

Fungal symbiont effects on dune plant diversity depend on precipitation

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Summary

1. Historically, mutualisms have been considered to be less important than antagonisms in affecting the composition of ecological communities. In plant communities, beneficial microbes may feature as keystone mutualists in structuring community composition. Understanding the direction and magnitude of mutualist effects at the community scale may be critical for making accurate predictions on plant responses to climate change, particularly for mutualists that ameliorate climate-induced stressors. Such mitigation could shift outcomes between mutualist-enhanced species diversity and mutualist-reduced diversity, depending on whether a mutualist accelerates habitat modification or competitive exclusion by its partner species.

2. Here, we tested the relative importance and interactive effects of altered precipitation and symbiosis between an epichloid fungal endophyte and a dominant grass species for dune plant communities along the Great Lakes, USA. In 2010, we imposed field manipulations of endophyte presence in the foundation dune grass *Ammophila breviligulata* in combination with rainout shelters and rainfall additions. We monitored natural rates of colonization by new plant individuals over 3 years.

3. Under the current precipitation regime, endophyte symbiosis in *A. breviligulata* reduced colonizing plant diversity, species richness and evenness. This effect depended on the amount of precipitation, with the symbiosis having weaker effects on plant diversity under both augmented and reduced rainfall treatments.

4. Despite the overall negative effect of endophyte symbiosis on plant diversity, plant responses to the endophyte were species specific. A federally threatened forb, *Cirsium pitcheri*, increased in abundance when the symbiosis was present, regardless of precipitation regime. Endophyte symbiosis in *A. breviligulata* caused minor reductions in the abundance of other grass species; however, augmented precipitation benefited other grasses.

5. Synthesis. We show that microbial mutualisms can have strong effects on community structure in a native ecosystem and that the amount of precipitation has the potential to alter how these keystone species interactions affect community composition. Predictions on future plant community structure, for both restored and native dunes, can be improved by accounting for the presence of fungal symbionts in the foundation plant species.

Key-words: *Ammophila*, community, dune geomorphology, endophyte, *Epichloe*, keystone mutualism, ontogenetic, plant–fungal

Introduction

Relative to antagonistic species interactions, mutualisms and other positive interactions have long been considered to be less important factors in the structure and composition of ecological communities (May 1982; Bruno, Stachowicz &

Bertness 2003). However, mutualisms are ubiquitous (Bronstein 1994) and have the potential to affect communities in both positive and negative ways (Hacker & Gaines 1997; Stachowicz 2001; Afkhami, Rudgers & Stachowicz 2014). When the benefits of mutualism are shared among community members, the presence of a mutualist may cause net increases in community diversity. For example, the presence and structure of pollinator networks equalized the fitness of competing plant species,

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thereby promoting plant diversity (Bastolla *et al.* 2009). Similarly, losses of mutualists caused cascading declines in diversity in a plant–animal interaction web (Rodríguez-Cabal *et al.* 2013). Alternatively, mutualisms may reduce species coexistence and diversity. This effect can occur when a mutualist preferentially increases the competitive ability of its partner, thereby promoting competitive exclusion. For example, in tall grass prairies, nutritional mutualisms with mycorrhizal fungi increased the competitive supremacy of the dominant grass species (Hartnett & Wilson 2002).

In plant communities, beneficial microbes could feature as keystone mutualists in structuring community composition. Most plants form symbioses with fungi and bacteria, many of which function as mutualists under certain contexts (Bacon & White 2000; Smith & Read 2008). Depending on the system and context, pairwise studies show support for the hypothesis of mutualist-enhanced plant species coexistence (Wagg *et al.* 2011) or mutualist-reduced coexistence (e.g. Clay, Marks & Cheplick 1993). Furthermore, more than three decades ago, Janos (1980) proposed that symbiotic fungi could play important roles in driving plant successional dynamics. However, the majority of experiments conducted thus far have lacked the community-scale perspective necessary to test for such systemwide effects (but see Rudgers & Clay 2007; Yurkonis *et al.* 2014).

One group of symbiotic microbes in plants that may have particularly strong community-level effects is the epichloid endophytic fungi. These fungi occur systemically in plants, often conferring protection against abiotic (drought, heat) or biotic stressors (herbivores, pathogens) (Rodríguez *et al.* 2009). Endophyte benefits can be exceptionally large, resulting in several-fold increases in plant survival, biomass and/or reproduction (Clay & Schardl 2002). Strong community-level effects have now been documented in one system. In tall fescue grass, *Lolium arundinaceum* (syn., *Schedonorus arundinaceus*), the presence of epichloae slowed the rate of plant succession from grassland to forest by 60–80%, with suppressive effects on both plant species diversity (Rudgers *et al.* 2007) and arthropod diversity (Rudgers & Clay 2008). Due to the economic importance of forage and turf grasses, the majority of studies on epichloae have focused on individual plants or monospecific stands in agronomic settings, and their ecological roles in native ecosystems remain largely unstudied (Cheplick & Faeth 2009), but see Hol *et al.* (2007) for effects of a different type of endophyte.

Understanding the direction and magnitude of mutualist effects on community structure may be fundamental to predicting ecological responses to climate change. Species interactions can have indirect effects on plant responses to climate change that either exacerbate or ameliorate the direct effects of a changing climate (Tylisanakis *et al.* 2008). However, relatively few studies have addressed the roles of microbial mutualists in plant responses to altered climates (Compant, van der Heijden & Sessitsch 2010; van der Putten 2012). For example, while epichloid endophytes can confer drought tolerance to plants (Malinowski & Belesky 2000), possibly improving plant performance in warmer, drier climates, little work has

directly tested fungal endophyte benefits in the context of future climate manipulations (reviewed by Kivlin, Emery & Rudgers 2013). Thus far, studies have focused on agronomic systems (Newman *et al.* 2003; Hunt *et al.* 2005; Brosi *et al.* 2011; Ryan *et al.* 2014; but see Emery & Rudgers 2013). Ultimately, benefits provided by microbial mutualists may ameliorate the negative, direct effects of climate change on plants (Singer, Travis & Johst 2013). Such mitigation could shift community-level outcomes between mutualist-enhanced species diversity and mutualist-reduced diversity, depending on whether a mutualist weakens or accelerates competitive exclusion by its partner species in a future climate.

Here, we investigated the influence of an epichloid endophyte symbiosis on native dune plant communities along the Great Lakes. The Great Lakes contain 21% of all fresh surface water on the planet (Gleick 1993) and 84% of fresh surface water in North America (Tilzer & Serruya 1990). Sand dunes cover much of the shoreline, forming the most extensive freshwater dunes in the world and covering >1,000 km² in Michigan alone (Albert 2000). Great Lakes sand dunes are dominated by the early colonist and ecosystem engineering sand dune grass, *Ammophila breviligulata*, which stabilizes moving sand during the early stages of dune succession and contributes to early soil carbon enrichment (Olson 1958; Martin 1959; Cheplick 2005). Biotic engineering of dunes can be rapid, altering dune geomorphology within months to years (Godfrey 1977; Lichter 1998; Stallins 2003). After dunes are stabilized, other plant species colonize and out-compete *A. breviligulata*, succeeding ultimately to a mixed deciduous-pine forest (Lichter 1998, 2000). In previous work, we discovered an undescribed species of endophyte in the genus *Epichloë* (Clavicipitaceae) at low frequencies in natural *A. breviligulata* populations of the Great Lakes, but at very high frequencies in the commercial stocks used for dune restoration (Emery & Rudgers 2010; Emery, Bell-Dereske & Rudgers in press). The community and ecosystem level consequences of this symbiosis remain largely unknown. In similar dune systems in Europe that are dominated by the sister species, *A. arenaria*, a generalist endophytic fungus (*Acremonium strictum*) reduced host-plant suppression by plant-feeding nematodes (Hol *et al.* 2007).

Understanding the interacting effects of climate change and microbial symbiosis has important applications for dune conservation and restoration. Dunes maintain ecosystem integrity along the aquatic–terrestrial border, provide habitat for endemic plant species and shelter neighbouring wetlands. Native dune vegetation also protects human developments by stabilizing soil and protecting interior lands from storms (McHarg 1972; Ranwell & Boar 1986). 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 (Jones, Gladstone & Hacking 2007; Schlacher *et al.* 2008).

Climate models project increases in evapotranspiration rates and drops in lake levels in the Great Lakes (McBean &

Motiee 2008; Angel & Kunkel 2010; IPCC 2012), potentially increasing water stress for dune plants. Under the highest CO₂ emission general circulation models, the Great Lakes region is expected to experience altered rates of annual precipitation (Kling *et al.* 2003; IPCC 2012) and an increase of 5 °C in annual temperature by 2070–2099 (Hayhoe *et al.* 2010). The most recent regionally downscaled projections include a range of possible outcomes, from a 35% increase in growing season precipitation (PCM model) to a 45% decrease (GFDL model) (Vavrus & Van Dorn 2010). Earlier climate models predicted up to 30% declines in soil moisture during the growing season (IPCC 2001; Kling *et al.* 2003). Drought may be a particularly important element of climate change in the Great Lakes region. The survival of native dune plants has been shown to be water limited along Lake Michigan (Lichter 2000; Lyne Ensign, Webb & Longstaffe 2006), and water was more limiting than nutrients in a study of Canadian dunes (Houle 1997).

To evaluate the relative importance and interactive effects of altered precipitation and endophyte symbiosis in the foundation grass species *Ammophila breviligulata* on dune plant communities, we tested the following questions using a field experiment on the shore of Lake Michigan. (i) How does endophyte symbiosis in *A. breviligulata* affect early dune plant colonization and plant diversity? (ii) Do early plant colonists in dune communities respond to altered rates of precipitation? (iii) Are there interactive effects of endophyte symbiosis and precipitation on dune plant community development?

Materials and methods

STUDY SITE

The 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, ~200 m from the shoreline of Lake Michigan (Fig. 1). 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 sparsely vegetated gravel bed.

EXPERIMENTAL DESIGN

During late May 2010, we established a 2 × 3 factorial experiment to alter the presence or absence of endophyte symbiosis in *A. breviligulata* populations in the context of a climate manipulation (reduced, ambient, or augmented precipitation). Replication consisted of 15 plots (2 × 2 m) per treatment combination, each initially containing 25 transplanted *A. breviligulata* individuals. The 90 plots were arranged in seven rows of 8–15 plots each, matching the contour of the dune blowout (Fig. 1). Each plot was randomly assigned to a treatment combination.

Rainfall manipulation

Across ten general circulation models from the IPCC Fourth Assessment Report, the predicted change for the study region ranged from a

19% decrease to 12% increase in growing season precipitation by 2041–2070 (Fig. S1, Supporting Information, <http://www.cccsn.ec.gc.ca/>), and a 31% decrease to 19% increase by 2071–2100 (Fig. S2) compared to baseline data back projected by each GCM for 1971–2000. We constructed modified Sala rainout shelters to manipulate growing season precipitation (Yahdjian & Sala 2002). Shelters (2 × 2 × 2 m) were constructed from metal conduit secured with screws in order to withstand the high winds of the fore dune environment. Shelters were hammered into the sand such that the gutters were ~0.75 m above the ground surface. The gutters removed ~30% of ambient rainfall from the reduced rainfall plots. We added that collected rain to the augmented water plots after each rain event. Both augmented and ambient rainfall plots had mock shelters with gutters oriented upside-down to control for any effects on light levels, without altering the amount of ambient rainfall (Fig. 1). Each year, the gutters 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 (confirmed with lactic acid aniline blue stain and microscopy) collected at a nearby site in Sleeping Bear Dunes National Lakeshore (44°51.472', –86°3.834') during fall 2006. We germinated seedlings on 1% water agar and inoculated half with endophyte isolates grown on potato dextrose agar. Preliminary genotyping showed no genetic variation in mating type or alkaloid genes among endophyte isolates (C.A. Young, N. Charlton & J. A. Rudgers unpubl. data), which is not uncommon in the asexual, vertically transmitted epichloae clade. We used a sterile needle to either wound (sham inoculate, E- treatment) or insert hyphae into the meristem of each seedling (E+ treatment) at 3–5 days of age (Leuchtmann & Clay 1988). This method had an 8% success rate, comparable to inoculations of epichloae into other grass species (Chung *et al.* 1997). We used only successfully inoculated plants for the E+ treatment. Following inoculation, seedlings were grown in the glasshouse in a 50:50 mix of sterile play sand and Metro-Mix 220 (Sun Gro Horticulture, Agawam, MA, USA). As plants matured, we cloned genotypes by gently separating tillers from the original stock plants and replanting them into pots; these stock plants were grown in the glasshouse for 6 months. Cloning enabled us 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 (three genotypes m⁻²) to naturally occurring levels (Fant *et al.* 2008). Plants had, on average, five 30-cm-long leaves and 1–2 tillers when they were planted. During 2012–2013, estimated above-ground biomass per plot (based on allometric equations for tiller number) ranged from 3.4 g m⁻² to 19.2 g m⁻², with an average of 8.1 ± 0.2 g m⁻² in 2012 and 10.0 ± 0.3 g m⁻² in 2013.

DATA COLLECTION

To assess precipitation treatment effectiveness, we repeatedly measured volumetric water content using a hand-held TDR probe (Aquatrerr M 300, Costa Mesa, CA, USA). On each date, measurements were recorded at 20 cm and 40 cm depths for three locations next to *A. breviligulata* plants near the centre of each plot. Dates of observation included 26 July 2011; 12 and 28 June 2012; 10 July 2012; 3, 17–18 June 2013; 1, 9 and 15 July 2013; 1 and 7 August 2013 and 5, 10–11 September 2013. A small number of outlier observations

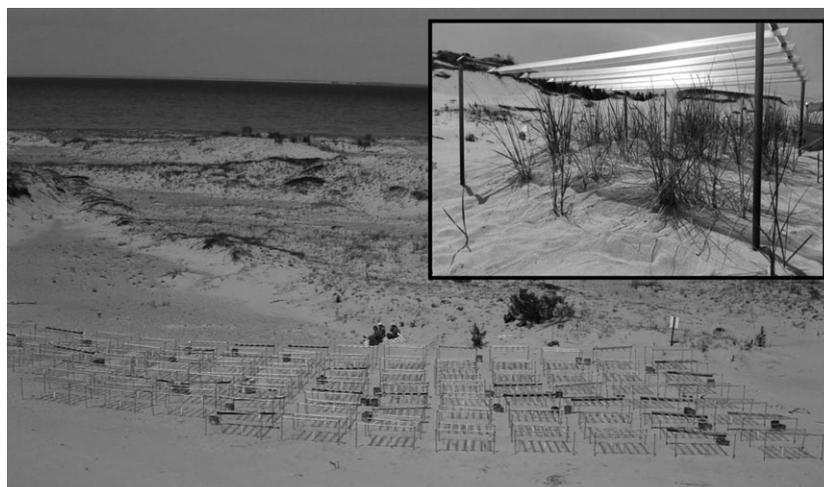


Fig. 1. Photograph of the experiment in Leelanau State Park, Leelanau Co., Michigan showing plot and rainout shelter arrangement within the dune blowout. Lake Michigan is in the background. Co-authors are shown for scale. Inset shows the side view of a single plot photographed in 2013.

(3.5% of the total) were excluded from the analysis; these observations were likely associated with faulty calibration or insertion of the probe. Data for each soil depth and date were averaged within a plot then analysed with repeated measures ANCOVA including the fixed effects of endophyte, precipitation, and endophyte \times precipitation, the date of observation, and plot as a random factor nested within the endophyte and precipitation treatments (Proc MIXED, SAS version 9.2; SAS Institute, Inc., Cary, NC, USA). Treatment effects were tested over variation among, not within, plots.

We counted and identified all newly colonizing plant individuals in plots on 18 May 2012 and 18 July 2013 (Table S1). Most recruiting species reproduce only from wind-dispersed seeds (Higman & Penskar 1999; Lichter 2000). For each census, we estimated colonizing plant species diversity and evenness using the Simpson index (inverse), excluding *A. breviligulata* (Begon, Townsend & Harper 2006). Newly colonizing *A. breviligulata* seedlings would include both seeds that dispersed into the plots and seeds produced by resident vegetation. Although we collected mature inflorescences from the planted individuals, some seeds dispersed prior to collection. Thus, we estimated *A. breviligulata* seedling recruitment as the number of colonists divided by the estimated number of seeds produced in the prior year. Seed production was estimated by counting the number of seeds produced per inflorescence for 5–10 inflorescences per plot and multiplying the number of seeds per inflorescence by the total number of inflorescences produced per plot. Responses of the original, planted *A. breviligulata* individuals will be presented elsewhere, along with plant trait data.

PLANT COMMUNITY ANALYSIS

We tested for treatment effects on plant species diversity, species richness, evenness, number of colonizing individuals, recruitment of colonizing *A. breviligulata* and abundance of the dune endemic *Cirsium pitcheri* (Pitcher's thistle) using repeated measures general linear mixed models. Statistical models included the fixed factors of endophyte treatment, precipitation treatment, the endophyte \times precipitation interaction and the repeated effect of year (2012 or 2013), with plot (nested in endophyte \times precipitation) as a random factor (Proc GLIMMIX, SAS v. 9.3; SAS Institute Inc.). As spatial blocking

factors, we included column (north–south gradient) and/or row (east–west gradient) as categorical variables when they explained significant variation in our response variables. For *post hoc* means comparisons, we used sequential Bonferroni tests. Total abundance of colonists (excluding *A. breviligulata*) was log-transformed to meet assumptions of normality of residuals. We examined simple Pearson correlations between plant community metrics and estimated biomass of the planted *A. breviligulata* in the plots to test whether patterns were consistent with the hypothesis that above-ground dominance of the foundation species reduced colonist diversity or abundance.

We examined overall shifts in plant community composition using PERMANOVA (Primer v. 6.1, Clarke & Gorley 2007), with counts of the number of individuals of each plant species as the response variables, and the fixed factors of endophyte treatment, precipitation treatment, and endophyte \times precipitation, and the blocking factors of row and column to account for spatial positions of plots in the field. For each year of observation, we analysed abundance data using the Bray–Curtis distance metric, 9999 randomizations, and permutation of residuals under a reduced model. We analysed the 2 years separately for two reasons. Firstly, some plant species were not present in both years, increasing the stress of repeated measures ordination. Secondly, plots lacking any colonists were non-overlapping between years, which reduced the sample size for a repeated measures model because plots with no colonists are not included in PERMANOVA. In 2012, the data matrix included seven plant species and 85 plots that had non-zero total colonist abundance, and in 2013, the data matrix included eight plant species and 78 plots. To visualize differences in plant composition, we used non-metric multidimensional scaling analysis with 500 restarts using a random starting configuration (Primer v. 6.1). If treatment effects were significant in the PERMANOVA, we then used SIMPER to determine the contributions of each plant species to dissimilarity among treatments (Primer v. 6.1). In addition to the NMS/PERMANOVA approach, we also examined the responses of plant functional groups (grasses, forbs, or woody species) using repeated measures general linear mixed models, as described above for community diversity metrics. Finally, because our data spanned 2 years of recruitment, we could estimate juvenile establishment rates by dividing the number of juveniles present in 2013 by the number of seedlings present in 2012. We complemented this analysis with data on

juveniles taken during mid-May 2013, using a repeated measures model to analyse the juvenile to seedling ratios for both May and July 2013.

Results

TREATMENT EFFECTIVENESS

Relative to ambient precipitation, the reduced rainfall treatment caused a stronger reduction in soil moisture at greater soil depth. At shallow soil depth, ambient and augmented treatments significantly differed in soil moisture, whereas at greater soil depth, reduced and ambient treatments significantly differed (Fig. S3). Specifically, at 20 cm depth, rainout shelters resulted in an average of 3% greater soil moisture in ambient rainfall plots than in reduced plots and 9% higher soil moisture in augmented plots than in reduced plots (Fig. S3a, Precipitation, $\chi^2_{2,84} = 19.39$, $P < 0.0001$). At 40-cm soil depth, we recorded an average of 6% higher soil moisture in ambient rainfall plots than in reduced plots and 9% higher soil moisture in augmented plots than in reduced plots (Fig. S3b, Table S2, Precipitation, $\chi^2_{2,84} = 13.04$, $P = 0.0015$). Presence of the endophyte did not significantly affect soil moisture across all dates (Table S1, Endophyte, 20 cm depth $\chi^2_{1,84} = 2.36$, $P = 0.12$; 40 cm $\chi^2_{1,84} = 0.4$, $P = 0.53$).

DUNE PLANT DIVERSITY

Presence of the *Epichloë* endophyte in *A. breviligulata* plants reduced colonizing plant species diversity, richness and evenness under the current climate conditions of our ambient precipitation treatment (Fig. 2; Endophyte \times Precipitation, Table 1). The strongest suppression of diversity occurred under ambient precipitation levels (Fig. 2a) with 26% lower diversity in the presence of the endophyte than in its absence. This diversity reduction weakened under reduced precipitation and the pattern reversed, although not significantly so, under augmented rainfall (Fig. 2a). Under current climate, plant species richness in endophyte plots was on average 29% lower than in endophyte-free plots over the years 2012–2013 (Fig. 2b). But again, these differences were only significant under ambient precipitation (Endophyte \times Precipitation, Table 1). In the presence of the endophyte, plant diversity and richness were highest under augmented precipitation, whereas in the absence of the endophyte, richness peaked under ambient precipitation. As with richness, the response of plant species evenness to endophyte presence also occurred only under ambient precipitation (Fig. 3, Year \times Endophyte \times Precipitation, Table 1). Specifically, under ambient precipitation in 2013, endophyte presence reduced evenness by 20% relative to endophyte-free plots, whereas the endophyte effect on evenness was non-significant in 2012 (Fig. 3). Across all plots, evenness was higher in 2013 than in 2012 (Fig. 3). Correlation analyses revealed negative relationships between *A. breviligulata* estimated biomass and the abundance (Pearson $r = -0.34$, $P = 0.001$, $n = 90$ plots) and evenness ($r = -0.23$, $P = 0.029$) of colonists in 2013, but

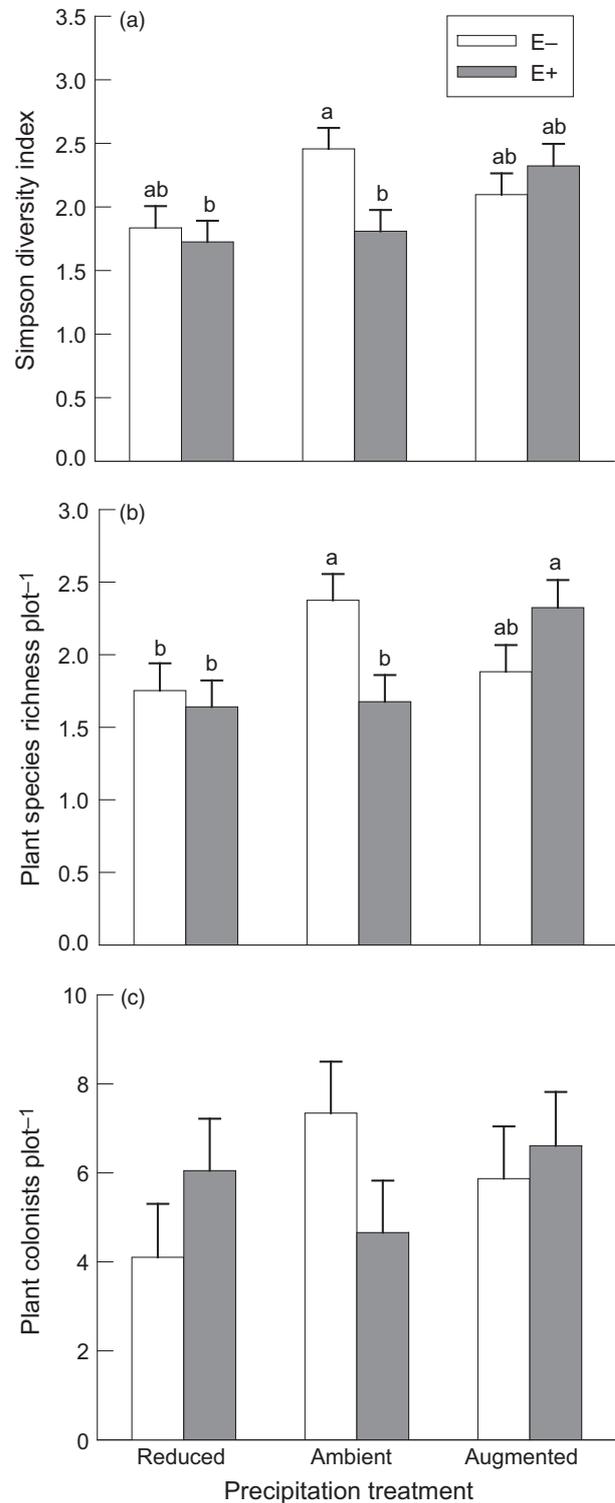


Fig. 2. Plant community responses to the combined influences of precipitation and endophyte presence in *Ammophila breviligulata*. Data were averaged over the 2 years of the experiment (2012–2013) and include (a) Simpson diversity index (inverse) per plot, (b) plant species richness per plot and (c) the number of colonizing individual plants per plot, excluding *A. breviligulata*. Bars show LS means \pm SE from repeated measures general linear mixed models. Different letters indicate significant differences according to *post hoc* sequential Bonferroni tests. Statistical results are given in Table 1.

Table 1. Statistical results for repeated measures general linear mixed models examining the effects of the presence of an endophyte symbiont in *Ammophila breviligulata* (Endophyte, E+/E-), precipitation manipulation (reduced, ambient, or augmented rainfall), and the repeated effect of year of observation (2012, 2013) on the Simpson diversity index, species richness, evenness, the abundance of colonizing plants and the abundance of colonizing *Cirsium pitcherii*. Spatial location within the dune blowout (Column) was included as a covariate. Significant effects ($P < 0.05$) are shown in bold

Source	df	Simpson diversity index		Plant species richness		Evenness		Number of colonists		Number of <i>Cirsium pitcherii</i>	
		χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Endophyte	1,70	1.67	0.1959	0.67	0.4137	1.19	0.2758	0.18	0.6731	1.38	0.2393
Precipitation	2,70	7.62	0.0221	5.61	0.0604	1.73	0.4211	6.15	0.0462	0.09	0.9562
Endophyte × Precipitation	2,70	6.62	0.0365	9.03	0.0109	1.05	0.5907	6.89	0.0318	2.74	0.2537
Column	14,70	26.79	0.0205	36.81	0.0008	9.48	0.7988	42.36	0.0001	48.98	<0.0001
Year	1,70	24.01	<0.0001	0.78	0.3761	38.65	<0.0001	2.86	0.0911	21.04	<0.0001
Year × Endophyte	1,70	0.05	0.8265	0.01	0.9289	2.11	0.1464	0.52	0.4729	7.26	0.0071
Year × Precipitation	2,70	1.70	0.4281	0.56	0.7553	3.01	0.2216	1.71	0.4261	1.35	0.5083
Year × Endophyte × Precipitation	2,70	0.51	0.7764	4.94	0.0847	6.47	0.0394	1.59	0.4520	3.55	0.1693
Year × Column	14,70	9.14	0.8221	12.72	0.5490	12.87	0.5371	14.51	0.4127	60.72	<0.0001

correlations were non-significant for the 2012 data (abundance: $r = 0.02$, $P = 0.85$; evenness: $r = -0.09$, $P = 0.39$).

DUNE PLANT RECRUITMENT

Plots varied considerably in the number of recruits, from 0–43 individuals plot⁻¹ in May 2012 and from 0–22 individuals plot⁻¹ in July 2013. Under ambient precipitation, endophyte presence in *A. breviligulata* reduced colonists by 37%, from 7.3 individuals per plot to 4.6 individuals per plot (Fig. 2c, *post hoc* tests were non-significant; Endophyte × Precipitation, Table 1). In contrast, under reduced precipitation, colonists were 32% more abundant in the absence of the endophyte (6.0 individuals per plot) than in its presence (4.1 individuals). Neither the endophyte nor the precipitation treatment altered the ratio of juveniles per plot in 2013 to seedlings per plot in 2012 (not including colonizing *A. breviligulata*, Endophyte $\chi^2_{1,70} = 2.07$, $P = 0.15$; Precipitation $\chi^2_{2,70} = 0.11$, $P = 0.94$, Endophyte × Precipitation $\chi^2_{2,70} = 2.17$, $P = 0.34$, Column $\chi^2_{14,70} = 14.68$, $P = 0.40$). Finally, recruitment of *A. breviligulata* from seed, independent of any treatment effects on seed production of the resident plants, was higher in the presence of the endophyte (0.30 ± 0.05 recruits in 2013 per seed produced in 2012) than in the absence of the endophyte (0.07 ± 0.06 recruits per seed) (Endophyte $\chi^2_{1,64} = 7.79$, $P = 0.0053$) with no effects of the precipitation regime (Precipitation $\chi^2_{2,64} = 0.70$, $P = 0.70$, Endophyte × Precipitation $\chi^2_{2,64} = 0.68$, $P = 0.71$), but strong spatial variation across the plot matrix (Column $\chi^2_{14,64} = 23.40$, $P = 0.0540$, Row $\chi^2_{2,64} = 18.15$, $P = 0.0059$).

DUNE PLANT SPECIES COMPOSITION

Compositional analysis showed overall shifts in plant species composition in response to precipitation and marginally in response to endophyte presence. Community shifts were statistically significant only in 2013, following three seasons of

water manipulation (Fig. 4; Endophyte *pseudo-F*_{1,52} = 2.34, $P = 0.0576$; Precipitation *pseudo-F*_{2,52} = 2.12, $P = 0.0382$; Endophyte × precipitation *pseudo-F*_{2,40} = 0.87, $P = 0.5450$; Column *pseudo-F*_{14,52} = 1.67, $P = 0.0081$; Row *pseudo-F*_{6,52} = 1.79, $P = 0.0190$) and were not statistically significant in 2012 (Endophyte *pseudo-F*_{1,59} = 1.18, $P = 0.3280$; Precipitation *pseudo-F*_{2,59} = 0.58, $P = 0.7878$; Endophyte × precipitation *pseudo-F*_{2,59} = 1.39, $P = 0.1908$; Column *pseudo-F*_{14,52} = 1.79, $P = 0.0016$; Row *pseudo-F*_{6,52} = 1.76, $P = 0.0216$). SIMPER analysis revealed three plant species were the strongest drivers of the plant composition response to altered rainfall. Two forbs, *Cirsium pitcheri* (Pitcher's thistle) and *Artemisia campestris* (beach wormwood), and one grass species, *Schizachyrium scoparium* (little bluestem), each contributed ~24–32% of the dissimilarity among precipitation treatments. Abundances of *S. scoparium* and *A. campestris* were highest under augmented precipitation, whereas *C. pitcheri* was most abundant under reduced precipitation. SIMPER analysis also showed these three species each accounted for 26–30% of the variation between endophyte treatments, with both *A. campestris* and *S. scoparium* being more abundant in the absence of the endophyte. In 2013, these three plant species were the dominant colonists, representing 31.3% (*A. campestris*), 30.6% (*S. scoparium*), and 28.0% (*C. pitcheri*) of all recruits. In 2012, *A. campestris* was similarly dominant (38.6% of all recruits) but was followed by *C. pitcheri* (26.4%). Two grass species were less abundant in 2012, ranking third and fourth in dominance (6.6% *Calamovilfa longifolia*, 4.3% *S. scoparium*).

Analyses of plant responses by functional group indicated declines in the grasses (excluding *A. breviligulata*) under reduced precipitation ($\chi^2_{2,70} = 7.00$, $P = 0.0302$). We observed ~140% higher grass abundance under augmented precipitation (mean plants plot⁻¹ ± SE, 1.6 ± 0.4) than reduced precipitation (mean ± SE, 0.7 ± 0.4), on average across the 2 years, with intermediate grass abundance in the ambient treatment (mean ± SE, 1.3 ± 0.4). Endophyte

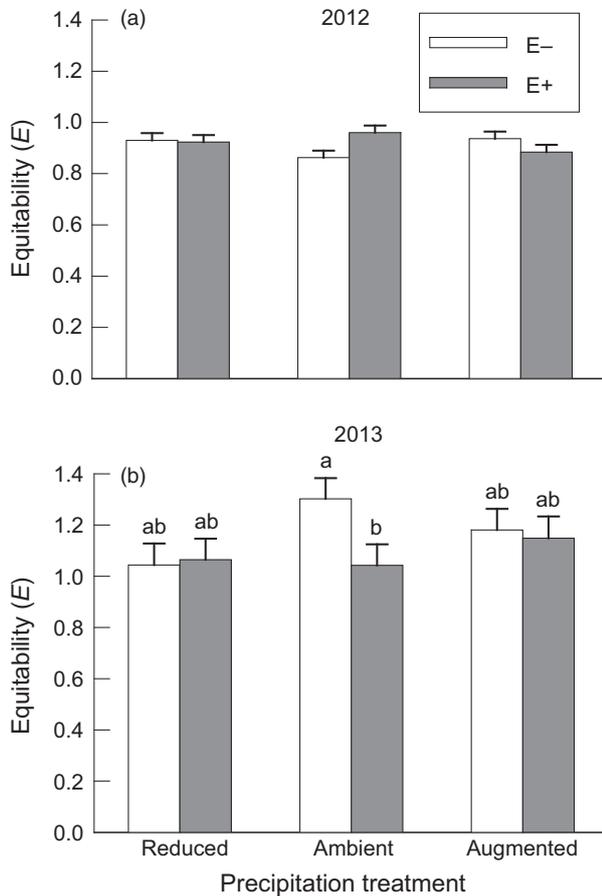


Fig. 3. Plant species evenness (equitability based on the Simpson index) responses to the combined influences of precipitation and endophyte presence in *Ammophila breviligulata* in years (a) 2012 and (b) 2013. Bars show LS means \pm SE from repeated measures general linear mixed models. Different letters indicate significant differences according to *post hoc* sequential Bonferroni tests within a year. Statistical results are given in Table 1.

presence in *breviligulata* had no effect on the abundance of the grass functional group (Endophyte $\chi^2_{1,70} = 1.05$, $P = 0.3062$, Endophyte \times Precipitation $\chi^2_{2,70} = 3.65$, $P = 0.1609$). No other plant functional group showed a significant response to the treatments, suggesting that the majority of plant responses were species specific rather than specific to a functional group.

RARE DUNE ENDEMIC

The federally threatened, Great Lakes dune endemic *Cirsium pitcheri* was 49% more abundant in plots with the endophyte present in 2012 (Fig. 5, Endophyte \times Year, Table 1). There was no significant effect of the endophyte on *Cirsium pitcheri* in 2013. Positive effects of the *Epichloë* symbiosis in *A. breviligulata* on *C. pitcheri* were consistent across the precipitation manipulation, as indicated by the non-significant endophyte \times precipitation interaction (Endophyte \times Precipitation \times Year, Table 1). The abundance of juvenile *C. pitcheri* followed the same trend, but the endophyte effect was

non-significant (Fig. 5, Endophyte, $\chi^2_{1,70} = 0.44$, $P = 0.51$; Endophyte \times Precipitation $\chi^2_{2,70} = 2.91$, $P = 0.23$), possibly indicating that endophyte effects on *C. pitcheri* were strongest at the seedling stage.

Discussion

HOW DOES ENDOPHYTE SYMBIOSIS IN *A. BREVILIGULATA* AFFECT EARLY DUNE PLANT COLONIZATION AND PLANT DIVERSITY?

Under current climate conditions, the presence of the mutualism between native *A. breviligulata* and its *Epichloë* endophyte reduced the species richness, evenness and diversity of colonizing dune plants. A similar effect was previously documented in the agronomically important (and non-native) grass species, tall fescue (*Lolium arundinacium*, syn., *Schedonorus arundinaceus*), for which the presence of an epichloid endophyte caused up to 60% reductions in plant species richness (Clay & Holah 1999; Rudgers *et al.* 2007). To our knowledge, this is the first report of a fungal endophyte altering plant community composition when present in a native host plant.

The endophyte effect on plant diversity may be due to a combination of increases in the competitive dominance of *A. breviligulata*, changes in habitat quality and ecosystem processes (such as sand accumulation) and shifts in the abundance of non-plant community members. Since *Epichloë* symbiosis increases the survival and tiller density of *A. breviligulata* (Emery & Rudgers 2013; Emery, Bell-Dereske & Rudgers in press), we predicted that increased competitive dominance of the host grass would be shown by a negative relationship between *A. breviligulata* above-ground biomass and colonist diversity/abundance. Significantly negative correlations for evenness and the number of colonists in 2013 supported this hypothesis. In Atlantic coastal dunes, recruitment of annual plants was negatively correlated with the density of *A. breviligulata* (Cheplick 2006). Our results suggest that similar dynamics occur in Great Lakes dunes. In addition to changes in the competitive dominance of *A. breviligulata*, the presence of the endophyte could affect the plant community by altering plant traits that affect ecosystem processes such as decomposition (e.g. in tall fescue Lemons, Clay & Rudgers 2005) or sand accumulation (e.g. plant traits affect sand accumulation rates, Zarnetske *et al.* 2012). For example, the result that *A. breviligulata* seedlings were more successful at recruiting into plots with the endophyte suggests that endophyte symbiosis may ameliorate habitat conditions for its host plant. In addition, endophyte presence may affect other community members such as herbivores (Rudgers & Clay 2008) or mycorrhizal fungi (Mack & Rudgers 2008), with cascading effects on co-occurring plant species. For example, *Ammophila* species are well documented to associate with mycorrhizal fungi (e.g. Rodriguez-Echeverria *et al.* 2008), and other soil microbes are important controls on plant parasites in *Ammophila*-dominated European dunes (e.g. Piskiewicz *et al.* 2007).

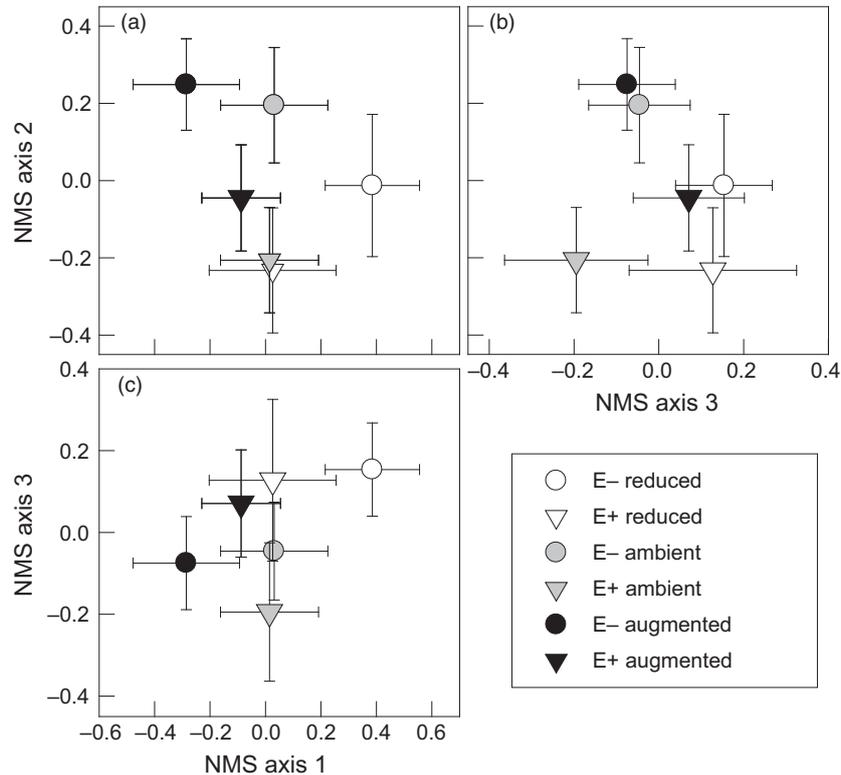


Fig. 4. Shifts in dune plant species composition in response to endophyte presence (E+) or absence (E-) in *Ammophila breviligulata* and to altered precipitation treatments: reduced by 30%, ambient, or augmented by 30%. Points show centroids \pm bidirectional standard errors along each of three NMS axes, allowing visualization of plant community separation. (a) shows data along NMS axes 1 and 2, (b) along axes 3 and 2 and (c) along axes 1 and 3. For 2013 (data shown here), 3D NMS stress = 0.09, indicating low risk of erroneous ordination.

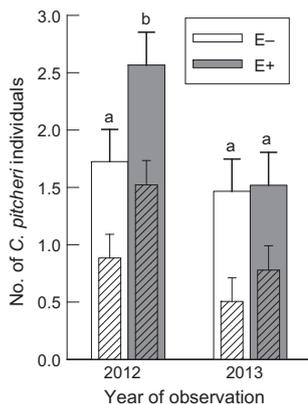


Fig. 5. Response of *Cirsium pitcheri* to endophyte presence (E+) or absence (E-) in *Ammophila breviligulata*. Solid bars show the LS mean number of individuals observed per plot \pm SE, from repeated measures general linear mixed models. Hashed bars show the LS mean number of juveniles (rosettes) observed per plot \pm SE. Different letters indicate significant differences in total abundance (solid bars only) ($P < 0.05$) as indicated by *post hoc* sequential Bonferroni tests. Statistical results are shown in Table 1.

Despite the negative effect of the endophyte mutualism on plant diversity under ambient precipitation, endophyte presence in *A. breviligulata* increased recruitment of a federally threatened forb species, the Great Lakes endemic *Cirsium*

pitcheri. An endophyte-mediated increase in sand accumulation may be one mechanism underlying higher *C. pitcheri* abundance. Our ongoing work shows ~25% greater sand accumulation when the endophyte is present (Emery, Bell-Dereske & Rudgers in press). Hamze & Jolls (2000) found that *C. pitcheri* seeds do not germinate without some sand burial, but seedling emergence is inhibited by sand cover >4 cm deep (Chen & Maun 1999). Additionally, survival, growth and below-ground biomass of *C. pitcheri* are stimulated by moderate levels of sand burial (~25% of their height) (Maun, Elberling & D'Ulisse 1996; Rowland & Maun 2001). A second possible mechanism driving mutualist-enhanced recruitment of *C. pitcheri* could be greater seed trapping rates. *Cirsium pitcheri* seeds are ~5–7.5 mm long and most fall within 5 m of the parent plant. Detached seed heads transported by wind and shifting sand provide the major mode of long-distance dispersal for this species (Loveless & Hamrick 1988). Greater tiller densities of endophyte-symbiotic *A. breviligulata* could increase the likelihood that detached seed heads become trapped on the dune, accumulating higher *C. pitcheri* seed densities. Altered soil moisture is a third possible mechanism. Both Hamze & Jolls (2000) and Chen & Maun (1999) speculated that the benefit of shallow sand burial on *C. pitcheri* germination was due to in part to greater soil moisture. However, we did not find increases in soil volumetric water content under endophyte presence, and SIMPER

analysis showed that *C. pitcheri* was most abundant when precipitation was reduced. Disentangling potential mechanisms underlying the facilitative effect of endophyte presence on *C. pitcheri* will require experiments that decouple the effects of the endophyte on rates of seed trapping from its effects on seed germination, seedling survival and plant growth.

As individual plants mature from seedlings into juveniles, then to reproductive adults, the magnitude and direction of the endophyte effect may change. For example, while the endophyte in *A. breviligulata* promoted the total number of *C. pitcheri* individuals, its effects on juvenile *C. pitcheri* were non-significant, suggesting that the benefits of endophyte presence attenuate at later life stages. Similar, life-history stage-specific effects of endophyte symbiosis have been reported for endophyte host plants (e.g. Rudgers *et al.* 2012), but these are the first data, to our knowledge, to suggest that an endophyte's effects on other community members can also be stage specific or size specific.

Under ambient precipitation, the endophyte effect on community evenness shifted between years. During the 2012 growing season, the presence of the endophyte had a marginally facilitative effect on plant community evenness under current climate, whereas in 2013, the endophyte significantly reduced evenness. This interannual difference could be due to yearly variation in abiotic factors or plant species dispersal rates. Alternatively, differences between years could reflect the successional process, possibly a shift from *A. breviligulata* facilitating seed trapping during 2012 to *A. breviligulata* competing more intensely with established colonists during 2013. Correlation analysis supported the latter hypothesis, as *A. breviligulata* biomass was significantly negatively correlated with plant species evenness in 2013, but showed no significant pattern in 2012, when *A. breviligulata* biomass was, on average, ~20% lower. Another possible explanation is that interactions among the colonists that arrived in 2012 could lead to a filtering effect during the second year of recruitment. The composition of the subdominant species pool has been shown to drive subsequent recruitment and assembly of plant communities in other ecosystems (Gibson *et al.* 2013).

DO EARLY PLANT COLONISTS IN DUNE COMMUNITIES RESPOND TO ALTERED RATES OF PRECIPITATION?

Altered rates of precipitation shifted dune plant species composition. The three species that showed the greatest responses included one grass (*Schizachrium scoparium*, little bluestem) and two herbaceous species, *Artemisia campestris* (beach wormwood) and *Cirsium pitcheri* (Pitcher's thistle). Both *S. scoparium* and *A. campestris* were more abundant under increased precipitation. Similarly, Lichter (2000) found that recruitment of dune plants was extremely water limited: without water addition, no *S. scoparium* seeds emerged. In contrast to the other two species, the federally threatened dune endemic *C. pitcheri* was most abundant when precipitation was reduced. In other ecosystems, there is evidence that climate change can significantly disrupt communities by having

disproportionately large effects on certain species (Lavergne *et al.* 2010). A deeper understanding of plant life-history traits may help to predict the characteristics of future communities, as our results suggest that the effects of future precipitation regimes in Great Lakes dunes will differ among plant species.

When dune plant species were classified into functional groups, only the grasses strongly responded to altered precipitation. The 140% increase in grass abundance under augmented relative to reduced precipitation may derive from higher soil moisture increasing the rate of grass germination (Lichter 2000). The lack of a significant effect of altered precipitation on other plant functional groups could be due to species differences within functional groups. For example, among herbaceous forbs, *A. campestris* increased with precipitation, whereas *C. pitcheri* declined. Under future increases in growing season precipitation (Vavrus & Van Dorn 2010), we would expect grass species to become more dominant members of the dune plant community. Such a shift in community composition could have effects that cascade to dune ecosystems via altered carbon, water and/or nutrient dynamics (e.g. Ampleman, Crawford & Fike 2014). However, it is important to note that there is a great deal of variation in precipitation projections depending on the climate model (Figs S1 and S2). It is possible that the Great Lakes region will experience shifts in precipitation exceeding the 30% decrease or increase imposed in our treatments. In addition, months outside the growing season may see even greater shifts in seasonal precipitation (Figs S1 and S2), with effects on dune communities that could not be captured by our study.

ARE THERE INTERACTIVE EFFECTS OF ENDOPHYTE SYMBIOSIS AND PRECIPITATION ON DUNE PLANT COMMUNITY DEVELOPMENT?

The strength of the mutualist-caused reduction in plant diversity depended on the precipitation regime and was only statistically significant under ambient rainfall. Based on our results, it appears that changes in growing season precipitation could alter or override the effects of endophytes on dune plant diversity in the future. Our previous work in growth chamber studies found overriding effects of temperature and precipitation changes on *A. breviligulata* growth and survival, independent of endophyte presence (Emery & Rudgers 2013).

It remains unclear what mechanisms underlie the endophyte-caused suppression of plant diversity only under certain precipitation regimes. One possibility is that the endophyte enhances the ability of *A. breviligulata* to compete for soil water, and we did observe reduced soil moisture in endophyte plots on some dates (Emery, Bell-Dereske & Rudgers in press, Table S2, Endophyte \times Precipitation \times Date). However, under this hypothesis, we would expect the strongest suppression of diversity under reduced, rather than ambient precipitation. Correlational evidence suggests that endophyte-suppressive effects may be stronger when the host plant is more dominant. For example, evenness and colonist abundance negatively correlated with *A. breviligulata* biomass

only in 2013, when *A. breviligulata* plants were larger. Thus, a lack of endophyte effect under reduced precipitation could result from the lower biomass of *A. breviligulata* under water limitation.

An additional suite of possible mechanisms relate to changes in non-plant members of the dune community. Pathogen and parasite accumulation on *A. breviligulata* (e.g. as for *A. arenaria*, van der Putten & Peters 1997) could increase under higher soil moisture and reverse any competitive advantage conferred by endophyte symbiosis. Such a mechanism could explain why the mutualist-mediated reduction in diversity disappeared when we increased rainfall. Interactions between the endophyte and beneficial soil microbes, such as arbuscular mycorrhizal fungi, may also be important for driving endophyte \times precipitation interactions. Foliar endophyte presence can alternately increase (Novas *et al.* 2011) or reduce arbuscular mycorrhizal colonization of grass roots (Mack & Rudgers 2008), depending on the species studied. Mycorrhizal fungi can be important for plant establishment in dune ecosystems world-wide (e.g. Koske & Gemma 1997; de la Pena *et al.* 2006; Rodriguez-Echeverria *et al.* 2008; Emery & Rudgers 2011) and mycorrhizas are sensitive to water availability (Kivlin, Emery & Rudgers 2013). Thus, shifts in the soil microbial assemblage, including mycorrhizal fungi, could underlie the responses of recruiting plant species in our system. Finally, endophyte presence and precipitation both can affect rates of nutrient cycling and litter degradation, as has been shown in other grass-dominated systems (e.g. Franzluebbers & Hill 2005; Lemons, Clay & Rudgers 2005). For instance, across the south-eastern US, endophyte presence in stands of tall fescue grass increased soil organic carbon by 6% and total nitrogen by 5% relative to adjacent endophyte-free stands (Iqbal *et al.* 2012). Whether and how the above-ground *Epichloë* endophyte and precipitation may alter these more cryptic, potential drivers of dune plant diversity remain to be investigated.

Endophyte treatments differed in which precipitation regime showed the highest diversity and richness of colonizing plants. When the endophyte was absent from *A. breviligulata*, a peak in diversity and species richness occurred under ambient precipitation (Fig. 2). In contrast, with the endophyte present, plant diversity and richness were highest under augmented precipitation. These results could indicate endophyte-mediated shifts in which resources are most limiting for colonizing plants. For example, deeper soil moisture showed a larger difference between reduced and ambient precipitation regimes than did shallow soil moisture (Fig. S3); this deeper resource could be more limiting in the absence of the endophyte, driving a plant diversity peak under ambient precipitation. In contrast, shallow soil moisture responded more strongly to precipitation augmentation than deep moisture; shallow moisture could be more limiting than deep moisture when the endophyte is present due to shifts in *A. breviligulata* root allocation. Additional mechanisms underlying differences in diversity and richness peaks may include endophyte-mediated differences in habitat modification by *A. breviligulata* or shifts in the abundance of non-plant community members.

Conclusion

In summary, we found that under current climate conditions, the presence of a grass-endophyte mutualism reduced the diversity of plant species colonizing during primary dune succession. To our knowledge, this work is the first to show that endophyte symbiosis can alter plant succession and diversity in a native ecosystem. However, the magnitude of the diversity reduction depended on the amount of rainfall, highlighting the importance of interactive abiotic and biotic drivers in dune succession and the potential for climate change to alter mutualist-mediated shifts in plant communities. A better understanding of the complex interactions involving cryptic, but potentially keystone, microbial symbionts may help to predict the characteristics of future communities under changing climates.

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Conflict of Interest

J.A.R., L.B.D., K.M.C. and S.M.E. have no conflict of interests to declare.

Data accessibility

Data deposited in the Dryad repository: (Rudgers *et al.* 2014).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. List of colonizing plant species found in experimental plots.

Table S2. Statistical results from analysis of the soil moisture response to experimental treatments.

Figure S1. Predicted change in average daily precipitation by 2041–2070 for (a) A1B emission scenario, (b) A2 emissions scenario and (c) B1 emissions scenario.

Figure S2. Predicted change in average daily precipitation by 2071–2100 for (a) A1B emission scenario, (b) A2 emissions scenario and (c) B1 emissions scenario.

Figure S3. Soil water content at (a) 20 cm (b) 40 cm in experimental plots.