

# Fungal symbiosis and precipitation alter traits and dune building by the ecosystem engineer, *Ammophila breviligulata*

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**Abstract.** Ecosystem engineer species influence their community and ecosystem by creating or altering the physical structure of habitats. The function of ecosystem engineers is variable and can depend on both abiotic and biotic factors. Here we make use of a primary successional system to evaluate the direct and interactive effects of climate change (precipitation) and fungal endophyte symbiosis on population traits and ecosystem function of the ecosystem engineering grass species, *Ammophila breviligulata*. We manipulated endophyte presence in *A. breviligulata* in combination with rain-out shelters and rainfall additions in a factorial field experiment established in 2010 on Lake Michigan sand dunes. We monitored plant traits, survival, growth, and sexual reproduction of *A. breviligulata* from 2010–2013, and quantified ecosystem engineering as the sand accumulation rate. Presence of the endophyte in *A. breviligulata* increased vegetative growth by up to 19%, and reduced sexual reproduction by up to 46% across all precipitation treatments. Precipitation was a less significant factor than endophyte colonization for *A. breviligulata* growth. Reduced precipitation increased average leaf number per tiller but had no other effects on plant traits. Changes in *A. breviligulata* traits corresponded to increases in sand accumulation in plots with the endophyte as well as in plots with reduced precipitation. Sand accumulation is a key ecosystem function in these primary successional habitats, and so microbial symbiosis in this ecosystem engineer could lead to direct effects on the value of these dune habitats for humans.

**Key words:** *Ammophila*; *dune geomorphology*; *endophyte*; *epichloae*; *keystone mutualism*; *Neotyphodium*; *plant-fungal interactions*; *positive interaction*.

## INTRODUCTION

Identifying factors that influence populations of ecosystem engineers is vital for conserving biodiversity and ecosystem function. Ecosystem engineer species influence their community and ecosystem by creating or altering the physical structure of habitats (Jones et al. 1994, Wright and Jones 2006). The function of ecosystem engineers is variable and can depend on both abiotic and biotic factors (e.g., Balke et al. 2012). For many ecosystems, one of the primary factors influencing populations of ecosystem engineers is climate change (Wolters et al. 2000, Menge et al. 2008). For example, eelgrass (*Zostera marina*), which engineers coastal estuarine systems, lost 44% of shoot density under simulated increases in temperature (Ehlers et al. 2008), corresponding to a potential loss of productivity, nutrient cycling, and sediment stabilization (Hughes and Stachowicz 2009). Similarly, ocean acidification due to increases in atmospheric CO<sub>2</sub> is reducing coral calcification, leading to a predicted fourfold decrease in coral cover over the next 80 years (Madin et al. 2012).

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Interactions with other members of the community, such as mutualists and competitors, can modify how ecosystem engineers respond to climate change (Singer et al. 2013), and so have a large influence on diversity and ecosystem function. For example, mutualists that alter traits of an ecosystem engineer species that ameliorate environmental stress or facilitate diversity in other trophic levels (e.g., coral reefs) may increase community diversity and ecosystem function (Bruno et al. 2003), especially in early-successional systems (Purschke et al. 2013). Because ecosystem engineers fundamentally alter the ecosystem, assessing the potential for interactive responses to abiotic and biotic factors has strong potential to improve predictions on the ecological consequences of climate change.

Systemic fungal endophytes represent one interaction that may have considerable ecosystem impacts under changing climates because these fungi can increase host plant resistance to drought, thermal stress, herbivory, and salinity (e.g., Malinowski and Belesky 2000, Redman et al. 2001, Clay and Schardl 2002, Marquez et al. 2007). The epichloae endophytes grow in the intercellular spaces of leaves and stems of ~20–30% of all graminoids (Leuchtmann 1992, Rodriguez et al. 2009). While their roles as mutualists can be context dependent (Cheplick and Faeth 2009), these endophytes are known to alter a number of plant traits including root biomass

and architecture, leaf nutrient content, and stomatal conductance, particularly under water stress (Lyons et al. 1990, Malinowski and Belesky 2000, Kannadan and Rudgers 2008). Such changes have the potential to modify plants' effects on the physical environment during the ecosystem engineering process.

Systemic grass endophytes have been primarily studied in two agriculturally important forage and turf grasses, *Lolium arundinaceum* (tall fescue syn., *Schedonorus arundinaceus*) and *L. perenne* (perennial ryegrass), where they can have dramatic community and ecosystem consequences, promoting dominance of host grasses, reducing plant and arthropod diversity, altering rates of decomposition and nutrient cycling, increasing soil carbon sequestration, and slowing plant succession (Franzluebbers et al. 1999, Lemons et al. 2005, Rudgers et al. 2007, Rudgers and Clay 2008, Iqbal et al. 2012). However, the effects of endophytes on natural ecosystems remain largely unknown (Saikkonen et al. 2006, Rudgers et al. 2009). By altering key traits such as root or shoot biomass (e.g., van der Stoel et al. 2002), clonal and sexual reproduction (e.g., Pan and Clay 2003, Faeth 2009), or litter quality (e.g., Omacini et al. 2004), plant symbionts may alter habitat structure (Eviner 2004) and the ecosystem engineering process (Wolters et al. 2000).

Here we make use of a primary successional system to evaluate the direct and interactive effects of climate change (precipitation) and endophyte symbiosis on plant traits and ecosystem function of the engineering grass species, *Ammophila breviligulata*, in a factorial field experiment. We hypothesized that the presence of the endophyte, *Epichloë* sp. (Clavicipitaceae), would increase plant growth and ecosystem function (sand stabilization), with the strongest benefits realized under the lowest levels of growing-season precipitation.

## METHODS

### *Study system*

Great Lakes dune plant communities are classic models of primary succession (Cowles 1899), but understanding of the mechanisms regulating that succession is limited (Little and Maun 1996). Dunes are dominated by the ecosystem engineering grass species, *Ammophila breviligulata* Fernald (American beachgrass), which stabilizes moving sand during early succession, thus directly altering the physical habitat and facilitating colonization of later-successional species (Olson 1958, Lichter 2000, Cheplick 2005). Two traits in particular, tiller diameter and tiller density, have been directly linked to dune building ability by *A. breviligulata* in wind tunnel experiments (Hacker et al. 2012, Zarnetske et al. 2012). Our previous work revealed that an undescribed species of systemic endophyte, *Epichloë* sp. (Clavicipitaceae), is present in *A. breviligulata* populations of the Great Lakes and especially frequent in material planted for restoration (Emery et al. 2010). However, the ecological consequences of this symbiosis remain largely unknown.

Drought may be a critical element of climate change in dune ecosystems, as many native Great Lakes dune plants are water-limited (Lichter 2000, Lyne Ensign et al. 2006). Climate models predict increases in evapotranspiration rates and drops in lake levels in the Great Lakes (IPCC 2007, Gronewold et al. 2013). Published regionally downscaled projections span a range from 35% increased (PCM model) to 45% reduced (GFDL model) growing-season precipitation (Vavrus and Van Dorn 2010). However, across 10 general circulation models from the IPCC Fourth Assessment Report, the predicted change for the study region ranged from a 30.9% decrease to 18.6% increase in growing-season precipitation by 2071–2100 compared to model baseline projections from 1971–2000 (Appendix A). The ability of dune ecosystems to withstand increasing environmental stress will depend in part on the ability of *A. breviligulata* to survive, and maintain or even increase traits important for dune building and soil stabilization.

### *Study site*

Our experiment site was located in Leelanau State Park, Leelanau County, Michigan, USA ( $45^{\circ}10.964' N$ ,  $85^{\circ}34.578' W$ ). We set up the experiment on a large blowout on the leading edge of the second fore dune, ~200 m from the Lake Michigan shore (Appendix B: Fig. B1). The blowout was largely devoid of vegetation and showed ongoing sand movement at the time of establishment.

### *Experimental design*

During late May 2010, we established a  $2 \times 3$  factorial field 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 ( $2 \times 2$  m) per treatment combination, each containing 25 transplanted *A. breviligulata* individuals. The 90 plots were arranged in seven rows of 8–15 plots each, matching the contour of the blowout (Appendix B: Fig. B2). Each plot was randomly assigned to a treatment combination.

**Rainfall manipulation.**—We constructed modified rain-out shelters to manipulate growing-season precipitation (Yahdjian and Sala 2002). Shelters ( $2 \times 2 \times 2$  m) were built from metal conduit secured with screws in order to withstand the high winds of the foredune 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 then 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 gutter presence without altering ambient rainfall. Each year, the gutters were reinstalled at the beginning of the growing season (late May) and removed as plants senesced (mid-Sept.).

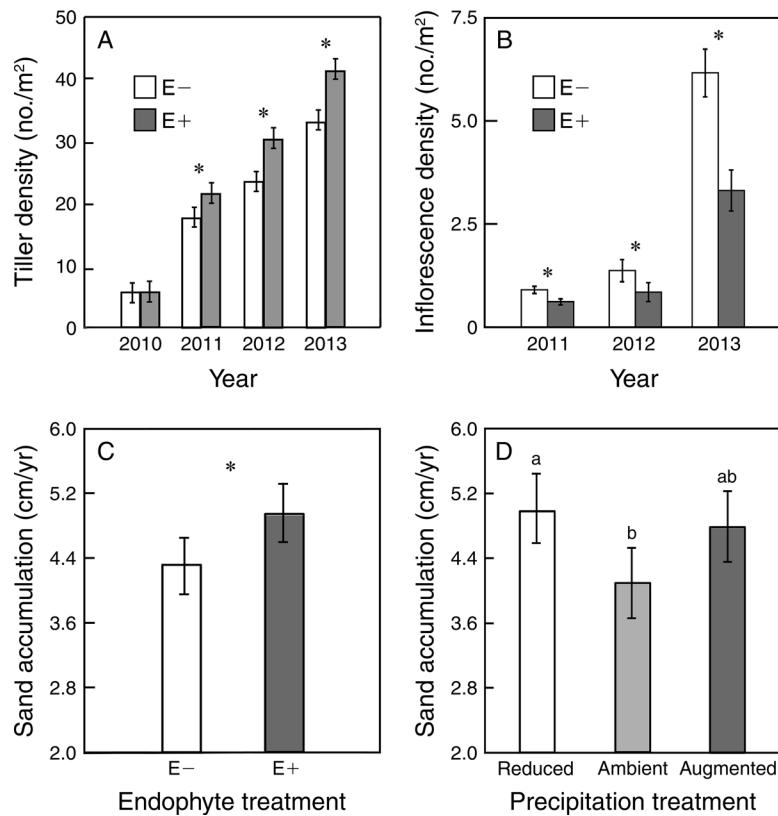


FIG. 1. Effects of (A) endophyte treatment (E–, without endophytes; E+, with endophytes) and year on tiller densities 2010–2013, (B) endophyte treatment and year on inflorescence densities 2011–2013, (C) endophyte treatment on sand accumulation rates 2010–2013, and (D) precipitation treatment on sand accumulation rates 2010–2013. Asterisks indicate significant Tukey post-hoc comparisons in the models at  $P < 0.05$ ; different lowercase letters in (D) indicate significantly different treatments. Bars show least-square means ( $\pm$ SE) from models that include the covariates of spatial location of the plot.

**Endophyte manipulation.**—To manipulate endophyte presence, we used endophyte-free seeds collected at nearby Sleeping Bear Dunes National Lakeshore ( $44^{\circ}51.472'$  N,  $86^{\circ}3.834'$  W) during fall 2006. Due to the unpredictable occurrence of the endophyte, we germinated seedlings on 1% water agar and inoculated half with endophyte isolates grown on potato dextrose agar. Preliminary genotyping showed no genetic variation among endophyte isolates in mating type or alkaloid genes (C. A. Young, *unpublished data*), which is not uncommon in asexual, vertically transmitted, epichloae species. We used a sterile needle to either wound (sham-inoculate, E– treatment) or insert hyphae into the meristem of each seedling (E+ treatment) at three to five days of age (Leuchtmann and Clay 1988). We used only successfully inoculated plants for the E+ treatment. Inoculations had an 8% success rate, comparable to other studies (Chung et al. 1997). Seedlings were grown in the greenhouse in a 50:50 mix of sterile play sand and Metro-Mix 220 (Sun Gro Horticulture, Agawam, Massachusetts, USA). As plants matured, we cloned genotypes by separating tillers from the original stock plants. After ~6 months of greenhouse propagation, we planted the same set of 12 *A. breviligulata*

*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<sup>2</sup>) to naturally occurring levels (Fant et al. 2008). Plants had, on average, five 30 cm long leaves and one or two tillers at time of planting (May 2010).

#### Data collection

**Plant traits.**—In September 2010, we recorded survival of the 25 tillers transplanted into each plot. From 2011–2013, individual transplants were no longer distinguishable, and we recorded *A. breviligulata* traits at the plot level during each year. Tiller density and sexual reproduction were recorded as the total number of tillers and total number of inflorescences per  $2 \times 2$  m plot at the end of the growing season (September; Fig. 1). We quantified tiller size as the average number of leaves and length of the longest leaf for five haphazardly chosen tillers per plot. In May 2013, we also recorded the number of newly germinated *A. breviligulata* seedlings per plot. These seedlings likely recruited from seeds that dispersed from inflorescences in the plot

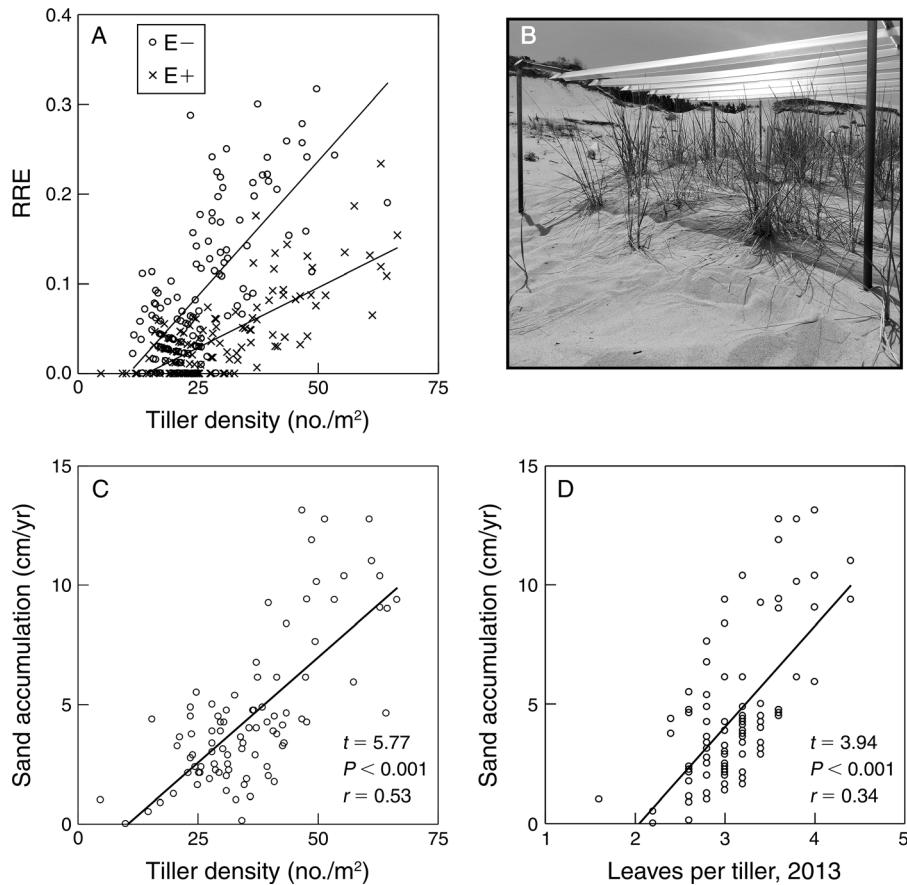


FIG. 2. (A) Relationships between tiller density and allocation to sexual reproduction (relative reproductive effort, RRE) in plots with ( $\times$ ) and without (circles) the endophyte. E+ slope = 0.00067, intercept = -0.038; E- slope = 0.0015, intercept = -0.063. (B) Photograph of single plot showing sand accumulation. (C and D) Linear relationships between yearly sand accumulation and tiller density and leaves per tiller.

before we collected them, although we cannot rule out immigration from nearby plots or natural vegetation. In May 2014, we measured basal tiller diameter to the nearest 0.1 mm for five haphazardly chosen tillers per plot using digital calipers. We also measured basal tiller diameter for one haphazardly selected live tiller per pot of plants maintained in the greenhouse that were the original source material for our field experiment; these plants were grown in equivalently sized pots (10.16 cm square  $\times$  10.16 cm deep) in a 50:50 mixture of sterilized sand and Metromix 360 (Sun gro Horticulture).

**Ecosystem function.**—At the initiation of the experiment, we placed a 60 cm long, 5 cm diameter PVC pipe in the center of each plot and marked the sand surface level on the pipe (Fig. 2B). In May and September of 2012 and 2013, we quantified sand stabilization in each plot by marking new sand levels and measuring changes since the previous measure. We calculated the average sand accumulation rate (cm/yr) by adding sand accumulation measures across all sampling dates and dividing by the number of years sampled (2010–2013).

#### Data analyses

We tested for treatment effects on *A. breviligulata* traits using repeated-measures general linear mixed models. We analyzed effects on each trait separately, as traits were not strongly correlated (correlation coefficients ranged from 0.14–0.56 for trait pairs).

The statistical models included the fixed factors of endophyte treatment, precipitation treatment, the endophyte  $\times$  precipitation interaction, and the repeated effect of year when multiple years of data were collected for a response variable (2010–2013 for tiller density; 2011–2013 for other responses). Plots was included as a random factor nested in endophyte and precipitation treatments. For sand stabilization and 2014 field tiller diameter, we used similar models without year. As covariates in all models, we included the spatial, categorical factors of column and row to represent plot location within the blowout (Appendix B: Fig. B2), which more accurately represented the sloping habitat gradient than a block term. For greenhouse tiller diameter, we analyzed data with one-way ANOVA using endophyte status as a fixed factor.

We followed these analyses with separate tests to address potential mechanisms operating in our system. To evaluate whether there was a tradeoff between sexual reproduction and clonal growth in *A. breviligulata*, we used a general linear model with tiller density, endophyte status, and their interaction as factors and relative reproductive effort (RRE; Miller et al. 2006), which is the proportion of total tillers that developed reproductive structures, as the response variable. This metric ranges between 0 and 1, with 0 indicating that a plant allocates all tillers to growth and 1 indicating that a plant allocates all tillers to reproduction. We also evaluated relationships between plant traits and sand accumulation using a general linear model, with 2013 data on tiller density, leaf length, leaves per tiller, and 2014 field data on tiller diameter as independent variables, along with their interactions with endophyte treatment. These variables were standardized to z-scores so that mean = 0, SD = 1 for each predictor variable. Response variables of tiller density and inflorescence number were square-root transformed to better meet model assumptions. All analyses were conducted using Systat version 12 (SYSTAT Software 2007).

## RESULTS

### *Soil moisture regimes*

During 2011–2013, our rain-out shelters significantly altered soil moisture. At 20 cm soil depth, augmented rainfall plots had an average of 9% higher soil moisture than reduced rainfall plots, and ambient plots had 3% greater soil moisture than reduced plots. At 40 cm soil depth, augmented plots had 9% higher soil moisture than reduced plots, and ambient plots had 6% higher soil moisture than reduced plots (J. A. Rudgers, *unpublished data*).

### *Plant traits*

The presence of the endophyte increased tiller density by 3–19% during the four-year experiment (Fig. 1A, Appendix C). The endophyte also decreased sexual reproduction (number of inflorescences per plot) by 32–46% (Fig. 1B). Inflorescence number in 2012 was positively correlated with the abundance of germinating *A. breviligulata* seedlings in 2013 ( $r = 0.375$ ,  $P < 0.001$ ), indicating that inflorescence production was a valid surrogate for reproductive success. Average leaf length was not affected by either the endophyte or precipitation, but tillers produced 5–6% more leaves under reduced compared to augmented rainfall (Appendix C; reduced,  $3.31\% \pm 0.06\%$  [mean  $\pm$  SE]; ambient,  $3.17\% \pm 0.05\%$ ; augmented,  $3.13\% \pm 0.06\%$  more leaves). In the greenhouse, endophyte presence explained 23% of the variation in basal tiller diameter ( $F_{1,38} = 11.4$ ,  $P = 0.0017$ ). Endophyte-inoculated plants had 20% smaller diameters than endophyte-free plants ( $E+ = 3.8 \pm 0.20$  mm;  $E- = 4.7 \pm 0.17$  mm). However, neither the endophyte nor precipitation affected tiller diameter in the field (endophyte  $F_{1,64} = 0.78$ ,  $P = 0.38$ ; precipitation

$F_{2,64} = 0.19$ ,  $P = 0.83$ ; endophyte  $\times$  precipitation  $F_{2,64} = 0.93$ ,  $P = 0.40$ ; row  $F_{6,64} = 5.92$ ,  $P < 0.001$ ; column  $F_{14,64} = 1.26$ ,  $P = 0.26$ ).

### *Ecosystem function*

Both endophyte presence and precipitation regime affected sand accumulation (endophyte  $F_{1,64} = 6.40$ ,  $P = 0.014$ ; precipitation  $F_{2,64} = 4.88$ ,  $P = 0.011$ ). Despite our prediction that endophyte benefits would be strongest under the least precipitation, there was no significant endophyte  $\times$  precipitation interaction ( $F_{2,64} = 0.88$ ,  $P = 0.421$ ; row  $F_{6,64} = 16.46$ ,  $P < 0.001$ ; column  $F_{14,64} = 35.58$ ,  $P < 0.001$ ). Plots with E+ plants had 16% more sand accumulation than did plots with E- plants (Fig. 1C). Plots with reduced precipitation had 19% more sand accumulation than ambient precipitation plots; augmented plots were intermediate (Fig. 1D).

### *Mechanisms*

There was no evidence for a tradeoff between vegetative growth and inflorescence production, as plots with high tiller density also had high RRE (Fig. 2A). However, the presence of the endophyte altered the relationship between sexual and clonal reproduction, with high tiller density E+ plots having lower RRE than did high tiller density E- plots (tillers  $F_{1,266} = 288.9$ ,  $P < 0.001$ ; endophyte  $F_{1,266} = 2.92$ ,  $P = 0.089$ ; tillers  $\times$  endophyte  $F_{1,266} = 42.32$ ,  $P < 0.001$ ).

Both tiller density and average leaves per tiller were positively associated with sand accumulation (tiller density  $t = 5.65$ ,  $P < 0.001$ , slope  $\beta = 0.53$ ; leaves per tiller  $t = 3.83$ ,  $P < 0.001$ ,  $\beta = 0.34$ ; mean leaf length  $t = 0.10$ ,  $P = 0.92$ ,  $\beta = 0.01$ ; tiller diameter  $t = 0.02$ ,  $P = 0.99$ ,  $\beta < 0.001$ ; model  $r^2 = 0.60$ ). While plants with the endophyte had higher tiller densities overall, endophyte status did not significantly alter the slopes of these relationships (all interactions with endophyte,  $P > 0.25$ ). Tiller density explained 28% of the variation in the sand accumulation rate, while leaf number per tiller explained 12% (Fig. 2C and D).

## DISCUSSION

This is the first field study, to our knowledge, to show that endophyte symbiosis can result in net benefits to *A. breviligulata*. Presence of the endophyte in *A. breviligulata* increased above-ground vegetative growth across all precipitation treatments. We found similar results in a short-term growth chamber experiment, where endophyte-inoculated *A. breviligulata* plants had 14–23% greater survival and growth compared with endophyte-free plants (Emery and Rudgers 2013). From the plant's perspective, increased vegetative growth is especially advantageous in harsh ecosystems such as sand dunes, where new tillers have better chances of survival than new seedlings (e.g., Pennings and Callaway 2000). However, the endophyte also reduced sexual reproduction. On sand dunes, seeds and seedlings of *A. breviligulata* are often quickly buried or blown away

and do not contribute greatly to population persistence (Maun 1984, 1985). Longer-term data will ultimately allow us to quantify the contribution of endophyte symbiosis to demographic transitions and net population growth (e.g., Miller and Rudgers 2014), but given the prior demographic work on this plant species, we do not expect the endophyte-mediated decline in sexual reproduction to have a strong influence at the population scale.

While we did not find evidence for a direct trade-off between sexual reproduction and vegetative growth, an individual E– tiller more than doubled allocation to reproduction as compared to an individual E+ tiller (E– slope = 0.0015, E+ slope = 0.00067, Fig. 2A). We suggest this may be a plant response to increased stress in the absence of the endophyte. Some perennial plants respond to stress by increasing allocation to reproduction (e.g., Kozlowski and Pallardy 2002, Reekie and Bazzaz 2005), although further observations or greenhouse experiments would be required to directly test for stress-induced reproductive allocation in *A. breviligulata*. Other studies on grasses have similarly failed to find trade-offs between sexual reproduction and vegetative investment (e.g., Reekie 1991, Ott and Hartnett 2011). Endophyte-enhanced vegetative growth and reduced sexual reproduction have been shown in other plant-endophyte systems as well (e.g., Groppe et al. 1999, Ahlholm et al. 2002). For example, the endophyte *Epichloë glyceriae* reduced sexual reproduction but increased stolon numbers in infected *Glyceria striata* (Pan and Clay 2003). In contrast, in the grass, *Cinna arundinacea*, endophyte symbiosis reduced plant survival, but increased reproduction in ways that enhanced population growth rates over endophyte-free populations (Rudgers et al. 2012). As the *Epichloë* sp. endophyte appears to be exclusively vertically transmitted at our sites (we have never observed stromata), and vertical transmission through seeds is often imperfect (Afkhami and Rudgers 2008), increased vegetative growth, rather than seed transmission, is likely to be the more important contributor to endophyte fitness (Pan and Clay 2003).

The increase in tillering due to the presence of the endophyte had immediate impacts on ecosystem function in Great Lakes sand dunes. Plots with the endophyte had ~16% greater sand accumulation than plots without the endophyte, and this shift was due in part to the endophyte-mediated increase in numbers of tillers (explaining 28% of the variation in yearly sand accumulation). Previous work conducted on populations on the U.S. Pacific Coast showed that *A. breviligulata* tiller density was a major predictor of dune building (Hacker et al. 2012, Zarnetske et al. 2012), so it is not surprising that plants with more vigorous vegetative growth would accumulate sand faster. Somewhat surprisingly, tiller diameter was not a significant predictor of sand accumulation in our study, despite this being an important trait for sand accumu-

lation in other studies (Hacker et al. 2012, Zarnetske et al. 2012). This may in part be due to our limited sampling of field tiller diameters and confounding effects of sand burial. However, tiller diameter measures from the greenhouse showed that endophyte-free plants had larger tiller diameters than symbiotic plants. Because this pattern was inconsistent with field observations of higher sand accumulation in plots with the endophyte present, we suggest that tiller diameter may not play a large role in ecosystem functioning in our system.

Sand accumulation is a key ecosystem function in these primary successional habitats that depend on stabilization of dunes to increase biodiversity (Lichter 1998). Along the U.S. Pacific Coast, increased sand accumulation by species with high tiller densities was associated with taller, narrower dunes (Zarnetske et al. 2012), which can control ecosystem services such as coastal protection, erosion control, water purification, maintenance of wildlife, and tourism opportunities (Barbier et al. 2011). Thus, the presence of microbial symbiosis in the Great Lakes region could lead to taller, narrower dunes, with unknown consequences for humans and wildlife.

Precipitation appears to be a less significant factor than endophyte presence for *A. breviligulata* growth. Reduced precipitation slightly increased leaf number per tiller, while there were no effects of precipitation on tiller density, tiller diameter, leaf length, or reproduction. While our precipitation manipulation significantly altered soil moisture in plots (J. A. Rudgers, *unpublished data*), the average change in soil moisture was small, and our soil moisture values overall were high compared to reported values in similar habitats. For example, Ensign et al. (2006) found that soil moisture in dune habitats along Lake Huron naturally ranged from 2% to 16%, while our soil moisture values ranged between 1% and 30%. Since dune ecosystems are known to have very low soil moisture due to high soil porosity, even in areas with high rainfall (Baldwin and Maun 1983), our precipitation reduction may not have been stressful to the plants. However, the increase in sexual reproduction by endophyte-free plants could indicate some physiological stress (Griffiths and Bonser 2013). Importantly, the predicted range of soil moisture in this region under future climate change falls within the range of soil moistures found in our experiment (Appendix A), suggesting future precipitation regimes may have only small effects on *A. breviligulata* populations. It may also be that *A. breviligulata*, like its relative *A. arenaria*, tolerates drought stress better than other dune species (Dixon et al. 2004).

While precipitation appears to have minimal effects on aboveground traits of *A. breviligulata* populations, we did find significant effects of altered precipitation on sand accumulation. Plots with reduced precipitation had 19% more sand accumulation than ambient precipitation plots, perhaps due to increased number of leaves

per tiller, which explained 12% of the variation in sand accumulation rate and could slow wind velocity thereby increasing sand deposition (Udo and Takewaka 2007). Reduced soil moisture is also linked to increased aeolian processes, which could increase dune building activity (Ravi et al. 2011). We did not measure belowground root biomass or root traits, which are known to influence sand binding in *A. breviligulata* (Emery and Rudgers 2013), and could contribute to sand accumulation by retaining sand when seasonal winds result in erosive forces. Future work could benefit from manipulations of external sand inputs to test whether sand burial or erosion alter plant traits associated with sand capture, generating biophysical feedbacks (Murray et al. 2008, Corenblit et al. 2011).

Finally, we found no evidence for context-dependency in the endophyte effect on plant growth or ecosystem function. The endophyte-mediated increases in plant growth and ecosystem function (sand stabilization) did not occur under the reduced water regime, but were similar across precipitation treatments. Mutualistic species interactions are often context-dependent (Bronstein 1994, Chamberlain et al. 2014), and based on prior studies of epichloae in grasses (e.g., Malinowski and Belesky 2000, Kannadan and Rudgers 2008, Davitt et al. 2011), we predicted the benefits of symbiosis would increase when plants experienced a more stressful environment, such as precipitation extremes. Alternatively, under abiotic stress, endophytes could also become a liability due to excessive resource demands (Cheplick 2007). However, little research has directly tested fungal endophyte benefits under manipulations of future climates (reviewed in a recent meta-analysis by Kivlin et al. [2013]) and, in our system, the direct benefits of the endophyte appear consistently independent of climate. It may be that other climate variables, such as increased temperature or CO<sub>2</sub> levels, would show interactive effects with this symbiosis (Hunt et al. 2005, Kivlin et al. 2013). However, in an earlier growth chamber experiment with *A. breviligulata*, we found no evidence for context dependency in the endophyte effect on plant growth or survival under warmer temperatures (Emery and Rudgers 2013), providing further support that endophyte effects in this system are largely context independent.

The direct benefits of endophyte symbiosis for *A. breviligulata* and dune building processes could have important conservation implications. *Ammophila breviligulata* is widely planted throughout the Great Lakes and Atlantic Coast regions to stabilize disturbed dunes, and the endophyte is present in much of the plant material available for restoration (Emery and Rudgers 2010). As more dune habitats are disturbed due to human or climate impacts, raising awareness of the potential benefit of restoring not only the plant but also its microbial symbionts will become important for maximizing ecosystem function in these ecosystems.

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## SUPPLEMENTAL MATERIAL

## Ecological Archives

Appendices A–C are available online: <http://dx.doi.org/10.1890/14-1121.1.sm>