

## Biotic and abiotic predictors of ecosystem engineering traits of the dune building grass, *Ammophila breviligulata*

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**Abstract.** Ecosystem engineers are species that fundamentally influence their community and ecosystem by creating or altering the physical structure of habitats. However, the function of ecosystem engineers is variable and can depend on abiotic and biotic factors. In this study, we characterized the ecosystem engineering traits of plant size and tiller density for the dune grass, *Ammophila breviligulata*, in 37 sites across a broad geographic gradient in the western Great Lakes region. We also measured 20 biotic and abiotic factors related to climate, soil chemistry, and fungal symbionts associated with these survey sites and assessed their relationships with *A. breviligulata* population traits and dune plant species richness. Climate factors, especially temperature and precipitation, were positively associated with *A. breviligulata* tiller size, while soil organic matter was the only factor associated with tiller density. Several factors, including temperature, soil nitrogen, and mycorrhizal colonization, were associated with plant richness across our sites. Our results suggest that climate can influence at least some ecosystem engineering traits (i.e., plant size) of an important dune building species, although general conclusions from our work indicate that the population trait tied most closely with dune building ability—population density—is not strongly influenced by climate at the regional scale. This offers insight for conservationists interested in preserving intact dune ecosystems in a changing climate, but further work is needed to reconcile conflicting lab and field studies.

**Key words:** *Ammophila breviligulata*; *Epichloë*; geomorphology; Great Lakes region; marram grass; succession.

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### INTRODUCTION

Ecosystem engineers are species that fundamentally influence their community and ecosystem by creating or altering the physical structure of habitats (Jones et al. 1994, Wright and Jones 2006). The presence and abundance of ecosystem engineers often outweighs the influences of other factors such as edaphic factors or trophic interactions in communities (Jones et al. 1997, Cuddington et al. 2007, Hastings et al. 2007).

Ecosystem engineers can interact in complex ways with other species in the community; for example they can increase diversity in an ecosystem by ameliorating harsh physical habitat conditions, but can also decrease diversity through direct biotic interactions like competition (Hastings et al. 2007).

For ecologists interested in conserving biodiversity and ecosystem function, identifying factors that influence populations of ecosystem engineers is vital. The function of ecosystem

engineers is variable and can depend on both abiotic and biotic factors (e.g., Balke et al. 2012). For example, the ability of the engineering polychaete *Dipatra* to build tubes and stabilize substrate depends on abiotic ocean sediment dynamics (Berke 2012). In contrast, corals show evidence of biotic drivers, where mutualistic amphipods and polychaetes increase coral branching, providing better habitat for reef fish (Bergsma 2012). Few studies have examined the relative importance of biotic versus abiotic factors on ecosystem engineers (Wright and Jones 2006).

One way to predict variation in ecosystem engineering ability is to compare populations across environmental or biogeographic gradients (Crain and Bertness 2006, Balke et al. 2012). For many engineering species, climate is hypothesized to have strong effects on engineering capabilities (Wolters et al. 2000, Menge et al. 2008). However, few studies on climate variability and ecosystem engineering have been conducted (Lavelle et al. 1997, Ling et al. 2008). The potential for climate to affect ecosystem engineers is demonstrated by work showing that *Zostera marina* (common eelgrass), which engineers coastal estuarine systems, lost 44% of shoot density under simulated increases in temperature (Ehlers et al. 2008). In light of climate change predictions for the next century (IPCC 2007), understanding how climate interacts with other factors to influence ecosystem engineering species can improve predictions of the ecological consequences of climate change.

One ecosystem engineering species that may be particularly sensitive to climate variability is *Ammophila breviligulata* Fernald (American beach grass; Poaceae). *Ammophila breviligulata* is a highly clonal, perennial, C<sub>3</sub> grass and the dominant pioneer plant in temperate coastal and lacustrine dunes of North America (Maun 1984). *Ammophila breviligulata* is unique in its ability to thrive in habitats with sand burial greater than 1 m per year, (Maun and Lapierre 1984), where its extensive root network can stabilize moving sand during the early stages of dune succession and contribute to early soil carbon enrichment (Olson 1958, Martin 1959, Cheplick 2005). *Ammophila 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). In the Great Lakes region, *A. breviligulata*-dominated habitat forms the most extensive freshwater dunes in the world, covering more than 1,000 km<sup>2</sup> in Michigan alone (Albert 2000). These dunes form the infrastructure for maintaining ecosystem integrity along the aquatic-terrestrial border, provide habitat for endemic plant species, and shelter neighboring wetlands. Native dune vegetation such as *A. breviligulata* also protects human developments by stabilizing soil and protecting interior lands from storms (McHarg 1972, Ranwell and Boar 1986). This is especially important as coastal and lacustrine ecosystems are expected to be among the most vulnerable to climate change due to increases in the intensity of severe weather events, such as storms and droughts, which will accelerate erosion and reduce dune stability (Jones et al. 2007, Schlacher et al. 2008). Under the highest CO<sub>2</sub> emissions general circulation models, the Great Lakes region is expected to experience altered rates of annual precipitation (IPCC 2012) and an increase of 5°C in annual temperature by 2070–2099 (Hayhoe et al. 2010), which pose a direct threat to dune habitats that are already water limited (Lichter 2000, Ensign et al. 2006). Understanding how climate influences population traits of *A. breviligulata* is a first step in predicting how dune habitats will respond to climate change.

Two traits in particular, tiller size and tiller density, have been directly linked to dune building ability by *A. breviligulata* in controlled wind tunnel experiments (Hacker et al. 2012, Zarnetske et al. 2012). By stabilizing sand, *A. breviligulata* can also facilitate the establishment of other plant species (Cowles 1899, Zarnetske et al. 2010). On the other hand, high tiller density has been associated with lower plant diversity in Atlantic Coast dunes (Cheplick 2005). Thus, an understanding of what factors influence population tiller size and density at a landscape scale will improve predictions on the degree of ecosystem function provided by this species.

While few studies have explored factors influencing population traits of *A. breviligulata* at a landscape scale, small-scale lab and field studies have shown that survival and growth are affected by a number of factors (e.g., Seneca and Cooper 1971). This species is temperature sensi-

tive, with plants being especially susceptible to mortality above 35°C (Seneca and Cooper 1971). Even a 5°C increase in temperature was enough to kill 45% of tillers in a growth chamber experiment (Emery and Rudgers 2013).

Additionally, *A. breviligulata* tiller growth is affected by two fungal symbionts. Arbuscular mycorrhizal fungi (AMF) have been shown to increase tiller initiation by 31% compared to uncolonized plants in Atlantic Coast dunes (Gemma and Koske 1997). In a previous study, we discovered an undescribed species of class I endophyte in the genus *Epichloë* (Clavicipitaceae) at low frequencies in natural *A. breviligulata* populations of the Great Lakes, but in very high frequencies in commercial stocks often used in dune restoration efforts (Emery et al. 2010). These fungi occur systemically in plants, often conferring protection against abiotic or biotic stressors such as drought, heat, herbivores, and pathogens (Rodriguez-Echeverria et al. 2009). *Ammophila breviligulata* plants colonized by the endophyte had a 22% increase in tiller initiation compared to uncolonized plants in growth chamber and field experiments (Emery and Rudgers 2013; Emery and Rudgers, unpubl. data), supporting results from studies on endophyte effects on growth of other plant hosts (e.g., Clay 1990). Separating climate effects from biotic effects and other abiotic factors becomes increasingly relevant to making predictions about future ecosystem function in dune habitats.

The goals of this study were to: (1) characterize population traits of *A. breviligulata* associated with ecosystem engineering, specifically tiller size and density, across a biogeographic gradient; (2) assess the predictability of these engineering traits as a function of climate and biotic interactions; and (3) assess the predictability of plant community diversity based on measured biotic and abiotic factors. Based on small-scale studies of *A. breviligulata* described above (Seneca and Cooper 1971, Emery and Rudgers 2013), we expected that tiller density and size would be positively associated with higher latitudes and cooler, wetter climates. We also expected that the presence of potential mutualists such as endophytes and AMF would increase tiller density and size (e.g., Clay 1990, Gemma and Koske 1997, Kivlin et al. 2013). We expected that *A. breviligulata* tiller density would be the strongest

factor negatively associated with plant community diversity, as *A. breviligulata* is known to be a vigorous competitor in early successional dune ecosystems (Cheplick 2005). We tested these hypotheses in a survey of *A. breviligulata* populations across a broad geographic region.

## METHODS

### Study areas

Thirty-seven populations located in state and national parks were sampled for this study, spanning 6° of latitude and 8° of longitude along three lakeshores in the western Great Lakes (Fig. 1; Appendix A). All sampling sites were located in natural foredune habitat, the first vegetated dune ridge from the shore (no more than 100 m from water's edge), and were dominated by *A. breviligulata* (>60% cover). These sites were all located on beach-margin dunes formed during the late Holocene, with aeolian sand deposits overlaying spodosols (Arbogast and Loope 1999). As state or federal protected areas, disturbance in these sites was minimal and limited to beach-visitor foot traffic on designated trails.

### Biotic measurements

In each site, we haphazardly located a 20 m × 25 m area to measure *A. breviligulata* traits and ecosystem engineering capabilities. We collected 50 tillers per site (one tiller clipped at the soil surface every 5 m along five 25-m transects) and measured tiller height as a metric of tiller size. To survey plants for the foliar endophyte, *Epichloë*, we cut stem cross-sections from each collected tiller and screened them with commercial immunoblot kits, visually scoring color development to indicate endophyte presence (Phytoscreen: Agriagnostics, Watkinsville, GA; Appendix B: Fig. B1). This method is comparable to histological staining (Hiatt et al. 1999). We estimated *A. breviligulata* tiller density in each site by counting and averaging tiller numbers within 10 1-m<sup>2</sup> quadrats (two quadrats placed at the 5- and 20-m distance along each of the five 25-m transects). Presence, but not abundance, of other vascular plant species within each 20 m × 25 m site was also recorded based on visual inspection of the entire site. Plant richness was always less than 14 species per site, with common native species

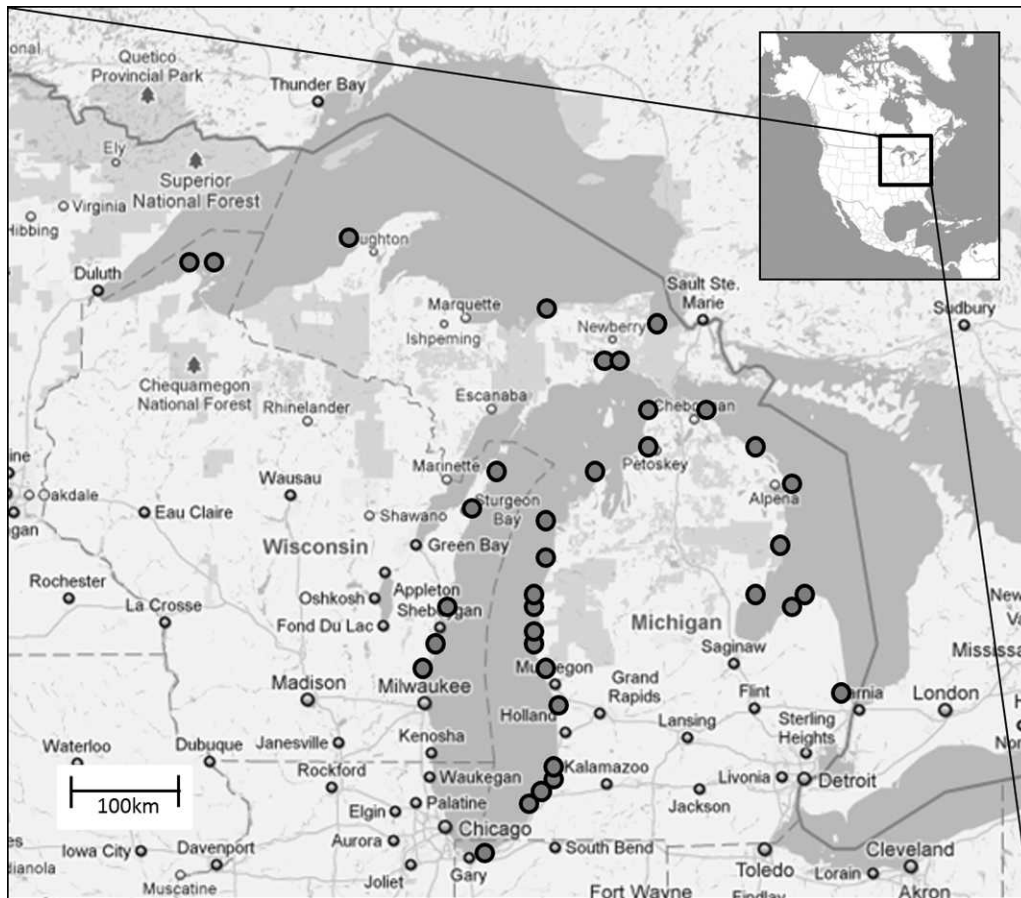


Fig. 1. Map of field sites sampled. A complete list of sites with latitude/longitude coordinates is provided in Appendix A. Source: Google Maps.

including *Artemisia campestris*, *Calomovilfa longifolia*, and *Prunus pumila*. Non-native species found in sites included *Centaurea maculosa* and *Saponaria officinalis*, though these were never common.

For AMF measurements in each site, we collected and composited 50 2 cm × 30 cm soil cores (one from the base of each collected tiller), representing the active root profile (Maun 1984). Arbuscular mycorrhizal fungi activity in *A. breviligulata* roots was quantified by picking fine root pieces from composited soil samples. These roots were rinsed and 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). From each site, ten 1 cm

root sections were mounted on a microscope slide. Using a compound microscope (Leica Microsystems, Wetzlar, Germany) at 200× magnification, the percentage of roots colonized by AMF was recorded using the gridline intercept method (McGonigle et al. 1990) for AMF hyphae, arbuscules, or vesicles with 100 views per slide ((number of views with AMF structures visibly present in roots/total number of views) × 100). These structures are indicative of fungi in the phylum Glomeromycota (Appendix B: Fig. B1; Morton et al. 1993). Three sites had <100 views (39, 64, or 88) due to low quantities of root material.

To estimate activity of AMF in the soils of each site, the length of extraradical hyphae was quantified from 20-g soil subsamples. Each subsample was mixed with 500 ml DI water in

a 100-ml beaker and stirred at 80% speed for 2 min with a magnetic stirrer (Fisher Scientific 11-500-49SH). Before solid material settled, the solution was poured through 500- $\mu$ m and 212- $\mu$ m sieves to separate sand and large organic material from the hyphal suspension. Residue from the 212- $\mu$ m 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- $\mu$ m sieve and rinsed with DI water until water ran clear from the sieve. The residue on the 38- $\mu$ m 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 microanalysis vacuum filter holder fitted with a 0.45- $\mu$ m mesh nylon membrane. The membrane was then rinsed and dried under vacuum and mounted onto a slide. Hyphal length was recorded as percentage of intercepts per field of view using the gridline-intercept method (McGonigle et al. 1990) under a stereomicroscope (Nikon SMZ1500 at 70 $\times$ ).

#### Abiotic measurements

To collect climate data associated with each site, we accessed the PRISM Climate Group database (2012; <http://prism.oregonstate.edu>) and the National Oceanic and Atmospheric Administration's National Data Buoy Center CoastWatch Great Lakes Node C-MAN stations (<http://coastwatch.glerl.noaa.gov/marobs/>). We downloaded monthly precipitation data, summer months' daily average high temperatures (June–August), and winter months' daily average low temperatures (December–February) from 2007–2011 for each site's latitude and longitude from PRISM to calculate the 5-year average for each climate variable. These climate variables are predicted to be most sensitive to climate change in the region (Kling et al. 2003). We downloaded monthly average windspeed and monthly maximum windspeed for 2011 from the closest permanent coastal station to each sampling site from the NOAA database.

While we were specifically interested in the relationship between climate and *A. breviligulata* population traits, we measured a number of other abiotic variables to account for variability among sites in terms of soil chemistry and dune

location. We collected subsamples (100 ml) from the composited soils collected at each site (described above) and sent them to University of Kentucky Soil Testing Laboratories (Lexington, KY) for analysis of phosphorous, potassium, soil pH, calcium, magnesium, zinc, percent soil organic matter, and total nitrogen using Mehlich III extraction and LECO combustion. These variables all have the potential to influence or be influenced by the plant community (Aber and Melillo 1991). We used Google Earth v. 7.1.2.2041 (Google 2013) to estimate dune height and dune distance to water from each sampling site based on GPS coordinates.

#### Data analysis

Because many of the climate and soil variables were correlated, we conducted a principal components analysis (PCA) to reduce dimensionality and control for collinearity among predictor variables (see Graham 2003) using the software program PC-ORD (McCune and Meford 1999). Twenty predictor variables were used in the PCA (Table 1). We used the correlation cross-products matrix, which centers and standardizes the variables by their standard deviation to account for different units of measurement among variables. Based on a randomization test and stopping rules outlined in Peres-Neto et al. (2005), three principal components were determined to adequately describe data structure. However, we also included the fourth component, which was correlated with AMF colonization (Table 1).

We used linear multiple regression to evaluate relationships between PCA axes and *A. breviligulata* tiller height and density, as well as dune plant species richness. For each PC axis that was a significant predictor for a given variable, we then conducted simple linear regressions between the response variable and the measured factors that were weighted most heavily on the PC axis. All variables met model assumptions of normality and equal variances based on the Shapiro-Wilks and Levene's tests and inspections of residuals against predicted, and all regression analyses were performed in Systat v. 12 (SYSTAT 2007).

## RESULTS

The PC axes used in regression analyses explained 62% of the variation in measured

Table 1. Variable loadings on the first four principal components of the PCA, with the variables with the highest absolute loadings per principal component in bold. Eigenvalues and percent variance extracted in the full data by each principal component are: PC1, 5.15, 25.5%; PC2, 3.07, 15.4%; PC3, 2.55, 12.8%; PC4, 1.76, 8.6%.

Measured variable	PC1	PC2	PC3	PC4
Latitude	<b>0.9175</b>	0.2027	-0.0283	0.0134
Longitude	-0.1621	-0.0568	-0.4221	0.3857
Distance from water (m)	-0.6458	-0.0277	0.1136	0.3447
Dune height (m)	-0.5749	0.1555	0.1756	0.1303
Ave. monthly windspeed (m/s)	-0.5132	0.3696	-0.0184	0.0503
Ave. monthly max windspeed (m/s)	-0.0774	0.6077	-0.1732	-0.0793
Ave. monthly precipitation (mm)	<b>-0.9155</b>	-0.131	0.0865	0.0283
Ave. daily summer max. temperatures (°C)	<b>-0.893</b>	-0.095	0.1083	-0.0755
Ave. daily winter min. temperatures (°C)	<b>-0.8974</b>	-0.1429	-0.1733	0.122
Soil phosphorus (ppm)	0.2121	-0.5208	0.2762	-0.14
Soil potassium (ppm)	0.1813	-0.5603	0.5166	0.1175
Soil pH	-0.4554	-0.3851	<b>-0.65</b>	-0.1449
Soil calcium (ppm)	-0.191	0.2838	<b>-0.7953</b>	-0.0388
Soil magnesium (ppm)	0.3045	<b>-0.7477</b>	-0.4154	-0.1867
Soil zinc (ppm)	-0.2648	<b>-0.702</b>	0.2409	0.1318
Soil organic matter (%)	0.1949	-0.5216	<b>-0.6953</b>	-0.2446
Total soil N (%)	0.1351	-0.4576	-0.1636	<b>0.6641</b>
Endophyte colonization (%)	-0.2543	-0.1322	0.2211	-0.2878
AMF hyphal length (% views)	0.3006	-0.1294	0.0139	<b>0.6048</b>
AMF root colonization (%)	0.2816	0.2893	-0.2514	<b>0.5862</b>

variables, and corresponded to four general groups: (1) latitude/precipitation/temperature, (2) soil magnesium/zinc, (3) soil organic matter/calcium/pH, and (4) soil nitrogen/AMF (Table 1). Full site data are reported in Appendix A.

Average *Ammophila breviligulata* tiller height, which ranged from 0.48 to 0.78 m across sites, was significantly predicted by PC1 and PC4 (Table 2), corresponding to a negative relationship between latitude and tiller height (Fig. 2A) and a positive association with average monthly precipitation and summer and winter temperatures (Fig. 2B, C). Despite the significant relationship between PC4 and *A. breviligulata* tiller height, none of the individual variables associated with PC4 were related to tiller height (soil nitrogen:  $p = 0.287$ ; AMF root colonization:  $p = 0.899$ ; AMF extraradical hyphae:  $p = 0.769$ ).

Tiller density of *A. breviligulata*, which showed large variability among sites ranging from 8.5 to 162.2 tillers  $m^{-2}$ , was predicted by PC3 only (Table 2), which corresponded to a negative association with soil organic matter (Fig. 3). Soil pH and soil calcium, which also weighed strongly on PC3, were not significantly associated with tiller density on their own ( $p = 0.18$ ,  $p = 0.15$ , respectively).

Plant species richness ranged from 1 to 13 species per site, with an average of 5.65 species,

and was significantly predicted by PC1, PC3, and PC4 (Table 2), corresponding to a negative association with average daily summer maximum temperatures and positive associations with soil nitrogen and AMF root colonization (Fig. 4A–C). Other individual variables associated with PC1 and PC4 were not significantly correlated with plant species richness (latitude:  $p = 0.11$ ; precipitation:  $p = 0.19$ ; winter temperatures:  $p = 0.33$ ; AMF extraradical hyphae:  $p = 0.10$ ). Similarly, no single variable associated with PC3 was significantly associated with plant richness (soil calcium:  $p = 0.196$ ; soil organic matter:  $p = 0.10$ ; soil pH:  $p = 0.21$ ). Tiller densities of *A. breviligulata* were negatively correlated with plant species richness (Fig. 4D).

## DISCUSSION

In this survey, abiotic factors were more important predictors of *A. breviligulata* ecosystem engineering traits than were biotic factors. Climate factors, especially temperature and precipitation, were positively associated with *A. breviligulata* tiller size, though these factors were, surprisingly, not important in predicting tiller density. Plants located at higher latitudes were smaller than those at lower latitudes. For example, plants at the most southern site, Indiana Dunes National Lakeshore, were 48%

Table 2. Results of multiple regressions predicting *A. breviligulata* tiller height and density, and overall plant species richness; overall GLM model fit statistics for tiller height were  $F = 8.02$ ,  $p = 0.001$ ,  $R^2 = 0.50$ ; for tiller density were  $F = 3.02$ ,  $p = 0.032$ ,  $R^2 = 0.27$ ; and for plant species richness were  $F = 6.41$ ,  $p = 0.001$ ,  $R^2 = 0.45$ . Significant predictor variables ( $p < 0.05$ ) are in bold.

Predictor variable	Tiller height		Tiller density		Plant species richness	
	Partial correlation	<i>T</i> statistic ( <i>p</i> )	Partial correlation	<i>T</i> statistic ( <i>p</i> )	Partial correlation	<i>T</i> statistic ( <i>p</i> )
PC1	<b>0.517</b>	<b>4.14 (0.001)</b>	0.231	1.53 (0.135)	<b>-0.286</b>	<b>2.16 (0.037)</b>
PC2	0.091	0.73 (0.471)	0.206	1.37 (0.181)	-0.116	0.884 (0.383)
PC3	0.247	1.98 (0.057)	<b>0.422</b>	<b>2.80 (0.009)</b>	<b>-0.436</b>	<b>3.31 (0.002)</b>
PC4	<b>0.405</b>	<b>3.23 (0.003)</b>	-0.006	0.43 (0.966)	<b>0.399</b>	<b>3.03 (0.005)</b>

larger than plants at the most northern location at McClain State Park (MI). This finding was somewhat surprising, as *A. breviligulata* has been shown to be quite sensitive to warm temperatures in lab studies (Emery and Rudgers 2013). However, it is likely that local adaptation has occurred in the region, as suggested by studies on genetic diversity (Fant et al. 2008). Future work could test the hypothesis that genotypes from warm, southern sites have evolved greater heat tolerance than populations from higher latitudes. Results from a lab study by Seneca and Cooper (1971) support this idea, as they found that seeds collected from *A. breviligulata* populations in North Carolina did not need cold treatments for germination and also produced plants with a higher temperature tolerance than seeds collected from Michigan populations.

Another hypothesis is that *A. breviligulata* shows plastic responses to climate; plants may be smaller at northern latitudes due to a shortened growing season (e.g., 109 frost-free days on average at Pictured Rocks National Lakeshore vs. 164 frost-free days at Indiana Dunes National Lakeshore; wcc.nrcs.usda.gov). Common garden experiments could tease apart these possible mechanisms.

No measured variable was a good predictor of tiller density except soil organic matter, which showed a negative relationship. It is not obvious whether this is a cause or an effect of tiller density. Soil organic matter increases with time in primary successional systems due to the facilitative effects of early plant colonizers (e.g., Chapin et al. 1994, Lichter 1998), so it is possible that soil organic matter responds directly to *A. breviligulata*.

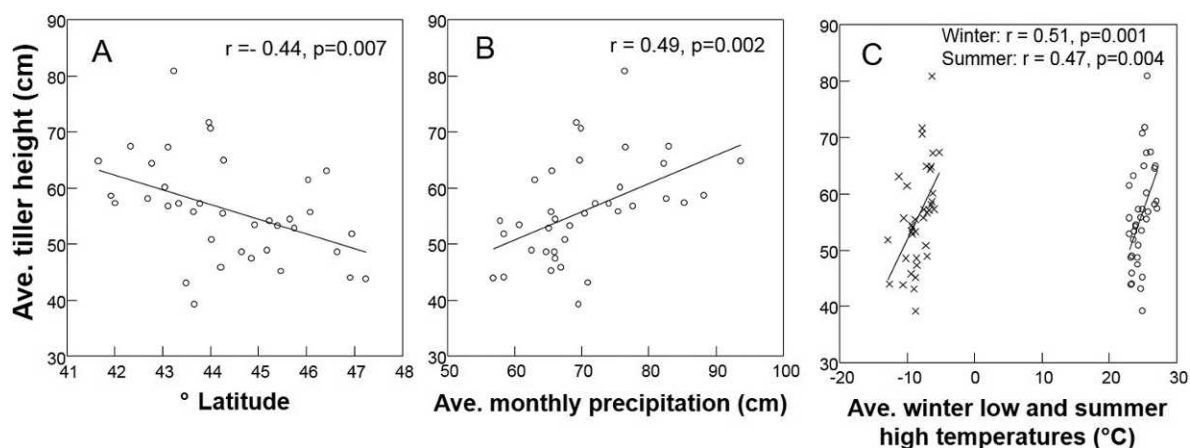


Fig. 2. Simple linear relationships between *A. breviligulata* tiller height and (A) latitude, (B) monthly precipitation, and (C) winter (crosses) and summer (open circles) temperatures.

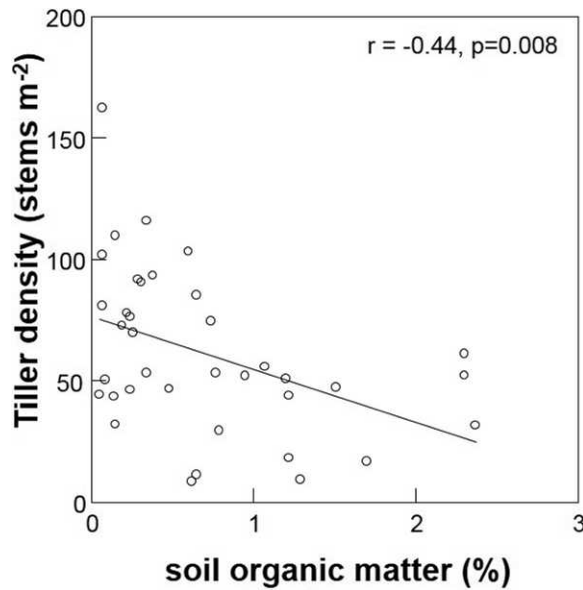


Fig. 3. Simple linear relationship between *A. breviligulata* tiller density and percent soil organic matter.

*lata* density. Alternatively, since *A. breviligulata* is one of the first plant species to colonize bare sand (most often via clonal spread into disturbed dunes; Maun 1984), increases in soil organic matter may simply follow as *A. breviligulata* populations decline during later stages of dune succession. Thus, a negative relationship between tiller density and soil organic matter may indicate a decline in *A. breviligulata* with time since initial colonization. Indeed, the increase in other plant species richness as *A. breviligulata* tiller density declines may indicate that *A. breviligulata* density directly correlates with succession.

It is still unclear what other factors might influence *A. breviligulata* tiller density in the Great Lakes region. In a study of *A. breviligulata* populations along the Pacific Coast of the US, Hacker et al. (2012) found that sand supply rates (cm sand deposition per month) differed among sites and were better predictors of tiller densities than were local climate factors. *Ammophila breviligulata* is adapted to the dune environment and actually dependent on moving sand and burial to maintain vigorous populations (Maun and Lapierre 1984). While we did not measure sand supply rates directly, we did use wind speed, distance

from shore, and dune height as surrogate measures of sand supply (Young et al. 2011). None of these factors were significantly associated with tiller density, in contrast to some other studies on dunes placing more significance to wind on the presence or absence of vegetation (e.g., Wilson and Sykes 1999, Miller et al. 2008, Fenu et al. 2013). It is predicted that wind speeds will increase over the Great Lakes in the future as a result of increasing air and water temperatures (Desai et al. 2009), so future monitoring of these populations' responses to wind may be warranted.

Fungal symbiont presence, which has been shown to alter tillering in *A. breviligulata* in other studies (e.g., Gemma and Koske 1997), also had no association with tiller densities in our survey. This lack of pattern might derive from the rather narrow range of variation in aboveground endophyte colonization found in our survey (only 0–30% of tillers in any one population showing endophyte presence), although variation in AMF colonization were substantially higher (0–71% of all tillers in a given population). As one caveat, our measures of climate variability may have been at the wrong scale for influencing plant population traits, where microclimate at the soil surface may be more important, but not strongly coupled to weather station or climate model data (Lortie and Cushman 2007, Graae et al. 2012)

Both biotic and abiotic factors were important predictors of plant species richness across our sites. We expected, and found, that *A. breviligulata* tiller density was negatively related to plant species richness. *Ammophila breviligulata* is known to be a vigorous competitor in foredune ecosystems and high densities of *A. breviligulata* were similarly associated with lower abundances of other species on coastal dunes in New York (Cheplick 2005). In European sand dunes dominated by the related species *Ammophila arenaria*, other native plants only outcompete *Ammophila* once pathogens build up in the soil causing *Ammophila* dieback (Van der Putten and Peters 1997). Such a mechanism could also explain the negative relationship between *A. breviligulata* density and plant diversity in our sites, although all of our samples were collected from active foredunes showing no evidence of dieback. Plant species richness was also positively associated with AMF root colonization, one measure of AMF



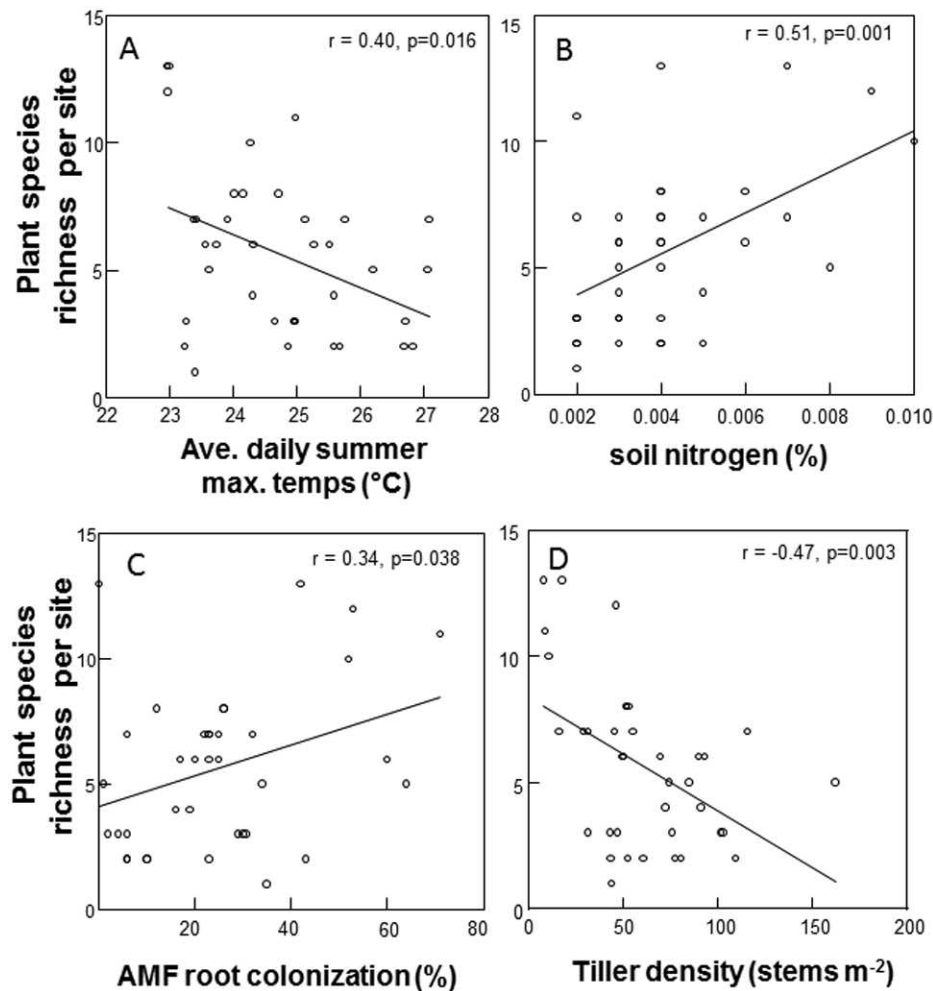


Fig. 4. Simple linear relationship between plant species richness per site and (A) average daily summer maximum temperature, (B) percent soil nitrogen, (C) percent AMF root colonization, and (D) *A. breviligulata* tiller density.

activity in soil. It has repeatedly been demonstrated that AMF can increase plant species richness in a variety of ecosystems (Hartnett and Wilson 2002, Hart et al. 2003), including sand dunes (Koske and Gemma 1997, Cakan and Karatas 2006, but see Sikes et al. 2012).

Soil nitrogen was the third factor that was significantly positively associated with plant species richness. Soil nitrogen is known to be limiting in sand dune ecosystems, and generally increases with dune successional age (Olson 1958, Jones et al. 2008). However, it has been suggested that plant available nutrients are not limiting for vegetation to re-establish in other systems (e.g., Baer et al. 2004, Maron and Marler

2008) and a manipulative experiment in the Great Lakes region found that soil nitrogen was not a limiting factor in plant species establishment (Lichter 2000). Plant diversity, soil nitrogen, AMF activity, and *A. breviligulata* tiller densities are all known to change across successional gradients, and our findings support other studies of the natural successional development of soils and plant communities in dune habitats (e.g., Olson 1958, Sikes et al. 2012).

Average daily maximum summer temperature was the final variable significantly associated with plant species richness, with higher plant richness found in sites with cooler summers. This result was somewhat surprising as higher diver-

sity is usually associated with warmer climates at a global scale (Clarke and Gaston 2006), and at the regional scale (e.g., Stropp et al. 2009, Tang et al. 2012). However, in our system, warmer climates were also associated with taller *A. breviligulata* tillers that may do a better job of outcompeting native plants through greater resource use. Plant diversity is a key component of ecosystem function in many habitats (Loreau et al. 2001), including in dunes (Crawford and Rudgers 2012, 2013). For sand dunes and other primary successional ecosystems dependent on ecosystem engineering species, increases in plant diversity may only occur once the dominant ecosystem engineer starts to die back (i.e., the “inhibition” model of succession; Connell and Slayter 1977) and so could be of secondary importance when considering dune building as a valuable ecosystem function.

During the next century, climate changes are expected to result in increasing temperatures and altered precipitation patterns worldwide, with important consequences for community structure and ecosystem processes (IPCC 2012). For many ecosystems, one of the primary avenues of climate impacts may be through changes to ecosystem engineering species (Wolters et al. 2000). Our results suggest that climate can influence at least some ecosystem engineering traits (i.e., plant size) of an important dune building species. However, the general conclusion from our work is that the population trait tied most closely with dune building ability—tiller density—was not strongly influenced by climate at the regional scale. This pattern offers insight for conservationists interested in preserving intact dune ecosystems in the Great Lakes region, although further work is needed to reconcile conflicting lab and field studies.

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## SUPPLEMENTAL MATERIAL

## APPENDIX A

Table A1. Site location data.

Site	°Latitude	°Longitude	Average <i>Ammophila</i> height (cm)	Average <i>Ammophila</i> density (stems m <sup>-2</sup> )	Plant species richness per site	Distance from water (m)	Dune height above water (m)
Big Knob State Forest, MI	46.039	-85.593	61.4	46.6	12	71.84	3
Brimley State Park, MI	46.416	-84.559	63.06	50.2	6	15.24	1
Cheboygan State Park, MI	45.65	-84.403	54.42	52	8	61.44	1
Duck Lake State Park, MI	43.344	-86.409	57.14	76.2	3	72.85	19
Grand Haven State Park, MI	43.048	-86.243	60.08	93.2	6	61.76	3
Grand Mere State Park, MI	42.004	-86.554	57.28	29.4	7	81.59	4
Harrington Beach State Park, WI	43.496	-87.793	43.08	52.1	8	13.33	0.5
Harrisville State Park, MI	44.648	-83.297	48.56	53	8	25.74	1
P. H. Hoelt State Park, MI	45.465	-83.88	45.16	9.2	11	54.85	2
P.J. Hoffmaster State Park, MI	43.127	-86.276	67.24	72.7	4	69.09	9
Hog Island Point State Forest, MI	46.079	-85.298	55.66	8.5	13	36.14	0.5
Holland State Park, MI	42.778	-86.211	64.4	103.1	3	81.97	3
Indiana Dunes National Lakeshore, IN	41.664	-87.06	64.82	53.1	2	62.93	4
Kohler-Andrae State Park, WI	43.671	-87.712	39.2	47.2	3	30.05	0.5
Lakeport State Park, MI	43.122	-82.491	56.7	16.7	7	54.82	4
Leelanau State Park, MI	45.182	-85.582	48.9	55.7	7	30.27	2
Ludington State Park, MI	44.015	-86.495	50.76	91.6	4	41.2	1
McClain State Park, MI	47.236	-88.616	43.82	31.9	3	20.01	3
Charles Mears State Park, MI	43.784	-86.44	57.26	69.7	6	65.51	2
Muskegon State Park, MI	43.238	-86.343	80.88	109.6	2	113.14	7
Negwegon State Park, MI	44.855	-83.322	47.4	11.2	10	33.35	0.5
Newport State Park, WI	45.234	-86.986	54.1	31.6	7	5.61	0.5
Orchard Beach State Park, MI	44.279	-86.319	64.94	115.8	7	21.74	6
Petoskey State Park, MI	45.408	-84.913	53.28	50.6	6	33.7	2
Pictured Rocks National Lakeshore, MI	46.645	-86.206	48.58	61	2	23.76	0.5
Point Beach State Forest, WI	44.217	-87.509	45.8	46.1	7	16.62	0.5
Port Crescent State Park, MI	44.002	-83.069	70.64	101.7	3	24.2	3
Apostle Islands National Lakeshore WI, Little Sand Bay	46.948	-90.887	51.84	162.2	5	27.32	1
Saugatuck State Park, MI	42.702	-86.207	58.1	77.8	2	36.24	3
Silver Lake State Park, MI	43.652	-86.54	55.78	43.5	3	41.92	2
Albert E. Sleeper State Park, MI	43.98	-83.209	71.66	90.3	6	29.66	1
Apostle Islands National Lakeshore WI, Stockton Island	46.917	-90.546	43.98	44.2	1	28.69	2
Tawas Point State Park, MI	44.255	-83.444	55.44	80.7	2	11.84	0.5
Van Buren State Park, MI	42.333	-86.31	67.38	85.2	5	29.98	0.5
Warren Dunes State Park, MI	41.924	-86.594	58.58	74.5	5	71.41	13
Whitefish Dunes State Park, WI	44.926	-87.187	53.37	43.9	2	27.56	0.5
Wilderness State Park, MI	45.751	-84.891	52.82	18.1	13	27.32	1

Table A2. Climate data.

Site	Ave. monthly windspeed (m/s)	Ave. maximum monthly windspeed (m/s)	Ave. monthly precipitation (mm)	Ave. summer daily max. temp.(°C)	Ave. winter daily min. temp. (°C)
Big Knob State Forest, MI	4.28	19.71	63.02	22.98	-10.00
Brimley State Park, MI	4.14	20.49	65.61	23.57	-11.18
Cheboygan State Park, MI	1.88	17.09	66.15	24.02	-9.31
Duck Lake State Park, MI	5.80	19.18	72.08	24.96	-6.72
Grand Haven State Park, MI	5.80	19.18	75.76	25.52	-6.15
Grand Mere State Park, MI	3.45	19.55	85.38	27.08	-6.03
Harrington Beach State Park, WI	3.41	18.06	70.93	24.72	-9.00
Harrisville State Park, MI	3.03	20.48	65.97	24.16	-8.65
P. H. Hoelt State Park, MI	4.90	23.40	65.50	24.98	-8.74
P.J. Hoffmaster State Park, MI	5.80	19.18	76.54	25.59	-6.22
Hog Island Point State Forest, MI	4.27	20.43	65.48	22.97	-10.49
Holland State Park, MI	6.11	21.53	82.26	26.71	-6.51
Indiana Dunes National Lakeshore, IN	5.77	25.68	93.75	26.82	-6.44
Kohler-Andrae State Park, WI	5.95	19.86	69.58	24.98	-8.72
Lakeport State Park, MI	4.65	20.98	77.69	25.76	-6.99
Leelanau State Park, MI	5.36	22.43	62.64	23.38	-7.02
Ludington State Park, MI	3.70	17.84	67.56	24.31	-7.23
McClain State Park, MI	3.63	20.39	56.88	23.26	-10.62
Charles Mears State Park, MI	3.70	17.84	74.09	24.33	-7.54
Muskegon State Park, MI	5.80	19.18	76.43	25.68	-6.33
Negwegon State Park, MI	3.03	20.48	66.14	24.27	-8.54
Newport State Park, WI	4.28	22.45	57.89	23.91	-9.23
Orchard Beach State Park, MI	5.43	24.38	69.73	25.13	-7.10
Petoskey State Park, MI	5.36	22.43	68.34	23.74	-8.77
Pictured Rocks National Lakeshore, MI	3.27	18.36	64.74	23.25	-10.17
Point Beach State Forest, WI	3.99	20.59	66.97	23.43	-9.39
Port Crescent State Park, MI	4.92	25.16	69.93	24.96	-7.76
Apostle Islands National Lakeshore WI, Little Sand Bay	3.87	25.51	58.39	23.62	-12.82
Saugatuck State Park, MI	6.11	21.53	82.66	26.69	-6.43
Silver Lake State Park, MI	3.70	17.84	75.55	24.65	-7.56
Albert E. Sleeper State Park, MI	6.71	24.22	69.26	25.27	-7.73
Apostle Islands National Lakeshore WI, Stockton Island	6.02	21.71	58.41	23.41	-12.56
Tawas Point State Park, MI	2.44	17.73	70.48	25.58	-8.79
Van Buren State Park, MI	4.55	21.72	83.02	26.20	-5.24
Warren Dunes State Park, MI	6.25	21.96	88.23	27.06	-6.35
Whitefish Dunes State Park, WI	4.07	20.78	60.75	24.86	-9.34
Wilderness State Park, MI	4.69	20.42	65.10	23.00	-9.22

Table A3. Soil chemistry.

Site	Phosphorus (ppm)	Potassium (ppm)	Calcium (ppm)	Zinc (ppm)
Big Knob State Forest, MI	4	13.5	340.5	0.7
Brimley State Park, MI	2.5	15.5	58	1.45
Cheboygan State Park, MI	3	10	637.00	0.95
Duck Lake State Park, MI	3.5	10.5	453.5	0.6
Grand Haven State Park, MI	3.5	13.5	1,085.00	1.65
Grand Mere State Park, MI	1	8.5	1,968.50	0.8
Harrington Beach State Park, WI	4	21	755.50	1.35
Harrisville State Park, MI	2	10.5	2,024.00	0.65
P. H. Hoeft State Park, MI	0	5.5	3,437.00	0.25
P.J. Hoffmaster State Park, MI	3	10	279	1.05
Hog Island Point State Forest, MI	2	13	348	0.6
Holland State Park, MI	1.5	11.5	2,679.00	0.85
Indiana Dunes National Lakeshore, IN	1	10	826.50	0.9
Kohler-Andrae State Park, WI	3.5	12	641.50	1.1
Lakeport State Park, MI	0	9	4,971.50	0.85
Leelanau State Park, MI	0	4	6,170.00	0.2
Ludington State Park, MI	2	10.5	595.00	0.4
McClain State Park, MI	3.5	12	551.50	0.7
Charles Mears State Park, MI	3	11	232	0.7
Muskegon State Park, MI	3	10	274.5	0.75
Negwegon State Park, MI	2	9.5	316	0.75
Newport State Park, WI	3.5	8.5	532.00	0.55
Orchard Beach State Park, MI	1.5	11.5	495.5	0.3
Petoskey State Park, MI	0	6	7,648.50	0.3
Pictured Rocks National Lakeshore, MI	4.5	11.5	809.00	0.85
Point Beach State Forest, WI	2	8	794.50	0.4
Port Crescent State Park, MI	1	6	71.5	0.4
Apostle Islands National Lakeshore WI, Little Sand Bay	1.5	11	45.5	0.3
Saugatuck State Park, MI	2	9	1,018.50	0.8
Silver Lake State Park, MI	2	12	143	0.5
Albert E. Sleeper State Park, MI	3.5	11.5	212	0.7
Apostle Islands National Lakeshore WI, Stockton Island	0	10	23.5	0.15
Tawas Point State Park, MI	1.5	12	82.5	0.4
Van Buren State Park, MI	3	9.5	1,174.50	1.4
Warren Dunes State Park, MI	0.5	8.5	2,099.50	0.7
Whitefish Dunes State Park, WI	14	7	423.5	0.85
Wilderness State Park, MI	0	7.5	2,195.50	0.3



Table A4. Soil data.

Site	% soil organic matter	% total nitrogen	Soil pH	% stems colonized by EF	AMF extra-radical hyphal length (no. views)	% roots colonized by AMF
Big Knob State Forest, MI	0.48	0.009	8	0.0	73.5	53.0
Brimley State Park, MI	0.09	0.004	6.4	0.0	12	20.0
Cheboygan State Park, MI	0.95	0.006	8.9	26.0	6.5	12.0
Duck Lake State Park, MI	0.24	0.004	8.4	0.0	0	29.0
Grand Haven State Park, MI	0.38	0.004	8.9	12.0	7.5	23.0
Grand Mere State Park, MI	0.79	0.004	8.8	4.0	3.5	6.0
Harrington Beach State Park, WI	2.3	0.004	8.9	0.0	11.5	26.0
Harrisville State Park, MI	0.77	0.004	8.8	0.0	0	26.0
P. H. Hoeft State Park, MI	1.29	0.002	8.9	4.0	3.5	71.0
P.J. Hoffmaster State Park, MI	0.19	0.005	8.8	0.0	0	16.0
Hog Island Point State Forest, MI	0.62	0.007	8.1	0.0	4.5	42.0
Holland State Park, MI	0.6	0.003	8.9	0.0	4.5	2.0
Indiana Dunes National Lakeshore, IN	0.34	0.002	8.5	16.0	0	6.0
Kohler-Andrae State Park, WI	1.51	0.003	8.7	2.0	0	6.0
Lakeport State Park, MI	1.7	0.005	8.9	0.0	8	23.0
Leelanau State Park, MI	1.07	0.002	9	0.0	7.5	32.0
Ludington State Park, MI	0.29	0.003	8.5	0.0	37	19.0
McClain State Park, MI	0.15	0.002	8.1	6.0	0	30.7
Charles Mears State Park, MI	0.26	0.003	8.2	4.0	1.5	25.0
Muskegon State Park, MI	0.15	0.004	8.1	0.0	1.5	43.0
Negwegon State Park, MI	0.65	0.01	8.2	0.0	4	52.0
Newport State Park, WI	2.37	0.007	8.5	0.0	2.5	25.0
Orchard Beach State Park, MI	0.34	0.004	8.8	10.0	5.5	22.0
Petoskey State Park, MI	1.2	0.003	9	0.0	5	60.0
Pictured Rocks National Lakeshore, MI	2.3	0.005	8.9	0.0	18.5	6.0
Point Beach State Forest, WI	0.24	0.003	8.7	4.0	1.5	23.0
Port Crescent State Park, MI	0.07	0.002	7.4	0.0	11.5	30.0
Apostle Islands National Lakeshore WI, Little Sand Bay	0.07	0.003	6.8	0.0	4	34.0
Saugatuck State Park, MI	0.22	0.004	8.7	2.0	12.5	10.0
Silver Lake State Park, MI	0.14	0.002	8.3	4.0	0	4.0
Albert E. Sleeper State Park, MI	0.31	0.006	8.4	0.0	11	17.0
Apostle Islands National Lakeshore WI, Stockton Island	0.05	0.002	6.8	0.0	11.5	35.0
Tawas Point State Park, MI	0.07	0.002	7.3	20.0	6	23.0
Van Buren State Park, MI	0.65	0.008	8.7	0.0	5	64.0
Warren Dunes State Park, MI	0.74	0.004	8.9	14.0	1.5	1.0
Whitefish Dunes State Park, WI	1.22	0.003	8.5	0.0	0	10.0
Wilderness State Park, MI	1.22	0.004	8.8	0.0	13	0.0

## APPENDIX B

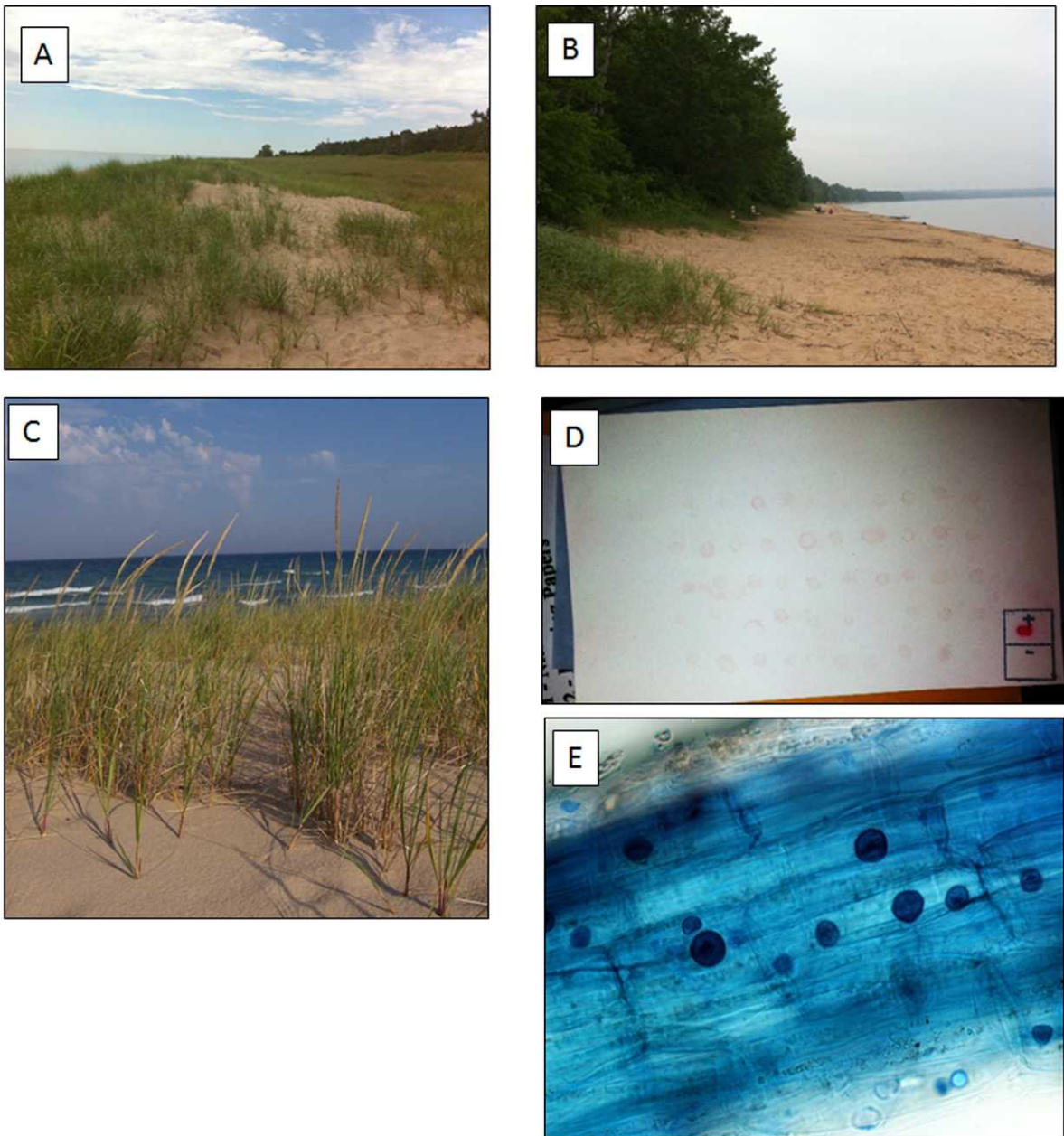


Fig. B1. (A) The site at Koehler-Andrae State Park, WI. (B) The site at Negwegon State Park, MI. (C) The study species, *Ammophila breviligulata*. (D) An example of endophyte screening using immunoblot kits. The red indicates endophyte presence. (E) An example of root tissue containing AMF.