Beach Restoration Efforts Influenced by Plant Variety, Soil Inoculum, and Site Effects

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ABSTRACT

EMERY, S.M. and RUDGERS, J.A., 0000. Beach restoration efforts influenced by plant variety, soil inoculum, and site effects. *Journal of Coastal Research,* 00(0), 000–000. West Palm Beach (Florida), ISSN 0749-0208.

Recent research has highlighted the importance of soil biota in the establishment success of target plant species in restorations. This has led to restoration efforts that include soil amendments containing mycorrhizal fungi as well as other soil organisms. Although several studies have demonstrated interactions between plant *species* and soil biota, few studies have looked at interactions between plant *variety* and soil biota, particularly in the context of restoration. When several sources of plant material are available to land managers, it may be important to account for interactions between plant variety and soil inoculum. Here we present results from an experiment examining interactions between plant variety and soil inoculum for beach restoration. At two lakeshore beaches in Chicago, IL, we planted two varieties of the native beach grass *Ammophila breviligulata*, a common plant used in beach restoration. We included a soil inoculum treatment for half of the plots, using soil from nearby mature plant communities. The "Cape" variety of *Ammophila* had double the survivorship and growth of the regional variety of *Ammophila* "Vans." Soil inoculum significantly increased survivorship and growth of the regional variety in one site, but not of the "Cape" variety. Mycorrhizal spore abundance and diversity, as well as plant root colonization, did not differ between treatments, indicating that some other soil organisms may be providing benefits to plants in this study. Our work shows that performance of key species for beach restoration can vary up to 300% depending on complex interactions between soil biota and plant variety.

ADDITIONAL INDEX WORDS: Ammophila breviligulata*, mycorrhizae, endophyte, sand dunes, Great Lakes, soil amendments.*

INTRODUCTION

Great Lakes sand dunes and beaches represent the most extensive freshwater sand habitat in the world (Albert, 2000). These habitats provide the physical substrate for maintaining ecosystem integrity along the aquatic–terrestrial border for much of the Great Lakes region (Albert, 2000; Arun *et al.,* 1999). Like sand dunes throughout the world, these systems provide habitat for endemic plant species, shelter neighboring wetlands, and serve as tourist attractions. Native dune vegetation also protects human developments by stabilizing soil, and will be important habitat in mediating the negative impacts of global climate change, including changing lake and sea levels, severe storms, and greater erosion (Cochard *et al.,* 2008). Because these habitats are highly susceptible to human and natural disturbances, restoration efforts have become common. Since the 1950s, *Ammophila breviligulata* (hereafter referred to as simply *Ammophila*), a highly clonal, perennial, cool-season (C_3) grass and the dominant native pioneer plant species in temperate coastal and lacustrine dunes of North America (Gleason and Cronquist, 1991), has been planted for restoration of both natural and artificial dune habitats

throughout North America (Maun, 1984; Maun and Krajnyk, 1989; Seneca and Cooper, 1971). Land managers continue to explore methods to increase success of these restoration efforts in the Great Lakes region (Maun and Krajnyk 1989).

Recent research in restoration ecology has highlighted the importance of soil biota in the establishment success of many target plant species (Callaham, Rhoades, and Heneghan, 2008; De Deyn *et al.,* 2003; Thrall *et al.,* 2005; Young, Petersen, and Clary, 2005). For example, mycorrhizal fungi, which form symbiotic, usually beneficial, relationships with terrestrial plants, can have an important role in increasing establishment of native plants in restorations by increasing nutrient acquisition and the competitive ability of plants they colonize (Rillig, 2004). In fact, restoration efforts that include soil inoculum containing native mycorrhizal communities can often increase native plant diversity (Heneghan *et al.,* 2008; Requena *et al.,* 2001; Smith, Charvat, and Jacobson, 1998).

Mycorrhizal fungi have been shown to be beneficial for sand dune restoration in several coastal systems, though very little is known about Great Lakes dune systems. On Florida dunes, native mycorrhizal fungi were twice as beneficial as a one-species commercial inoculum for stimulating growth of the native dune grass *Uniola paniculata* (Sylvia, Jarstfer, and Vosatka, 1993). Similarly, a field restoration experiment along Massachusetts sand dunes demonstrated that a soil amendment containing native mycorrhizal communities could in-

crease tillering and flowering of the native dune grass *A. breviligulata* (Gemma and Koske 1997). For primary successional systems, such as sand dunes, it can take up to 6 years for significant symbiont networks to naturally establish (Gemma and Koske 1997), further indicating that adding soil biota such as mycorrhizal fungi to restoration projects may "jump start" the successional process.

Although evidence to date indicates that addition of soil symbionts, such as mycorrhizal fungi, to restorations is generally, but not always, beneficial (e.g., Renker *et al.,* 2004; Schwartz *et al.,* 2006), little work has investigated potential interactions between soil symbionts and individual plant identity. For example, whereas several ecological studies have demonstrated interactions between plant *species* identity and additions of mycorrhizal fungi (e.g., Bever *et al.,* 1996; Klironomos, 2003), few studies have examined interactions between plant *variety* and mycorrhizal fungi. Further, studies that have examined plant variety × mycorrhizae interactions often show conflicting results. A study by Streitwolf-Engle *et al.* (2001) found no plant variety × mycorrhizae effect when comparing benefits of mycorrhizae across 17 genotypes of *Prunella vulgaris*. Alternatively, six different varieties of wheatgrass, *Agropyron cristatum*, inoculated with the same mycorrhizal fungi, showed differences in growth, tissue phosphorus levels, and water use efficiency (Jun and Allen, 1991). To our knowledge, no study has considered the potential consequences of plant variety × soil symbiont interactions for Great Lakes dune restoration efforts. When several plant source options are available to land managers, it may be important to take into account interactions between plant variety and soil symbionts to achieve the most successful restoration.

Currently, at least seven different *Ammophila* varieties are available from the U.S. Department of Agriculture (USDA) (Gemma and Koske 1997), and a few other regional varieties are available from smaller plant nurseries in the eastern United States (Emery, Thompson, and Rudgers, 2010). One main role of *Ammophila* in beach and sand dune systems includes slowing of erosion through the stabilization of sand; this process contributes to a buildup of organic matter and allows soil microbial communities to develop (Lichter, 2000; Olson, 1958). Thus, soil biota may play an important role in later beach and dune community development and succession (Gemma, Koske, and Carreiro, 1989). Here we present results from field experiments designed to examine potential interactions between plant variety and soil inoculum for beach restoration.

MATERIALS AND METHODS

In May 2007, we established duplicate restoration experiments at two beach sites along the southern Lake Michigan shoreline. The first site was a protected natural area within Loyola Park, part of the Chicago City Parks System (42°00′24″ N, 87°39′22″ W), and surrounded by urban development. This area had recently started accumulating sand and park managers were interested in using native vegetation to continue to stimulate dune growth (A. Whelan, personal communication). Although not heavily vegetated, the site had

several weedy plant species, including *Cenchrus longispinus* (sandbur), *Xanthium strumarium* (cocklebur), and *Elymus repens* (quackgrass). The second site was along a narrow strip of beach at the base of a lake bluff in Millard Park, part of the Highland Park, IL, parks system (42°10′50″ N, 87°46′49″ W). This area had been steadily losing sand over the past several decades, and steel groins added in the 1950s had been unsuccessful in stopping beach erosion (R. Grill, personal communication). Park managers were interested in using native dune species to slow the continued erosion. The beach area here was generally unvegetated.

In each site, we set up 32 plots (2 m × 2 m), each separated by a 0.5-m border, for a 2 × 2 factorial experiment manipulating both plant variety and soil biota. Each treatment combination was replicated eight times. Plots were laid out linearly parallel to the shore, and were approximately 3 m from the water's edge. For the soil inoculation treatment, we thinly scattered and raked in 3 L of sand collected from the vegetated forest edge of the Millard Park site in 16 plots at each site (randomly assigned). This sand was collected immediately before plot setup by digging and mixing sand from several shallow holes (~30 cm in depth) near the site. Sand was raked to approximately 10-cm depth in the plots in attempts to expose newly planted grass tillers (see below) to the inoculum. This sand inoculum represented soil from a mature dune habitat, capable of supporting high plant diversity. Because the Loyola Park site was completely isolated from mature dune habitat, we used the inoculum from Millard Park at this site as well. We expected that the main benefit of this soil inoculum would be the introduction of arbuscular mycorrhizal fungi (AMF), although other soil symbionts may have been present as well. We raked the other 16 plots without adding the extra sand to control for any disturbance effects. We assumed that the inoculum altered only the live component of the soil with no direct effect on nutrient availability, as the total addition of inoculum comprised just 0.015% of total soil volume in the rooting zone (~50-cm depth) of each plot.

Immediately after the sand addition, we planted 25 tillers of *A. breviligulata* into each plot, spaced 0.5-m apart, according to recommended planting densities (Seneca and Cooper, 1971; U.S. Army Corps of Engineers, 1979). Tillers were dormant when planted, and had very few roots present. We did not fertilize tillers at any point during the experiment. We used separate planting tools for soil inoculum plots to avoid possible contamination, and we sterilized shovels between sites using 50% commercial bleach. In eight of the plots for each soil treatment, we planted the "Cape" variety of *Ammophila* (Vans Pines Nursery, West Olive, MI), a cultivar developed by the USDA from initial plant collections in New Jersey (Soil Conservation Service, 1977), and widely sold throughout the East Coast and Great Lakes region. In the other eight plots for each soil treatment, we planted the "Vans" variety of *Ammophila* (Vans Pines Nursery, West Olive, MI), a regional variety developed from native Michigan populations. Although roots were not sterilized, plants of both varieties came from the same nursery and were propagated in identical field conditions (Vans Pines Nursery, West Olive, MI, personal communication), so we assumed plants were exposed to similar soil biota during propagation. Besides plant genetic differences, the

"Cape" variety was also 100% infected with a systemic endophyte (Halisky and White, 1991), which was lacking in the "Vans" variety (Emery, Thompson, and Rudgers, 2010). Selective sampling of the "Cape" plants used in this experiment also confirmed 100% colonization by the endophyte (unpublished data). Some evidence suggests that this endophyte may convey drought and herbivore resistance to colonized plants (Clay and Schardl, 2002; Emery, Thompson, and Rudgers, 2010; Schardl, Leuchtmann, and Spiering, 2004), as has been demonstrated for other grass–endophyte interactions (Clay and Schardl, 2002; Kannadan and Rudgers, 2008; Schardl, Leuchtmann, and Spiering, 2004). Because the endophyte is vertically transmitted from maternal plants to seeds and has not been documented to spread contagiously from plant to plant (White *et al.,* 1992), it may be considered a component of plant genotype (though see Moy *et al.,* 2000; Tadych *et al.,* 2007 for possible exceptions); as it is inherited maternally, it will therefore be present in plant material propagated either clonally or from seed.

In September 2007 and 2008, we counted the surviving plants in each plot, counted any new tillers and flower heads present, and took five 15-cm-deep × 1.9-cm-diam soil cores near surviving tillers from each plot to measure mycorrhizal spore abundance and mycorrhizal root colonization. Although *Ammophila* does not heavily rely on seed production as a reproductive strategy, viable seeds are often present in flowering heads and offer one measure of fitness (Laing, 1958). Total tiller counts per plot were used instead of estimating average tiller production per plant because of the difficulty of identifying unique individuals in plots after the first growing season without physically digging up plants. To quantify spore abundance, we used wet-sieving and sucrose density gradient centrifugation (Walker, Mize, and McNabb, 1982) on a 50-ml subsample of sand collected in the five cores from each plot. We then counted and identified spore morphospecies under a microscope in the lab. Morphospecies identification was based on the size and color of spores, and served as a rough measure of diversity. To measure mycorrhizal root colonization of *Ammophila*, we sieved the remaining soil sample from each plot to collect root fragments, boiled these root fragments in 10% KOH for 30 minutes, and stained using the ink (Shaeffer black) and vinegar method (Vierheilig *et al.,* 1998). Ten 1-cm root sections from each plot were then mounted on microscope slides and visually scored for AMF colonization. Each root section was categorized as either 0%, 5%, 25%, 50%, 75%, or 100% colonized on the basis of total root area occupied by AMF hyphae, and root section scores were averaged for a given plot (Giovannetti and Mosse, 1980). We returned to sample the experiment in 2009, but rising lake levels had flooded the experiments over the winter, washing away most plants at both sites.

We used general linear models to examine the effects of plant variety and soil inoculum on plant survival and growth, with response variables calculated at the plot level (*e.g.,* number of plants surviving per plot). Most response variables (surviving number of plants, number of tillers, number of flowers, number of AMF spores) were square-root transformed to improve normality of residuals. Initial analyses indicated a significant site effect, so we have chosen to present results from the two

sites separately. All analyses were performed in SYSTAT v.12 (SYSTAT Software Inc., 2007).

RESULTS

Ammophila Survival and Growth

In both sites, the "Cape" variety of *Ammophila* had almost double the survival and growth rate (new tiller growth) of the "Vans" variety (Table 1, Figure 1). The Cape variety was the only one to flower at both sites in 2008 (Figure 2). The soil inoculum treatment had no significant effect on *Ammophila* survival, growth, or flowering at the Loyola site, but increased survival and growth of "Vans" plants at the Millard site almost threefold (Table 1, Figures 1, 2).

Mycorrhizal Fungi

The soil inoculum treatment had a minimal effect on AMF in the plots. In 2007, total spore numbers were almost doubled (though not statistically significant) with soil inoculum at the Millard site, but not the Loyola site, and there were no differences in AMF morphospecies richness with soil inoculum at either site (Table 1, Figure 3a, b). This lack of significance may be due in part to low power to detect these differences in AMF abundance given the fairly large variation among plots. A retrospective power analysis using the program PASS (Hintze, 2007) indicated low power levels for this study (0.36 for the soil inoculum factor). By 2008, there were no differences in AMF spore abundance or morphospecies richness across treatments at either site (Table 1, Figure 3c, d). Plant variety had no effect on AMF spore abundance or morphospecies richness in either 2007 or 2008, nor was there any interaction between plant variety and the soil inoculum treatment on AMF responses (Table 1).

Mechanisms other than AMF may explain the enhanced growth of the "Vans" variety with soil inoculum at Millard Park. When AMF spore abundance in 2007 was added as a covariate to the model of plant survival at Millard Park in 2007, soil inoculum still had a significant effect on survival (plant variety: $F_{1, 32} = 29.5$, $p < 0.001$; soil inoculum: $F_{1, 32} = 4.37$, $p = 0.046$; variety * soil: $F_{1, 32} = 5.86$, $p = 0.02$; total spore abundance: $F_{1, 32} = 0.137$, $p = 0.71$), suggesting that AMF spore abundance was not a key driver. However, this pattern was not true for plant growth, as all factors except plant variety became nonsignificant when AMF abundance was added as a covariate (plant variety: $F_{1, 32} = 29.3$, $p < 0.001$; soil inoculum: $F_{1, 32} = 0.07$, $p = 0.80$; variety * soil: $F_{1, 32} = 0.36$, $p = 0.55$; total spore abundance: $F_{1, 32} = 1.04$, $p = 0.31$). Further, AMF root colonization of *Ammophila* did not differ among soil treatments at either the Loyola or the Millard site (Table 1). At the Millard site, "Cape" plants had significantly lower (2007) or similar (2008) root colonization compared with "Vans" plants (Figure 4), suggesting that AMF are not directly contributing to the enhanced growth of this variety.

DISCUSSION

Results from this study showed that soil inoculum can benefit the "Vans" variety of *Ammophila* in the first year of plant

Table 1. *Analysis of variance results for effects of plant variety and soil inoculum on plant survival, growth, and flowering, AMF abundance and diversity, and AMF root colonization. Significant effects are in bold.*

| Source of variation	Plant Survival 2007 $F_{1,32}$	p-value	Plant Growth 2008 $F_{1,32}$	p-value	Flowering, 2008 $F_{1,32}$	p-value	AMF Abundance 2007 $F_{1,32}$	p-value	AMF Abundance 2008 $F_{1,32}$	p-value	AMF Morphorichness 2007 $F_{1,32}$	p-value	AMF Morphorichness 2008 $F_{1,32}$	p-value	AMF Root Colonization 2007 $F_{1,32}$	p-value	AMF Root Colonization 2008 $F_{1,32}$	p-value
Loyola Park																		
Plant variety	**7.4**	**0.01**	**8.9**	**0.006**	**8.3**	**0.007**	0.83	0.37	2.4	0.14	0.001	0.98	0.76	0.39	2.36	0.14	3.44	0.08
Soil inoculum	0.03	0.86	0.02	0.88	0.68	0.42	1.69	0.21	0.03	0.86	1.80	0.19	0.02	0.90	0.18	0.68	1.61	0.22
Variety * soil	0.9	0.42	2.95	0.10	0.68	0.42	0.01	0.94	0.15	0.70	0.98	0.34	1.9	0.18	0.64	0.44	0.03	0.86
Millard Park																		
Plant variety	**30.9**	**<0.001**	**28.9**	**<0.001**	**16.4**	**<0.001**	0.15	0.71	3.2	0.08	0.68	0.42	1.65	0.21	**5.65**	**0.03**	2.63	0.12
Soil inoculum	**5.3**	**0.03**	1.22	0.27	0.73	0.79	2.15	0.15	0.14	0.98	0.25	0.63	0.80	0.38	1.09	0.31	1.70	0.20
Variety * soil	**5.9**	**0.02**	**4.42**	**0.05**	0.73	0.79	0.25	0.62	2.4	0.13	1.20	0.28	0.68	0.42	0.87	0.36	2.6	0.12
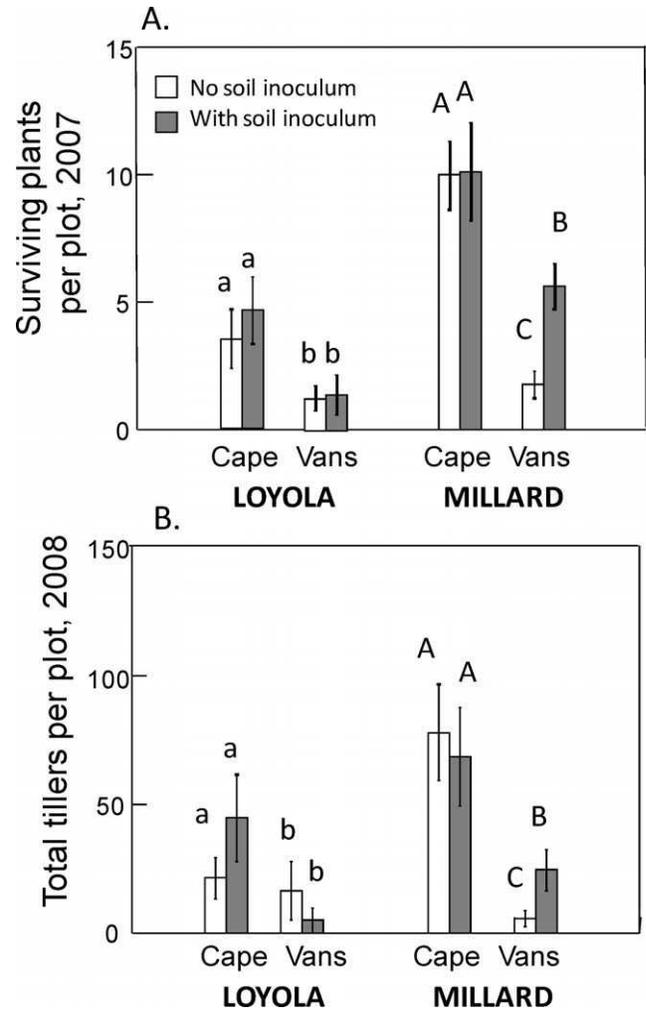
Figure 1. (A) Numbers of surviving *Ammophila* plants after one growing season (2007). (B) Total numbers of tillers per plot in 2008. Different letters indicate significant differences within sites according to a post hoc Tukey honestly significant differencetest. Error bars indicate ±1 SE.

establishment, but that soil inoculum provided no benefit to the "Cape" variety of *Ammophila*. This pattern was not consistent across sites, which may be due to the prerestoration conditions of these regions. The Millard site, where significant inoculum effects were seen, was an unvegetated, narrow beach with erosion problems, whereas the Loyola site was accumulating sand and had a pre-existing weedy plant community. It is not surprising that soil inoculum had stronger effects in the unvegetated beach, where presumably soil conditions have been more sterile, as has been documented in other North American dune systems (Gemma and Koske, 1997). Additionally, although we did not explicitly measure soil nutrients in the two sites, it is possible that differences in nutrient availability underlie the different effects of the soil inoculum (Johnson *et al.*, 2003). In particular, soils with increased nitrogen levels tend to decrease abundance of mycorrhizae (Egerton-Warburton and Allen 2000). It is also possible that the
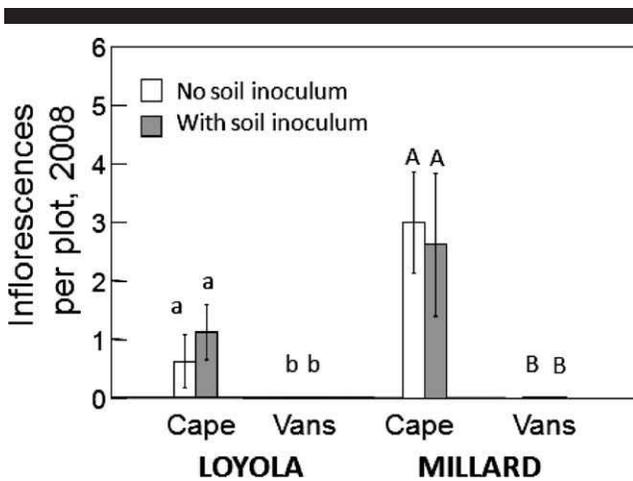
Figure 2. Number of *Ammophila* inflorescences per plot, 2008. Different letters indicate significant differences within sites according to a post hoc Tukey honestly significant difference test. Error bars indicate ±1 SE.

inoculum collected from the Millard site may have had a stronger effect at that site because of adaptation to local conditions, despite the relative proximity of the two sites used in this study. This local soil effect has been shown in other dune restoration studies. For example, local populations of sea oats (*U. paniculata*) grew best with local soil inoculum in dune restorations in Florida (Al Agely and Sylvia 2008). Additionally, the regional plant variety "Vans" may be more adapted to Great Lakes soil biota than the East Coast variety "Cape". The different patterns between the two sites indicate the challenges of making generalizations about the role of soil symbionts in restoration efforts.

The strong difference in performance of the two varieties of *Ammophila* was unexpected, especially when considering issues of regional adaptation. The "Cape" variety had almost double the survival and growth of the "Vans" variety, independent of soil inoculum. If local adaptation was important (e.g., Jones, 2003; McKay *et al.*, 2005), we would expect the "Cape" variety, which was developed from East Coast *Ammophila* populations (Soil Conservation Service 1977), to have lower establishment
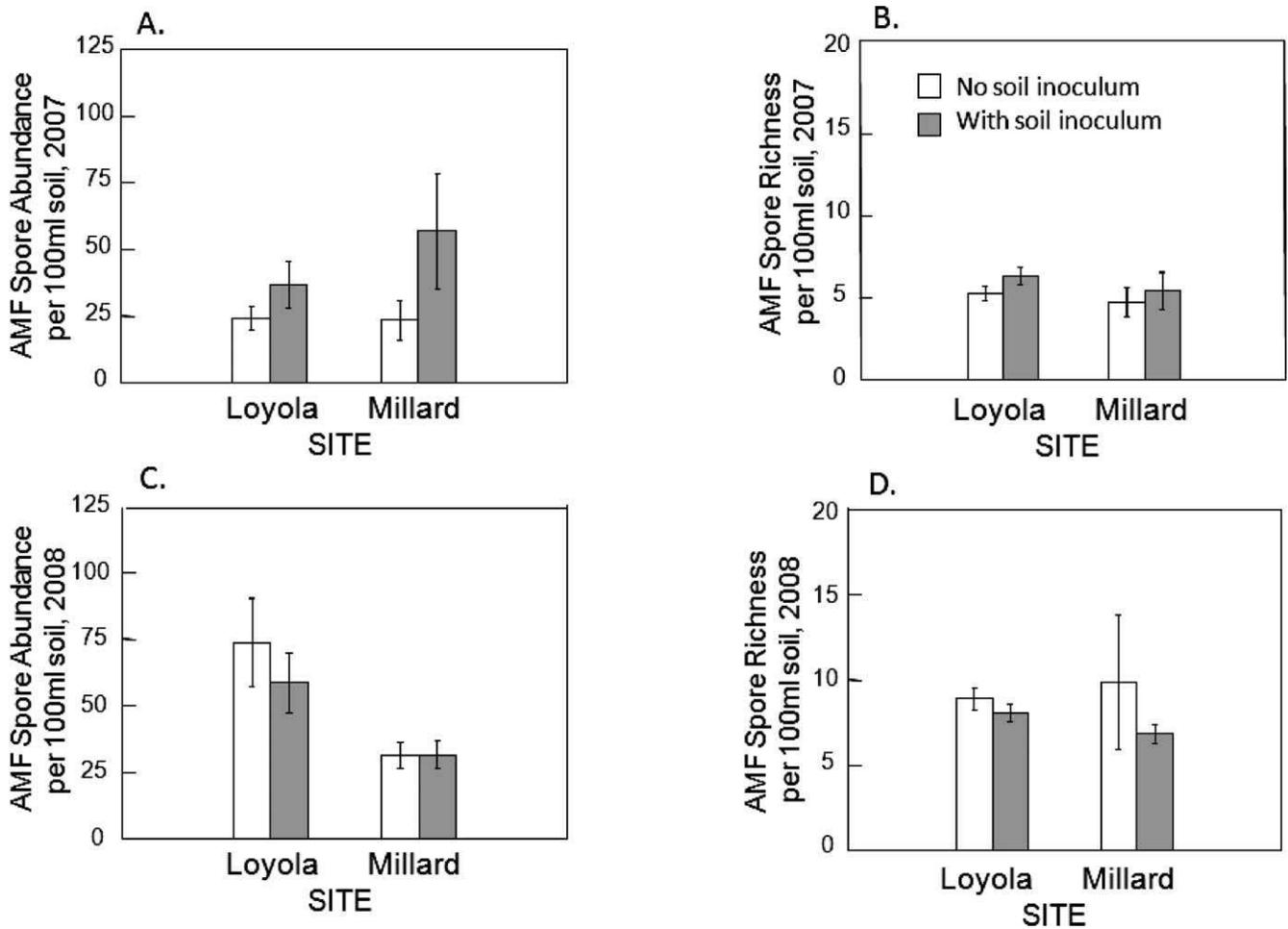

Figure 3. Arbuscular mycorrhizal fungi (AMF) spore abundance per plot in 2007 (A) and 2008 (C). No significant effects of soil inoculum were present for either the Loyola site or the Millard site. Spore morphospecies richness in 2007 (B) and 2008 (D). No significant effects of soil inoculum were present for either the Loyola site or the Millard site. Error bars indicate ±1 SE.
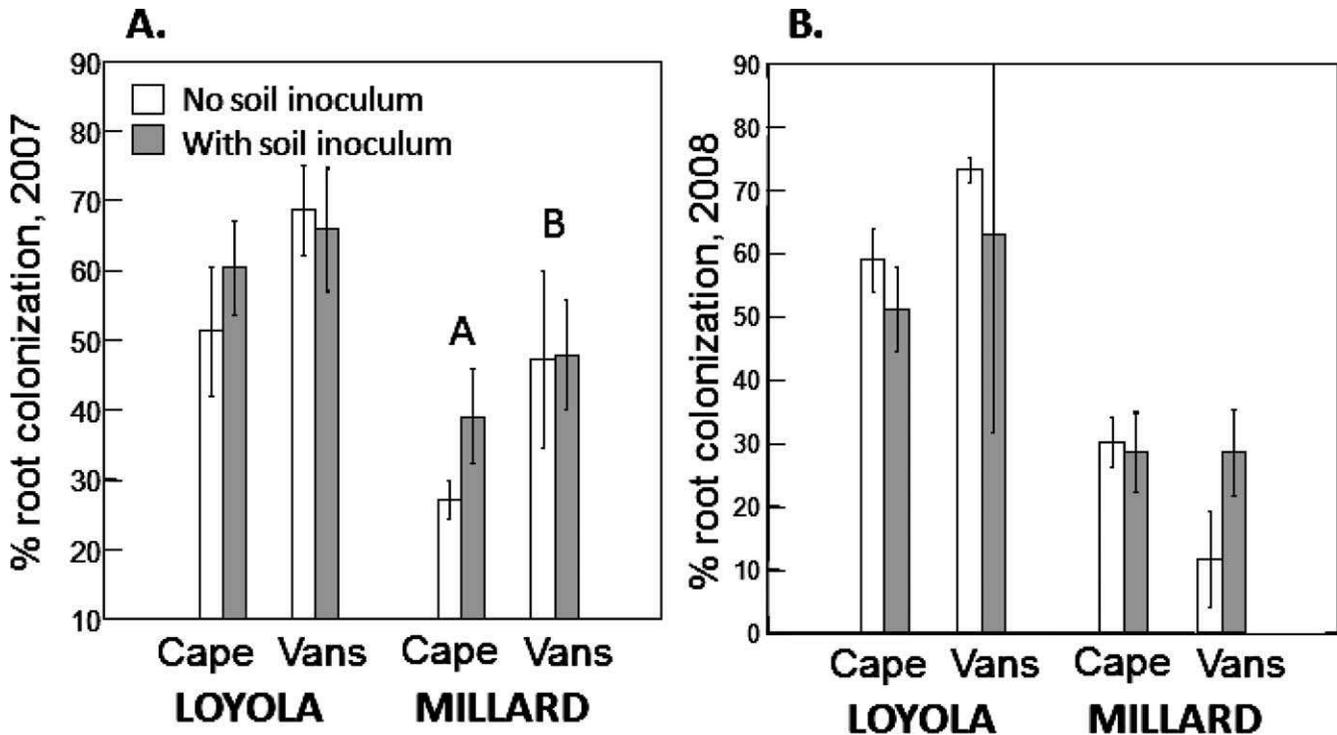
Figure 4. Percentage of *Ammophila* roots colonized by mycorrhizae in (A) 2007 and (B) 2008. Different letters indicate significant differences within sites. There were no significant effects of soil inoculum. Error bars indicate ±1 SE.

success than the "Vans" variety, which was developed from Great Lakes populations. Although the "Cape" variety may just be more vigorous because of a history of artificial selection and clonal propagation by the USDA through their "Comparative Observation Evaluation" (Natural Resources Conservation Service, 2006), another possible explanation for the improved success of the "Cape" variety may be the presence of a systemic endophyte (Halisky and White, 1991), which is lacking in the Michigan variety (Emery, Thompson, and Rudgers, 2010). Although not explicitly studied in this system, systemic endophytes in other systems have been shown to increase plant competitive ability and drought tolerance (Clay and Schardl, 2002; Malinowski *et al.,* 1997; Rudgers *et al.,* 2007). This endophyte may also explain some of the significant interactions between plant variety and soil inoculum. The "Cape" variety had 20% lower levels of mycorrhizal colonization than the "Vans" variety at the Millard site during the early stage of establishment in 2007 (Figure 4), which may indicate an ability of the endophyte to reduce other fungi initially coinfecting the plant. Such antagonistic interactions between endophytes and AMF have been demonstrated in other grass species (Mack and Rudgers, 2008; Omacini *et al.,* 2006).

Although there was a demonstrated benefit of soil inoculum for the "Vans" variety, it is unclear whether mycorrhizae are causing this benefit. Spore abundance across plots was not a significant predictor of *Ammophila* survival. It may be that plant root growth is stimulated because of a chemotropic response when roots come in contact with mycorrhizal spore

germ tubes, rather than actual root colonization from mycorrhizae (Gemma and Koske, 1989). Additionally, molecular methods of quantifying mycorrhizal abundance and diversity may give a clearer picture of mycorrhizal communities, despite our methods being widely used for similar studies (e.g., Gemma and Koske, 1997; Greipsson and El-Mayas, 2000; Requena *et al.*, 2001) However, other soil organisms may also be providing benefits to plants in this study. For example, bacterial endophytes can increase the performance of plants (Chanway, 1998). In *Ammophila arenaria*, the sister species to *A. breviligulata*, soil inoculation with azotobacter increased plant growth (Dalton *et al.,* 2004; Wahab and Wareing, 1980). In addition, *Ammophila* species produce a mucilaginous rhizosheath surrounding roots; in other grass species, the rhizosheath can be an important site for the colonization of nitrogen-fixing bacteria that promote plant growth (Bergmann *et al.,* 2009; Wullstein, Bruening, and Bollen 1979), which may be particularly important in nitrogen-limited dune systems (Gilbert, Pammenter, and Ripley, 2008; Olson, 1958). The potential role for soil bacteria is also supported by observations that bacterial communities, as well as fungal communities, increase in diversity and abundance along dune successional gradients (Webley, Eastwood, and Gimingham 1952). Finally, it may be that our results are due to differential effects of soil pathogens, rather than beneficial soil organisms. Soil pathogens have been found to affect *A. arenaria* in dune systems (Beckstead and Parker, 2003; Knevel *et al.,* 2004), though in our system the inoculum either increased plant growth (Mill-

ard) or had no effect (Loyola), making this explanation unlikely. Our sampling protocols were specifically focused on mycorrhizal communities because prior evidence had linked AMF to *Ammophila* growth and to the process of dune succession (Gemma and Koske, 1997; Little and Maun, 1996; Maun, 1998; Perumal and Maun, 1999). Additionally, we did not sample soil communities before initiation of the experiment. Therefore, we were unable to explicitly address the role of other soil organisms in this study. However, the inoculum made up such a small amount of the total volume of the sand in the plots, it seems unlikely that inoculum effects were due to differences in nutrients or organic matter addition.

In conclusion, results from this study indicate that *Ammophila* varieties can be differentially affected by soil inoculum. Whole soil inoculum from native, mature plant communities increased the survival of a regional variety of *Ammophila* used in beach restorations at one of our sites (Millard). Although results can be site dependent, in no case were soil inocula harmful to plant growth. This suggests that soil inocula are generally good management techniques on these sand dunes. Although the mechanism explaining the benefits of the whole soil inoculum are still unknown, our results indicate no clear effect of mycorrhizal-plant interactions. Instead, this benefit may be due to other soil microbes. In general, for Great Lakes beaches and sand dunes, the widely available "Cape" variety of *A. breviligulata* has almost twice the survival and growth of the regional variety. If use of nonlocal varieties is not a concern, "Cape" *Ammophila* may be the best choice for quickly revegetating beach habitats. However, if genetic diversity or local adaptation is important, other revegetation strategies may be preferred (Fant *et al.*, 2008). Results from this study join others showing that complex interactions between plants and soil biota can often make site-specific restoration recommendations difficult (Eviner and Hawkes, 2008; Sylvia, Jarstger, and Vosatka, 1993).

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