structure?

Plant community responses to precipitation suggested that subordinate plant species compete with each other for water. The precipitation regime did not alter the effects of the microbial mutualist on the plant community ($P > 0.40$). Instead, precipitation effects on changes in plant evenness, excluding *Ammophila*, depended on the presence of the interspecific interactions as well as on the year of observation (community × precipitation × year $X^2 = 10.22$, $P = 0.037$; Table S3.2). In 2015, extreme precipitation events

decreased the loss of subordinate evenness by 38% compared to ambient precipitation only when interspecific interactions were permitted (Fig. 3.6c). This increased evenness under extreme precipitation was largely due to lower biomass of *S. scoparium* (Fig. 3.7d, 3.8, and S3.4). Additionally, the effects of interspecific interactions were the strongest under ambient precipitation across nearly all response variable measured (Fig. 3.6 and 3.7).

DISCUSSION

Using our novel experimental design, we compared the strengths of interspecific interactions, microbial mutualism, and precipitation regime as drivers of plant community composition. Interspecific interactions were the strongest driver of plant community composition and productivity, although the microbial mutualism also had strong effects on community composition. Relatively few field studies directly compare the strength of competition to microbial mutualism for plant communities, although arbuscular mycorrhizal (AM) fungi have received the most attention (Klironomos *et al.* 2011). Most studies on AM fungi were conducted in the greenhouse and have found that outcomes are dependent on the level of AM fungal dependence among members of the focal plant community (Lin *et al.* 2015). One of the first studies to compare the relative strength of competition versus microbial mutualism in the field, found that the removal of the two facultative mycotrophic dominant grass species (accounting for >80% of the cover) increased the diversity and growth of the subordinate community more than AM fungi exclusion alone (Smith *et al.* 1999). A recent neighbor removal study found that the relative importance of competition and symbiotic fungi on species competitive responses

depended on life-stage of the individual. Overall plant-plant interactions had a stronger effect on target plant growth, but the effects of neighbor presence shifted from slightly positive during the seedling stage to strongly negative during the adult stage (Bennett & Cahill 2016). Both of these studies used removal to test competitive interactions and fungicide to reduced AM fungal abundance, both techniques may have non-target effects on the plant and soil community (Goldberg & Barton 1992; Allison *et al.* 2007). A general conclusion that seems to be emerging from our studies and others is that interspecific interactions are typically a stronger driver of plant community composition than are microbial mutualists.

Although the negative effects of interspecific competition have been the focus of most of the past research in plant communities, in the nutrient poor, high disturbance dune ecosystem studied here, interspecific plant-plant interactions were less limiting for the subordinate plant species than were intraspecific interactions. Our modified experimental null model approach allowed us to evaluate intra- versus inter-specific interactions at the community scale (beyond pairwise plant interactions) and avoided the potential pitfalls of dominant species removal experiments, which is limited to testing competitive effects of dominants on the rest of the plant community (Goldberg & Barton 1992). Modern species coexistence theory predicts that species within a community can coexist if they are more limited by intra- than inter-specific interactions (Chesson 2000). Given the relatively positive effects of interspecific interactions on our subordinate plant community, coexistence among these species is likely. On the other hand, the community dominant, *Ammophila,* was more limited by inter- than intra-specific interactions, leading to the prediction that it ultimately will be competitively excluded. The potential decline

of *Ammophila* and subsequent increase in other species abundance is consistent with the typical successional trajectory in Great Lakes dune communities (Olson 1958; Lichter 1998). Although our study was not designed specifically as a test of coexistence theory, it could be complemented by future work that alters plant density directly testing the density dependent effects of intra- versus inter-specific competition necessary for predicting coexistence (Chesson 2000). For example, response surface designs that alter densities of competing plant species have been used recently to examine the role of plant-soil feedbacks in plant coexistence (Chung & Rudgers 2016) and could be used to test endophyte effects on species coexistence. The increasing effect size of interspecific interactions over time, as the community filled in and increased in density, suggests that the density-dependent effects of interspecific interactions may be less limiting than density-dependent intraspecific interactions, promoting species coexistence.

Prior studies of the effects of endophytes on plant communities have largely ignored how the endophyte modifies the strength of plant-plant interactions and focused on instances when both intra- and interspecific plant interactions are present. When interspecific interactions were present, the endophyte decreased plant diversity, a result that mirrors past studies in both managed/unmanaged and non-native/native ecosystems. The best studied plant-endophyte system is an introduced forage grass, for which presence of the endophyte decreased plant diversity and productivity of the subordinate plant community (Clay & Holah 1999; Rudgers & Clay 2008). In another agronomic grass species, endophyte presence decreased the richness and abundance of co-occurring weed species (Saikkonen *et al.* 2013). An exception to these negative effects of endophytes on plant diversity is work by Afkhami and Strauss (2016) who found that

endophyte presence *increased* the diversity of the plant community by reducing biomass of an invasive grass species in the same genus as the endophyte host grass. The authors hypothesized that a native grass-endophyte symbiosis increased the competitive response of a dominant invasive grass, although this hypothesis was not tested directly (Afkhami & Strauss 2016). In our system, the effects of both interspecific interactions and microbial mutualism on community composition occurred primarily through increased relative abundance of *Ammophila*, the most dominant plant species and the only host for the focal endophyte. Consistent with our previous research in this system, the endophyte symbiosis increased the biomass of *Ammophila* (Emery *et al.* 2010; Emery & Rudgers 2013; Emery *et al.* 2015). However, the endophyte lead the host to be more sensitive to the negative effects of interspecific competition contrary to tall fescue studies (Yurkonis *et al.* 2014). Increased biomass of the host plant is likely to be the primary driver of the negative effect of endophyte presence on plant diversity (Clay & Holah 1999; Saikkonen *et al.* 2013). On the other hand, reduced mammalian and insect herbivory, trophic interactions (reviewed in Rudgers & Clay (2007)), altered soil microbial communities (Matthews & Clay 2001; Rudgers & Orr 2009), and/or allelopathy (Orr *et al.* 2005) are possible alternative mechanisms for negative effects of endophytes on plant diversity. Importantly, other than Afkhami and Strauss (2016) and our work on *Ammophila* (this study and Rudgers *et al.* (2015)), all other prior tests of endophyte effects on communities have used agronomic and/or non-native host-endophyte symbioses.

If the endophyte plays a large role in altering community composition, then we would expect endophyte effects to be stronger when interspecific effects are present. Endophyte presence increased evenness of the subordinate plant community in 2015 by

19% overall and the effect was strongest when plant species were allowed to interact. This result suggests that the effect of the endophyte (beyond changes in host plant biomass) may become stronger over time or in particular years. Underlying this increased evenness is the suppression of *S. scoparium*, which is one of two grass species that becomes the dominant (reaching >15% cover by ~150yo) as *Ammophila* begins to decline. Additionally, by the end of the experiment *S. scoparium* was the second most dominant species in our community and showed the great increase in biomass when competing with other plant species. Therefore, the endophyte driven reduction in the *S. scoparium* growth led to a decrease in the biomass of the subordinate community and suggests that the endophyte may be altering the successional trajectory of the community. A possible mechanism for this effect is altered soil nutrients and moisture, specifically a reduction in soil nitrogen and an increased soil moisture when the endophyte was present. Thus, endophyte presence in the dominant plant species may alter interspecific resource competition among subordinate plant species. In a companion experiment examining the effects of *Ammophila-Epichloë* symbiosis on plants that naturally colonized during primary succession, we found an endophyte-mediated reduction in the diversity of plant species that recruited into plots (Rudgers *et al.* 2015). Since the present experiment used mature individuals for the subordinate community, the observation of opposing effects of mutualism in our two experiments suggests the endophyte may have divergent effects during different life history stages within the dune plant community.

Altered precipitation regime had few effects on the plant community, but overall reduced the strength of interspecific interactions with altered plant-plant interactions most apparent in evenness and beta diversity in the final year. A lack of strong effects of

increased precipitation is consistent with previous studies in tallgrass prairies (Collins *et al.* 2012) and desert grasslands (Collins *et al.* 2016). Although, strong effects of altered intra-annual precipitation regimes on plant communities and ecosystem services have been recorded in tallgrass prairies (Knapp *et al.* 2002), the majority of studies find that grassland are resilient to effects of intra-annual variability in precipitation (Unger & Jongen 2015). Additionally, the effects of altered precipitation regimes on plant community compositions may depend strongly on the length of the experiment (Jones *et al.* 2016) or pulse dynamics such as fire disturbance (Collins *et al.* 2016). Increased precipitation in our system seems to reduce the strength of interspecific interaction. The largest driver of the subordinate community's response was the growth of *S. scoparium*, which had the greatest biomass when competing with other plant species under ambient precipitation. These large precipitation events increased the evenness of the other subordinate plant species when interspecific interactions were present. The large precipitation events may have alleviated some of the negative effects of resource competition through increased soil moisture and nutrients such as sulfur. On the other hand, large rain events decreased the amount of soil phosphorus, which may have been a limiting factor for *S. scoparium*. Overall, the dune plant community seems resilient to future variation in intra-annual precipitation regimes.

Observed shifts in the composition of dune plant communities provide an important result for restoration efforts in Great Lakes dunes, suggesting alternative strategies depending on management goals. If a rapid increase in the diversity of the plant community is the restoration goal, managers should establish communities with species spatially interspersed to increase evenness of the subordinate community relative to that

of *Ammophila*. However, if the goal of a restoration effort is to increase the productivity of the entire community, increase sand accretion, and accelerate dune building, then planting *Ammophila* with the endophyte in mostly monoculture would be a better choice (Maun & Lapierre 1984; Emery *et al.* 2015).

CONCLUSION

Plant-plant interactions were the strongest drivers in the plant community, but both the microbial mutualist and the precipitation regime altered the strength of interspecific interactions. Subordinate plant species experienced stronger negative effects of intra- than inter-specific interactions, whereas the reverse occurred for the dominant dune plant. Furthermore, contrary to our expectations, the presence of the microbial mutualist increased the sensitivity of the dominant grass to interspecific interactions. Along with altering its host response to competition, the microbial mutualist increased the facilitative effect of it host as evidenced by increased evenness of the subordinate community when the endophyte is present. On the other hand, altered precipitation reduced the strength of interspecific interactions in the plant community. Thus, we predict that the effects of interspecific interactions on the dune plant species will depend on future precipitation regimes. Irrespective of the strong influence of plant-plant interactions alone, we also found a role for microbial mutualism in structuring the plant community via large increases in host biomass and altered competitive hierarchies among subordinate plant community.

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Figures

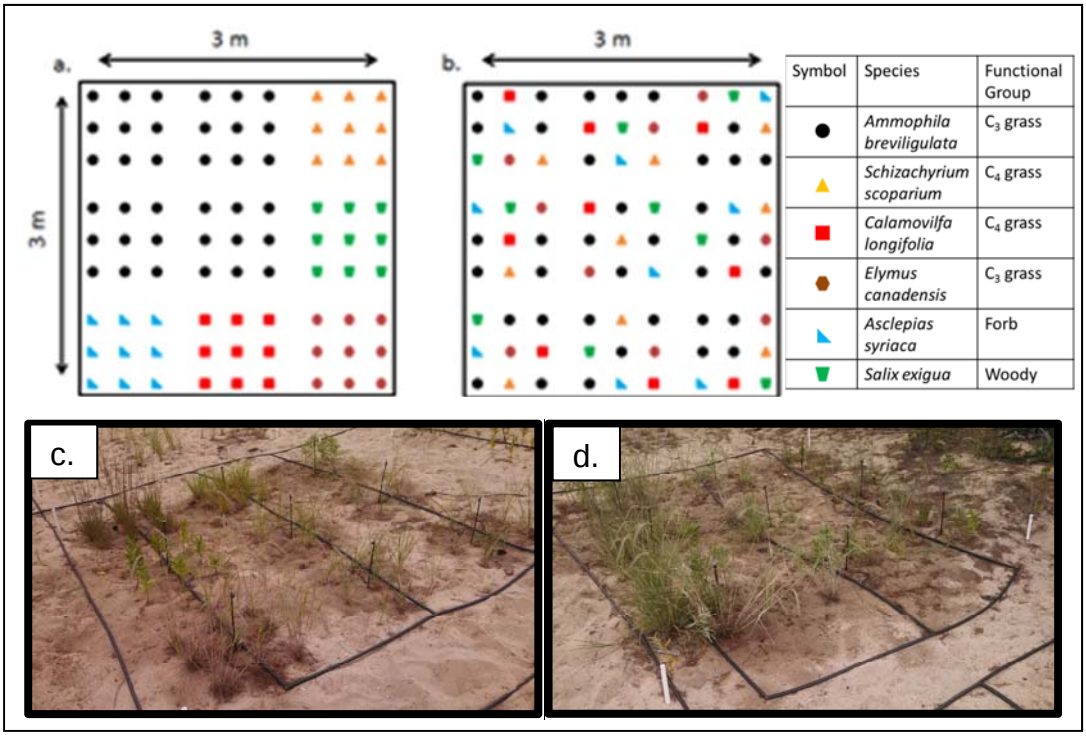

Figure 3.1. Diagram of plant interaction treatments and photographic examples of experimental plots: Plots are composed of nine subplots with nine individuals (symbols) in each subplot composed of either a) conspecific with only intraspecific plant-plant interactions (Monoculture), or b) mixed plant species with both intra- and inter-specific interactions (Mixture). Photo of irrigation plots with either c) Monoculture or d) Mixture interaction treatments.

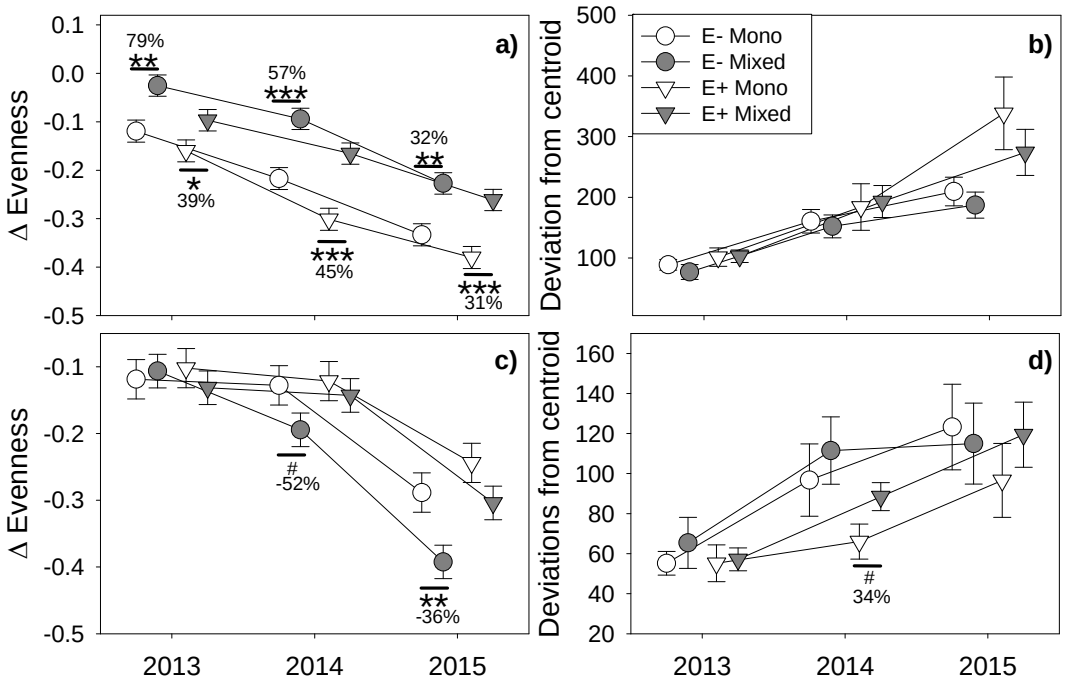

Figure 3.2. Change in evenness (Pielou) of the plant community a) with and c) excluding *Ammophila* and beta diversity b) with and d) excluding *Ammophila* in the analyses of plots with the endophyte (E+: triangles) or endophyte free (E-: circles) and with only intraspecific interactions (Mono: open symbols) or with both intra- and inter-specific interactions (Mixed: dark grey symbols). Symbols show means ± SE. Numbers above and below the symbols are percentage difference between means. "#", "*", "**" and "***" represent $P < 0.10$, $P < 0.05$, $P < 0.01$ and $P < 0.001$ following uncorrected *a priori* pairwise comparisons, respectively.

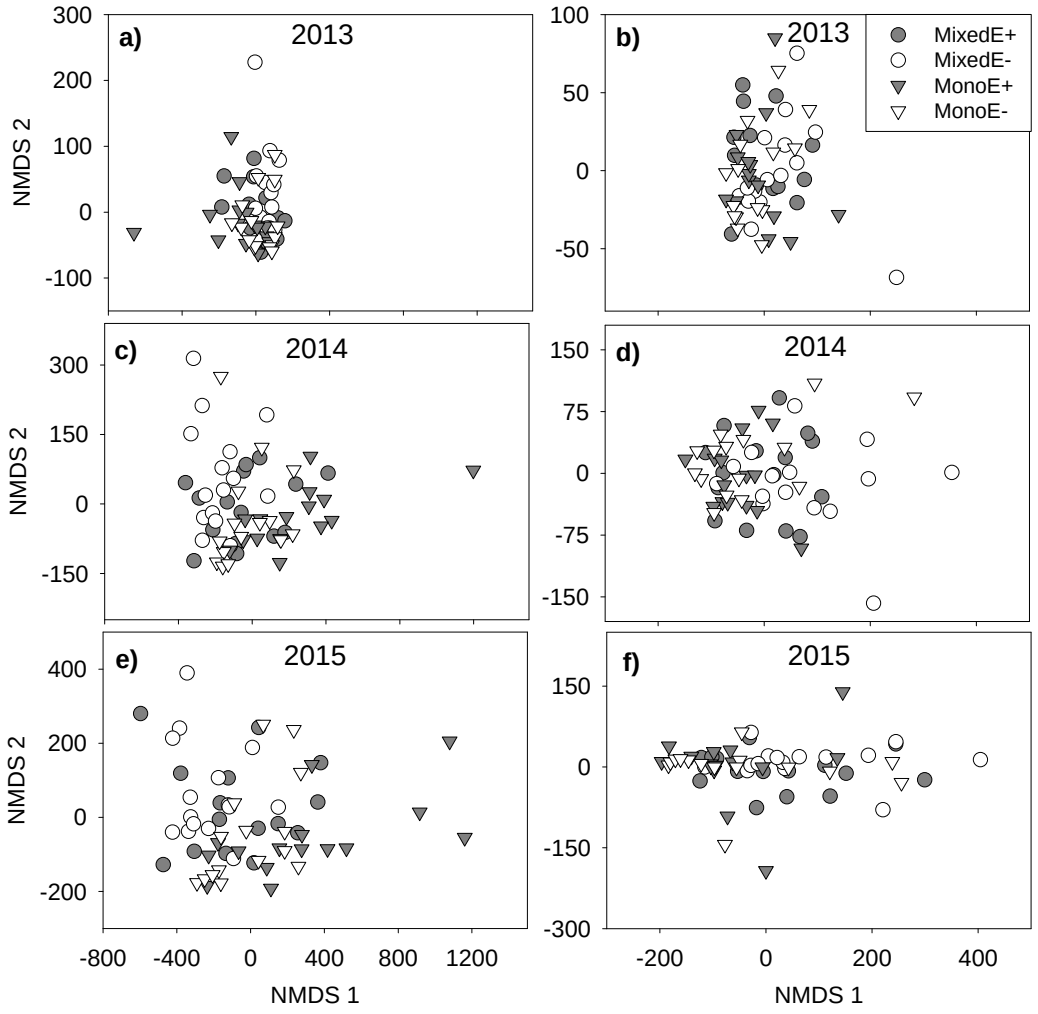

Figure 3.3. Non-metric multidimensional scaling (NMDS) plots of plant community composition [a), c), and e)] with and [b), d) and f)] excluding *Ammophila* in plots with the endophyte (E+: grey symbols) or endophyte free (E-: open symbols) and with only intraspecific interactions (Mono: circles) or with both intra- and inter-specific interactions (Mixed: triangles).

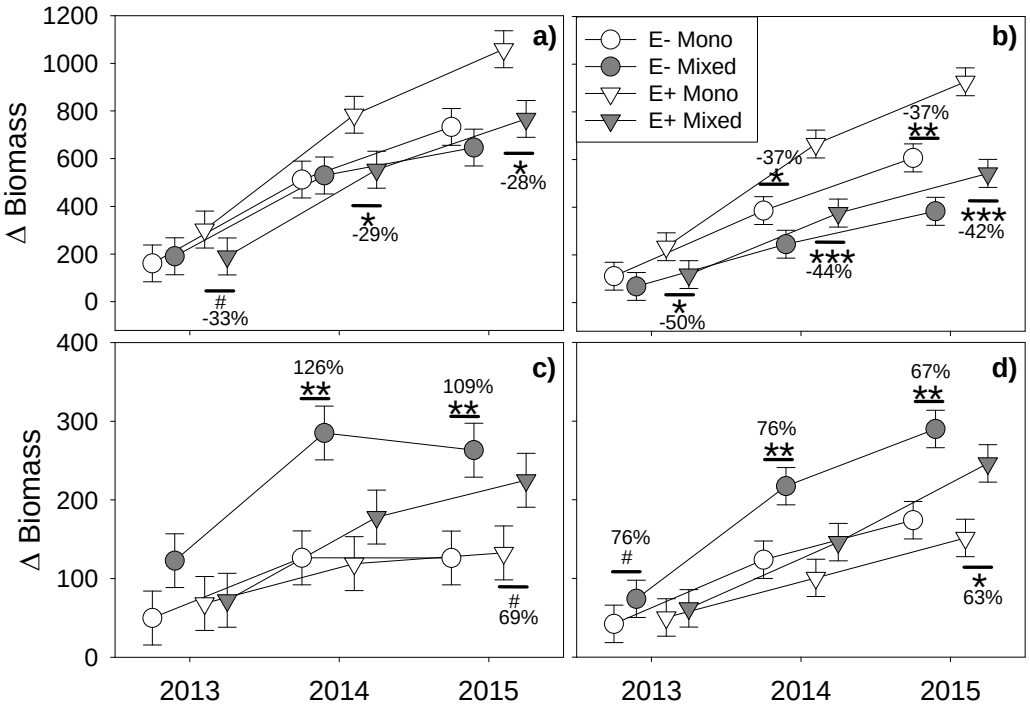

Figure 3.4. Change in biomass of a) the plant community, b) *Ammophila*, c) plant community excluding *Ammophila,* and d) *Schizachyrium scoparium* in plots with the endophyte (E+: triangles) or endophyte free (E-: circles) and with only intraspecific interactions (Mono: open symbols) or with both intra- and inter-specific interactions (Mixed: dark grey symbols). Symbols show means ± SE. Numbers above and below the symbols are percentage difference between means. "#", "*", "**" and "***" represent $P < 0.10$, $P < 0.05$, $P < 0.01$ and $P < 0.001$ following uncorrected *a priori* pairwise comparisons, respectively.

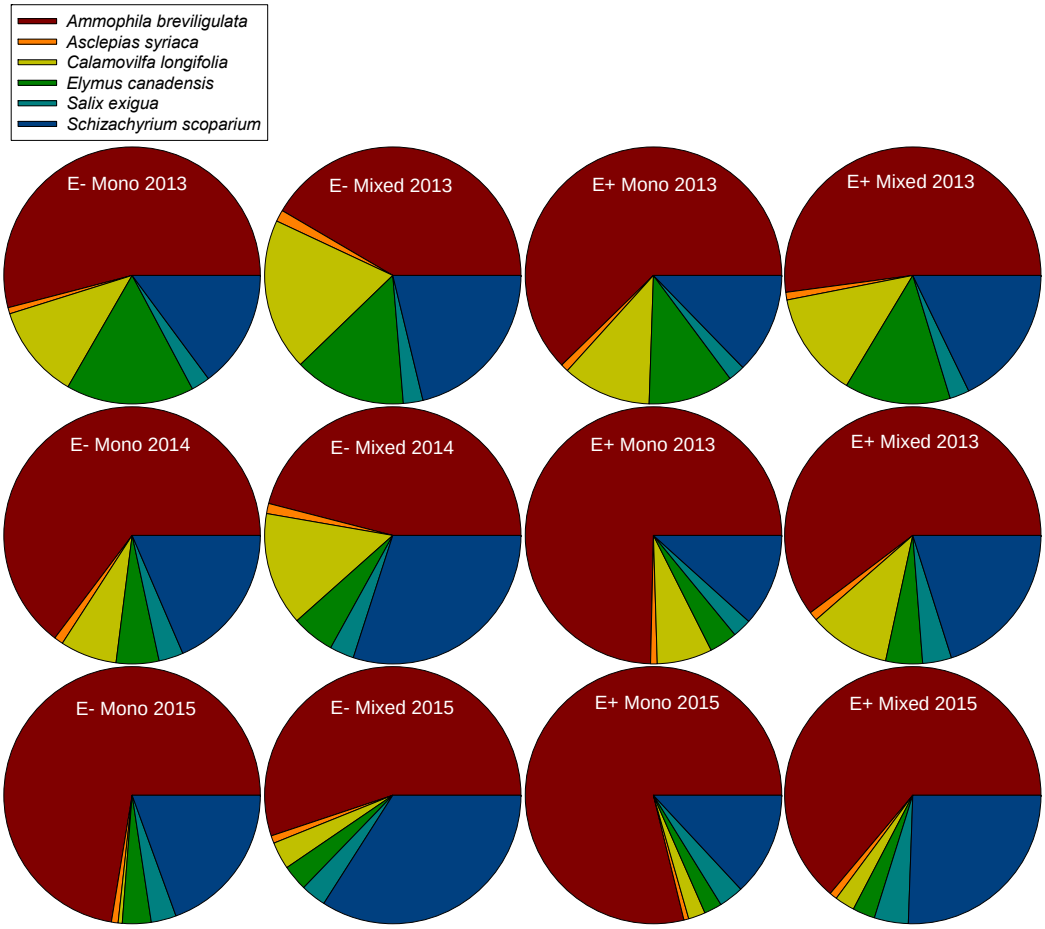

Figure 3.5. Relative composition of the plant community with the endophyte (E+) or endophyte free (E-) and with either only intraspecific interactions (Mono) or with both intra- and inter-specific interactions (Mixed) during 2013-2015.

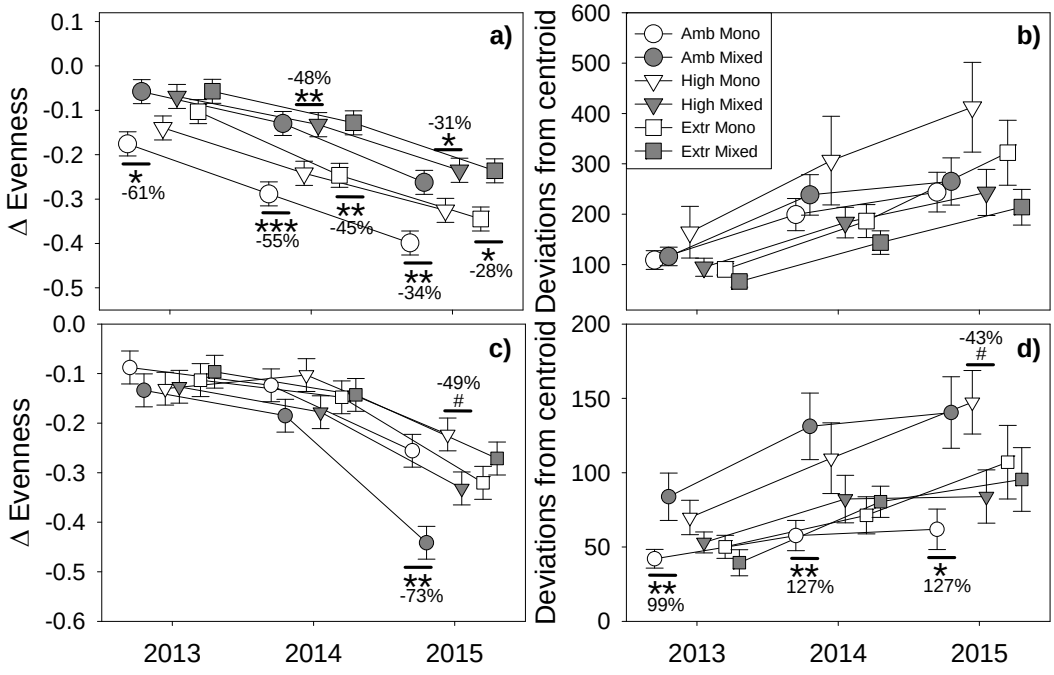

Figure 3.6. Change in evenness (Pielou) of the plant community a) with and c) excluding *Ammophila* and beta diversity b) with and d) excluding *Ammophila* in the analyses in plots with ambient precipitation (Amb: circles), a 35% increase in precipitation in weekly events (High: triangles), or large monthly pulses (Extr: squares) with only intraspecific interactions (Mono open symbols) or with both intra- and inter-specific interactions (Mixed dark grey symbols). Symbols show means ± SE. Numbers above and below the symbols are percentage difference between means. "#", "*", "**" and "***" represent $P < 0.10$, $P < 0.05$, $P < 0.01$ and $P < 0.001$ following false discovering rate adjusted pairwise comparisons, respectively.

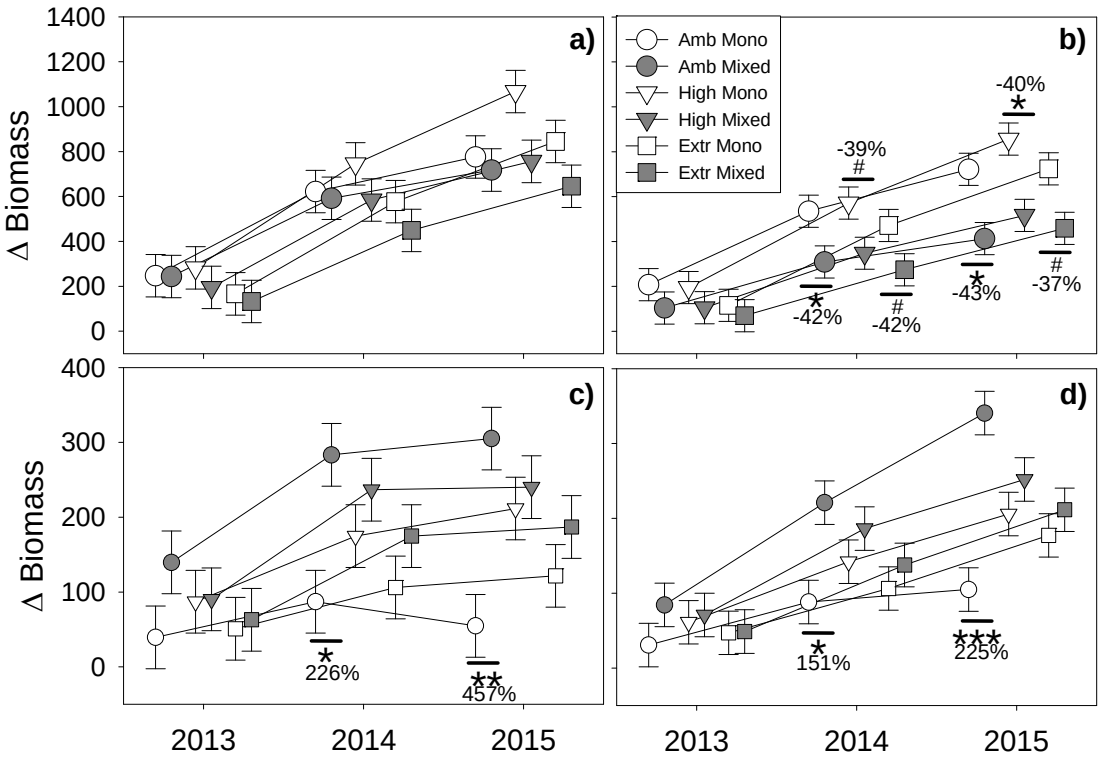

Figure 3.7. Change in biomass of a) the plant community, b) *Ammophila*, c) plant community excluding *Ammophila,* and d) *Schizachyrium scoparium* in plots with ambient (Amb: circles) precipitation, a 35% increase in precipitation in weekly events (High: triangles), or large monthly pulses (Extr: squares) with only intraspecific interactions (Mono open symbols) or with both intra- and inter-specific interactions (Mixed dark grey symbols). Symbols show means ± SE. Numbers above and below the symbols are percentage difference between means. "#", "*", "**" and "***" represent $P < 0.10$, $P < 0.05$, $P < 0.01$ and $P < 0.001$ following false discovering rate adjusted pairwise comparisons, respectively.

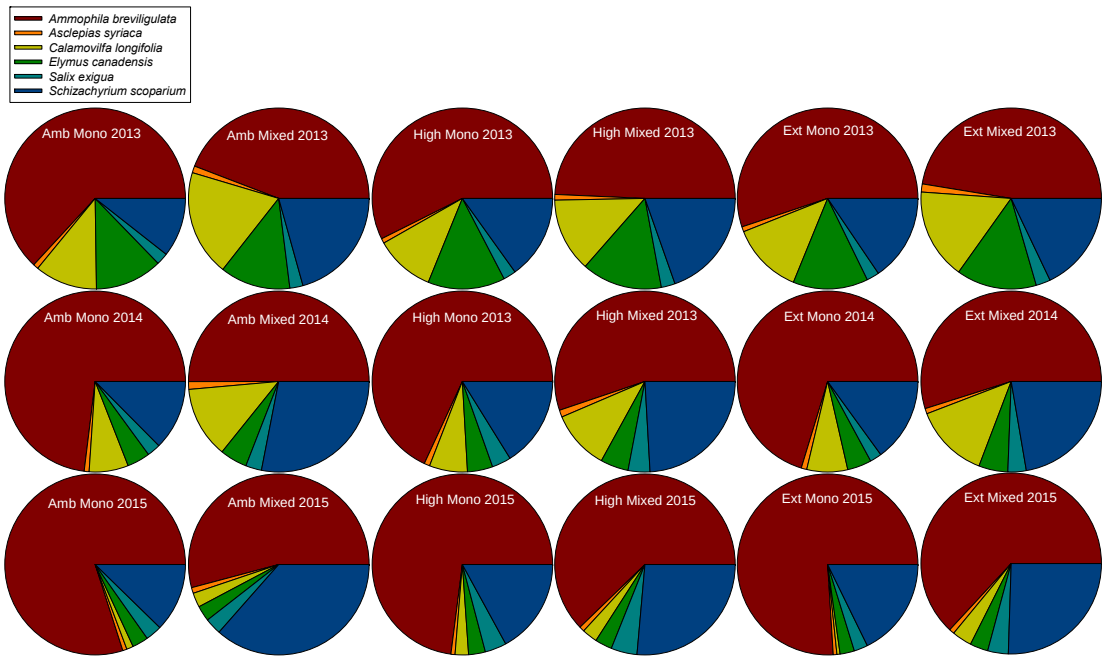

Figure 3.8. Relative composition of the plant community with ambient precipitation (Amb), a 35% increase in precipitation in weekly events (High), or large monthly pulses (Ext) with only intraspecific interactions (Mono) or with both intra- and inter-specific interactions (Mixed)..

Supplemental Tables

Table S3.1. Allometric equations used to estimate species-specific biomass in the experimental community.

Allometric equations						
Species	**Equation**	**N**	***F***	***P***	r^2	***Range (g)***
Ammophila breviligulata	y = 3.6679×TillerNumber	20	414.3	<0.0001	0.95	1.72-35.43
Asclepias syriaca	y = 0.07476×LeafNumber	11	40.22	<0.0001	0.78	0.04-3.21
Calamovilfa longifolia	y = 0.25105×LeafNumber	9	91.26	<0.0001	0.91	0.81-8.22
Elymus canadensis	y = 0.6859×TillerNumber	10	17.52	0.003	0.65	0.39-8.19
Salix exigua	y = 0.032277×Height×BranchNumber	18	274.9	<0.0001	0.94	0.24-4.41
Schizachyrium scoparium	y = 0.63154×EstTillerNumber	10	277.3	<0.0001	0.97	0.44-50.29

Table S3.2. Statistical results from mixed effects models examining the effects of plant-plant interaction (Mixture or Monoculture), endophyte presence (E+ or E-), precipitation (ambient or 35% increase in precipitation in weekly events or in large monthly pulses) and year of collection, with plot as a random factor, on changes in Pielou's evenness (including or excluding *Ammophila*), *Ammophila* biomass, or community productivity (including or excluding *Ammophila*). Significant factors are bolded.

	Change in Plant Evenness			Change on Plant evenness excl. *Ammophila*		Change in *Ammophila* biomass	
Effect	*df*	X^2	*P*	X^2	*P*	X^2	*P*
Interaction	1	**30.07**	**<0.001**	**4.14**	**0.042**	**16.05**	**<0.001**
Endophyte	1	**8.99**	**0.003**	1.92	0.166	**10.26**	**0.001**
Precipitation	2	2.24	0.327	0.93	0.628	1.43	0.488
Year	3	**483.86**	**<0.001**	**229.53**	**<0.001**	**101.69**	**<0.001**
Site	1	**4.43**	**0.035**	0.13	0.714	**6.93**	**0.008**
Inter×Endo	1	0.002	0.961	0.13	0.719	1.10	0.294
Inter×Precip	2	1.31	0.519	5.33	0.070	0.25	0.883
Endo×Precip	2	0.16	0.924	4.79	0.091	0.06	0.972
Inter×Year	2	**8.06**	**0.018**	**7.27**	**0.026**	**15.01**	**<0.001**
Endo×Year	2	4.23	0.120	**6.72**	**0.035**	3.87	0.144
Precip×Year	4	4.27	0.371	7.65	0.105	**13.05**	**0.011**
Inter×Endo×Precip	2	0.56	0.757	0.15	0.927	0.29	0.866
Inter×Endo×Year	2	1.89	0.389	3.32	0.190	0.09	0.955
Inter×Precip×Year	4	1.38	0.848	**10.22**	**0.037**	0.43	0.980
Endo×Precip×Year	4	1.17	0.883	2.75	0.600	1.43	0.838
Inter×Endo×Precip×Year	2	0.42	0.981	4.03	0.402	0.78	0.941

	Change in Productivity			Change in Productivity excl. *Ammophila*	
Effect	*df*	X^2	*P*	X^2	*P*
Interaction	1	2.33	0.311	**7.64**	**0.006**
Endophyte	1	3.16	0.075	0.88	0.348
Precipitation	2	2.33	0.311	2.14	0.342
Year	3	**1298.37**	**<0.001**	**118.04**	**<0.001**
Site	1	2.65	0.103	0.20	0.657
Inter×Endo	1	2.47	0.116	1.26	0.261
Inter×Precip	2	0.45	0.797	4.56	0.102
Endo×Precip	2	0.86	0.650	1.74	0.419
Inter×Year	2	5.00	0.082	14.78	**<0.001**
Endo×Year	2	3.98	0.137	4.65	0.098
Precip×Year	4	**12.11**	**0.017**	4.02	0.403
Inter×Endo×Precip	2	0.22	0.897	0.03	0.986
Inter×Endo×Year	2	2.36	0.307	1.54	0.463
Inter×Precip×Year	4	2.14	0.710	6.15	0.188
Endo×Precip×Year	4	0.72	0.948	3.65	0.455
Inter×Endo×Precip×Year	2	2.64	0.620	3.30	0.509

Table S3.3. Mean and standard errors of beta diversity (distance to centroid) of plant community composition using Euclidean dissimilarity from with only intraspecific interactions (Monoculture) or with both intra- and inter-specific interactions (Mixture), with or without the endophyte (E+ or E-) and plots under ambient precipitation or 35% increase in precipitation in weekly events weekly events (High) or in large monthly pulses (Extreme). Statistical results for t-tests pairwise comparisons. Significant results are bolded.

Beta Diversity (Distance to centroid)

All plant species

	Interactions						Endophyte					
	Monoculture		Mixture				E-		E+			
Year	Mean	STE	Mean	STE	*t-value*	*P*	Mean	STE	Mean	STE	*t-value*	*P*
2013	12.87	19.74	95.87	10.023	1.31	0.288	94.47	7.84	130.27	19.57	1.70	0.136
2014	231.77	34.34	192.71	19.79	0.99	0.450	186.79	15.85	250.77	34.92	1.67	0.117
2015	322.37	43.23	245.17	26.08	1.530	0.178	235.73	21.50	358.24	46.73	**2.38**	**0.025**

	Precipitation									
	Ambient		High		Extreme		Amb vs. High		Amb vs. Extr	
Year	Mean	STE	Mean	STE	Mean	STE	*t-value*	*P*	*t-value*	*P*
2013	128.48	12.73	131.43	8.23	82.08	29.09	0.09	0.964	**3.06**	**0.008**
2014	250.90	28.07	254.88	51.74	193.05	20.54	0.07	0.966	1.66	0.136
2015	310.42	34.87	336.72	63.16	291.62	41.15	0.60	0.638	0.36	0.744

Excluding *Ammophila*

	Interactions						Endophyte					
	Monoculture		Mixture				E-		E+			
Year	Mean	STE	Mean	STE	*t-value*	*P*	Mean	STE	Mean	STE	*t-value*	*P*
2013	55.74	5.47	61.82	7.44	0.66	0.601	63.28	7.63	56.48	5.38	0.73	0.568
2014	81.82	10.62	101.26	11.36	1.28	0.294	114.94	5.38	80.76	5.89	**2.38**	**0.046**
2015	112.18	13.91	117.35	13.45	0.27	0.835	128.82	15.85	117.81	12.32	0.55	0.638

	Precipitation									
	Ambient		High		Extreme		Amb vs. High		Amb vs. Extr	
Year	Mean	STE	Mean	STE	Mean	STE	*t-value*	*P*	*t-value*	*P*
2013	69.91	10.01	62.43	6.62	45.38	5.75	0.62	0.647	2.12	0.070
2014	113.00	16.50	1010.21	13.53	77.85	8.89	0.55	0.645	1.88	0.149
2015	145.41	20.41	118.75	1.14	103.41	16.08	1.06	0.364	1.63	0.239

Table S3.4. Statistical results from PERMANOVA examining the effects of plant-plant interaction (Mixture or Monoculture), endophyte presence (E+ or E-), precipitation (ambient or 35% increase in precipitation in weekly events or in large monthly pulses) and year of collection, with plot as a random factor, on changes in plant community composition (including or excluding *Ammophila*) using Euclidean dissimilarity. Significant factors are bolded.

	Plant community			Plant community excluding *Ammophila*	
Effect	*df*	*Pseudo-F*	*P*	*Pseudo-F*	*P*
Interaction	1,107	**11.95**	**<0.001**	**7.16**	**0.006**
Endophyte	1,107	**8.58**	**<0.001**	1.29	0.264
Precipitation	2,107	0.71	0.541	0.71	0.545
Year	2,107	**151.35**	**<0.001**	**84.78**	**<0.001**
Site	1,107	**5.56**	**0.014**	1.38	0.232
Inter×Endo	1,107	1.26	0.267	0.96	0.341
Inter×Precip	2,107	0.46	0.728	2.24	0.090
Endo×Precip	2,107	0.20	0.926	0.91	0.424
Inter×Year	2,107	**5.56**	**0.011**	**5.23**	**<0.001**
Endo×Year	2,107	**8.34**	**<0.001**	1.80	0.121
Precip×Year	4,107	0.94	0.459	0.51	0.873
Inter×Endo×Precip	2,107	0.11	0.975	0.10	0.992
Inter×Endo×Year	2,107	0.37	0.811	0.25	0.947
Inter×Precip×Year	4,107	0.43	0.884	1.84	0.069
Endo×Precip×Year	4,107	0.36	0.931	1.05	0.390
Inter×Endo×Precip×Year	4,107	0.09	1.000	0.37	0.961
Plot	36,107	**6.39**	**<0.001**	**5.44**	**<0.001**

Table S3.5. Statistical results from mixed effects models examining the effects of plant-plant interaction (Mixture or Monoculture), endophyte presence (E+ or E-), precipitation (ambient or 35% increase in precipitation in weekly events or in large monthly pulses) and year of collection, with plot as a random factor, on change biomass of *Asclepias syriaca, Calamovilfa longifolia, Elymus canadensis, Salix exigua, and Schizachyrium scoparium*. Significant factors are bolded.

	Change in *A. syriaca* biomass			Change in *C. longifolia* biomass		Change in *E. canadensis* biomass	
Effect	*df*	X^2	*P*	X^2	*P*	X^2	*P*
Interaction	1	0.41	0.521	**4.32**	**0.038**	0.22	0.636
Endophyte	1	0.37	0.543	0.14	0.706	1.24	0.265
Precipitation	2	0.05	0.973	0.54	0.764	1.75	0.416
Year	3	**64.66**	**<0.001**	**153.35**	**<0.001**	**441.67**	**<0.001**
Site	1	1.94	0.164	2.94	0.087	3.72	0.054
Inter×Endo	1	1.41	0.235	**3.90**	**0.048**	0.04	0.842
Inter×Precip	2	0.86	0.651	0.83	0.660	1.04	0.596
Endo×Precip	2	0.70	0.706	2.77	0.250	0.81	0.669
Inter×Year	2	0.07	0.968	5.69	0.058	4.60	0.100
Endo×Year	2	0.88	0.644	3.33	0.190	0.01	0.994
Precip×Year	4	5.37	0.251	5.88	0.208	3.40	0.493
Inter×Endo×Precip	2	1.22	0.543	0.41	0.816	0.08	0.959
Inter×Endo×Year	2	**9.77**	**0.008**	0.86	0.649	0.70	0.703
Inter×Precip×Year	4	3.44	0.487	2.91	0.572	1.71	0.789
Endo×Precip×Year	4	4.72	0.317	5.34	0.254	1.15	0.886
Inter×Endo×Precip×Year	2	3.20	0.524	2.92	0.572	4.85	0.303

	Change in *S. scoparium* biomass			Change in *S. exigua* biomass	
Effect	*df*	X^2	*P*	X^2	*P*
Interaction	1	**10.21**	**0.001**	0.81	0.368
Endophyte	1	0.92	0.339	2.15	0.143
Precipitation	2	1.44	0.486	3.83	0.147
Year	3	**55.48**	**<0.001**	**58.12**	**<0.001**
Site	1	**0.23**	**0.634**	5.86	0.016
Inter×Endo	1	0.78	0.376	0.08	0.781
Inter×Precip	2	5.38	0.069	0.82	0.664
Endo×Precip	2	1.70	0.428	0.03	0.985
Inter×Year	2	**18.62**	**<0.001**	0.30	0.860
Endo×Year	2	8.30	0.016	3.35	0.187
Precip×Year	4	0.71	0.950	**6.82**	**0.146**
Inter×Endo×Precip	2	0.02	0.991	0.41	0.784
Inter×Endo×Year	2	0.87	0.647	0.49	0.784
Inter×Precip×Year	4	6.90	0.141	0.35	0.986
Endo×Precip×Year	4	9.45	0.051	2.23	0.693
Inter×Endo×Precip×Year	2	1.00	0.910	2.26	0.687

Table S3.6. Mean and standard errors of nutrients (micro grams/10cm^2/day) captured by plant root simulator ion exchange probes in 2013 and 2014 from plots with or without the endophyte (E+ or E-) and under either ambient precipitation or 35% increase in large monthly pulses (Extreme). Plant communities had both intra- and interspecific interactions (Mixture).

Nutrient flow (micro grams/10cm^2/day)								
Endophyte								
	2013				2014			
	E-		E+		E-		E+	
Element	Mean	STE	Mean	STE	Mean	STE	Mean	STE
Total N	0.30	0.059	0.15	0.031	0.33	0.051	0.31	0.023
P	0.0094	0.0023	0.0085	0.0011	0.013	0.0018	0.013	0.0017
K	0.18	0.011	0.18	0.021	0.53	0.029	0.61	0.094
Ca	19.29	1.30	15.21	1.68	23.07	1.69	18.33	1.61
S	0.95	0.11	0.66	0.14	0.54	0.12	0.41	0.10
Mg	1.83	0.15	1.30	0.13	2.20	0.19	1.72	0.13
Fe	0.021	0.0010	0.022	0.0017	0.019	0.0008	0.018	0.0009
Mn	0.0060	0.0012	0.0057	0.0011	0.010	0.0004	0.009	0.0003
Zn	0.0072	0.0007	0.0061	0.0005	0.0087	0.0012	0.0095	0.0021
Al	0.20	0.013	0.23	0.013	0.055	0.0009	0.054	0.0012
Pb	0.0032	0.0007	0.0038	0.0012	0.0050	0.0004	0.0043	0.0008
Precipitation								
	2013				2014			
	Ambient		Extreme		Ambient		Extreme	
Element	Mean	STE	Mean	STE	Mean	STE	Mean	STE
Total N	0.20	0.050	0.24	0.053	0.25	0.025	0.38	0.040
P	0.011	0.0018	0.0067	0.0013	0.016	0.0016	0.011	0.0015
K	0.20	0.019	0.16	0.014	0.67	0.083	0.46	0.026
Ca	17.02	1.49	17.25	1.81	19.73	1.19	21.67	2.25
S	0.72	0.090	0.86	0.16	0.20	0.014	0.75	0.088
Mg	1.45	0.15	0.64	0.17	1.70	0.076	2.23	0.21
Fe	0.022	0.0010	0.022	0.0017	0.020	0.0008	0.017	0.0008
Mn	0.0061	0.0014	0.0056	0.0008	0.0099	0.0004	0.0094	0.0005
Zn	0.0070	0.0006	0.0063	0.0007	0.011	0.0021	0.0073	0.0006
Al	0.21	0.0095	0.22	0.017	0.056	0.0010	0.053	0.0009
Pb	0.0031	0.0006	0.0039	0.0013	0.0046	0.0005	0.0047	0.0007

Table S3.7. Statistical results from mixed effects models examining the effects of endophyte presence (E+ or E-), precipitation (ambient or 35% increase in precipitation in weekly events or in large monthly pulses) and year of collection, with plot as a random factor, on volumetric water content (% VWC) at 20 cm and 40 cm depth. Significant factors are bolded.

		VWC at 20 cm		VWC at 40 cm	
Effect	*df*	X^2	*P*	X^2	*P*
Endophyte	1	**4.75**	**0.029**	0.26	0.607
Precipitation	2	**25.90**	**<0.001**	**7.81**	**0.020**
Year	1	**4.96**	**0.026**	**15.95**	**<0.001**
Site	1	3.62	0.057	2.36	0.125
Endo×Precip	2	5.17	0.075	3.44	0.179
Endo×Year	1	0.13	0.715	1.81	0.178
Precip×Year	2	**15.23**	**<0.001**	3.20	0.202
Endo×Precip×Year	2	0.37	0.829	1.48	0.477

Supplemental Figures

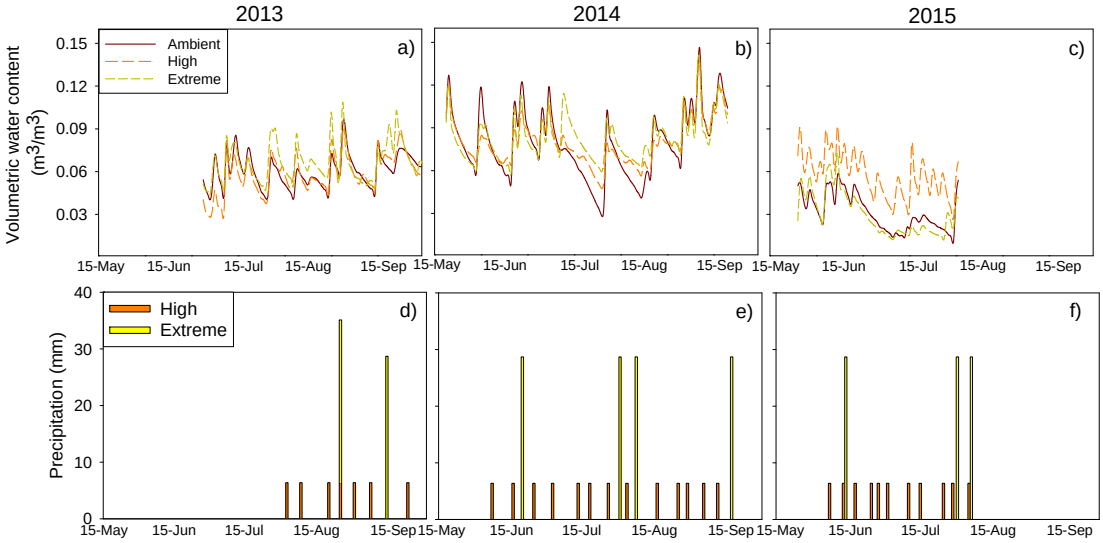

Figure S3.1. Average soil volumetric water content (m^3/m^3) in a) 2013, b) 2014, and c) 2015 from plot with Precipitation Treatments: including ambient (dark red) precipitation, a 35% increase in precipitation in weekly events (High: long dash burnt orange), or large monthly pulses (Extreme: short dash dark yellow). Bar graph of timing of precipitation treatments throughout the growing seasons during d) 2013, e) 2014, and f) 2015. A 35% increase in precipitation in weekly events (High: burnt orange bars), or large monthly pulses (Extreme: dark yellow bars).

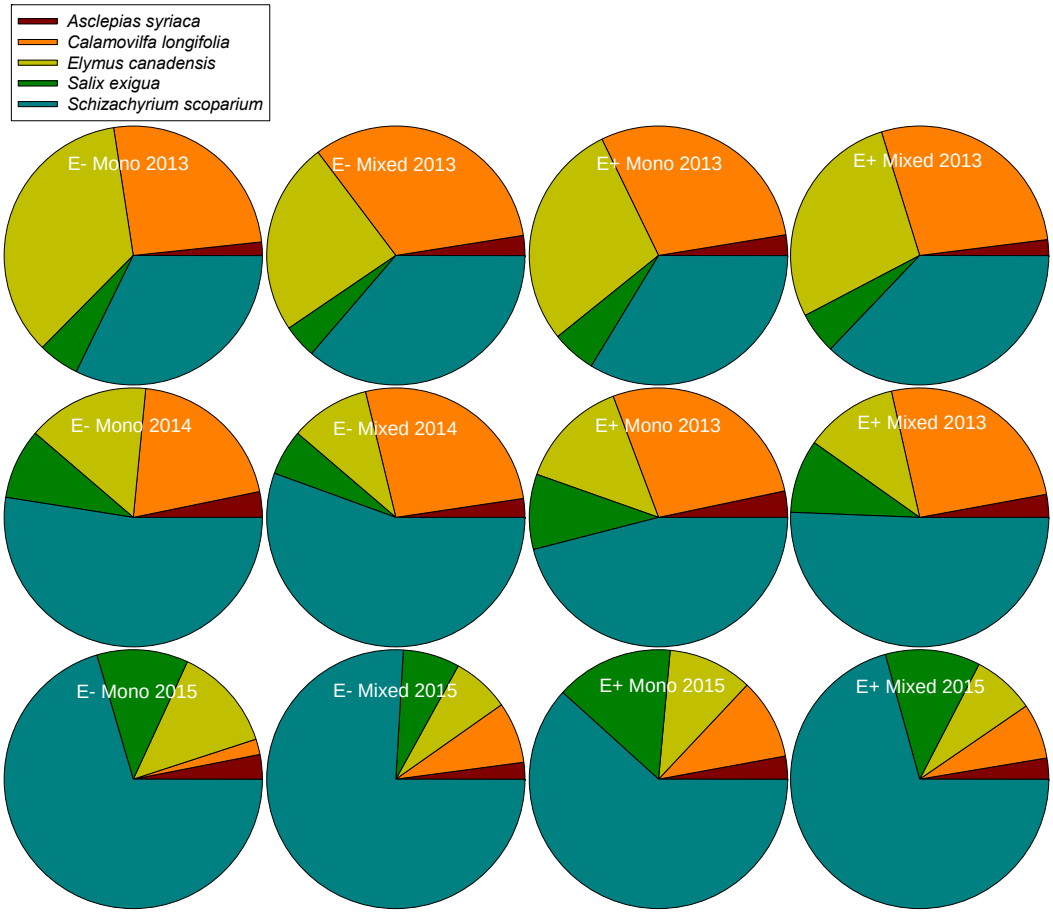

Figure S3.2. Relative composition of the subordinate plant community with the endophyte (E+) or endophyte free (E-) with only intraspecific interactions (Mono) or with both intra- and inter-specific interactions (Mixed) during 2013-2015.

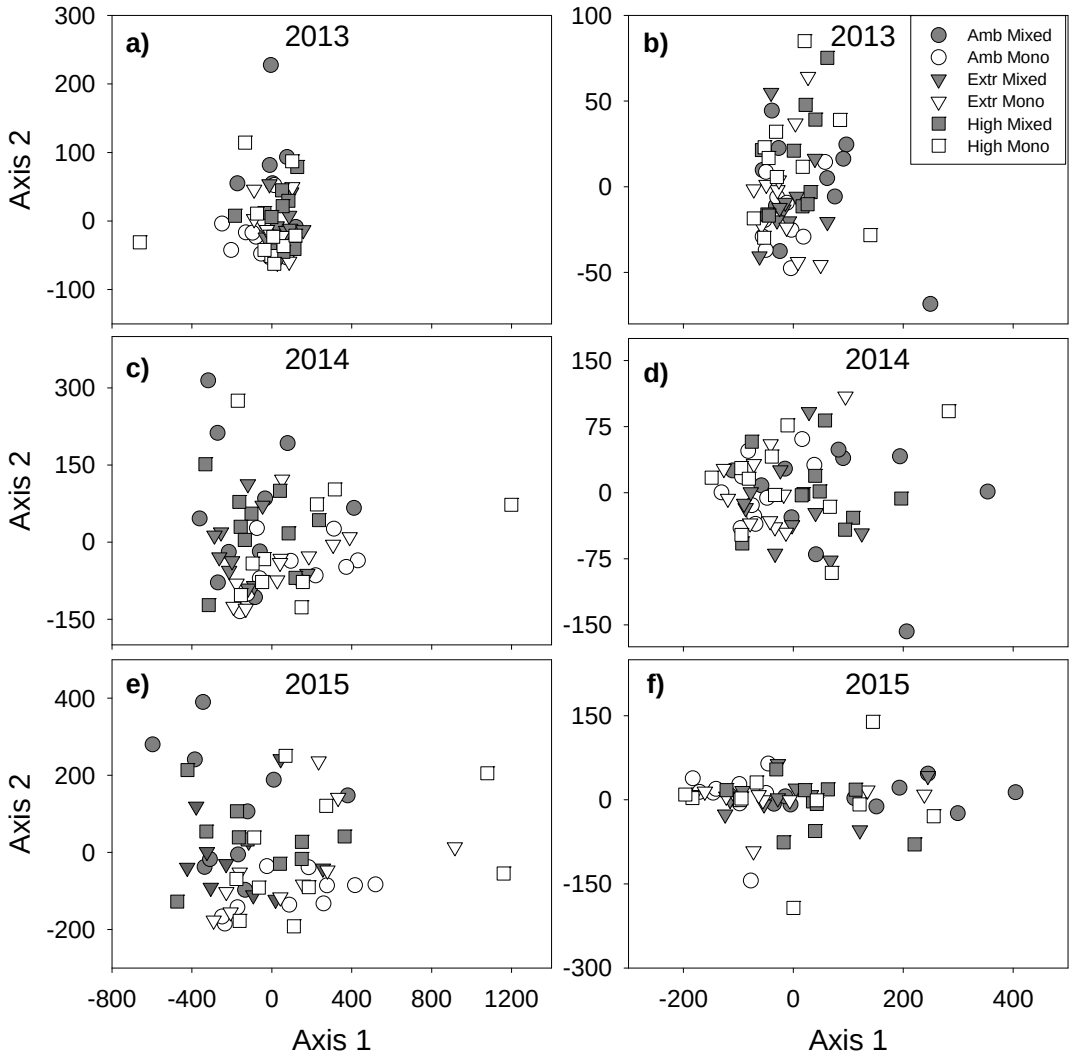

Figure S3.3. Non-metric multidimensional scaling (NMDS) plots of plant community composition [a), c), and e)] with and [b), d) and f)] excluding *Ammophila* in plots with ambient (Amb: circles) precipitation, a 35% increase in precipitation in weekly events (High: squares), or in large monthly pulses (Extr: triangles) with only intraspecific interactions (Mono open symbols) or with both intra- and inter-specific interactions (Mixed dark grey symbols) during 2013-2015.

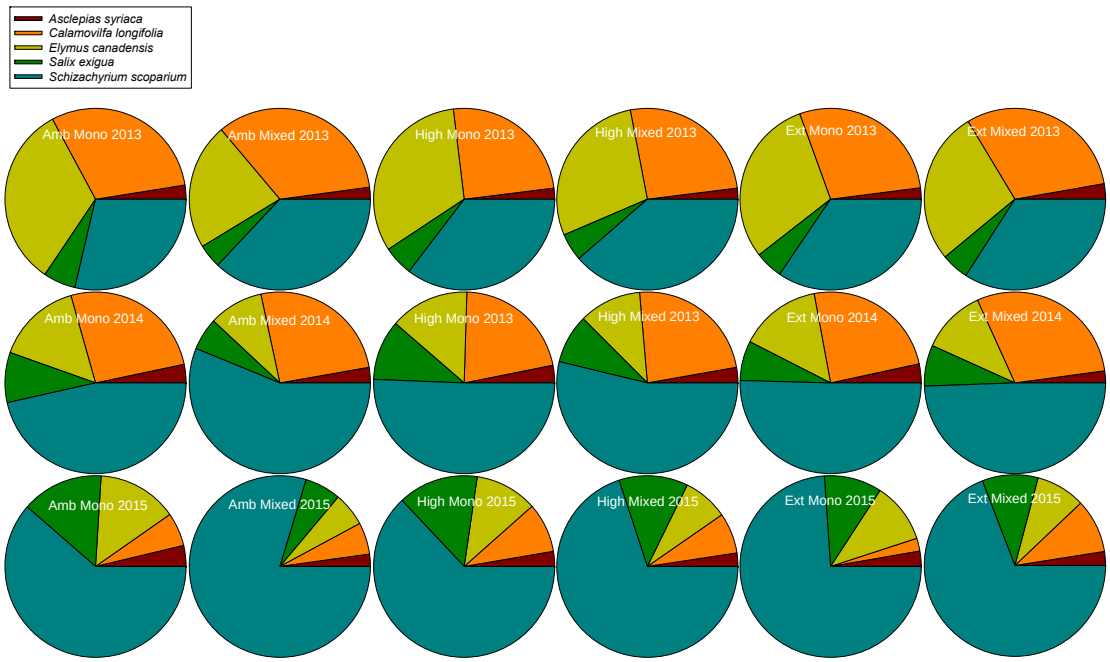

Figure S3.4. Relative composition of the subordinate plant community with ambient precipitation (Amb), a 35% increase in precipitation in weekly events (High), or large monthly pulses (Ext) with only intraspecific interactions (Mono) or with both intra- and inter-specific interactions (Mixed).

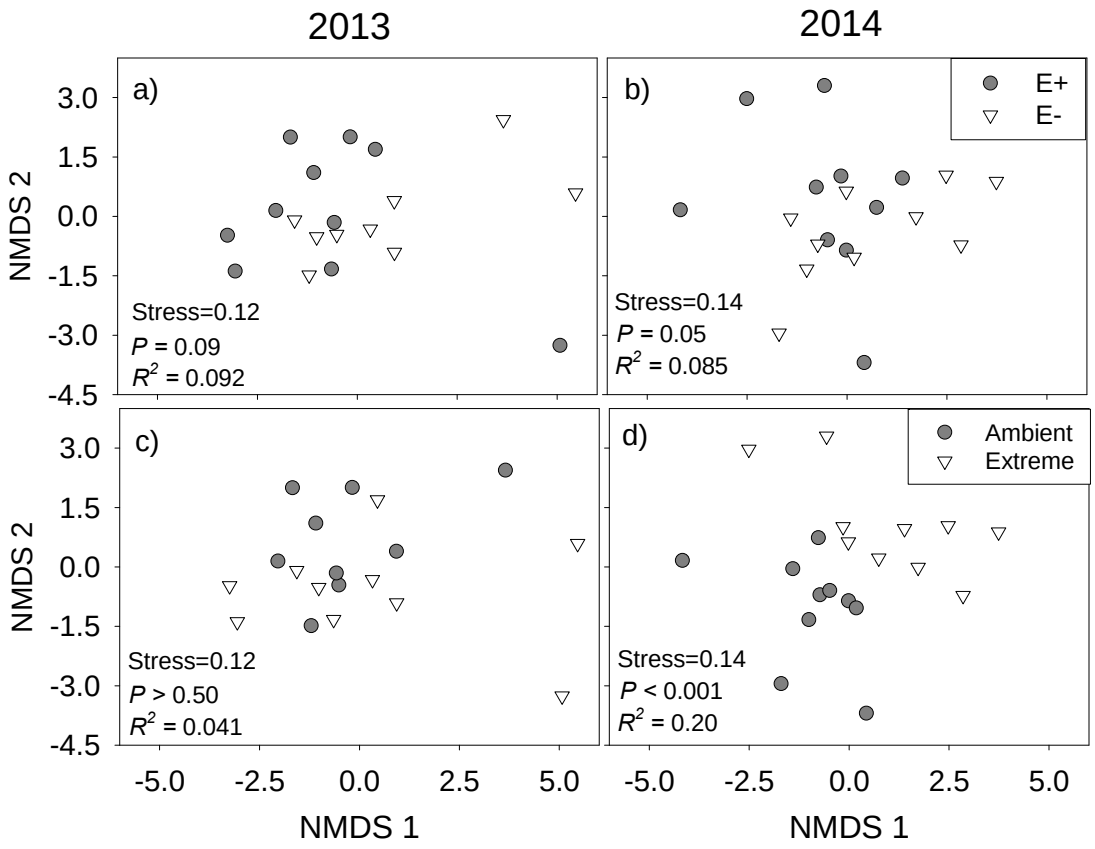

Figure S3.5. Non-metric multidimensional scaling (NMDS) plots of soil nutrient profiles a-b) Endophyte Treatments: with *Ammophila* hosting *Epichloë* (E+: gray symbols) or endophyte free (E-: open symbols) c-d) Precipitation Treatments: with ambient (gray circles) precipitation or 35% increase in precipitation in large monthly pulses (Extreme: open triangles).

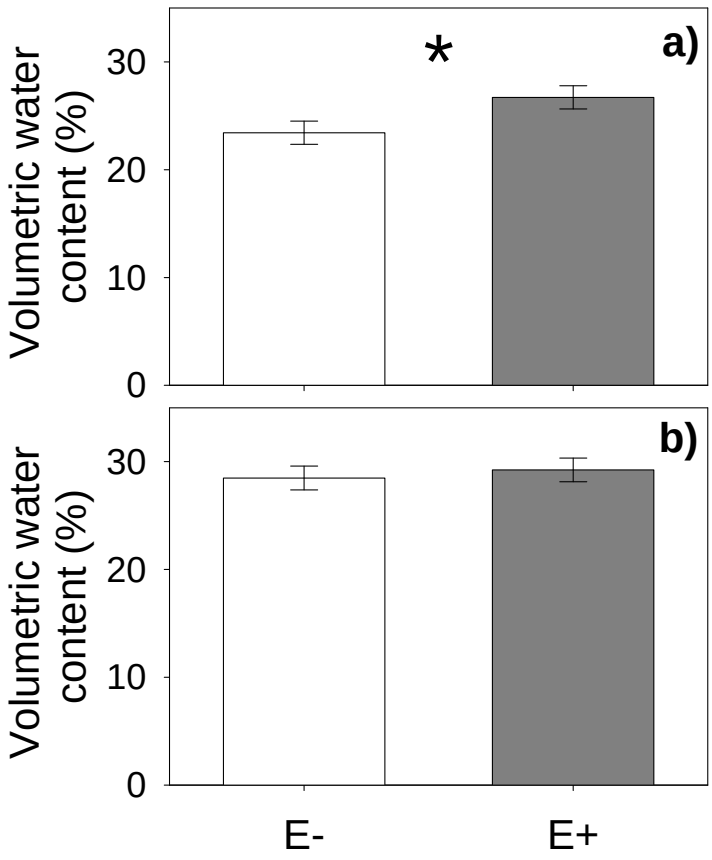

Figure S3.6. Average soil volumetric water content (%) at a) 20 cm and b) 40 cm depth across 2013-2014 in *Ammophila* Monocultures subplots either hosting the endophyte (E+: dark grey fill) or endophyte free (E-: unfilled). Bars show means + SE. "*" represent $P < 0.05$ significance.