

Impacts of simulated climate change and fungal symbionts on survival and growth of a foundation species in sand dunes

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Abstract For many ecosystems, one of the primary avenues of climate impact may be through changes to foundation species, which create habitats and sustain ecosystem services. For plants, microbial symbionts can often act as mutualists under abiotic stress and may mediate foundational plant responses to climate change. We manipulated the presence of endophytes in *Ammophila breviligulata*, a foundational sand dune species, to evaluate their potential to influence plant responses to climate change. We simulated projected climate change scenarios for temperature and precipitation using a growth chamber experiment. A 5 °C increase in temperature relative to current climate in northern Michigan reduced *A. breviligulata* survival by 45 %. Root biomass of *A. breviligulata*, which is critical to dune stabilization, was also strongly reduced by temperature. Plants inoculated with the endophyte had 14 % higher survival than endophyte-free plants. Contrary to our prediction, endophyte symbiosis did not alter the magnitude or direction of the effects of climate manipulations on *A. breviligulata* survival. However, in the absence of the endophyte, an increase in temperature increased the number of sand grains bound by roots by 80 %, while in symbiotic

plants sand adherence did not significantly respond to temperature. Thus, plant–endophyte symbiosis actually negated the benefits in ecosystem function gained under a warmer climate. This study suggests that heat stress related to climate change in the Great Lakes may compromise the ability of *A. breviligulata* to stabilize dune ecosystems and reduce carbon storage and organic matter build-up in these early-successional systems due to reduced plant survival and root growth.

Keywords *Ammophila breviligulata* · Arbuscular mycorrhizal fungi · Ecosystem engineer · Endophyte · Mutualism

Introduction

During the next century, climate changes are expected to result in increasing temperatures and altered precipitation patterns worldwide, with possible consequences for community structure and ecosystem processes. For many ecosystems, one of the primary avenues of climate impacts may be through changes to foundation species, which create habitats and sustain ecosystem services (Ehlers et al. 2008; Gedan and Bertness 2010). Because foundation species fundamentally structure the community and ecosystem, understanding their responses to abiotic factors can improve predictions on the ecological consequences of climate change.

Climate change can have both direct effects on foundation species through altered abiotic conditions (e.g., temperature effects upon eelgrass; Ehlers et al. 2008) and indirect effects through altered biotic interactions (Tylianakis et al. 2008; Van der Putten 2012). For plants, microbial symbionts that act as mutualists under abiotic stress (Bacon

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and White 2000; Rodriguez et al. 2008) may ameliorate the effects of climate disruptions, yet these relationships have been relatively understudied. Our previous work with the foundation species *Ammophila breviligulata* (American beachgrass) found that a systemic fungal endophyte (epichloae, Clavicipitaceae) was present in populations in the Great Lakes, although the ecological consequences of this symbiosis are largely unknown (Emery et al. 2010). Endophytic fungi in other grass species can show considerable ecological impacts by increasing plant resistance to drought, thermal stress, herbivory, or salinity (Crawford et al. 2010; Marquez et al. 2007). Alternatively, under abiotic stress, endophytes could become a liability due to excess resource demands (Cheplick 2007). Endophytes in some well-studied species are also known to alter plant traits, for example by increasing root biomass or modifying root architecture, particularly under water stress (Kannadan and Rudgers 2008; Malinowski and Belesky 2000). It remains unclear whether symbiont-mediated changes in the functional traits of foundation plant species will affect key ecosystem processes.

In plants, above-ground symbionts could also influence ecosystem processes—and ultimately affect the ecosystem-level response to climate change—through their influence on below-ground plant–microbe interactions. For example, in some plants, the presence of foliar endophytes reduces colonization of host plant roots by mycorrhizal fungi (e.g., Chu-chou et al. 1992; Mack and Rudgers 2008), although positive correlations between above- and below-ground fungal symbionts have also been reported (Novas et al. 2011). Mycorrhizal fungi can contribute to soil aggregate stability and carbon sequestration (e.g., Tisdall 1991) and also mediate the response of host plants to stress (Augé 2001). If similar ecosystem-level effects occur in foundation dune grasses, this could have implications for dune conservation under climate change.

Here we examined the effects of increased temperature and altered water availability on the foundation dune grass, *A. breviligulata*. We manipulated the presence of systemic foliar endophytes to test for their mediation of plant responses to altered temperature and water availability. Using a growth chamber experiment, which allowed us to control abiotic stressors and to collect detailed trait data, we addressed the following questions. (1) Does increased temperature or altered water availability reduce survival, growth or functional traits associated with ecosystem engineering in *A. breviligulata*? (2) Do foliar endophytes increase *A. breviligulata* performance or alter functional traits associated with ecosystem processes? (3) Can foliar endophytes alter the magnitude or direction of *A. breviligulata* responses to climate-related stressors? (4) Do foliar endophytes or climate manipulations alter associations between *A. breviligulata* and mycorrhizal fungi, with potential consequences for ecosystem processes?

Methods

Study organisms

Ammophila breviligulata (American beach grass) is a highly clonal, perennial, C₃ grass and a dominant pioneer plant in temperate coastal and lacustrine dunes of North America (Maun 1984). *A. breviligulata* is native to the Atlantic Coast from Newfoundland to North Carolina, and its range also includes the shores of all five Great Lakes and Lake Champlain (Gleason and Cronquist 1991). This species stabilizes moving sand during the early stages of dune succession and contributes to early soil carbon enrichment; thus, it is considered a foundation species and ecosystem engineer (Cheplick 2005; Martin 1959). Sand stabilization is most likely a function of the extensive root and rhizome system of this species (Seliskar 1994). In addition, work in other systems has linked several below-ground traits, including longer and denser fine roots and root hairs, density of fungal hyphae, and greater root mass, with increased soil stability and reductions in soil erosion (Eviner and Chapin 2003; Tisdall 1991).

In previous work, we found that an undescribed species of systemic endophyte in the genus *Epichloë* (Clavicipitaceae) was present in *A. breviligulata* populations of the Great Lakes (Emery and Rudgers 2010), although the ecological consequences of this symbiosis remain largely unknown. Earlier, White et al. (1992) found *Epichloë* in *A. breviligulata* on the East Coast of the United States. Commercially available *A. breviligulata*, including the commonly sold USDA varietal ‘Cape,’ are 100 % symbiotic with this endophyte (Emery and Rudgers 2010).

For this experiment, we collected seeds of *A. breviligulata* from Sleeping Bear Dunes National Lakeshore (SBDNL) in Michigan (44.73, –86.15). Surveys of endophyte frequency found no endophytes in the source population for this study, although endophytes were found in nearby populations (Emery and Rudgers 2010) and a recent survey indicated that 40 % of Great Lakes populations have at least low levels of endophyte presence (Emery, unpublished data). Collected seeds were stored at 4 °C until germinated.

Endophyte inoculation

To test for effects of endophyte symbiosis in *A. breviligulata*, we inoculated endophyte-free plants with an endophyte isolated from the USDA varietal ‘Cape’ *A. breviligulata*. Genotyping work has shown that all endophytes isolated from *A. breviligulata* thus far, including Great Lakes genotypes, are identical (C.A. Young, unpublished data). As Cape is reliably 100 % colonized (Emery et al. 2010), it was logistically easier to isolate the endophyte

from these plants compared with field collected plants. Additionally, while removal of endophytes from seeds has been a common practice in experiments with other grass species (e.g., Rudgers et al. 2005), *A. breviligulata* has extremely low seed set (Emery and Rudgers, unpublished data); thus, artificial inoculation represented the best method for manipulating endophyte presence while controlling for plant genotype. To isolate the Cape endophyte, we cut 1 cm stem sections from living Cape plants (nursery sources: Church's Nursery, Cape May, NJ; Peat and Sons Nursery, Westhampton, NY; Cape Farms, Lewes, DE), sterilized the surface in 1 % Tween solution for 5 min and in 50 % household bleach solution for 10 min, and then placed the sections on cornmeal malt agar petri plates. Plates were then sealed and stored at room temperature for 3 weeks until the endophyte hyphae grew out of the stem tissue onto the agar plate. The fungus was then batch-cultured using standard techniques (Bacon and White 1994).

Seeds were surface sterilized in a 50 % household bleach, then were set in 1 % water agar in sealed petri dishes and kept at room temperature until germination. When seedlings were 2–4 days old, we inoculated them by puncturing the base of the seedling stem with a flame-sterilized insect pin and then inserted hyphae into the meristem. Seedlings were either inoculated with sterile water (control) or with a plug of endophyte hyphae grown on potato dextrose agar, following Latch and Christensen (1985). Seedlings were then planted into sterile potting soil (Metro-mix 220; Sunagro Horticulture, Bellevue, WA), watered, and covered with plastic to maintain humidity and encourage colonization by hyphae. Approximately 8 % of inoculated seedlings were successfully colonized, as revealed by both light microscopy on stained sections of the inner leaf sheath and immunoblot detection methods (Hiatt et al. 1999, 2002). Mature plants were maintained in a greenhouse and encouraged to tiller by planting in shallow 10 cm square pots filled with a 50:50 mix of sterile sand and potting soil (Metro-mix 220). As new tillers grew, we gently separated them from the main plant, keeping roots intact, and transplanted them into new pots for clonal propagation.

Growth chamber experiment

Set-up

In June 2010, 60 EF⁻ plants (propagated from 12 genotypes) and 60 EF⁺ plants (propagated from another set of 12 genotypes) were planted in 2.8 l “Tall One” pots (10 cm wide × 36 cm deep; Stuewe and Sons, Corvallis, OR) with a mixture of 75 % screened and washed play sand (Quikrete Inc., Atlanta, GA) and 25 % MetroMix 200. We added 50 ml of sand collected from Sleeping Bear Dunes National Lakeshore to the root zone of plants to supply native soil biota.

Temperature treatment

Half of each group of plants was placed in a Conviron growth chamber (model PGR15; Winnipeg, Manitoba, Canada) set at 25 °C/11 °C, representing the current average summer high and low temperatures for June in the SBDNL region. The remaining plants were placed in a growth chamber set at 30 °C/16 °C, representing a predicted future climate of a 5 °C increase in summer high temperatures, consistent with several climate change models (IPCC 2007). For example, in Michigan summer maximum temperatures are expected to increase 4–8 °C by 2095 (Kling et al. 2003). In the Sleeping Bear Dunes National Lakeshore region specifically, AR4 projections predict anywhere from a 1.9 to 8.2 °C increase in summer maximum temperatures by 2080 (<http://ccsn.ec.gc.ca>). Both growth chambers had equal light measurements (~600 μmol/m²/s) and were set for the same light/dark cycles reflecting available daylight in June for Michigan (16 h light). After the first month, temperature settings were adjusted in both chambers to reflect average July high and low temperatures (27 °C/14 °C, 15 h daylight; plus 5 °C increase in the future climate chamber). After the second month, temperature settings were adjusted in both chambers to reflect average August high and low temperatures (26 °C/14 °C, 14 h daylight; plus 5 °C increase in the future climate chamber). The experiment ran for 90 days. The entire experiment was then repeated from October to December 2010, with the growth chamber temperature assignments switched to attempt to control for any variation between the chambers.

Water treatment

The plants in each chamber were split into three groups for watering treatments. We created three levels of water availability corresponding to normal current precipitation in the Great Lakes. (1) Average/ambient: watering with 440 ml every 2 days based on average long-term summer precipitation amounts (0.11 cm/day) and frequency (16–18 rain events per month) reported in NOAA climatic summaries, corrected by surface area of pots, increased by 30 %. (2) 570 ml water every 2 days (McBean and Motiee 2008), or reduced by 30 %. (3) 310 ml water every 2 days, reflecting the high end of climate model predictions (IPCC 2001; Kling et al. 2003). Although precipitation is less predictable in the Great Lakes region in many climate scenarios (range of 21 % increase to 24 % decrease in total summer precipitation predicted by AR4 projections; www.ccsn.ec.gc.ca), this will mostly come from increases in severe storm events, with longer intervals between rain events and possible increased erosion associated with severe storms (Sousounis and Grover 2002). The interaction between increased temperatures and more variable/

extreme precipitation events will likely increase drought stress in this region (Kling et al. 2003). While we intentionally controlled the amount of water addition, rather than controlling soil moisture, in order to allow temperature to affect evapotranspiration, as would occur in the field (see similar methods in De Boeck et al. 2008), high soil moisture values in the first weeks of the experiment led us to reduce watering to twice a week for the remainder of the experiment.

Average volumetric soil water content in the water treatments ranged from 8.94 to 20.21 % (increased), 7.62–15.91 % (average), and 7.27–10.77 % (reduced). These were comparable to values from a complementary field experiment in Michigan where average volumetric water content (%) in ambient rainfall plots at 20 cm depth was 15.2–27.0 % using a digital soil moisture meter (Aquater M300; Costa Mesa, CA). The soil moisture probe has a ± 2 % error.

Response variables

Plant survival and growth

At the beginning of the experiment, we measured the lengths of all leaves as a measure of initial plant size to use as a covariate. At the end of the experiment, we determined survival and then clipped all stem and leaf tissue, which was dried at 65 °C and weighed to the nearest 0.01 g. Roots were gently rinsed to remove sand, processed for root traits (see *Root traits* section), then dried at 65 °C, and weighed to quantify below-ground biomass. For analysis, we conducted MANCOVA on log-transformed above- and below-ground biomass, including the fixed factors of endophyte (presence/absence), temperature (current/future), water (reduced/average/increased), round (first/second), and all interactions; log-transformed initial plant size (sum of leaf lengths) was a covariate. If treatments were significant, we followed with protected ANCOVA on individual responses. For survival, we applied a log-linear model (same factors as MANCOVA), with a binomial distribution for survival (0/1). Analyses were conducted in SAS version 9.3 (SAS Institute, Inc., Cary, NC). Because statistical results were qualitatively the same as analyses without genotype and because high mortality restricted replication of genotype effects for trait measurements on some treatment combinations, we did not include plant genotype in final analyses nor did we interpret differences in responses among genotypes.

Sand adherence

Before roots were rinsed, ten 1 cm sections of root were cut from active root tips, placed into 1.5 ml mini-centrifuge

tubes, and air dried for 2 weeks. A single root section was then placed into a beaker of 50 ml DI water, and forceps were used to gently loosen all sand adhering to the root. The sand solution was poured through a 25 mm glass microanalysis vacuum filter holder fitted with P8 grade filter paper. Filter paper was air dried, and sand grains on the filter paper were brushed onto a black petri dish and digitally photographed at 75 \times (Nikon SMZ1500 stereoscope). Sand grains were manually counted from digital photographs. For analysis, we conducted ANCOVA on square-root-transformed sand grain adherence, following methods for biomass. For exploratory analysis of relationships between sand adherence and traits (see next section), we additionally included each trait singly as a covariate in the ANCOVA model and examined the Spearman rank correlation matrix among traits and sand grain adherence.

Root traits

Washed roots were placed in a clear plastic container with water on a flatbed scanner and scanned. The image analysis program WinRhizo (Regent Instruments, Canada) was used to measure average root diameter, total root length, total root volume, root area, total number of root tips, and root tips per cm of root length. We additionally included the root:shoot ratio, calculated from dry biomass. Response variables were combined in MANCOVA on root traits, including the fixed factors of endophyte, temperature, water, round, and all interactions with log-transformed initial plant size as a covariate. Root length, area, volume, and total number of root tips were square-root transformed, average root diameter and tips per cm were log-transformed, and the root:shoot ratio was arcsin square-root transformed to achieve normality of residuals and homogeneity of variances. In addition, root traits were standardized to mean = 0, SD = 1 to eliminate differences in the scale of measurement and weight traits equally in multivariate analysis. If treatments were significant, we tested individual responses with protected ANCOVA.

Arbuscular mycorrhizal fungi

Five root sections (≥ 3 cm length) were clipped from haphazardly chosen locations from rinsed roots after they were scanned. Root sections were placed into tissue cartridges (M510 Slimsette; Simport Scientific, Beloeil, Quebec, Canada) and then soaked in hot 10 % KOH for 30 min and stained using the ink (black; Scheaffer Pen, Shelton, CT) and vinegar method (Vierheilig et al. 1998). Ten 1 cm root sections from each pot were mounted on microscope slides and visually scored for arbuscular mycorrhizal fungi (AMF) colonization. Using a compound microscope (Leica Microsystems, Wetzlar, Germany) at 200 \times magnification,

colonization rates were scored as the percentage of fields of view with AMF hyphae, arbuscules, or vesicles present out of a total of 40 fields of view per plant. This method provides an accurate measure of relative treatment effects, but may overestimate the total level of root colonization (McGonigle et al. 1990). No dark septate endophytes were observed, and brown fungi (possible saprotrophs) were rare (<10 % of plants). Rates of root colonization by AMF ranged from 12.5 to 90.0 %.

To estimate the length of extraradical hyphae, 20 g of well-mixed soil per pot was mixed with 500 ml DI water in a 100 ml beaker and stirred at 80 % speed for 2 min with a magnetic stirrer (Fisher Scientific 11-500-49SH). 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 on the magnetic stirrer. A 20 ml sample was removed from ~1 cm below the water surface and drained through a 25 mm glass micro-analysis 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 assessed using the gridline-intercept method (McGonigle et al. 1990) under a stereomicroscope (Nikon SMZ1500 at 70 \times) and is reported as percentage of intercepts per field of view. AMF response variables were standardized to mean = 0 and standard error = 1, then combined in MANCOVA, as described for plant biomass. If treatments were significant, we tested individual responses with protected ANCOVA. Prior to standardization, percentage root colonization was arcsin square-root transformed, and extraradical hyphal length was log-transformed.

Results

Plant survival and growth

A 5 °C increase in temperature relative to current climate for northern Michigan reduced *Ammophila breviligulata* survival by 45 % (Fig. 1a, Table 1). Endophyte symbiosis increased survival, but by 14 %, a smaller effect size than seen with temperature (Fig. 1b, Table 1). *A. breviligulata* was somewhat sensitive to both high and low extremes of water (marginally non-significant effect, Table 1), with 29 % higher survival under average water relative to reduced water, and 16 % higher survival under average

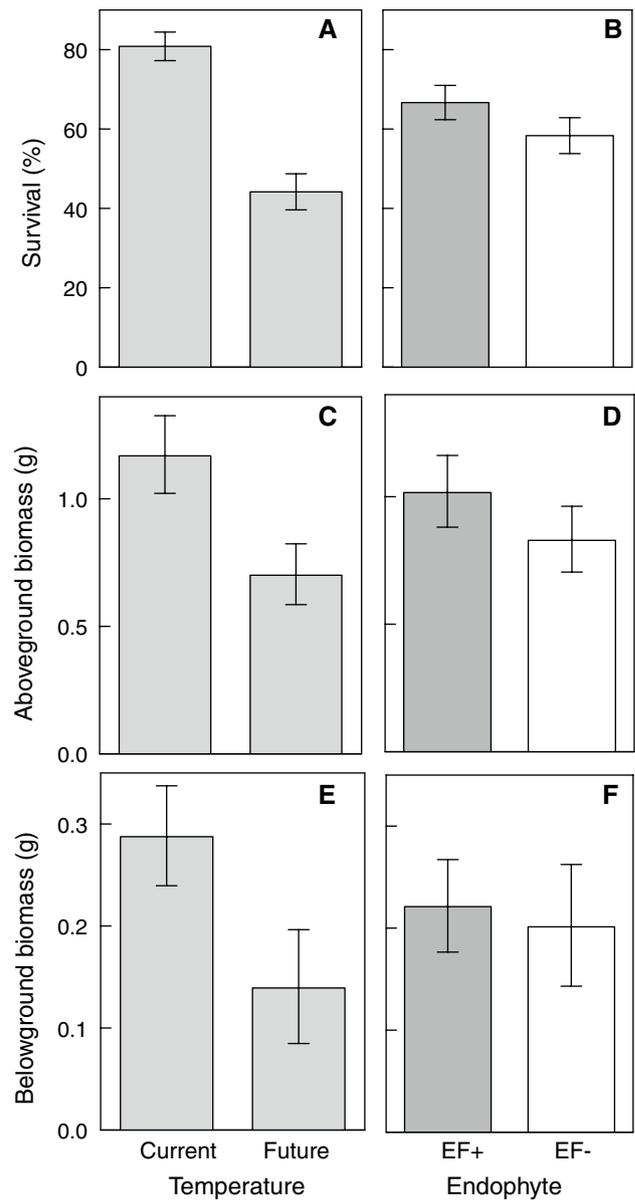


Fig. 1 *Ammophila breviligulata* survival (a, b), above-ground biomass (c, d) and below-ground biomass (e, f) in response to climate and endophyte manipulations. Bars show means with 95 % confidence limits

water relative to increased water (mean \pm s.e.: reduced: 56 ± 6 %; average: 72 ± 5 %; increased: 60 ± 6 %). Contrary to our prediction, endophyte symbiosis did not alter the magnitude or direction of climate effects on *A. breviligulata* survival (non-significant endophyte \times climate treatment interactions (Table 1).

The response of above-ground biomass to our treatments was similar in direction to that of survival. Under a future temperature scenario, above-ground biomass was reduced by 36 % relative to current temperature conditions (Fig. 1c, Table 1). The strength of the effect of temperature differed

Table 1 Statistical results for plant performance including survival, plant biomass (MANCOVA), and individually, above- and below-ground mass

Effect	Plant survival			Plant biomass MANCOVA				Above-ground mass		Below-ground mass	
	df	X^2	<i>P</i>	Pillai's trace	df	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Endophyte	1	6.08	0.0136	0.045	2, 206	4.83	0.0089	4.73	0.0309	0.13	0.7171
Temperature	1	41.84	<0.0001	0.169	2, 206	20.97	<0.0001	21.87	<0.0001	41.64	<0.0001
Endophyte × temperature	1	0.96	0.3267	0.004	2, 206	0.37	0.6932	0.12	0.7315	0.04	0.8357
Water	2	4.90	0.0861	0.049	4, 414	2.58	0.0371	2.53	0.0818	0.53	0.5878
Endophyte × water	2	0.54	0.7628	0.007	4, 414	0.37	0.8331	0.27	0.7644	0.00	0.9990
Temperature × water	2	3.88	0.1434	0.018	4, 414	0.96	0.4283	1.26	0.2865	0.96	0.3827
Endophyte × temperature × water	2	2.32	0.3141	0.010	4, 414	0.52	0.7220	0.11	0.8984	0.71	0.4908
Round	1	20.22	<0.0001	0.054	2, 206	5.87	0.0033	11.59	0.0008	8.86	0.0033
Endophyte × round	1	2.64	0.1040	0.012	2, 206	1.25	0.2881	0.61	0.4342	0.05	0.8261
Round × temperature	1	2.53	0.1115	0.037	2, 206	3.99	0.0200	4.12	0.0435	0.17	0.6845
Endophyte × round × temperature	1	0.94	0.3319	0.016	2, 206	1.67	0.1910	1.71	0.1923	3.31	0.0704
Round × water	2	0.32	0.8503	0.041	4, 414	2.17	0.0711	0.51	0.5998	3.13	0.0457
Endophyte × round × water	2	0.71	0.7012	0.003	4, 414	0.18	0.9499	0.08	0.9274	0.24	0.7875
Temperature × round × water	2	0.15	0.9285	0.010	4, 414	0.53	0.7134	0.88	0.4181	0.84	0.4350
Endophyte × temperature × round × water	2	2.05	0.3587	0.021	4, 414	1.07	0.3687	0.44	0.6445	0.39	0.6783
Initial plant size (covariate)	1	20.54	<0.0001	0.231	2, 206	30.87	<0.0001	53.53	<0.0001	16.16	<0.0001

Significant effects at $p < 0.05$ are shown in bold

between the two rounds of the experiment, with a stronger effect during the second round (50 % above-ground biomass reduction in future temperature) than in the first round (20 % reduction, Table 1). As with the survival response, the presence of the endophyte had a weaker effect on above-ground biomass than temperature, with a 23 % increase in biomass for inoculated plants (Fig. 1d, Table 1). We detected no context-dependency in the effect of the endophyte on above-ground biomass (Table 1). The water treatment significantly influenced the combined responses of above-ground and below-ground biomass (Table 1), although this effect was marginally non-significant in the ANCOVA on above-ground mass (Table 1). Above-ground biomass was ~20 % higher under average water than under either reduced or increased water treatments (back-transformed means [95 % CL] reduced: 0.84 g [0.70–0.99]; average: 1.05 g [0.90–1.21]; increased: 0.84 g [0.70–0.99]).

Root biomass of *A. breviligulata*, which is critical to stabilization of the dunes, was most strongly affected by temperature (Table 1). Temperature had nearly twice as strong an effect on below-ground biomass than on above-ground biomass, with a 60 % reduction under the future temperature treatment (Fig. 1e). The endophyte, which occurs only in above-ground plant tissues, had no significant influence on root mass (Fig. 1f, Table 1). As for above-ground biomass, there were declines in below-ground biomass under both high and low water availability, although the effect size was stronger in the second round (Table 1). During the second round, root mass of plants subjected to average water was 49 % higher than under increased water and 28 % higher than under reduced water (back-transformed means [95 % CL] reduced: 0.12 g [0.07–0.17]; average: 0.17 g [0.12–0.22]; increased: 0.08 g [0.04–0.13]).

Table 2 Statistical results for sand adherence (ANCOVA), root traits (MANCOVA), and arbuscular mycorrhizal fungi (AMF; MANCOVA)

Effect	Sand grain adherence			Root traits MANCOVA				AMF MANCOVA			
	<i>df</i>	<i>F</i>	<i>P</i>	Pillai's trace	<i>df</i>	<i>F</i>	<i>P</i>	Pillai's trace	<i>df</i>	<i>F</i>	<i>P</i>
Endophyte	1, 107	0.08	0.7793	0.049	7, 146	1.07	0.3846	0.017	2, 111	0.94	0.3944
Temperature	1, 107	7.95	0.0057	0.160	7, 146	3.98	0.0005	0.029	2, 111	1.68	0.1902
Endophyte × temperature	1, 107	4.23	0.0421	0.042	7, 146	0.92	0.4923	0.001	2, 111	0.06	0.9447
Water	2, 107	1.75	0.1795	0.114	14, 294	1.27	0.2264	0.105	4, 224	3.10	0.0163
Endophyte × water	2, 107	0.00	0.9981	0.076	14, 294	0.83	0.6393	0.079	4, 224	2.30	0.0596
Temperature × water	2, 107	1.59	0.2094	0.040	14, 294	0.43	0.9647	0.030	4, 224	0.86	0.4871
Endophyte × temperature × water	2, 107	0.24	0.7890	0.062	14, 294	0.67	0.8035	0.011	4, 224	0.31	0.8707
Round	1, 107	75.34	<0.0001	0.634	7, 146	36.19	<0.0001	0.106	2, 111	6.55	0.0020
Endophyte × round	1, 107	0.02	0.8854	0.023	7, 146	0.48	0.8451	0.012	2, 111	0.68	0.5072
Round × temperature	1, 107	1.30	0.2573	0.262	7, 146	7.40	<0.0001	0.046	2, 111	2.65	0.0754
Endophyte × round × temperature	1, 107	2.02	0.1579	0.082	7, 146	1.86	0.0801	0.001	2, 111	0.07	0.9320
Round × water	2, 107	1.40	0.2512	0.094	14, 294	1.04	0.4180	0.084	4, 224	2.46	0.0466
Endophyte × round × water	2, 107	0.21	0.8142	0.052	14, 294	0.56	0.8972	0.090	4, 224	2.64	0.0349
Temperature × round × water	2, 107	0.05	0.9512	0.113	14, 294	1.26	0.2297	0.036	4, 224	1.02	0.3985
Endophyte × temperature × round × water	2, 107	0.58	0.5634	0.110	14, 294	1.22	0.2589	0.014	4, 224	0.38	0.8195
Initial plant size (covariate)	1, 107	1.46	0.2295	0.038	7, 146	0.83	0.5616	0.007	2, 111	0.41	0.6616

Significant effects at $p < 0.05$ are in bold

Sand adherence

The response of sand binding to climate depended on the presence of the endophyte (Table 2). In the absence of the endophyte, there was an 80 % increase in the number of sand grains/cm of root tissue with a 5 °C increase in temperature (Fig. 2). However, for plants inoculated with the endophyte, sand adherence did not significantly respond to temperature (Fig. 2). Initial plant size had no effect on sand adherence (Table 2) and the sand adherence response did not correlate with plant biomass ($r_s > 0.05$), suggests that this measure of ecosystem function is not closely linked with plant biomass traits. However, sand adherence was positively correlated with average root diameter ($r_s = 0.45$, $P < 0.0001$, $n = 132$ plants) and with the number of root tips/cm of root tissue ($r_s = 0.48$, $P < 0.0001$), but was not correlated with any other root

traits that we measured. Additionally, sand adherence was negatively correlated with the amount of extraradical hyphae produced by mycorrhizal fungi ($r = -0.29$, $P = 0.0006$). We included these root and AMF traits (individually) in the model predicting sand adherence and found no evidence that the correlations among functional traits were strongly altered by climate manipulations or by the presence of the endophyte (traits × treatment interactions, all $P > 0.06$).

Root traits

Elevated temperature reduced plant investment in a combined analysis of root traits. A 5 °C increase in temperature relative to current climate reduced root length, root area, root volume, total number of root tips, and the root:shoot ratio by approximately 50 % (Table 2). The effect of

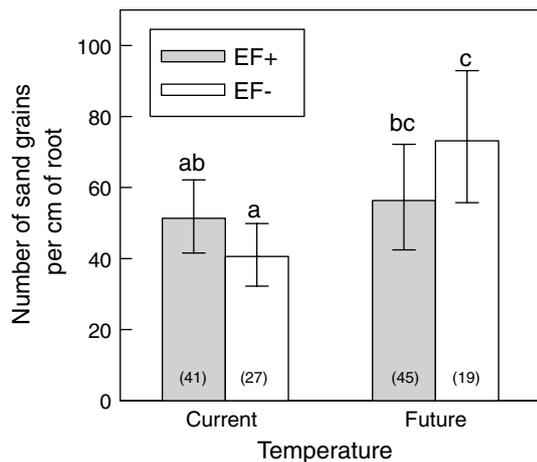


Fig. 2 Alteration of sand binding by climate and endophyte treatments. Bars show means with 95 % confidence limits. Sample sizes are given in parentheses and reflect differences in mortality among the treatments

temperature on average root diameter was marginally non-significant (ANCOVA, $P = 0.0643$), with a 5 % greater average root diameter in the warmed treatment relative to the current temperature scenario. The number of root tips/cm was the only root trait that significantly increased with temperature; plants made 16 % more tips/cm of root tissue with the 5 °C increase. Interestingly, the two traits that increased with future temperature, root tips/cm and average root diameter (non-significant increase in ANCOVA) were the only two traits that significantly correlated with sand adherence. Overall, the second round showed a stronger temperature effect on root traits than the first round of the experiment, but the direction of the effects of temperature were the same in both rounds (Table 2).

The magnitude and direction of the response in root traits to climate manipulations was not affected by the presence of the endophyte, as indicated by non-significant endophyte \times climate interactions (Table 2). The presence of the endophyte in above-ground plant tissues had no significant influence on root traits (Table 2). Water treatments also did not influence the expression of root traits (Table 2).

Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi (AMF) colonized plants from live sand collected from the dunes and added to pots. Water addition shifted fungal colonization from roots to soil (extraradical hyphae), and higher temperatures increased extraradical hyphal production. The increased water treatment reduced mycorrhizal colonization of roots relative to average water levels; plant subjected to reduced water had an intermediate level of colonization (back-transformed means [95 % CL] reduced: 33.9 % [28.1–40.0]; average: 37.1 %

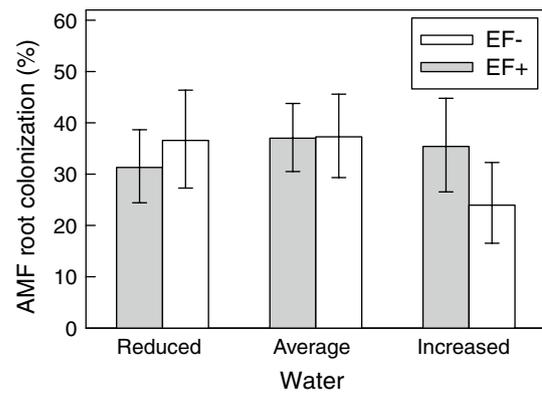


Fig. 3 Response of root colonization (% microscope views) by arbuscular mycorrhizal fungi to water and foliar endophyte manipulations. Bars show means with 95 % confidence limits

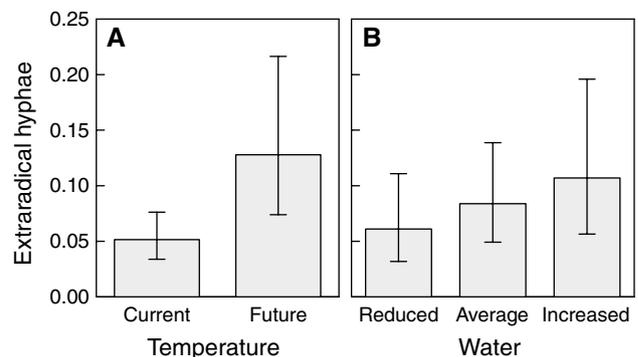


Fig. 4 Response of extraradical hyphae (% intersections/view) to **a** temperature treatments and **b** water manipulations. Bars show means with 95 % confidence limits

[32.0–42.5]; increased: 29.5 % [23.6–35.8]; Table 2). The presence of the endophyte helped plants to retain mycorrhizae in their roots under high water, as indicated by a significant round \times endophyte \times water interaction (Fig. 3, ANCOVA, $F_{1,112} = 5.12$, $P = 0.0074$). Under increased water, plants with the endophyte had 48 % higher rates of root colonization than endophyte-free plants; under average or reduced water there was little difference in colonization between endophyte-inoculated and endophyte-free plants. Extraradical hyphae responded to temperature and water, but not to the endophyte. Temperature increased production of extraradical hyphae, with the strongest increase in the second round, a fivefold change (Fig. 4a, ANCOVA temperature $F_{1,112} = 7.12$, $P = 0.0088$, round \times temperature $F_{1,112} = 4.24$, $P = 0.0419$). Water addition elevated extraradical hyphae production fourfold relative to the reduced water treatment (Fig. 4b, ANCOVA round \times water $F_{1,112} = 4.24$, $P = 0.0159$).

Discussion

Does increased temperature or altered water availability reduce survival, growth, or functional traits associated with ecosystem engineering in *Ammophila breviligulata*?

Temperature had a much stronger effect on *A. breviligulata* than did water availability. A 5 °C increase in temperature relative to current climate conditions for northern Michigan reduced survival by 45 %, root biomass by 60 %, and aboveground biomass by 36 %. Water treatments had smaller effect sizes, typically reducing biomass by ~20 %. Interestingly, *A. breviligulata* plants were sensitive to both augmentations and reductions in water availability. Plants had higher above-ground biomass under average water conditions than when water was reduced or supplemented.

The strong effect of temperature is somewhat surprising, as *A. breviligulata* is the dominant dune species not only in the Great Lakes region, but also along the North American Atlantic coast from Nova Scotia to North Carolina (Seneca and Cooper 1971), which represents a wide range in climate. However, *A. breviligulata* is a C3 species and expected to be physiologically more sensitive to high temperatures than common C4 dune grasses such as *Calamovilfa longifolia* and *Schizachyrium scoparium* (Elfman et al. 1986). Further, local adaptation may be strong in this species. A study by Seneca and Cooper (1971) compared temperature effects on *A. breviligulata* seed germination and seedling growth from populations in North Carolina and Michigan. The seedlings from Michigan populations were much less tolerant of high temperatures (80–90 °F; 26.7–32.2 °C) than seedlings from North Carolina populations. This study concluded that strong selective forces have resulted in low tolerance of high temperatures for northern populations of *A. breviligulata*, and that temperature could be the limiting factor for southward spread of this species. Our results suggest that *A. breviligulata* may be sensitive to climate warming, at least in northern populations.

Drought stress appears to be a less significant factor for *A. breviligulata*. It may be that *A. breviligulata*, like its congener *A. arenaria*, tolerates drought stress better than other dune species (Dixon et al. 2004). Dune ecosystems are known to have very low soil moisture, even in areas with high rainfall (Baldwin and Maun 1983), due to the sandy soils. Ensign et al. (2006) found that soil moisture in dune habitats along Lake Huron naturally ranged from 2 to 16 %, which encompasses the soil moistures in our experiment. While our experiment capitalized on the controlled conditions afforded by growth chambers, field manipulations of both temperature and precipitation (currently ongoing) will be essential for making quantitative climate change predictions.

Do foliar endophytes increase *A. breviligulata* performance and alter plant traits associated with ecosystem processes?

Here we have focused attention on the vegetative stage of mature plants, and we document endophyte benefits on the order of 14–23 % over the span of a 90 day growing season. The *Epichloë* endophyte increased plant survival and above-ground biomass, but had insignificant effects on below-ground biomass and root traits. Our results suggest that the endophyte in *A. breviligulata* should increase in dune ecosystems when it is present. However, for vertically transmitted symbionts that are associated with plants from seed to senescence, characterizing their interactions as mutualistic versus antagonistic requires an assessment of effects across the life cycle of the host (Rudgers et al. 2010). Because symbionts can have contrasting impacts on different plant life stages (e.g., reduced survival but enhanced seed production; Rudgers et al. 2012) and in different environments, understanding their net effect on host fitness requires not only characterizing the effects of symbionts for each life stage but also understanding the relative contributions of different life stages to rates of plant population growth (Gundel et al. 2008, 2011).

It remains unclear whether the endophyte in *A. breviligulata* may also influence plant performance or plant phenotypes at other life history stages, or whether the endophyte interacts with the biotic field environment of *A. breviligulata*. For example, benefits of endophyte colonization are expected to increase in the presence of herbivores, though a study by Crawford et al. (2010) showed that endophyte presence in *A. breviligulata* did not alter feeding preferences of two generalist herbivores under laboratory conditions, possibly due to a lack of loline production by the *Epichloë* species colonizing *A. breviligulata*. However, there are some specialist herbivores, such as *Trimerotropis huroniana*, on sand dunes in the Great Lakes region, and endophyte effects on palatability are often herbivore species-specific (Afkhami and Rudgers 2009). Few studies have tracked the consequences of fungal endophytes across the full life span of host plants under field conditions (Cheplick and Faeth 2009; Uchitel et al. 2011), and this remains a challenge for the study of endophyte ecology.

Can foliar endophytes alter the magnitude or direction of *A. breviligulata* responses to climate-related stressors?

Our results suggest that the endophyte will not modify the effects of climate change on the growth or survival of *A. breviligulata*. As is common in mutualistic species interactions (Bronstein 1994), we expected that benefits of the endophyte would be context-dependent. Based on prior studies of epichloae in grasses (e.g., Kannadan and

Rudgers 2008; Malinowski and Belesky 2000), we predicted the benefits of symbiosis to increase when plants experienced a more stressful environment, such as warmer temperatures or precipitation extremes. However, we found no evidence for context-dependency in the endophyte effect on plant growth or survival. While our earlier work suggested stronger benefits of the endophyte in *A. breviligulata* when plants experienced drought, this effect only occurred in the presence of insect herbivores. In addition, we did not directly manipulate endophyte presence in our earlier study; therefore, differences between plants may have been driven by plant genotype as well as by endophyte presence (Emery et al. 2010). Further, we have not considered the direct or interactive effects of elevated CO₂, another key component of future climate change. As a C3 plant, we expect that *A. breviligulata* would show an increase in above- and below-ground biomass, root-to-shoot ratios, photosynthetic rates, and water use efficiency in response to elevated CO₂ (e.g., Poorter and Navas 2003; Wang et al. 2012), which could partially counteract the effects of elevated temperature and decreased water availability.

Despite the lack of context-dependency in endophyte benefits to plant performance, we did find endophyte mediation of plant functional traits. The ecosystem service of sand stabilization provided by *A. breviligulata* under a warmer climate was altered by endophyte symbiosis. In the absence of the endophyte, simulated warming increased sand grain adherence to roots by 80 % compared to plants under ambient temperatures. However, plants inoculated with the endophyte had similar levels of sand-binding under both ambient and future temperature simulations. Thus, even when endophytes do not mediate the responses of plant performance to climate change, they may indirectly affect the ecosystem service of sand stabilization by altering functional traits. Further, *A. breviligulata* often grows best when being actively buried by sand; thus, increased sand binding may feedback to enhance survival (Voeselek et al. 1998).

Do foliar endophytes or climate manipulations alter associations between *A. breviligulata* and mycorrhizal fungi?

Prior studies on three grass species (*Lolium arundinaceum*, *L. multiflorum*, and *L. perenne*) have documented significant declines in root colonization and/or spore production by mycorrhizal fungi when endophytes were present in above-ground plant tissues (e.g., Chu-chou et al. 1992; Mack and Rudgers 2008; Omacini et al. 2006). Thus, we expected that the presence of the endophyte would reduce mycorrhizal fungi in *A. breviligulata*. Depending on the importance of arbuscular mycorrhizal fungi in stabilizing sand dune ecosystems, such an effect could be a critical,

but indirect, pathway by which foliar endophytes affect ecosystem services. In contrast to expectations, we found no significant overall effect of the *Epichloë* endophyte on root colonization or extraradical hyphal production associated with *A. breviligulata*. This contradicts results from a previous field study we conducted that showed lower AMF root colonization in endophyte-infected *A. breviligulata* (Emery and Rudgers 2011). Overall, AMF root colonization levels found in that field study match colonization levels in this study (30–40 % in both studies), indicating that AMF had adequate time to colonize roots. However, in the field study, plant population source was not controlled and may have influenced findings. In addition, under one climate manipulation—high water availability—the endophyte actually helped plants to maintain higher rates of root colonization by mycorrhizal fungi than in its absence. While field experiments will be helpful for extending our results to a larger spatial scale, thus far it appears that the endophyte in *A. breviligulata* is not antagonistic to mycorrhizal fungi, and may even increase colonization under some conditions. Consistent with this result, observations on a native brome in Patagonia reported positive correlations between an epichloae endophyte and mycorrhizal fungi (Novas et al. 2011). However, experiments manipulating endophyte presence have not yet been conducted in that system. Our results suggest that interactions between plants and their multiple symbionts are more complex than simple antagonisms between mutualistic partners, such as endophytes and mycorrhizal fungi, that share the same resource (e.g., plant-derived carbon; see also Chamberlain and Rudgers 2012; Whitney and Rudgers 2009).

Corresponding with prior studies (reviewed by Auge 2004; Augé 2001), we found that mycorrhizal fungi in *A. breviligulata* were sensitive to water availability. Interestingly, patterns of root colonization did not correspond with patterns of extraradical hyphal production. While the increased water treatment reduced root colonization (similar to the plant response), it enhanced extraradical hyphal production, suggesting a shift in fungal allocation from growth in roots to growth in wetter soils. A similar shift was reported in response to 22 years of experimental warming and associated soil drying in a sub-alpine meadow (Rudgers, unpublished data). Only extraradical hyphae responded to the manipulation of temperature, with increased production in the warmed treatment. Extraradical hyphae were negatively correlated with sand grain adherence, suggesting a possible reduction in sand-binding as a consequence of these temperature effects on mycorrhizal fungi. It remains unclear what the ecosystem-level consequences of changes in the root fungal community may be, but altered below-ground interactions provide an additional route by which endophytes may influence the dune building process.

Conclusion

This study suggests that heat stress related to climate change in the Great Lakes may compromise the ability of *A. breviligulata* to stabilize dune ecosystems and reduce carbon storage and organic matter build-up in these early-successional systems due to reduced root growth. Although endophyte symbiosis did not cause greater enhancements of plant performance under climate stress, symbiotic plants had higher survival and biomass than endophyte-free plants regardless of climate manipulations, suggesting that endophytes may provide one method to enhance the contribution of *A. breviligulata* to dune stabilization.

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