2011

IMPACTS OF ASIATIC SAND SEDGE ON NATIVE PLANTS AND ARBUSCULAR MYCORRHIZAL FUNGI IN A BARRIER DUNE

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IMPACTS OF ASIATIC SAND SEDGE ON NATIVE PLANTS AND ARBUSCULAR MYCORRHIZAL FUNGI IN A BARRIER DUNE

BY

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

BIOLOGICAL SCIENCES

UNIVERSITY OF RHODE ISLAND

2011

MASTER OF SCIENCE THESIS

OF

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2011

ABSTRACT

The recent expansion of the nonnative invasive Asiatic sand sedge (*Carex kobomugi* Ohwi) at East Beach State Park, Rhode Island, is reducing populations of the most important native, dune-building species and their associated arbuscular mycorrhizal fungi (AMF). In contrast to the native American beachgrass (*Ammophila breviligulata* Fern.) that is dependent upon AMF to thrive in nutrient-poor sand dunes, *C. kobomugi* does not form beneficial associations with the fungi. Furthermore, assessments suggest that the sedge is competitively superior in obtaining the essential nutrient phosphorous without AMF-facilitation. Analysis of data from transects of the dune system revealed significant negative correlations between distributions of *C. kobomugi* and *A. breviligulata* that are being extirpated. Percent cover of *A. breviligulata* was significantly reduced in areas of *C. kobomugi.* Other native plant species were not significantly reduced as a result of *C. kobomugi* expansion. Spore populations of AMF showed significant positive correlations with percent cover of *A. breviligulata* and significant negative correlations with percent cover of *C. kobomugi*. Mean spore abundance of AMF in areas of *C. kobomugi* was less than in areas dominated by *A. breviligulata*. The number of species of AMF was not significantly reduced as a result of *C. kobomugi* likely because of highly aggregated and infrequent distribution of some species' spores. Assessment of mycorrhizal inoculum potential (MIP) of soils taken from the field mirrored the spore-population data: mean root colonization of plants grown in field soil of *C. kobomugi* (12%-24%) was between three and five times lower than that of plants grown in field soil of *A. breviligulata* (55%-72%). This study was unique in

quantifying the effect of an invasive species on populations of mycorrhizal fungi in a dune habitat. It was novel in assessing the reduction of native plant and fungi species by *C. kobomugi* in Rhode Island. The replacement of AMF-forming species on dunes by a species that does not form AMF (and support spore production by these obligately biotrophic fungi) will have serious consequences when attempts are made to re-establish native species in the sites that are eventually cleared of *C. kobomugi*.

ACKNOWLEDGMENTS

I thank Dr. Richard Koske for his enthusiasm and guidance throughout this project. His personality and expertise helped to facilitate this study greatly, and as a mentor he helped to hone my skills as an active contributor to the scientific field.

I thank Dr. Keith Killingbeck, Dr. Laura Meyerson and Dr. Jane Gemma for suggestions that laid the foundation that I was able to expand upon. Their ideas helped to define and better communicate my findings. I thank my lab and teaching colleagues over the years for their support as well. This study would not have been possible without material assistance provided by the University of Rhode Island.

I thank Rick Enser for field notes pertaining to vegetation, Robert Paquette of the R.I. Department of Environmental Management Division of Parks and Recreation for permitting access to the dunes at East Beach. I thank Warren Hall of the Town of Middletown, and Andrew MacLachlan of the U.S. Fish and Wildlife Service for information concerning Sachuest Point and East Beach respectively.

Lastly, this thesis would not have been possible without the support from my friends and family.

PREFACE

This thesis is written in the manuscript style format approved by the Graduate School of the University of Rhode Island with modifications in style as required for publication in the specific journal listed. One manuscript is as follows:

MANUSCRIPT I. “Impacts of Asiatic sand sedge on native plants and arbuscular mycorrhizal fungi in a barrier dune (to be submitted in the Journal of Coastal Research).

Detailed methods for certain aspects of the study are presented in the Appendices.

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MANUSCRIPT I.

Impacts of Asiatic sand sedge on native plants and arbuscular mycorrhizal fungi in a barrier dune

by

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Running head: Impacts of an invasive sedge in RI dunes

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ABSTRACT

The recent expansion of the invasive Asiatic sand sedge (*Carex kobomugi* Ohwi) at East Beach State Park, Rhode Island, is reducing populations of the most important native, dune-building species and their associated arbuscular mycorrhizal fungi (AMF). In contrast to the native American beachgrass (*Ammophila breviligulata* Fern.) that is dependent upon arbuscular mycorrhizal fungi (AMF) to thrive in nutrient-poor sand dunes, *C. kobomugi* does not form beneficial associations with the fungi. Furthermore, assessments suggest that the invasive is competitively superior in obtaining the essential nutrient phosphorous without facilitation by mycorrhizae. Analysis of data from transects of the dune system revealed significant negative correlations between distributions of *C. kobomugi* and *A. breviligulata* that are being extirpated. Percent cover of *A. breviligulata* was significantly reduced in areas of *C. kobomugi.* Spore populations of AMF showed significant positive correlations with percent cover of *A. breviligulata* and significant negative correlations with percent cover of *C. kobomugi*. Assessment of mycorrhizal inoculum potential (MIP) of soils taken from the field mirrored the spore-population data. This study was unique in quantifying the effect of an invasive species on populations of mycorrhizal fungi in a dune habitat. The replacement of AMF-forming species on dunes by a species that does not form AMF will have serious consequences when attempts are made to re-establish native species.

ADDITIONAL INDEX WORDS

Ammophila breviligulata, *coastal dunes*, *disruption*, *disturbance*, *invasion*, *leaf phosphate*, *mutualism*, *mycorrhizal fungi*, *mycorrhizal inoculum potential*, *nonnative*, *spore abundance*, *percent cover*

INTRODUCTION

Mycorrhizal Fungi

Arbuscular mycorrhizal fungi (AMF) play a critical role in plant physiology and soil-ecological interactions in sand dunes (Koske *et al*., 2004; Maun, 2009). They form mutualistic relationships with the roots of a majority of plant species by the formation of specialized cells that enable the fungi to acquire carbohydrates from the host plants' roots while absorbing inorganic nutrients from the soil (Smith and Read, 1997). The increased exploitation of soil volume made possible by AMF is critical for the uptake of less mobile nutrients like phosphorous, zinc, and copper (Sorensen, Larsen, and Jakobsen, 2005). Studies by McGonigle and Fitter (1988) and Tinker, Jones, and Durall (1992) found that the uptake rate of phosphorous per unit root length in mycorrhizal plants can be 2-3 times higher than in non-mycorrhizal plants. AMF have been shown to increase drought tolerance and improve the overall structure and stability of soil (Auge, 2001; Koske, 1975). They enhance the uptake of inorganic nitrogen (Govindarajulu *et al*., 2005) and provide protection against harmful plant-pathogens (Cooper and Grandison, 1986; Newsham, Fitter, and Watkinson, 1994; Pozo *et al*., 2002). AMF have the ability to physically reduce the amount of root exudates responsible for nematode attraction, and they may possibly reduce nematode development in substrate and roots altogether (Maun, 2009). It has been demonstrated that AMF competitively exclude harmful plant-parasitic

nematodes from regions of roots of *A. breviligulata* specifically (Hussey and Roncadori, 1982).

Although major dune-building species are well adapted to a variety of abiotic stresses including frequent variations in wind, salt spray, temperature, moisture, sand movement, and organic matter deposition, their association with AMF to acquire critical nutrients is thought to be highly essential for survival (Maun, 2009). Sand dunes typically have relatively low nutrient concentrations that can vary with daily and seasonal wind speed and direction, as well as rainfall amount (Gemma and Koske, 1997; Maun and Baye, 1989; Mosse, 1973). A major source of nutrients in dune soil comes from the deposition of algae that are distributed randomly along the dune (Maun and Baye, 1989). Nutrients deposited as a byproduct of salt spray are quickly leached from the upper soil when it rains and, as a result, even in light of continuous deposition of nutrients from these sources, average levels of soil cations are low (van der Valk, 1974). Foredune areas especially are regions of extremely low nutrient concentrations as topographic rise restricts the deposition of detritus in general (Maun and Baye, 1989). Phosphorous generally occurs at deficient or near-deficient levels in sand dune substrates yet is one of the most critical nutrients for plant species (Atkinson, 1973; Gemma, Koske and Habte, 2002; Halvorson and Koske, 1988; Koske, unpublished observations). Available plant phosphorous and potassium levels in dune soils may be as low as 0.011% and 0.008%, respectively (Maun and Baye, 1989).

Physical aspects of the dune such as the sand mobility, the rate of nutrient deposition, fresh water availability, and distance from the shoreline, contribute to

overall functionality as habitat for any array of species and as a barrier to protect inland areas against storms (Maun, 2009; Maun and Baye, 1989). AMF-facilitated enhancement of dune structure further reduces the potential for degradation by constant wind erosion. Fungal hyphae of AMF enhance the geophysical structure of sand dunes by binding sand grains into larger aggregates thereby trapping organic matter and promoting microbial activity (Forster and Nicolson, 1981; Jehne and Thompson, 1981; Koske, Sutton and Sheppard, 1975; Sutton and Sheppard, 1976). Glomalin-related soil protein (GRSP), a component believed to be primarily synthesized by AMF, likely serves a larger role in binding sand grains. The formation of GRSP by AMF also retains organic matter and moisture that can be utilized by dune plant species (Rillig, 2004; Rillig and Steinberg, 2002).

Ammophila breviligulata and *Carex kobomugi*

Ammophila breviligulata is a cool-season deciduous perennial that propagates primarily via extensive rhizomes (Maun and Baye, 1989). It is a dominant pioneer species in dunes, with a habitat range in eastern coastal North America, from 35° N to 53° N, as well as in the Great Lakes region (Halvorson and Koske, 1988; Koske and Halvorson, 1981; Maun and Baye, 1989; Watkinson, 1988). *A. breviligulata* has a specific dependence on mycorrhizal fungi, both in terms of initial establishment and long-term colonization (Gemma and Koske, 1997; Koske and Polson, 1984; Maun and Baye, 1989; Nicholson, 1959; Puppi and Reiss, 1987). A functioning population of AMF is essential for this species to survive in sand dune

environments (Francis and Read, 1994; Gange, Brown and Sinclair, 1993; Gemma and Koske, 1989; Gemma and Koske, 1997; Little and Maun, 1996; Maun, 2009; Maun and Baye, 1989; Maun and Lapierre, 1984; Miller, 1987; 1995). In evaluating individual plants' roots throughout the species' distribution on the Atlantic coast, it has been reported that as much as 80% of roots of individual *A. breviligulata* can be colonized by AMF hyphae (100% being total root coverage) (Koske, 1987). Rhizomes of *A. breviligulata* grow horizontally seaward as much as 2 cm per day, and extend some overall distance of 1 to 2 m from the foredune and into the berm of the beach (Brodhead and Godfrey, 1977; Koske, personal communication). Koske and Halvorson (1981) showed that other dominant dune species, including *Solidago sempervirens* L., *Lathyrus japonicus* Willd., and *Myrica pensylvanica* Mirbel, were also colonized by AMF. Sylvia (1989) reported that transplants of sea oat (*Uniola paniculata* L.), another predominant sand dune and mycorrhizae-forming species in the warmer dunes of the U.S. Atlantic coast and Gulf of Mexico, grew 219% larger shoots and 53% more tillers when grown in soil containing AMF.

As a pioneer species, *A. breviligulata* plays a substantial role in the development and maintenance of sand dunes. The leaves slow on-shore winds, allowing sand to accrete, contributing to increased dune height. *A. breviligulata* is especially important because it frequently inhabits the foredune, an area critical in the early formation of dunes and highly susceptible to erosion and disturbance events (Maun, 2009; Maun and Baye, 1989; Maun and Lapierre, 1984). Additionally, *A. breviligulata* responds positively to increased deposition of sand, making it further well-suited for dune initiation, growth and stabilization of dunes over time (Maun

and Baye, 1989; Olson, 1958). Without such consistent sand accretion, *A. breviligulata* suffers from decreases in shoot weight, height and density. Though past studies have suggested that improved plant vigor due to sand burial was associated with a release from plant-pathogenic nematodes, Little and Maun (1996) found that plant vigor and the reduced impact from plant-parasitic biota in the substrate was more a result of enhanced tolerance and resistance provided by AMF that become more available with increased sand deposition.

Carex kobomugi is a robust primary colonizer of sand dunes that spreads rapidly by numerous rhizomes (Miyata and Haramoto, 1986; Miyata and Haramoto, 1987; Ishikawa *et al*., 1993). Before the species was formally recognized by Ohwi, it was included in *Carex macrocephala* Willd. ex Sprengel. Under this classification it was first reported in North America in the early 1900s in discharged ballast sand near Portland, Oregon (University of Washington Burke Museum, 2011). As a separately described species, it was first discovered on the east coast of the U.S. in 1929 at Island Beach State Park, New Jersey (Small, 1954). Though the introduction pathway to New Jersey is uncertain, it was suggested that the nonnative invasive was introduced via packing materials (Halsey, 2002; Small, 1954). However, due to the relative scarcity of the plant in its native range, using *C. kobomugi* in packing material in the past appears unlikely. Rather, seeds or rhizomes were likely transported in dry ballast (Wootton, 2007).

The ability of *C. kobomugi* to tolerate trampling, deter dune foot traffic with sharp, newly-emerged shoots and to resist disease and pests associated with *A. breviligulata*, such as marasmius blight (*Marasmiellius mesoporous* Singer) and soft

scale (*Eriococcus caroliniae* Williams), made it desirable to plant as a dune-stabilizing species in the past (Standley *et al*., 1983; Belcher *et al*., 1984; United States Department of Agriculture, 1983, 1984), thus expanding its range (Wootton *et al*., 2005). In the 1930s, *C. kobomugi* was deliberately planted in areas of southeastern Virginia for dune stabilization purposes, later escaping into non-target dune communities (Virginia Department of Conservation and Recreation, 2011). In the early 1990s, increased awareness of the proliferation of introduced species in the United States essentially halted the planting of *C. kobomugi* as a dune stabilizer, though fugitive populations continued to expand (Wootton, 2002).

Disturbance events that negatively affect native plants, such as naturally-occurring dune erosion or increased anthropogenic activity, likely further contributed to the increased spread of *C. kobomugi*. In areas in New Jersey, Wootton *et al*. (2005) reported exponential increases in overall population as high as 780% over the last 20 years. The largest and oldest stand of the invasive in North America at Island Beach State Park, New Jersey increased from an area of 2,000 m^2 to 90,032 m^2 between 1939 and 2005 (Belcher *et al*., 1984; Wootton *et al*., 2005). Its current range along the North American east coast extends from Massachusetts to North Carolina. At present the species is listed as invasive in Rhode Island, Connecticut, Maryland, New Jersey, and Virginia (Enser, 2005; Enser, 2006; MacLachlan, personal communication; Shisler, Wargo and Jordan, 1987). On the west coast of the United States, *C. kobomugi* has recently become established in southwestern Washington (University of Washington Burke Museum, 2011).

The populations of *C. kobomugi* first discovered at East Beach in 1981 (Champlin, 1994; Enser, 2006), have expanded significantly in the last 30 years. In 1983, *C. kobomugi* was reported as a single main population covering approximately 170 m^2 (Standley, 1983). After 2005, six distinct patches of *C. kobomugi* were documented in foredune areas, encompassing a total area estimated as 8,000 and 12,000 m^2 (Enser, 2005; Johnson, personal observation; MacLachlan, personal communication). In addition to dense foredune populations, *C. kobomugi* occurs in sparser populations beyond the crest of the dune, near backdune roads and clearings surrounding Ninigret Pond. In these areas *C. kobomugi* is more interspersed with other naturally occurring backdune species (Johnson, personal observation).

C. kobomugi typically propagates through vegetative means, as sexual reproduction yields seeds with low germination rates and high seedling mortality (Nobuhara and Miyazaki, 1974; Sasaki, 1987; Yamamoto, 1964). Plants are able to regrow fully from the vegetative remains or rhizomes left after manual removal (Lea and McLaughlin, 2002). Rhizomes can extend horizontally outward 0.5 m to 1.2 m from an individual, depending on degree of dune maturation (Ishikawa and Kachi, 1998). A survey in New Jersey by Small (1954) indicated that rhizomes of *C. kobomugi* are deeper, have shorter internodes, and root more profusely than *A. breviligulata.* Roots of *C. kobomugi* capable of producing new shoots occur at a depth of up to 60 cm (Ishikawa and Kachi, 1998; Nobuhara, 1967; Park, 1982; Wootton *et al*., 2003), as compared to roots of *A. breviligulata* that can extend vertically for 150 cm (Maun and Baye, 1989). In New Jersey, rhizomes of *C. kobomugi* have been documented at lower depths than those of *A. breviligulata,* though viable shoots

typically do not emerge from these rhizomes below a depth of 60 cm (Ishikawa and Kachi, 1998; Park, 1982; Small, 1954;). Rhizomes of *C. kobomugi* extend laterally from 50 to 250 cm depending on dune maturation (i.e., sand accumulation) and available resources (Miyata and Haramoto, 1987; Nobuhara, 1967). Although individual plants of *C. kobomugi* are short (10-30 cm) in comparison to those of *A. breviligulata* (>100 cm), *C. kobomugi* has more blades (5-10) per plant and can occur in dense stands of 200-512 plants per m^2 (Mehrhoff *et al*., 2003; Small, 1954; Wootton *et al*., 2005).

Staining and analysis of root samples from 6 individuals of *C. kobomugi* collected from multiple locations at East Beach indicate that it is non-mycorrhizal (Johnson, personal observation). A number of publications have indicated that other species in the Cyperaceae are facultative or non-mycorrhizal as well (Brundrett, 1991; Gerdemann, 1968; Miller, 2005; Newman and Reddell, 1987; Tester, Smith, and Smith, 1987). The long and fine root systems characteristic of sedges is thought to be an alternative to a dependence on AMF for nutrient acquisition and uptake (Brundrett and Kendrick, 1988; Miller *et al*. 1999). In general, the mycorrhizal status of species depends on genetic characteristics and on a series of environmental factors, such as the presence of AMF inoculum or soil moisture. In the assessment of mycorrhiza-forming ability of plants, careful measures must be taken to avoid misidentification of structures in roots formed by a variety of non-mycorrhizal soil-borne fungi whose appearance resembles that of the hyphae, arbuscules, and vesicles of AMF (Johnson, personal observation; Koske, personal communication).

Although a large number of studies on the interactions between invasive and

native plant species have been published, evaluating differences in phenology and attributes, (Baker, 1974, 1986; Rejmanek and Richardson, 1996), very few assess the effect of plant invasions on AMF (Vogelsang and Bever, 2009). As AMF are especially important to dependent host-plant species in obtaining nutrients and tolerating a variety of physical stresses (e.g., drought, salinity, excessive sunlight) in dune systems (Koske *et al*., 2004; Maun, 2009; Smith and Read, 1997), a reduction in the population of AMF in soil by an invasive species could have a significant ecological impact, and may be linked to the overall success of a non-mycorrhizal, nonnative invasive such as *C. kobomugi*.

The degraded mutualist hypothesis, as proposed by Vogelsang and Bever, (2009), describes a situation in which natives have been completely replaced by a competitively-dominant invasive, and are unable to re-colonize due to an absence of associated AMF. As non-mycorrhizal invasive plant species are less dependent on interactions with AMF to establish in novel areas (Allen and Allen, 1980; Pendleton and Smith, 1983; Reeves *et al*., 1979; Vogelsang and Bever, 2009), ecological dominance could likely shift from a mycorrhizal-dependent native to an invasive in a relatively short period of time. Furthermore, the longevity of the mycorrhizal communities themselves is put in jeopardy, as exotic species, even if facultatively mycorrhizal, are generally poorer hosts for the AMF mutualism than native, mycorrhizal-dependent species (Vogelsand and Bever, 2009).

Studies have shown that a change in the composition of AMF belowground alters plant carbon exudation and nutrient uptake, suggesting that the alteration of AMF has the capacity to dramatically influence the composition of aboveground

vegetation (Cavagnaro *et al*., 2005; Schwab, Leonard, and Menge, 1984). In California, Hawkes *et al*. (2006) found that exotic grasses (*Avena barbata* Link and *Bromus hordeaceus* L.) reduce species richness of mycorrhizal fungi, causing dramatic shifts in a mycorrhizal community of native grasses and lupines (*Nasella pulchra* (Hitchc.) Barkworth and *Lupinus bicolor* Lind). Vogelsang and Bever (2009) demonstrated that soils associated with a nonnative invasive thistle (*Carduus pycnocephalus* L.) did not promote growth of AMF as compared to soils conditioned with native herb species (*Gnaphalium californicum* (D.C.) Anderb.).

In addition to altering composition of host-plants, the direct, chemical disruption of AMF has also recently been identified as a means by which an invasive plant species achieves a competitive advantage in an established ecosystem. The best known example of this is a study by Stinson *et al*. (2006) demonstrating that a nonnative invasive species' active suppression of AMF fungi was connected to its ability to invade and successfully supplant native species in Northeastern U.S. forests. Garlic mustard (*Alliaria petiolata* (M. Bieb.) Cavara and Grande) significantly reduced growth and vigor of tree seedlings by killing mycorrhizal fungi in the soil (Stinson *et al*., 2006). Chemical compounds isolated from the invasive's root tissues, including allyl isothiocyanate, benzyl isothiocyanate, and glucotropaeolin, had allelopathic effects on native plants in the absence of AMF. Exposing AMF spores to these extracts severely reduced germination rates (Stinson *et al*., 2006).

Like the non-mycorrhizal garlic mustard it is possible that *C. kobomugi* produces allelopathic compounds to suppress AMF and established native species.

Though Li, Henry, and Seeram (2009) reported that several members of the genus *Carex* produce stilbenes and other bioactive polyphenols that are capable of such activity, it is unclear whether the *C. kobomugi* specifically uses exudates competitively. In stem-density experiments, Burkitt and Wootton (2010) found that native plants of diverse functional type (i.e., annuals, perennials, dicots, or monocots) were all equally negatively affected when interacting with *C. kobomugi*. They suggested that it may actively replace established, vigorously growing species, rather than solely colonizing recently disturbed areas. These authors further hypothesized that allelopathic chemicals could be the primary means of the invasive to outcompete natives, especially in consideration of its relatively uniform effect for such a wide functional range of species. Preliminary research found that germination of spores of *Gigaspora gigantea* treated with root-exudates from *C. kobomugi* was not significantly reduced (Johnson, personal observation; see Appendix).

Even without the use of direct allelopathic suppression, the replacement of AMF-dependent host plants by *C. kobomugi* may substantially reduce species diversity of mycorrhizae in sand dunes. Preliminary analysis of samples taken at East Beach in October 2008 and 2009 found that spore abundance of AMF was much lower in the *C. kobomugi*-dominated areas of the foredune than in the *A. breviligulata*-dominated areas (Koske and Gemma, unpublished observations; Johnson, personal observation). The research of this project was designed to investigate and document the relationship between the populations of *C. kobomugi* and *A. breviligulata* and the populations of AMF at East Beach. By surveying populations of AMF and plant species at foredune sites, and evaluating inoculum

potential of field soils in growth assays, it was possible to characterize and evaluate the replacement of *A. breviligulata* and the reduction of AMF resulting from the expansion of *C. kobomugi*. As *C. kobomugi* replaces populations of established *A. breviligulata,* a mycorrhizae-dependent species and primary dune-building native plant, the population of associated AMF also will decline, thus affecting the ability of future host-plants to acquire nutrients, and successfully colonize the area (Koske and Gemma, 1997; Miller, 1979; Reeves *et al*., 1979). Leaf tissues from *C. kobomugi* and native mycorrhiza-forming plant species, and available phosphorous in soils were analyzed to examine differences in phosphorous acquisition ability and further examine competitive interactions between the target plant species. These field phosphate measurements supplemented the main objective of investigating plant competition and the impact on mycorrhizal fungi.

The primary objective of this study was to address whether the rapidly expanding *C. kobomugi* reduced the dominant sand-dune building plant, *A. breviligulata*, as well as critical mycorrhizal fungi. This was tested by surveying percent cover of plant species and by sampling populations of AMF in soil along transects at foredune sites of *C. kobomugi*. Significant negative interactions between *A. breviligulata*, spore populations of AMF, and *C. kobomugi* supported the hypothesis that *C. kobomugi* effectively replaces and reduces native plant and fungi species. A secondary hypothesis was that *C. kobomugi* reduced future mycorrhizal inoculum potential of areas after it had invaded. This was investigated by collecting field soils from areas of *C. kobomugi* and *A. breviligulata*, and conducting growth trials using corn as an indicator species. A significant decrease in root colonization of

AMF of indicator plants in these studies supported the hypothesis that *C. kobomugi* reduces AMF populations in soils. The objective of the field plant leaf tissue phosphate assessment was to examine the ability of the invasive to acquire phosphorous, specifically without the AMF interaction that is essential for many native plants. Soil phosphorous was assessed to document baseline available phosphorous in dunes for comparison with leaf tissue phosphate of target plant species.

MATERIALS AND METHODS

Study Site

East Beach (approx. 41º N, 71º W) borders the Ninigret Conservation Area in Charlestown, Rhode Island (Figure 1). The beach forms a barrier between Block Island Sound of the Atlantic Ocean and Ninigret Pond, a tidal lagoon fed by an inlet on the northeastern end of a 4.8-km long spit. The beach comprises a substantial portion of the 0.7 km^2 East Beach State Park, attracting numerous tourists during the summer season and recreational fishermen throughout the year. The area is relatively undeveloped aside from a network of unpaved backdune access roads and a residential neighborhood at the southwestern end of the beach.

The dune extends for the length of the beach, and at its crest, the maximum overwash and beach sand deposits are approximately 3 m above mean sea level (Urish, 1982). Vegetation on the dunes at East Beach is typical of Mid- and North Atlantic coastlines of the U.S. (Godfrey, 1977; Koske and Halvorson, 1981). Study areas are well-established by *A. breviligulata* and include other less-dominant herbaceous perennials (*L. japonicus* and *S. sempervirens*) and woody shrubs (*Rosa rugosa* Thunb., and *M. pensylvanica*). The reed *Phragmites australis* (Cav.) Trin. ex Steud occurs in high density at the edge of nearby Ninigret Pond, indicating ample amounts of fresh ground water for the area (Stuckey, 1976; Urish, 1982). Populations of *Pinus thunbergii* Parl. occur commonly in both the foredune and backdune areas of the beach. *C. kobomugi* occurs at five distinct locations along the foredune (Figure 2) and in a number of areas in the backdune (Enser, 2005; Johnson, personal

observation). Each patch-site of *C. kobomugi* generally consists of a dense stand of the invasive interspersed with other plant species in low densities, and surrounded by common native species assemblages. Sites were designated E#1 through E#5, beginning with the southwestern-most patch. Study sites out of the five total foredune patch-sites of *C. kobomugi* were selected for sampling based on the quality of transition zones between different vegetation types (i.e., distinct areas of dense *C. kobomugi*, areas of a near-even ratio of cover between both *C. kobomugi* and *A. breviligulata*, and areas of dense *A. breviligulata*). In 2009 the study surveyed three distinct foredune patch-populations (E#2, E#3 and E#5) for spore abundance and richness of AMF, using one of the three patches (E#3) for percent cover assessment. Based on findings from the 2009 assessments, in 2010 the study was modified to intensely sample one patch-site (E#3) for spore abundance and richness of AMF as well as percent cover of plant species.

Permission to access and conduct surveys on the dunes for this study was obtained from the Rhode Island Department of Environmental Management Division of Parks and Recreation.

Vegetation Percent Cover Analysis

The vegetation percent cover method was used to document populations of *C. kobomugi* and *A. breviligulata*, and those areas of transition between the species. Three transects were made on the foredune, parallel to the shore length. These transects represented three elevation zones, denoted low, nearest the front of the dune,

mid, and high, nearest the crest of the dune. Generally they were spaced 6-7 m apart. The purpose of establishing elevation transects was to investigate whether elevation on the dune had any relevant effect on vegetative cover or spore abundance. Overall patch site measurements were a combination of assessments made by Enser (2005) and personal measurements using tape measures and marker objects. In 2009, transects were established from areas of dense *C. kobomugi* to areas of dense *A. breviligulata*. In 2010, transects were made from areas of dense *A. breviligulata* to areas of *C. kobomugi* and back into areas dominated by *A. breviligulata* (Figure 3).

A 1 m x 1 m quadrat, composed of 5-cm diameter polyvinyl chloride pipe, was dropped at each interval, with the bottom of the quadrat touching the top edge of the measuring tape. A photograph was taken of the interval at a height of approximately 1.8 m above the quadrat. A digital camera with a swivel-viewfinder was used to center the photographs above the quadrat effectively. In November 2009, photographs were taken at randomized intervals, following the same meter-intervals established for spore collection. Randomization of meter-intervals is explained in the spore collection portion of these methods. Thirty-six digital photographs were taken (12 photographs per transect, 3 elevation transects for one site) for one site (E#3). In October 2010, digital photographs were taken at standardized, 4-m intervals, as opposed to random meter-intervals, also using the dropped 1 m x 1 m quadrat (Figures 4-7). Ninety photographs were taken (30 per transect, 3 elevation transects) in the same patch site used for percent cover as in 2009 (E#3).

Using Photoshop editing software (Ver. 7.0, Adobe Systems Incorporated, San Jose, CA), photographs were digitally scaled and skewed to conform to a

superimposed grid with 100 total intersections. During image analysis, the topmost leaf at any intersection was used for percent cover (overlap was ignored). Trends in plant species were determined by assessing absolute percent cover (100% maximum) in 1 m^2 areas, at intervals along a transect. Leaves of both species had to be visually differentiated and identified. Those points that intersected stems or leaves of plants were tallied and divided by the total possible number of intersections, resulting in percent vegetative cover by individual species. The survey was conducted in November 2009 and repeated in October 2010. These months were selected for vegetation assessment, as perennial grasses senesce completely for the winter. *C. kobomugi* produces leaves that are typically curled and yellow-green compared to leaves produced by *A. breviligulata* that are straighter and darker-green in color (Ishikawa and Kachi, 1998; Johnson, personal observation; Maun and Baye, 1989).

Spore Abundance and Species Richness of AMF (2009)

Soil samples were collected in December of 2009 in accordance with seasonal peaks of sporulation for anticipated AMF species in the dune (Gemma and Koske, 1988; Gemma, Koske, and Carreiro, 1989; Lee and Koske, 1994). A stratified random sampling design was chosen based on sampling strategies suggested by Gemma, Koske and Carreiro (1989) and Tews and Koske (1986). Collection locations across transects were determined using the random number generator feature of Microsoft Excel (Ver. 2007, Microsoft Corporation, Redmond, WA). The total transect length, ranging from 0 m to 50 m, was divided into 4-meter sub-sections. Within each of these

sub-sections, a random value was selected, and this served to indicate the meter-interval where a soil sample was collected. Thirty-six soil samples were collected from each of three different patch sites, totaling 108 collection points. Samples were collected from root zones of *A. breviligulata* and *C. kobomugi* (20-30 cm) (Gemma, Koske, and Carreiro, 1989; Ishikawa and Kachi, 1998; Maun and Baye, 1989; Small, 1954). Collection holes were dug using a small shovel and 200 ml of substrate were retrieved with a plastic collection cup scraped along all sides of the hole to ensure a representative sample. The shovel and collection cup were wiped clean between collections to remove macro debris such as sand grains or root material. Based on the spores' size and sampling techniques used in the past this method was considered sufficient to reduce spore contamination between collection samples (Koske, personal communication). Sand was placed into sealable, polypropylene plastic bags (volume approximately 600 ml) and kept in refrigerated storage (5° C) until spore extraction and processing.

Spore Abundance and Species Richness of AMF (2010)

Soil sampling took place in November 2010, in accordance with the highest yearly occurring abundance peaks of sporulation by AMF (Gemma and Koske, 1988; Lee and Koske, 1994). As opposed to using three patch sites as in 2009, patch E#3 was intensively sampled as the primary site. For this sampling, three parallel elevation transects were established 6 m apart from one another. Other than reducing the number of sites assessed, and increasing the number of samples, the 2009 spore

collection and assessment methods were unaltered for 2010. Ninety soil samples were collected at this phase of the study (30 per transect, 3 transects).

Sampling for spore abundance of AMF in 2010 was conducted two weeks after a broad-spectrum herbicide (glyphosate) treatment was applied to the E#3 patch of *C. kobomugi* on the foredune (Johnson, personal observation; MacLachlan, personal communication). As spore counts only assessed healthy, viable spores (Lee and Koske, 1994; International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM), 2009) spore condition was assessed prior to counts to evaluate potential herbicide effects. The overall health, number and richness of AMF spores were considered normal based on the work of Lee and Koske (1994) and previous unpublished observations on samples from this dune (Johnson, personal observation; Koske, personal communication). Furthermore, as sporulation was essentially finished for the seasonal cycle period at the time of application, secondary effects of the herbicide on AMF was assumed negligible in terms of a reduction in overall number of spores (Gemma and Koske, 1988; Johnson, personal observation; Koske, personal communication). In general, the effects of pesticides on AMF are highly variable depending on pesticide type, AMF species, application rate, seasonal and environmental conditions (Jansa, Wiemken, and Frossard, 2006).

Spore Extraction

For efficiency in identifying and counting, spores were extracted from substrate with as minimal detritus and sand as possible. This was accomplished by wet sieving

and decanting in combination with a sucrose centrifugation-extraction method (Gerdemann and Nicolson, 1963; Walker, Mize, and McNabb, 1982). Two hundred ml of substrate from collection bags were transferred to a 1000-ml plastic beaker, and combined with approximately 200 ml of water in order to break up clods. This solution was agitated for approximately 30 seconds, until the soil had been sufficiently mixed. An additional 800 ml of water were added to suspend spores and root material for sieving. The material was decanted through two metal sieves, one coarse (#35, 500 µm) and one fine (#230, 63 µm). Material collected in the fine sieve was then washed into a plastic Petri dish lid. Once this solution was rinsed into a plastic 50-ml centrifuge tube, approximately 300 mg of kaolin clay were added, and the tube was centrifuged (IEC Clinical Centrifuge, Damon/International Equipment Company, Needham Heights, MA) for approximately 3 minutes near 3000 rpm (50 Hz). The supernatant was poured off and the inside of the topmost portion of the tube was wiped with a Kim Wipe (Kimberly-Clark Corporation, Neenah, WI) to remove excess debris. A 40% sucrose solution (gluten-free pure cane sugar in deionized water, agitated using a Corning magnetic stirrer for 5 minutes) was added, filling the centrifuge tube to approximately 45 ml. The tube was vortexed and shaken vigorously to re-suspend the pellet and then centrifuged at near 3000 rpm (50 Hz) for approximately 1 minute. The solution was filtered through a fine-mesh sieve (53 µm) onto a filter paper (5.5 cm diameter, medium/fast qualitative crystalline retention) and suspended in a ceramic filter container connected to a Buchner funnel vacuum filtration apparatus. The contents in the ceramic filter were rinsed with deionized water repeatedly until the filter paper was removed and placed in a plastic dish for

spore abundance and richness analysis.

Spore Identification and Analysis

Spore analysis was used to identify and quantify AMF spores retrieved from field sampling. Dead, parasitized, or spores filled with atypical contents were not used in assessments, as they are considered unable to produce typical mycorrhizal structures (INVAM, 2009). Only spores with a healthy, viable appearance as according to descriptions outlined by Lee and Koske (1994) and INVAM (2009) were used in the spore counts. Spores on filter paper were sorted by appearance morphology, collected using a metal inoculating needle with PVLG (8.33 g polyvinyl alcohol, 50 ml lactic acid, 5 ml glycerol, 50 ml water) and placed onto glass microscope slides. Spores were crushed by pressing the inoculating needle firmly on a cover slip. Crushing spores served to facilitate the recognition of differences in spore-wall structure, and was necessary for species identification. Characteristics such as color, shape, size, wall composition, and hyphal attachments were also used to further identify species under a compound microscope (40X-1000X magnification). Species identification was supplemented by descriptions by Schenck and Pérez (1990) and Koske (personal communication). A number of typical dune-inhabiting species of AMF were anticipated (Gemma and Koske, 1988; Gemma, Koske, and Carreiro, 1989; Halvorson and Koske, 1988; Koske, personal communication). The number of each species' spores on an individual filter paper was counted and recorded. Spores of individual species were totaled and combined to represent the total spore abundance of

AMF per 200 ml of substrate from a particular collection point. The number of species encountered in a sample was considered overall AMF species richness for that sample.

Mycorrhizal Inoculum Potential (MIP)

The objective of growth assays was to determine if areas of the dune with a history of *C. kobomugi* had reduced mycorrhizal inoculum potential (MIP) as compared to native (*A. breviligulata*-established) soils. The successful colonization by AMF of indicator plants' roots was used as an indicator of AMF functionality and availability in soil. The final MIP configuration (i.e., growing medium, watering schedule, fertilizer amount, time of harvest, and staining methodology) was determined by conducting preliminary trials and modifying previous MIP growth studies (Corkidi *et al*., 2004; Gemma and Koske, 1988; Giovannetti and Mosse, 1980; Tarbell and Koske, 2007).

Field soils were obtained from areas of highest plant density under the assumption that these areas had a relatively longer and more-concentrated exposure to the desired study-species. Soil was sampled from typical root zones of plant species (20-30 cm) and overlying surface sand was excluded as much as possible. Five ca. 1-L samples were taken at random locations throughout dense vegetation areas. Soils were collected from the field in polypropylene plastic bags and kept in refrigerated storage (5° C) for approximately 5 weeks to break dormancy and to promote germination of spores (Gemma and Koske, 1988; Gemma, Koske and Carreiro, 1989). Smaller volumes from these samples were later hand-mixed in lab to create representative soil

treatments. Treatments in 2009 (MIP #2) consisted of *C. kobomugi* field soil, *A. breviligulata* field soil, with the addition of a 50% *A. breviligulata* field soil with 50% *C. kobomugi* field soil treatment in 2010 (MIP #3). All soil treatments were mixed in a 1:1 ratio with multi-purpose Oil-Dri Safety Absorbent (Oil-Dri Corporation, Alpharetta, GA) to increase water retention. Soil treatments were then added to tapered plastic containers measuring 20.7 cm tall by 3.8 cm diameter (165 ml Cone-tainers, Ray Leach, Hummert International). To retain soil in each Cone-tainer, a flexible plastic mesh screen (1 mm^2 openings) was pushed to the bottom to overlay drainage holes. MIP #2 consisted of 10 plants each (2 treatments, 5 replicates per treatment). MIP #3 consisted of 15 plants (3 treatments, 5 replicates).

MIP studies consisted of the following aspects and methods. Corn (*Zea mays* L., Jubilee hybrid, W. Atlee Burpee and Co., Warminster, PA) was used in growth assays as it is the standard species for measuring the mycorrhiza-forming potential of soils (Gerdemann and Trappe, 1974; Moorman and Reeves, 1979; Reeves *et al*., 1979). Corn seeds were planted at a depth of approximately 5 cm in Cone-tainers, using a small wooden stick to prime planting holes. Plants were kept in a temperature-controlled (average 22°C) indoor growth room. Lighting came from two 1000-W metal halide lamps, controlled by an analog-light timer set to daily 15 h/9 h light/dark cycles. All Cone-tainers were rotated bidirectionally on a daily basis to ensure that lighting and heat was uniform across all soil treatments. After approximately 7 days (at emergence), seedlings were manually thinned to one plant. For a week after emergence, each plant was watered with 40 ml of deionized water as needed (approximately every other day). After this point, plants were watered weekly to rinse

accumulating salt from soils. On a daily basis they were watered with 40 ml of a dilute fertilizer solution prepared from a complete fertilizer with micronutrients. Fertilizer solution consisted of 75 ml of 40X stock solution (38 g of 50-8-50 Peter's HI-CAL, Grace-Sierra Horticultural Products Co., Milpitas, CA, 9.12 g $MgSO_4 \cdot 7H2O$, and 5.34 g KH_2PO_4; each component dissolved into 250 ml of deionized water, and then added together with enough deionized water to produce 1000 ml) dissolved in 3800 ml of deionized water. The pH of the fertilizer solution was checked before use, as pH levels below 6.0 are generally not sufficient for plants to access critical nutrients (Buckman and Brady, 1960). Fertilizer was adjusted to between pH 6.2 and 6.5 by adding 0.5 ml of 2.5% KOH to each 500 ml of fertilizer solution (40X stock in deionized water). Fertilizer was kept in refrigerated storage (5° C).

Plants were harvested at 6 weeks (approximately 42 days), a duration considered sufficient to allow development of AMF root colonization (Corkidi *et al*., 2004; Tarbell and Koske, 2007). For assessment of root colonization, root systems for each plant were washed, cleared and stained (Koske and Gemma, 1989). In general, sampled roots were approximately 15-20 cm in length. Roots were washed for 3 minutes with a hard stream of water to remove large debris. After washing, the top 5 cm (near base of plant) was discarded, and the bottom 5 cm (near base of Cone-tainer) was discarded. The middle 5 cm was used for assessment, as this is typically where AMF colonize (Koske, personal communication). Root colonization and staining was carried out in accordance with a combination of standard methods (Giovannetti and Mosse, 1980; Koske and Gemma, 1989; Moorman and Reeves, 1979) (see Appendix for detailed procedure). Stained root matter was placed in an 8 cm x 8 cm square Petri

dish, with a 1.5 cm x 1.5 cm grid of 25 total intersections. Modifications were made to the intersect method outlined by Giovannetti and Mosse (1980) to account for the highly variable number of roots and root diameters within each sample. As opposed to tallying the number of locations a root fragment intersected a vertical or horizontal line and further recording the fraction of those roots with AMF colonization, each intersection-point on the grid was assessed for "mycorrhizal presence". Under a dissecting microscope (30X magnification), intersection-points that crossed roots with vesicles, arbuscules, or characteristic hyphae of AMF were counted against those intersections lacking structures of AMF - this resulted in a fraction of positive and negative responses for colonization. The positive responses were divided by the total number of possible intersections and multiplied by 100 to yield percentages. This process was repeated three times within a sample (i.e., the root material was placed back in scintillation vial, agitated, and again poured onto the gridded Petri dish) and the resulting average number represented the overall root colonization by AMF for an individual plant. It was critical to differentiate between those structures characteristic of AMF, including hyphae, vesicles and arbuscules, and those structures characteristic of parasitic or saprotrophic fungi typically found in decaying plant matter.

Field Plant Leaf Tissue Phosphate

The field plant tissue phosphate assessment transect followed the mid dune elevation transect (0 m to 120 m) established for spore counts and vegetation cover surveys in 2010. Leaves of *A. breviligulata*, *C. kobomugi*, *L. japonicus*, and *S.*

sempervirens were sampled in October 2010. Due to the natural distributions of these species, it sometimes was necessary to move a maximum of 2 m from the transect (in a direction perpendicular to the dune) to obtain leaf samples. The ideal maximum number of samples of 270 (3 species, 3 leaves per species per interval, 30 intervals) was not achieved as this number did not account for the uneven distribution of plants on the dune - some locations across the transect did not have representative plants, as they were not naturally occurring at collection points (i.e., high density areas of *C. kobomugi* at times lacked other target species).

The most expanded, newly emerged leaf was sampled on each plant, as it was presumed that this would contain the highest tissue phosphate concentration (Gemma, Koske, and Habte, 2002; Koske, personal communication). Leaf tissue phosphorous was determined by adapting the molybdate blue/ascorbic acid procedures from Aziz and Habte (1987), Habte, Huang and Fox (1987) and Habte and Manjunath, (1987). Leaves were placed in labeled plastic scintillation vials or polypropylene plastic bags for transport back to the lab. For *L. japonicus*, the youngest, fully expanded pinnule was used. For *S. sempervirens*, 1.5 cm of the leaf tip was discarded, and the 1.5 cm following was used. *A. breviligulata* leaves were cut 2 cm from the base and tip, and a middle 3 cm piece was used. The same process was applied to *C. kobomugi* leaves as they share a similar growth form. Before beginning phosphate assessment, all glassware was washed and rinsed with deionized water to remove phosphate residue potentially left from soaps and soils. Reagent A (for composition see Appendix) was made prior to the assessment and stored in a dark bottle in the chemical hood, whereas Reagent B (molybdate blue reagent; for composition see Appendix) was made for

immediate use. Samples were dried in the oven at 70° C for 2 days and their dry weights were recorded. Each dried tissue sample was then placed in a glass test tube (18 mm x 150 mm) in a metal rack. Samples were ashed for 4 hours in a muffle furnace at 500° C. After ashing was complete, samples were allowed approximately 2 hours to cool. Ash was then dissolved by adding 10 ml of deionized water to each tube using a Brinkman bottletop dispenser apparatus. After all tubes had water added to them, 2.5 ml of activated Reagent B was added. Tubes were then vortexed and color was allowed to develop for approximately 20 min. Each assay used a phosphate standard consisting of 0.2197 g KH_2PO_4 in 250 ml deionized water in a volumetric flask (200 µg P/ml). One milliliter of stock phosphate standard was added to 99 ml of deionized water in a 100 ml volumetric flask, resulting in a solution of 2 µg P/ml. One ml of this 2 µg P/ml was then added to 9 ml deionized water and 2.5 ml of Reagent B. Two test tubes were made as phosphate standards. The absorbance of phosphate standards was roughly 150-200 nm (using a Turner SP-830 spectrophotometer), equivalent to approximately 0.16 µg P/ml. Five milliliters of test tube solutions were pipetted into spectrophotometer cubettes for absorbance assessment. The spectrophotometer was zeroed between each sample using a deionized water blank. The outside surfaces of cuvettes were wiped clean with Kim Wipes between samples to ensure that no particulate matter interfered with absorbance readings. Cuvettes themselves were thoroughly rinsed with deionized water between samples. If absorbance of a sample was over 600 nm, the sample was diluted and re-measured. A dilution consisted of 1 ml of the sample added to 9 ml of deionized water in a clean

test tube. This was then vortexed and re-measured. Calculations for tissue phosphate were corrected for the effect of the dilution.

Available Soil Phosphorous (2009)

Available soil phosphorous was determined based on the molybdate-blue procedure by Fox and Kamprath (1970). Soil from the spore counts in 2009 was used, from one site only (i.e., 3 transects). Approximately 1-2 g of soil per sample was dried in an oven at 70° C for 2 days in small aluminum dishes. After this, soil samples were ashed for 4 hours in a muffle furnace at 500° C and allowed approximately 2 hours to cool. The values 0.020 mg P/L and 0.200 mg P/L were concentrations of interest because these have previously been found to be the critical levels necessary to classify the mycorrhizal dependency of plant species (Habte and Manjunath, 1987). Findings from soil assessments in 2009 were compared with leaf phosphate assessments from 2010. As phosphorous inputs from the atmosphere, leaf litter and weathering of materials are considered very small, coupled with the small amount lost to leaching each year, it was assumed that available soil phosphorous in soils did not differ substantially between years (Maun, 2009).

Statistical Analysis

Statview (Ver. 5.0.1, SAS Institute Inc., Cary, NC) was used to analyze all data in this study. Initially, distributions of vegetation across transects were examined using

bivariate line representations to visually interpret trends. Spore population and percent cover data were investigated with linear and curvilinear regression analyses, as it was hypothesized that there were direct causal relationships between them. Where indicated in results, data for spore abundance were base-10 log-transformed (log (x+k) where k=1) to account for non-normal distribution in the field, and data from vegetation percent cover were square-root transformed to account for extreme outliers in data. MIP data were subject to one way analysis of variance (ANOVA), as data were in nominal groups. A confidence level of P = 0.05 was used to determine statistical significance in all cases. Microsoft Excel (Ver. 2007, Microsoft Corporation, Redmond, WA) was used to make a portion of the graphs used in this thesis. Photoshop editing software (Ver. 7.0, Adobe Systems Incorporated, San Jose, CA) was used to digitally transform vegetation photographs and to superimpose grids used in the percent cover assessment.

RESULTS

Vegetation Percent Cover

There were strong inverse associations between aboveground percent cover of *C. kobomugi* and of *A. breviligulata*: high cover values of one species across the transects were associated with low cover values of the other species. In general, the distributions of both species were inversely related to one another (Figures 8-11).

There were no significant differences in percent cover of *C. kobomugi* and *A. breviligulata* between dune elevation transects. In 2009, percent cover of *C. kobomugi* and *A. breviligulata* across each transect followed linear trends. *C. kobomugi* occurred at higher densities towards the beginning of transects (0 m) and lower densities towards the ends (50 m). In 2010, trends in percent cover of both species were curvilinear, as the transect length was increased from a radius of the site (50 m) to cover the entire length of the vegetation gradient (120 m). The highest densities of *C. kobomugi* were detected in the middle portion of transects (between 20 m and 100 m), whereas the highest percent cover of *A. breviligulata* was detected at either end. Investigation of percent cover data combined from 2009 and 2010 with linear regression analysis found that as *C. kobomugi* increased in percent cover along the dune, *A. breviligulata* decreased significantly ($P < 0.0001$) (Figure 12).

Native plant species outside the scope of this study, such as *L. japonicus, R. rugosa, S. sempervirens,* and *M. pensylvanica* were also present across transects, but

had far lower percent cover values, and were not significantly reduced by *C. kobomugi* (data not shown).

Spore Abundance and Species Richness of AMF

The relationship between percent cover, meters and spore abundance of AMF was investigated using linear and curvilinear regression analysis. Transects made in 2010 for surveying vegetation were standardized at 4 m intervals and did not precisely mirror the intervals selected for spore abundance surveys. But, after reviewing data from 2009 assessments, it was assumed in 2010 that the difference between vegetation cover and AMF distribution across a 4 m distance allowed for the coupling of both standardized and randomized data in this case.

In general, both 2009 and 2010 data suggest that increased percent cover of *C. kobomugi* is associated with decreased mean spore abundance of AMF and increased percent cover of *A. breviligulata* is associated with increased mean spore abundance of AMF. Linear regression analysis found that as percent cover of *C. kobomugi* increased, mean spore abundance of AMF decreased significantly ($P < 0.0001$) (Figure 13). Conversely, as aboveground percent cover of *A. breviligulata* increased, the mean spore abundance of AMF increased significantly ($P < 0.0001$) (Figure 14). In the 2009 transects, made from areas of dense *C. kobomugi* to *A. breviligulata*, mean spore abundance of AMF increased significantly with increasing meters across a transect (Figure 15 and 16). In the 2010 transects, sampling an entire patch site rather

than one radius, the relationship between mean spore abundance of AMF and meters was also significant, and followed curvilinear trends (Figure 17 and 18). In general, mean spore abundance is reported as the number of spores per 200 ml of collected field substrate. AMF species observed included *Acaulospora scrobiculata* Trappe, *Acaulospora lacunosa* Morton, *Acaulospora mellea* Spain and Schenck, *Acaulospora spinosa* Walker and Trappe, *Gigaspora gigantea* (Nicol. and Gerd.) Gerdemann and Trappe, *Glomus pustulatum* Koske, Friese, Walker and Dalpe, *Glomus aggregatum* Schenck and Smith emend. Koske, *Glomus etunicatum* Becker and Gerdemann, *Glomus microaggregatum* Koske, Gemma and Olexia, *Scutellospora calopsora* (Nicol. and Gerd.) Walker and Sanders, *Scutellospora erythropa* (Koske and Walker) Walker and Sanders, *Racocetra persica* (Koske and Walker) Walker and Sanders, Oehl, Souza and Sieverd., *Scutellospora pellucida* (Nicol. and Schenck) Walker and Sanders, and *Scutellospora reticulata* (Koske, Miller and Walker) Walker and Sanders. Though each species of AMF was analyzed individually in reference to percent cover of *C. kobomugi* and *A. breviligulata*, no one species declined more than another as a result (data not shown). Species richness of AMF data was investigated using linear regression analysis. Richness was defined as the mean number of species encountered from collected samples. Linear regression detected a statistically-significant trend between both percent cover of *C. kobomugi* and percent cover of *A. breviligulata* and species richness, but this was not biologically significant (Figure 19).

MIP Growth Experiment

In both MIP trials, corn plants grown in soil from the root zone of *A. breviligulata* had significantly greater root colonization than plants grown in soil from the root zone of *C. kobomugi* ($P < 0.0001$ for each) (Figure 20 and 21). Plants demonstrated higher root colonization in soil of *A. breviligulata* (55%-72%) than plants grown in soil of *C. kobomugi* (12%-24%) (Table 1). Mean root colonization of plants grown in the 50/50 combination soil of *A. breviligulata* and *C. kobomugi* was 48%, significantly less than the two other treatments ($P < 0.004$) (MIP 3).

Soil Phosphate

Differences in soil solution phosphate (mg P/L) across transects were not significant (Figure 22). Mean available soil phosphate detected at East Beach (0.029 mg P/L) was similar to values previously reported for unfertilized field soils (Fox and Kamprath, 1970; Habte and Manjunath, 1987; Gemma, Koske and Habte, 2002). Soil solution phosphate is reported as available phosphate for plants as determined by Fox and Kamprath (1970) and Habte and Manjunath (1987).

Field Plant Leaf Tissue Phosphate

Analysis of variance of leaf tissue phosphate taken from field plants in 2010 indicated significant differences among some species. *C. kobomugi* had significantly

higher leaf tissue phosphate than *L. japonicus* ($P = 0.0019$), *S. sempervirens* ($P = 0.0268$) and *A. breviligulata* ($P < 0.0001$). *A. breviligulata* was found to have significantly lower tissue phosphate than *S. sempervirens* ($P = 0.0166$) (Figure 23).

DISCUSSION

Effects of Invasive Plants on Belowground Biota and Processes

As most plant invasion research focuses on trends in aboveground distribution, a substantial gap in knowledge is left as to the effects on the composition of soil biota (Levine *et al*., 2003). Few studies examining the effect of invasive plants on belowground soil communities have been conducted and these have been among a very limited number of geographic regions (Wolfe and Klironomos, 2005). Recent research suggests that interactions with soil biota have the capacity to greatly influence community processes, as well as survival of native plants (Bever, 2003; Reinhart and Callaway, 2006; Vogelsang and Bever, 2009). After an invasive plant has established in a novel habitat, it has the ability to completely re-shape a soil community, changing a number of critical soil functions in an ecosystem, including mineralization of nutrients, aeration, and moisture retention (Bever *et al*., 1996; Wolfe and Klironomos, 2005).

Some invasive plants modify soil composition by releasing organic compounds and secondary metabolites. Li, Henry, and Seeram (2009) demonstrated that several members of the genus *Carex* produce stilbenes and other bioactive polyphenols that are potentially fungitoxic or allelopathic. Garlic mustard, a rapidly-expanding invasive in North American forests, kills AMF in soil by releasing root exudates (Stinson *et al*., 2006). Invasive plants are able to transform the soil community by introducing novel

nutrient acquisition abilities to a system. In volcanic sites on Hawaii, the invasive evergreen shrub *Morella faya* (Ait.) Wilbur and associated nitrogen-fixing bacterial-symbionts alter nitrogen cycling in soils, raising available soil nitrogen levels and potentially allowing other invasives to become established in previously nitrogen-limited areas (Vitousek and Walker, 1984). Invasives also have the capacity to directly change physical properties of the substrate, as seen in the hyperaccumulation of sodium in rangelands of the United States by the noxious weed, saltlover (*Halogeton glomeratus* (M. Bieb.) C.A. Mey.) (Duda *et al*., 2003; Wolfe and Klironomos, 2005).

It has been documented that by altering aboveground host-species density, invasive plants can substantially reduce a diverse AMF community (Vogelsang and Bever, 2009). The strong interdependence between AMF and host-plants likely contributes to this effect (Richardson *et al*., 2000; Hawkes *et al*., 2006). In plant communities dependent on mycorrhizal interactions, such as sand dunes, the alteration or reduction of AMF could promote the expansion of an invasive species that is non-mycorrhizal. Even in the event that nonnative invasives are at least facultatively mycorrhizal, they still may reduce densities of AMF by contributing fewer roots relative to those from appropriate, native plant-hosts (Vogelsang and Bever, 2009). In invaded areas of western North America, an invasive grass, *Bromus hordeaceus* L., decreases and shifts composition of species of AMF associated with the native *Avena barbata* Pott ex. Link, a species of wild oat (Hawkes *et al*., 2006). Kourtev, Ehrenfeld, and Haggblom (2002) documented a decrease in abundance of AMF of areas of the eastern United States invaded by ornamental barberry (*Berberis thunbergii* D.C.) and an increase in abundance of AMF associated with the invasive Japanese stilt grass

(*Microstegium vimineum* (Trin.) A. Camus) (Wolfe and Klironomos, 2005). These findings demonstrate the range of impacts that an invasive species can have on an established community of AMF, especially in a diverse organismal and geographic context. However, other than these recent cases, there is still very limited published research that specifically addresses the effect that invasive plant species have on AMF.

Because populations of AMF are directly linked to the survivability of their hosts, a nonnative invasive that does not support mycorrhizae poses a serious threat to the stability of a native plant-AMF community. The invasion of *C. kobomugi* at East Beach, RI represents a danger to host species, as well as their associated AMF. This study assessed populations of the nonnative invasive with specific interest in determining its ability in reducing or disrupting native species. Spores of AMF are essentially immobile, relying in part upon the spreading rhizomes and roots of host-plants to colonize new areas of the dunes. Thus, locations of maximum spore densities of AMF are correlated with a vigorous population of host plants (Gemma, Koske and Carreiro, 1989) and the variation in spore abundance across a dune varies with species (Gemma and Koske, 1988), vigor of the plant host (Koske and Halvorson, 1981), as well as the maturity of the dune (Puppi and Riess, 1987). In general, the composition and availability of appropriate host plants play a critical role in enabling mycorrhizal interactions - if native species are replaced by a poorer host, or a plant that may suppress the interaction, the community becomes in danger of becoming not only dominated by a non-native, but also a place where natives have difficulty re-colonizing. If these mycorrhizal-dependent host species are destroyed, future plant establishment and continued longevity of the area made possible by mycorrhizal

inoculum in soil, is effectively put in jeopardy (Gemma and Koske, 1997; Miller, 1979; Reeves *et al*., 1979).

Spore abundance of AMF is directly related to the overall mycorrhizal inoculum potential of sand dune soils. The colonization of plant roots at the beginning of the growing season is facilitated by a viable and seasonally-sporulating AMF (Lee and Koske, 1994). In sand dune habitats, growth conditions for host-plants include the availability of soil nutrients, the extent of wind and salt spray deposition, seasonal and daily temperature and moisture changes as well as sand movement and deposition. By affecting the vigor of the host plants, these same physical factors influence spore populations of AMF and soil inoculum potential values in the dune (Koske and Halvorson, 1981). In the dune soil AMF are dispersed primarily by the growth of roots and rhizomes and as rhizomes grow, spawning new plants and roots in novel areas, they subsequently establish new AMF populations (Gemma, 1987; Gemma and Koske, 1989). In general, the fluctuation of spores and the emergence of new host plants is an interdependent cycle - increased plant growth in response to the AMF mutualism leads to a greater number of AMF-plant interactions, resulting in greater overall AMF-growth and subsequently a greater number of spores. A higher number of spores is further supplemented by increased hyphal expansion, and an increase in colonized root fragments, resulting in a substantially higher inoculum potential for soils. Hence, whereas a reduction in spore abundance due to an invasive may indicate a more recent, localized effect, a reduction of overall inoculum potential may be interpreted as a long-term suppression of AMF and host-plants.

Reduction of *A. breviligulata* and AMF by *C. kobomugi*

A number of studies address the spread and mitigation of *C. kobomugi* in North America but none evaluate its potential to disrupt AMF (Burkitt and Wootton, 2010; Enser, 2006; Lea and McLaughlin, 2002; Wootton, 2002; Wootton, 2007; Wootton *et al*., 2003; Wootton *et al*., 2005;).The present study was the first to document a reduction of AMF populations by *C. kobomugi*, a quickly-expanding and relatively recent invasive to Rhode Island dune habitats. As AMF are essential to the growth and survival of many native species in sand dunes the great decline in spore populations and soil inoculum potential resulting from the invasion by *C. kobomugi* has serious implications for the dune, including its vegetation, value for recreation, and ability to protect the coast.

By replacing *A. breviligulata*, the dominant host for AMF in East Beach dunes, *C. kobomugi* appears to indirectly reduce spore populations of AMF and soil inoculum potential. The species richness of AMF was not reduced significantly as a result of increasing *C. kobomugi*, and this was possibly because either species of AMF did not occur regularly enough to be detected in sampling. It is also likely that the typical distribution of AMF in general is non-normal, as spores develop in aggregated patches relative to spreading clusters of host plant rhizomes. Certain species of AMF were so rarely encountered in samples that correlations along transects, much less across vegetation gradients, were not present or significant. To overcome the difficulty of assessing such distributions of spores, especially in light of the rhizomatous nature of

host plants, the number of samples could be increased in magnitude. Overall an individual species of AMF was not significantly reduced more or less than any other species of AMF.

The decrease in AMF by *C. kobomugi* may result from the direct effect of the release fungitoxic exduates or volatiles, a variety of which are produced by various *Carex* species (Li, Henry, and Seeram, 2009). However, preliminary studies adapted from Koske (1981), using sand-plant microcosms and assessing the ability of spores of *G. gigantea* to germinate in soil collected from the root zones of plants of *C. kobomugi*, did not indicate that the invasive had a direct negative effect on germination, number of germ tubes produced per spore, or length of hyphae formed per spore (see Appendix; Johnson, personal observation). Although not investigated in this study, it is possible that a reduction in the ability of AMF to form successful associations with native species was reduced by root exudates that inhibited post-germination stages (e.g., contact of hyphae with roots, synthesis of the mycorrhiza, and growth of new hyphae in the soil origination from the roots).

Findings of the MIP growth experiments demonstrated that plants grown in soils with a history of *C. kobomugi* had significantly lower root colonization than those plants grown in soil mixes or field soil collected from areas of *A. breviligulata*. Colonization of growth assay plants is determined by the viability of soil inoculum in terms of the abundance of spores and infective propoagules, such as previously-colonized root fragments or pieces of hyphae. Successful colonization by AMF is also controlled by abiotic factors, such as light, nutrients, soil pH and moisture (Tarbell and Koske, 2007). Stinson *et al*. (2006) used MIP assays to evaluate whether an invasive

specifically caused decline of AMF in native soils. In that investigation, a significantly lower colonization by AMF in soils conditioned by *Alliaria petiolata* suggested that not only was the invasive non-mycorrhizal in nature, but also that it reduced native plant performance by interfering with mycorrhizal associations. In the present study, the reduced colonization in MIP studies suggests that *C. kobomugi* effectively reduces inoculum potential in areas of the foredune, having consequences for the re-reestablishment of mycorrhizae-dependent native plants. As *C. kobomugi* does not form beneficial associations with mycorrhizal fungi, the plant is not growth-limited by populations of AMF, making it potentially far more pliant in its expansion in dune habitats as compared to AMF-dependent natives (Johnson, personal observation).

C. kobomugi was found to have a significantly higher mean leaf tissue phosphate concentration as compared to native plant species (*A. breviligulata, L. japonicus* and *S. sempervirens*). For native mycorrhizae-forming plants, phosphorous acquisition serves as a primary indicator of the functionality of AMF in soil (Fox and Kamprath, 1970). As phosphorous is deficient in sand dunes, most plant species that grow there require the AMF association to obtain it in any significant quantity (Gemma, Koske, and Habte, 2002; Habte and Manjunath, 1987; Koske, personal communication) however, the higher leaf phosphate concentrations detected in *C. kobomugi* suggests that it is highly capable of obtaining phosphorous from the soil, apparently independent of interactions with AMF. An alternative explanation, one that could be further investigated using seasonal sampling of phosphate, is that phosphorous use and storage among these plants differs greatly. The leaf tissue phosphate study was conducted multiple times over a two year period, the most recent

experiment's results reported in this study. It may be that *C. kobomugi* sequesters phosphate differently than native plants, especially in a phenologic sense, though this study was not meant to specifically focus on this mechanism – instead, sampling of leaf tissue phosphate was meant to supplement spore counts, and to determine in a broad sense the relative amounts of phosphate among species. These data were particularly useful as the nutrient is highly deficient in sand dunes, and natives are largely dependent on AMF to obtain it in any substantial quantity. Furthermore, available soil phosphate levels did not differ between *C. kobomugi*-dominated areas and *A. breviligulata*-dominated areas, suggesting the invasive does not interfere with AMF obtaining the nutrient for mycorrhizal species.

Increased phosphorous absorption could be accomplished by using finer roots, organized in a relatively more exploratory and denser pattern, as compared to other plants. The difference between phalanx-type and guerrilla-type rhizome spread could contribute to both plants' acquisition abilities in general. Guerrilla-type plants, such as *C. kobomugi*, are opportunistic and pioneering in their root development, growing quickly to search for and acquire nutrients (De Kroon and Knops, 1990). Conversely, phalanx-type plants, such as *A. breviligulata*, tend to maintain a fixed position and slowly colonize over time (Watkinson, 1988). If *C. kobomugi* can acquire critical nutrients in greater concentrations than AMF-dependent species, especially in deficient soils, it could likely gain a competitive advantage. As *C. kobomugi* does not require AMF to acquire phosphorous and does not form a mycorrhizal association with AMF, the AMF populations dies off in *C. kobomugi* dominated areas. Thus, serious consequences may exist for re-planted native mycorrhiza-dependent species

after the invasive has been mitigated and removed.

Effects of Replacement and Reduction of *A. breviligulata*

In its native range, *C. kobomugi* is distributed in a more seaward orientation and direction (e.g., areas of the dune associated with relatively harsher conditions) as compared to other native vegetation, due to its ability to withstand relatively harsher abiotic conditions (Ishikawa, Furukawa, and Oikawa, 1995). In Rhode Island, Enser (2006) suggested that this ability allows it to persist and expand into areas where less-tolerant natives cannot. At East Beach it appears that *C. kobomugi* shares a similar tolerance for salinity and desiccation associated with sand movement, and soil-water content stresses (Barbour, 1978; Ishikawa and Kachi, 1998; Kachi and Hirose, 1979; Maruyama and Miura, 1981; Nobuhara, 1967) as *A. breviligulata* and other dune species (Bertness and Ellison, 1987; Maun, 2009; Snow and Vince, 1984). In other words, the seaward expansion of *C. kobomugi* appears to be as limited by similar abiotic stresses, including anthropogenic activity, as populations of *A. breviligulata*. The effect of existing environmental heterogeneity on the distribution of natives and *C. kobomugi* in New Jersey has recently been explored by Burkitt and Wootton (2010), but further investigations in Rhode Island have yet to reveal how dune structure and specific conditions dictate the distribution of these plant species.

In general, the replacement of *A. breviligulata* by *C. kobomugi* may have considerable impact on the structure of sand dunes. It has been suggested that in contrast to tall, native plants that buffer the dune from the strong forces of wind and

salt spray, the low-growing *C. kobomugi* makes the dunes vulnerable to shifting sands and blowouts, and in effect, areas of secondary invasion (Virginia Department of Conservation and Recreation, 2011). The replacement of *A. breviligulata* by *C. kobomugi* could contribute to erosion and loss of protection of inshore areas because of its shorter roots (Hayes, 2009; Lea and McLaughlin, 2002). Consistently lower dunes may not be as effective as habitat or barrier, especially in light of the fact that Ninigret Pond exists within such close proximity to the shoreline.

Conversely, some evidence tends to suggest that *C. kobomugi* is as an effective dune stabilizer as *A. breviligulata* or even perhaps more effective, as periodic dieback of *A. breviligulata,* due to marasmius blight, coastal storms, erosional phases, or washover drift lines, make the species vulnerable to replacement (Wootton *et al.*, 2005). At East Beach it appears that the fine and extensive rooting of *C. kobomugi* binds sand into cohesive sod-like pieces (Johnson, personal observation). Photographs taken of roots in areas of dense *C. kobomugi* and dense *A. breviligulata* depict substantial differences in ability to bind sand solely with roots (Figures 24 and 25). Testable and quantifiable comparisons of dune-forming abilities between *C. kobomugi* and *A. breviligulata* need to be made in order to determine which is better at maintaining the geomorphology of dunes. Measurements of the maximum depth of live roots frequently are used to identify which species are the best dune stabilizers (Hayes, 2009; Lea and McLaughlin, 2002; Wootton *et al*., 2005), but other factors, including the aggregation of sand grains by hyphae of AMF, may be of equal or greater importance. Furthermore, an essential characteristic of dune-building and stabilizing species is the ability to tolerate anoxia associated with sand burial. In

general, this differential tolerance by certain species may be one of the principal causes of plant species zonation on coastal foredunes (Maun, 2004). As *A. breviligulata* is associated with sand deposition in mobile and early fixed dunes (1-25 cm per year), its tolerance for sand burial and makes it a well-suited dune stabilizer (Maun and Baye, 1989; Maun, 2009). Typically, burial depths of 5-20 cm maintain and enhance shoot density, percent cover, as well as belowground biomass for native dune grasses (Seliskar, 1994; Maun, 2009). Maun (2004) reported that *A. breviligulata* and *C. kobomugi* are vigorous in areas with average sand deposition of 17 to 28 cm/year, an amount typical for the first 40 m of the foredune. After that point (in the next landward 20 m of the dune) sand deposition decreased by 3-5 cm resulting in a significant decline of vigor and distribution of both species. Conversely, Wootton *et al*. (2005) reported that burying *C. kobomugi* in sand, a technique that has been shown increase vigor in *A. breviligulata* (Maun and Baye, 1989; Seliskar, 1994), causes extensive mortality (United States Department of Agriculture, 1983; Disraeli 1984). The possible causes for decline in plant vigor from excessive sand burial are unclear, and appear to be caused by a number of interactions, including increased desiccation, physiological deterioration of plant functions, and increased soil microorganism activity (Maun, 2004).

Spread of *C. kobomugi* and Implications for Future Mitigation

The robust growth form of *C. kobomugi*, and its propensity to expand via extensive rhizomes, makes it particularly difficult to manage. Widespread herbicide

treatments for *C. kobomugi* have the potential to destroy non-target species, leaving the dunes susceptible to becoming rapidly destabilized. Wootton *et al*. (2005) found that localized herbicide application failed to effectively eliminate *C. kobomugi* when applied on sites in New Jersey. Mechanical removal of *C. kobomugi* has great potential to disturb or even destroy non-target rhizomes of *A. breviligulata* and the associated beneficial mycorrhizal networks (Koske, personal communication). McGonigle and Miller (1993) found that in an agricultural environment, tillage or soil disturbance may disrupt the network of AMF hyphae in soils, and significantly reduce mycorrhizal inoculum potential. Rhizomes from *C. kobomugi* have been observed to spread into areas of dense *A. breviligulata*, and if they are not physically removed, new shoots have the capacity to develop there the following season (Johnson, personal observation). It is not certain to what depth plants of *C. kobomugi* would have to be buried by sand to eliminate the invasive from the dune. The species is very responsive to small amounts of sand deposition in natural habitats such as East Beach (Johnson, personal observation; Wootton *et al*., 2005). Because of the ability of *A. breviligulata* to tolerate sand deposition of up to 80 cm per year (Maun, 2009), burial of plants would seem to be a potentially effective (and costly) method for controlling *C. kobomugi* on East Beach dunes. The effect on dune AMF would be minimal because living roots and rhizomes of *A. breviligulata* already are associated with the fungi.

Compared to *A. breviligulata*, *C. kobomugi* seems well-suited of expanding well into the backdune, colonizing open sand space around non-dune plant species. Past studies have indicated that *C. kobomugi* fills in areas, primarily those spaces that become available as *A. breviligulata* becomes sparser in its distribution over time

(Belcher *et al*., 1984). The growth characteristics of *C. kobomugi* are likely the main cause for this trend, as the plants are shorter and have a greater number of leaves than *A. breviligulata*. Populations of *C. kobomugi* anecdotally documented in backdune areas potentially compound mitigation efforts at East Beach (Figure 26 and 27). Backdune populations were less dense relative to those of the foredune, and likely spread by seeds or small rhizome fragments, rather than advancement of a clonal rhizome network. Though the seeds of *C. kobomugi* have been shown to have a low germination rate and seedlings high mortality (Yamamoto, 1964; Nobuhara, 1974; Sasaki, 1987; Ishikawa *et al*., 1993), transport of seeds is quite plausible, given their small size. The germination of seeds of *C. kobomugi* is controlled by external dormancy, initiated by scarification and followed by a moist-chilling event caused by low soil temperature and warming to approximately 35° C (Ishikawa *et al*., 1993). In terms of these germination requirements, Wootton *et al*. (2005) has suggested that numerous hot summers associated with recent global warming may have promoted further expansion of the invasive in North America. Considering the lack of knowledge pertaining to patch age and at East Beach, populations of *C. kobomugi* in stable backdune areas should be regarded as equally critical as foredune populations in the successful management of the invasion. Possible explanations for expansion in these backdune areas could be overall higher available nutrient concentrations, or the reduction of physical stressors such as sand accumulation, wind, and salt spray typical of the foredune. The backdune expansion of *C. kobomugi* could also have been facilitated by a lack of competition from *A. breviligulata*. Reduced sand deposition characteristic to backdune areas is tantamount to decreased pathogen protection, and

nutrient availability associated with AMF (Hawk and Sharp, 1967; Shisler, Wargo, and Jordan, 1987), essentially making *A. breviligulata* grow relatively poorly there (Maun and Baye, 1989).

The relative ages of *C. kobomugi* patch sites could be of importance in further addressing its expansion. At East Beach, though the approximate age of the initial invasion is known (Champlin, 1994; Standley, 1983), relative ages of secondary patch sites are unknown. In a series of stem-density assessments conducted in New Jersey, Burkitt and Wootton (2010) determined that the size of secondary invasion sites was actually a poor proxy for age. Instead they proposed that larger beds may just represent areas where multiple introductions of *C. kobomugi* occurred in close proximity to one another and the smaller populations merged to form a single one. Ishikawa and Kachi (1998) demonstrated that the age of *C. kobomugi* could be approximated by means of counting branching shoots per node on rhizomes. Further analysis could also determine whether different patches of *C. kobomugi* are genetically distinct, providing further insight as to the expansion of the invasive at East Beach. Vegetative material taken from the large stands at East Beach and compared to populations at nearby Sachuest Point may provide an historical basis to the invasion by *C. kobomugi* in Rhode Island in general.

In addition to precipitating disturbance events, the high recreational accessibility at East Beach may also constitute an introduction pathway for *C. kobomugi*, with vehicles themselves acting as vectors for seeds and vegetative material. As *C. kobomugi* primarily propagates through rhizome or node tissue, if uprooted and caught in the tread of a vehicle, the invasive could initiate a secondary

invasion in a potentially distant location. At East Beach tire tracks suggest that vehicles occasionally stray onto foredune areas, specifically areas of *C. kobomugi* (Figure 28). Sachuest Point vegetation treatment maps obtained from Warren Hall and the Town of Middletown suggest that populations of the nonnative invasive are correlated with highly traveled and disturbed areas of the beach, such as paths or parking lots (Figure 29). Similarly, populations of *C. kobomugi* at East Beach seem to accompany paths and roads associated with high seasonal beach traffic (Johnson, personal observation).

Vehicles and high beach traffic have the capacity to destroy native dune vegetation, creating open niche space in which *C. kobomugi* can initiate secondary invasions. At East Beach, vehicles are allowed access to backdune road systems and are also permitted on the beach itself, frequently disturbing areas of the foredune. As the intertidal beach area is important as a nutrient cycling system, both in terms of algae and bacteria, one result of frequent vehicle activity is the exposure of sensitive organisms to desiccation. Though vehicles tend to travel in a corridor of minimal beach biota (Godfrey and Godfrey, 1981) when vehicles stray into the transition areas of foredune, crushing both seedlings and fragments of *A. breviligulata*, they potentially reduce the population of viable plants that act as stabilizers for new dunes (Figure 30). This straying also quickly deteriorates the sloping incline of the foredune that is necessary to prevent widespread and unchecked wind erosion. With strong enough prevailing winds, large blow-out areas can occur, inevitably threatening an entire dune system (Godfrey and Godfrey, 1981). The robust growth characteristics of *C. kobomugi* make it an ideal candidate for colonizing these areas, recently made

available by vehicle straying and beach traffic.

As a functioning population of AMF can have overarching effects on not only individual plants but also entire plant communities (Smith and Read, 1997), the disruption of the AMF community likely has significant, far-reaching effects for the future identity and functionality of East Beach dunes. The reduction in the population of AMF and MIP values in the East Beach dunes in response to invasion of the area by *C. kobomugi*, a non-mycorrhizal species (Johnson, personal observation), seems to be the result of a decline in the vigor and extent of the population of mycorrhiza-forming native species (especially the dominant *A. breviligulata*) that are necessary to maintain a large and diverse AMF community in the soil rather than the direct suppression of AMF activity (sporulation, germination of spores, hyphal growth, etc.) by exudates from the invasive. By replacing *A. breviligulata*, *C. kobomugi* disrupts the associated AMF populations therefore critically reducing the potential for natives to persist, and subsequently re-colonize areas. These findings are of particular importance to the successful re-planting of dunes after mitigation of the invasive. Concurrent with vegetation surveys in New Jersey that indicated overall native species diversity and density was reduced in areas of *C. kobomugi* when compared to non-invaded areas, (Wootton *et al*., 2005) it appears that *C. kobomugi* effectively replaces natives in the plant community at East Beach. Though difficult to quantify, a monoculture of *C. kobomugi* will certainly be a significant aesthetic loss and may substantially alter overarching ecosystem dynamics. Management methods are difficult to implement both due to the rapid and expansive rhizomatous growth of the invasive as well as the inherent sensitivity both of native dune plants and AMF hyphal networks. Widespread

climactic changes, such as sea level rise or temperature changes, coupled with anthropogenic pressures and prolonged shoreline erosion may result in the elimination of species from dune habitats, or the introduction of others (Maun, 2004). Thus, adaptive dispersal and tolerance mechanisms of *C. kobomugi* may be of critical importance for the future distribution of species at East Beach. Further studies investigating population age, supplemented with growth assays examining the effect of root extracts on AMF spores, and would likely contribute essential knowledge to the ecology and mitigation of *C. kobomugi* in established dune habitats.

ACKNOWLEDGEMENTS

This manuscript is a portion of a thesis submitted to the Graduate School of the University of Rhode Island in partial fulfillment of the requirements of the Master of Science degree completed under the direction of Dr. Richard Koske. I am also grateful for the insights and guidance of Keith Killingbeck, Laura Meyerson, Jane Gemma. I thank Warren Hall of the Town of Middletown, RI and Andrew MacLachlan of the Fish and Wildlife Service for their support. I thank Robert Paquette of the Rhode Island Department of Environmental Management for allowing access to foredune areas.

APPENDICES

Appendix A: Root Staining Procedure (modified and adapted from Koske and Gemma, 1989 and Schmidt and Reeves, 1984):

1. Separate roots from plant. Only the middle section (approximately 5 cm from base of plant, and approximately 5 cm from root tip) should be used, as this is the area having the highest AMF colonization (Koske, personal communication).
2. Rinse roots of large debris under a forceful stream of water, teasing away material with hands.
3. Clean roots by putting them in a small scintillation vial filled with 70% ethanol.
4. Pour off 70% ethanol and add 2.5% potassium hydroxide (KOH) to cover them. Screw cap to scintillation vial on, but not tightly. Place vials in porcelain tray and in oven at 65° C overnight.
5. After the vials have cooled, pour off 2.5% KOH and rinse roots with water. When rinsing roots use a small, coarse mesh screen to ensure that root material is not lost.
6. Pour 1% hydrochloric acid (HCl) into vials and place them back into the oven at 65 degrees for at least 1 hour.
7. Pour off 1% HCl but do not rinse the roots. Add trypan blue/glycerol staining solution (0.05% trypan blue in acidified glycerol) to cover the roots and place back into oven at 65° C overnight.
8. Pour off trypan blue/glycerol staining solution and add the destaining solution

(50% glycerol, 45% water, 5% hydrochloric acid (1%). Roots are ready for examination now, and they can be stored indefinitely in this solution, especially if in dark conditions.

Appendix B: Spore extraction (adapted and modified from Gerdemann and Nicolson, 1963 and Walker, Mize, and McNabb, 1982):

1. Soil into 1.5 L beaker. Add 200 ml water to break up clods and wash substrate.
2. Add water in hard stream to aerate and agitate.
3. Decant through two sieves, coarse and fine.
4. Wash material on fine sieve into small beaker.
5. Wash material from beaker into large centrifuge tube (50 ml capacity). Fill with deionized water until approximately 40 ml level.
6. Add kaolin (approximately 300 mg), centrifuge for 3 minutes at maximum speed.
7. Pour water off and wipe inside of centrifuge tube thoroughly to remove debris/dead spores.
8. Add 40% sucrose solution, shake well and vortex to resuspend pellet of kaolin.
9. Centrifuge for approximately 1 minute near maximum speed.
10. Filter supernatant through 53 um sieve.
11. Wash material on 53 um sieve onto filter paper, in Buchner filtration system.
12. Remove filter paper from Buchner funnel and place onto plastic Petri dish for further examination.
13. Remove spores from filter paper using inoculating needle with PVLG at tip.

14. Place spores onto slide with coverslip and crush to observe differences in cell wall morphology.

Appendix C: Leaf Phosphate Assessment (adapted and modified from Aziz and Habte, 1987; Habte, Fox, and Huang, 1987; Habte and Manjunath, 1987):

gdw = grams dry weight

0.16 µg P/ml = standard phosphate concentration

12.5 ml = volume of liquid in test tube (18 mm by 150 mm)

µP/g of leaf tissue = [(absorbance of sample)/(absorbance of standard)] x 0.16 x [(12.5)/(dry weight of sample)]

µg P/gdw of tissue = 12.5 ml x [(absorbance of sample)/ (leaf dry weight)] x [(0.16 µg P/ml) / (absorbance of standard)]

Molybdate blue reagent (Reagent A):

1. To 2.7 L of deoinized water add 0.35 g of antimony potassium tartrate and dissolve well.
2. Add 168 ml of concentrated sulfuric acid.
3. Add 14.43 g of ammonium molybdate [$(NH_4)6\ Mo7O24{\cdot}4H_2O$] and dissolve.
4. Add 120 ml of deionized water and store in a dark bottle.

Molybdate blue reagent (Reagent B):

1. Dissolve 0.428 g of ascorbic acid into 100 ml of Reagent A.
2. Add 2.5 ml of Reagent B to ever 10 ml of leaf solution in test tube.

Note: Reagent A and B are toxic and should be disposed of properly.

Appendix D: Spores of *G. gigantea* germination assessment (adapted and modified from Koske, 1981):

Sand was obtained from root zones of *A. breviligulata* and *C. kobomugi* at East Beach in Fall 2009. Sand was kept in refrigerated (5°C) storage for approximately 5 months to stimulate germination before spore extraction. Spores were obtained from the sand using a modified wet sieving and decanting procedure from Walker, Mize, and McNabb (1982). This extraction method was similar to that used in spore abundance assessments, but did not incorporate sucrose suspension to remove debris (essentially water aeration and two sieve-filtration onto collection dish). Only viable, healthy spores of *G. gigantea* were used (Lee and Koske, 1994). Sand plates were made to represent soil conditions in the field - 5 glass Petri dishes, each with 40 ml of sand from root zones of *A. breviligulata* and *C. kobomugi* were made. Petri dishes had a filter paper soaked with deionized water that was placed on the surface of the sand. Molecularporous membrane tubing (Spectrum Medical Industries, Inc., Los Angeles, California) was cut into two, 1 cm squares and placed onto the soaked filter paper. Five spores of *G. gigantea* were placed onto each membrane (10 spores per plate, 5 plates of each soil type, 50 spores per treatment). All plates were kept in an incubator (24°C) and evaluated for germination success (germinated vs. non-germinated) over a two week period. Comparing the number of successfully formed germ tubes served to indicate if soil or microcosm conditions affected overall germination of spores. This experiment was repeated three times to obtain germination results.

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MIP #	Soil treatment	Mean percent of roots colonized by AMF
MIP #2	Field soil with a history of *C. kobomugi*	12% ± 4%
	Field soil with a history of *A. breviligulata*	55% ± 9%
MIP #3	Field soil with a history of *C. kobomugi*	24% ± 3%
	Field soil with a history of *A. breviligulata*	72% ± 1%
	1:1 mix of *A. breviligulata* and *C. kobomugi* soil	49% ± 3%

Table 1. Mean root colonization of indicator corn grown in soil treatments from MIP #2 and MIP #3, with standard error of the mean [SEM]

Figure 1. A satellite image from April 2010 showing the study area. The inset map shows the approximate location of East Beach relative to the rest of the Rhode Island. Photograph courtesy of Google Map Satellite Imagery, Google, 2011. Accessed January, 2011.

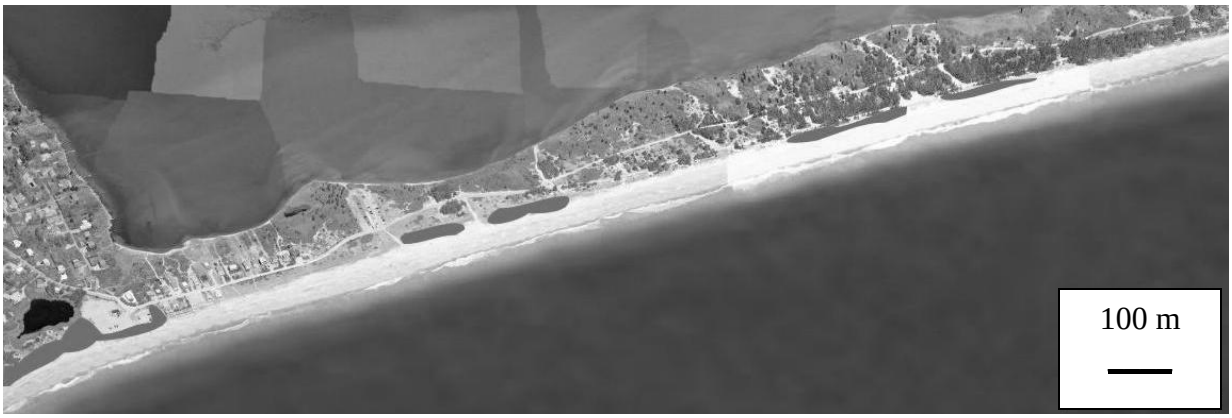


Figure 2. An oblique aerial photograph taken of East Beach in April 2008 with patch-sites of *C. kobomugi* (shaded areas). Photograph courtesy of Pictometry International MDA Geospatial Services and Rhode Island Geographic Information Services, 2008. Accessed January, 2011.

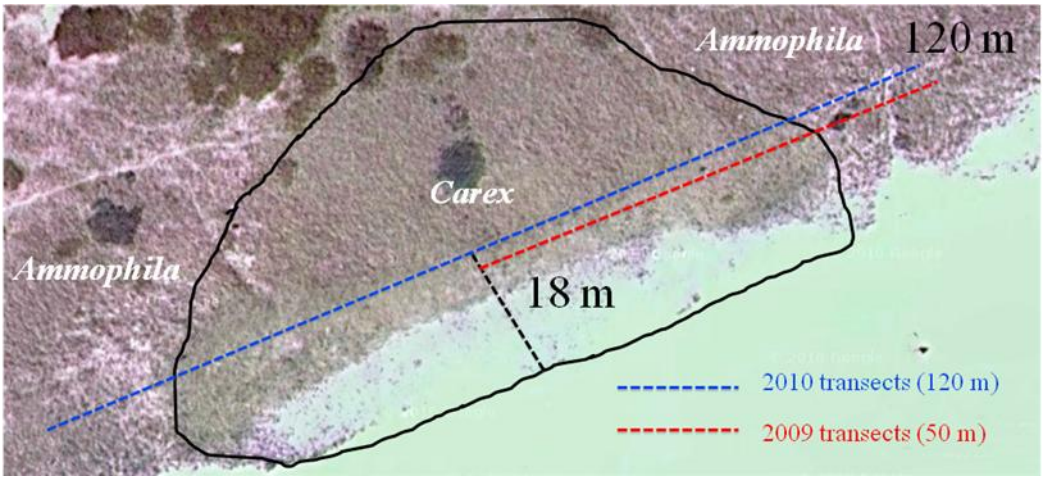


Figure 3. Differences in transect coverage between 2009 and 2010. In 2009, transects were made from areas of dense *C. kobomugi* to areas of dense *A. breviligulata* (50 m). In 2010, transects were made from areas of dense *A. breviligulata* to areas of *C. kobomugi* and back into areas of *A. breviligulata* (120 m). Photograph courtesy of Google Map Satellite Imagery, Google, 2011. Accessed January, 2011.

Figure 4. Percent cover photograph at Low Dune 56 m. This photograph exemplifies an area dominated by *C. kobomugi* (97% cover). Open space at this interval was virtually absent (3%).

Figure 5. Percent cover photograph at Mid Dune 108 m. This photograph exemplifies an area dominated by *A. breviligulata* (87%). Open space (10%) at this interval is relatively conserved.

Figure 6. Percent cover photograph at High Dune 80 m. This photograph exmplifies an interval of near even percent cover of *C. kobomugi* and *A. breviligulata* (34% and 36%, respectively).

Figure 7. Percent cover photograph at Low Dune 120 m. This photograph exemplifies an area dominated by *A. breviligulata* (41%). Open space at this interval was 59%, greater than that of the actual plant cover.

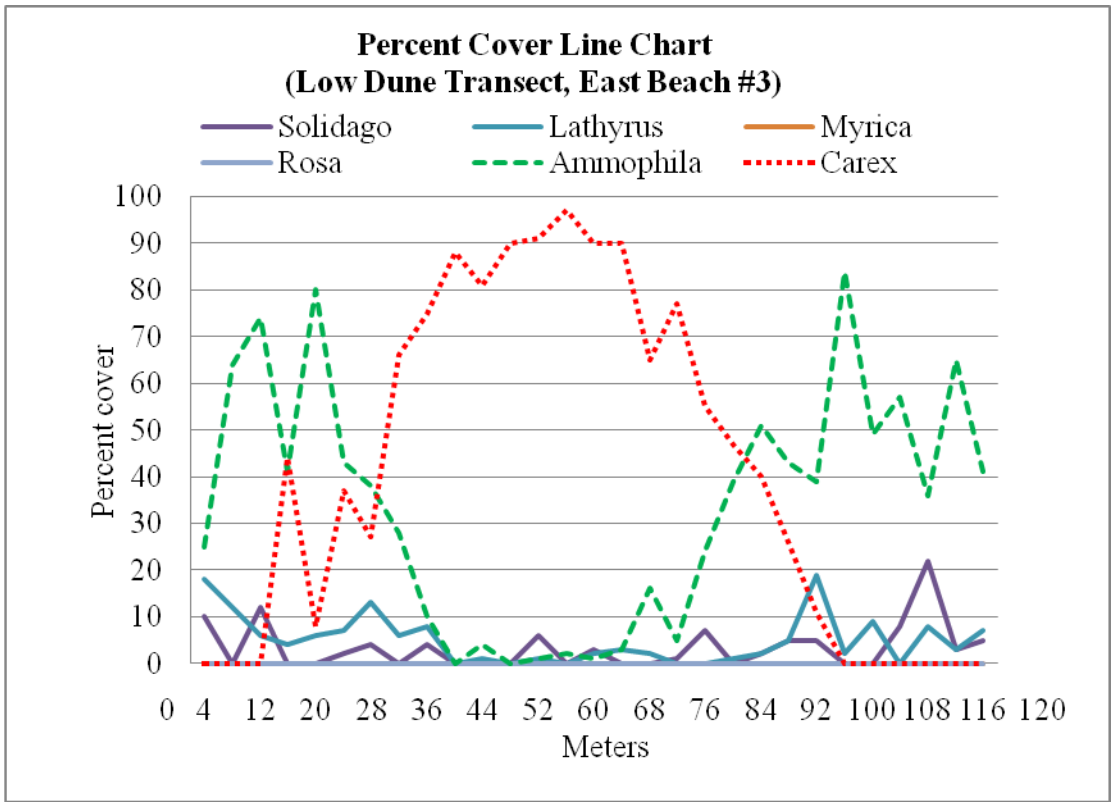


Figure 8. Percent cover trends of plant species in comparison to meters across a Low Dune transect. *Solidago* = S. sempervirens, *Lathyrus* = L. japonicus, *Myrica* = M. pensylvanica, *Rosa* = R. rugosa, *Ammophila* = A. breviligulata, *Carex* = C. kobomugi. Data are representative of assessments in 2010.

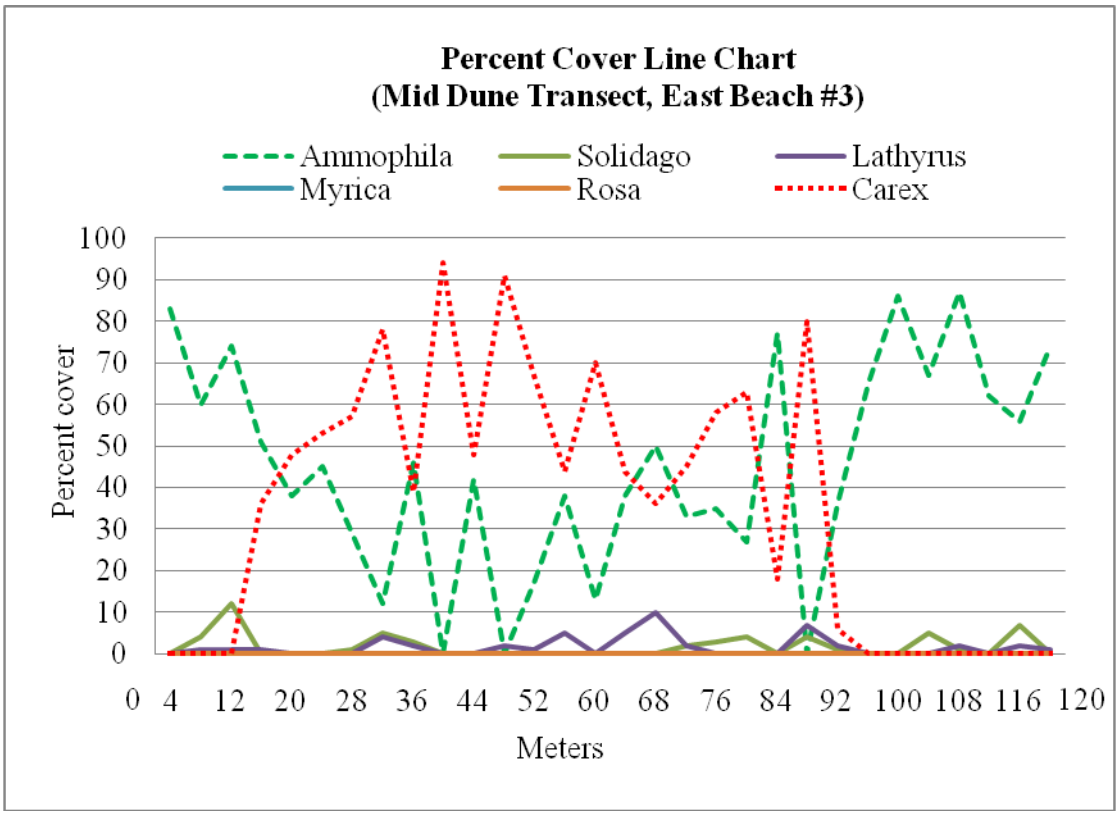


Figure 9. Percent cover trends of plant species in comparison to meters across a Mid Dune transect. Data are representative of assessments in 2010.

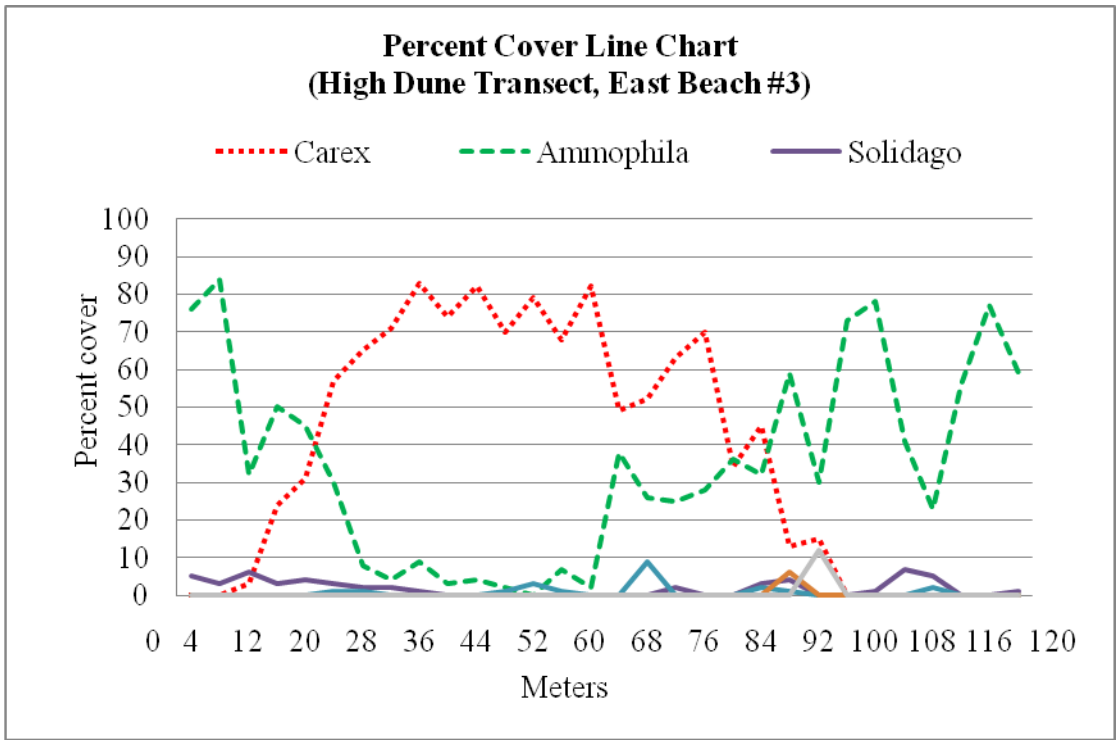


Figure 10. Percent cover trends of plant species in comparison to meters across a High Dune transect. *Solidago* = S. sempervirens, *Lathyrus* = L. japonicus, *Myrica* = M. pensylvanica, *Rosa* = R. rugosa, *Ammophila* = A. breviligulata, *Carex* = C. kobomugi. Data are representative of assessments in 2010.

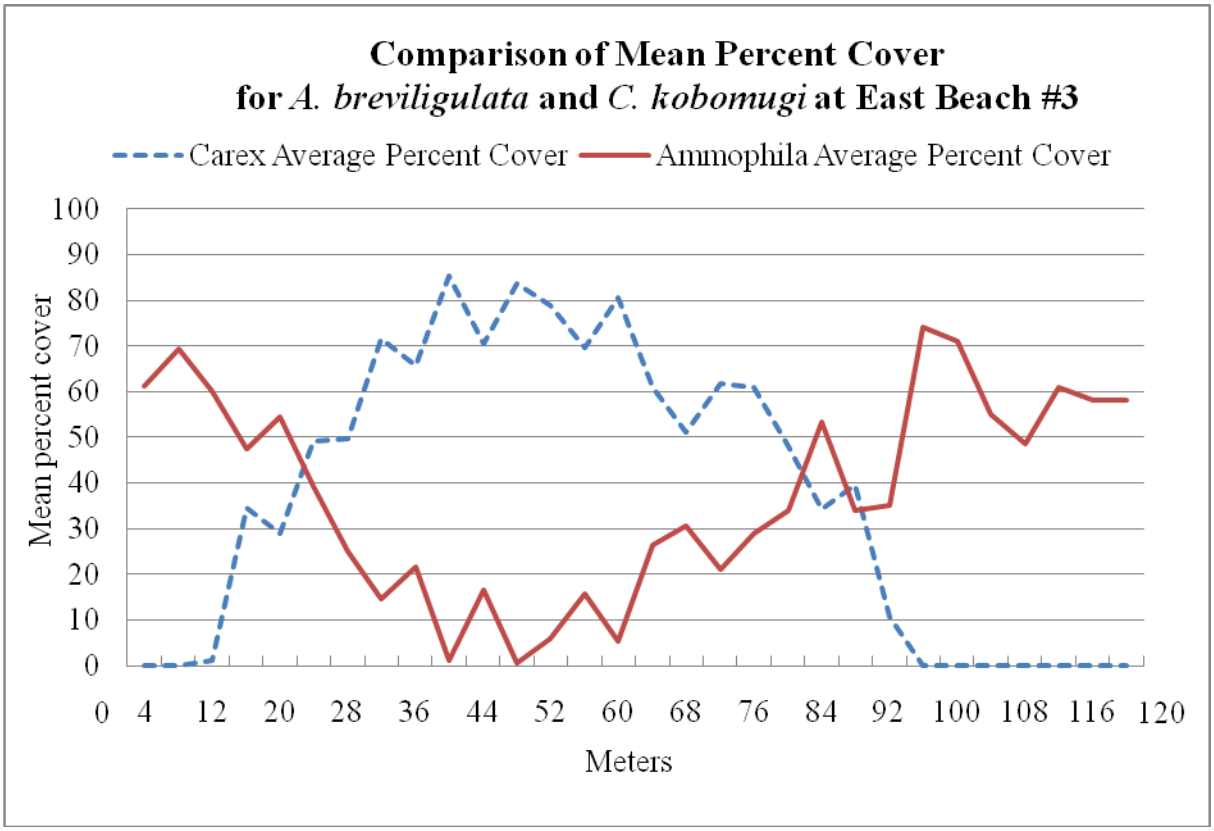


Figure 11. Mean percent cover of *A. breviligulata* and *C. kobomugi* in comparison to meters across all transects (Low, Mid and High). *Ammophila* = A. breviligulata, *Carex* = C. kobomugi. Data are from assessments in 2010.

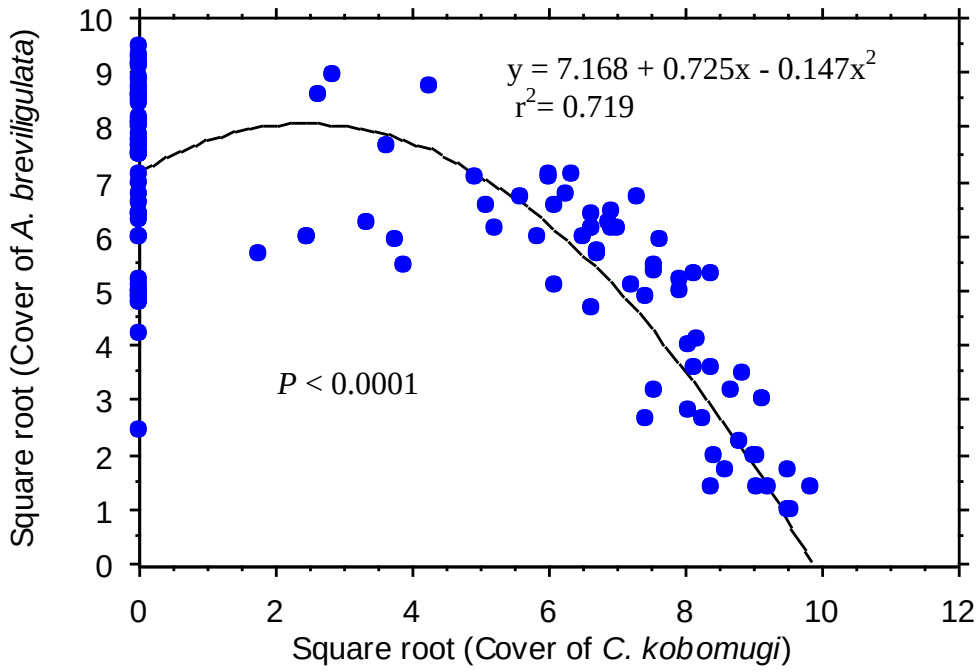


Figure 12. Percent cover of *C. kobomugi* and *A. breviligulata.* Data were square-root transformed. Data are from assessments in 2009 and 2010.

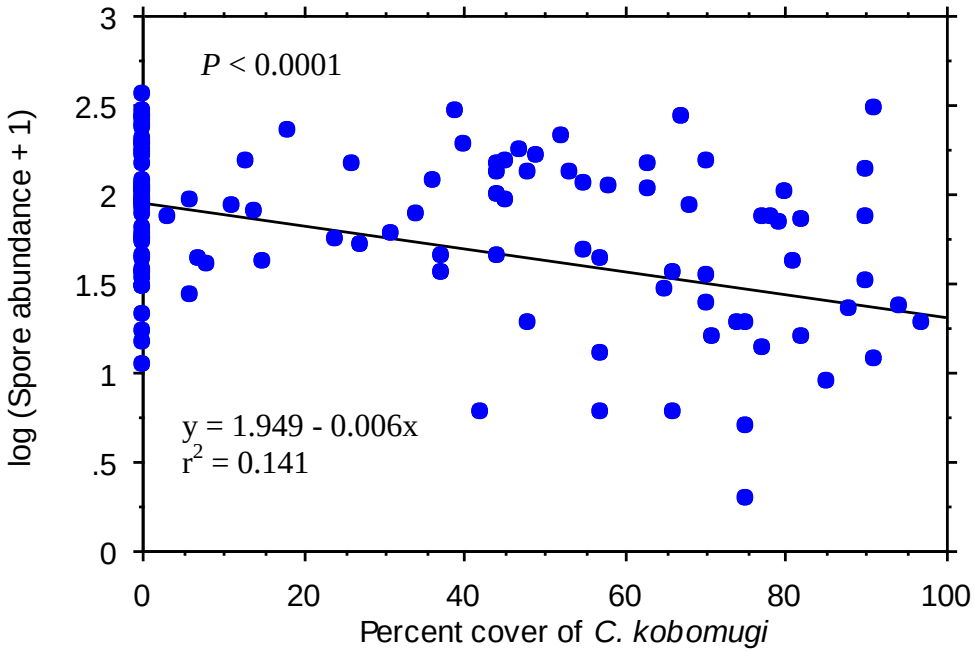


Figure 13. Percent cover of *C. kobomugi* and spore abundance of AMF. Spore abundance is the number of spores per 200 ml soil. Spore abundance was log transformed. Data are from assessments in 2009 and 2010.

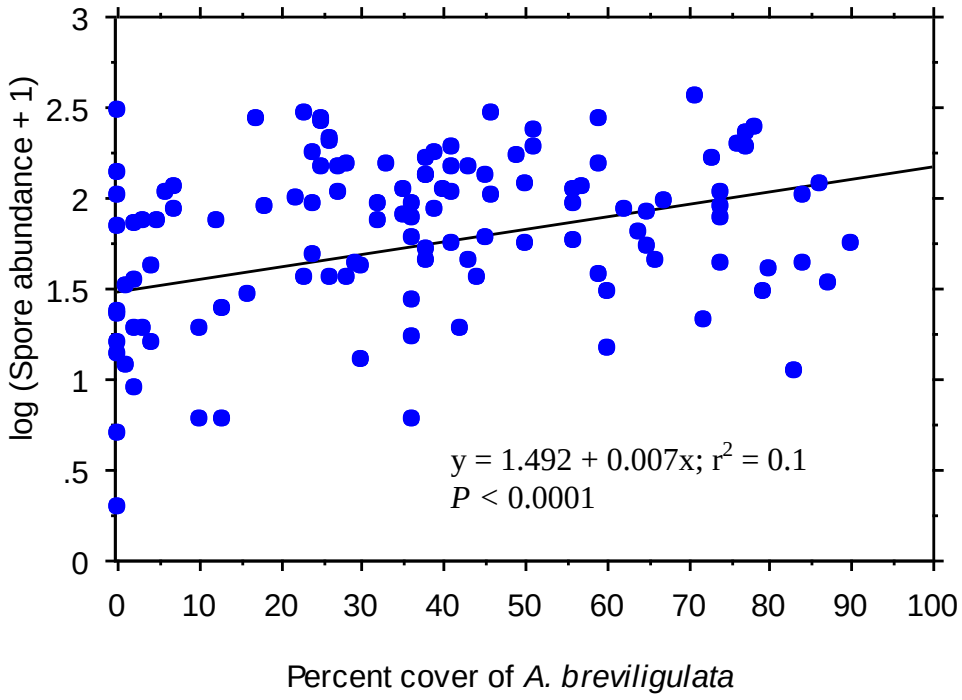


Figure 14. Percent cover of *A. breviligulata* and spore abundance of AMF. Spore abundance was log transformed. Data are from assessments in 2009 and 2010.

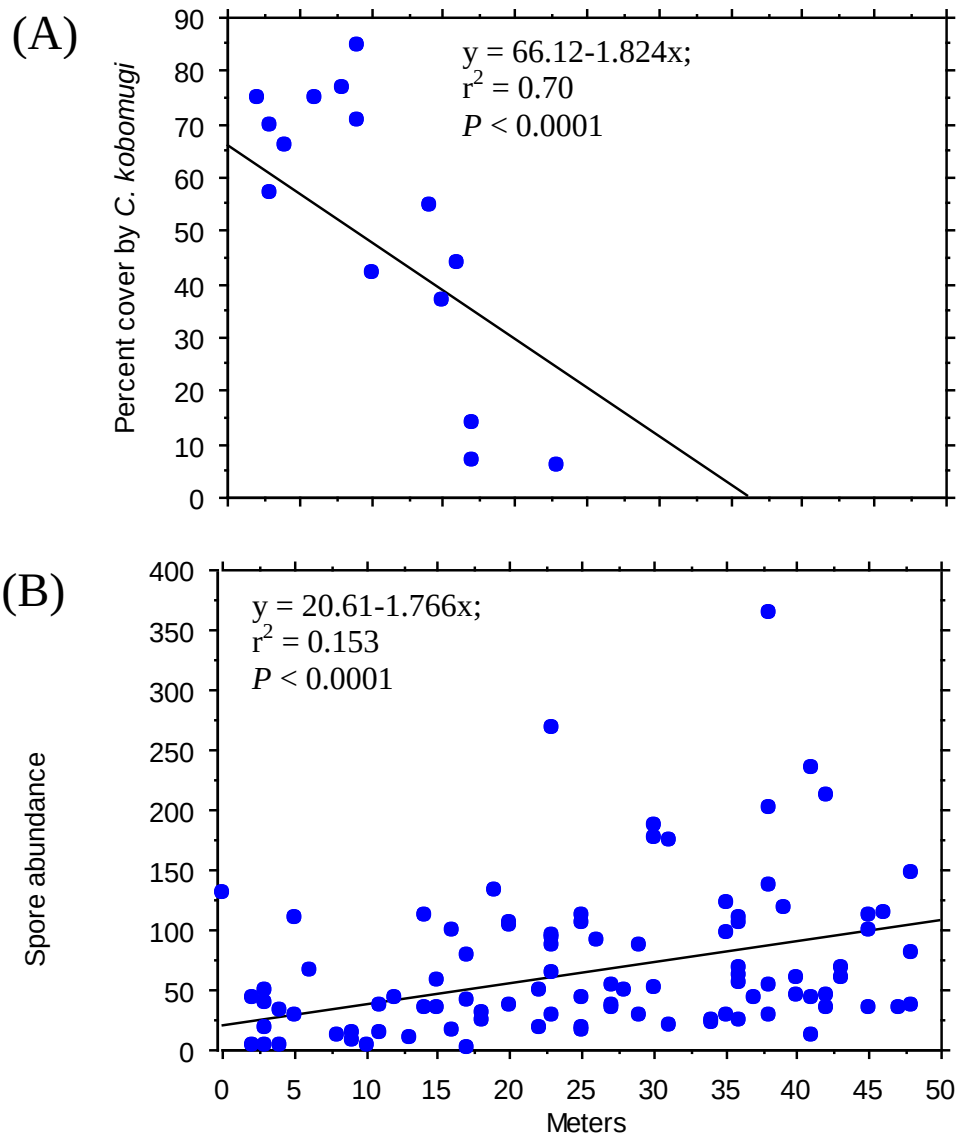


Figure 15. (A) Percent cover of *C. kobomugi* and (B) spore abundance of AMF in comparison to meters across all transects (Low, Mid and High). Spore abundance is the number of spores per 200 ml soil and data are representative of three patch sites. Vegetation percent cover data are representative one patch site. Data are from assessments in 2009.

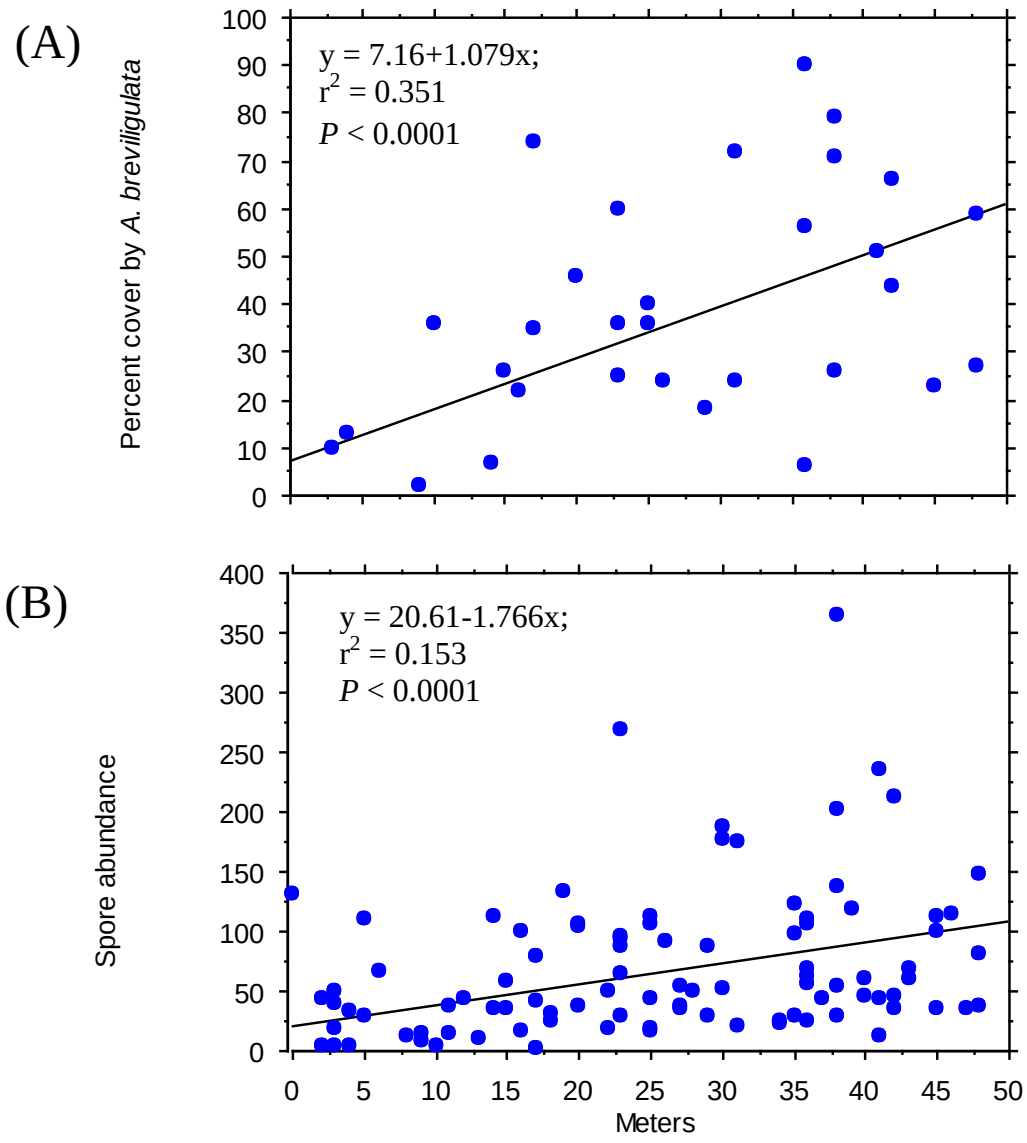


Figure 16. (A) Percent cover of *A. breviligulata* and (B) spore abundance of AMF in comparison to meters across all transects (Low, Mid and High). Spore abundance is the number of spores per 200 ml soil and data are representative of three patch sites. Vegetation percent cover data are from one patch site. Data are from assessments in 2009.

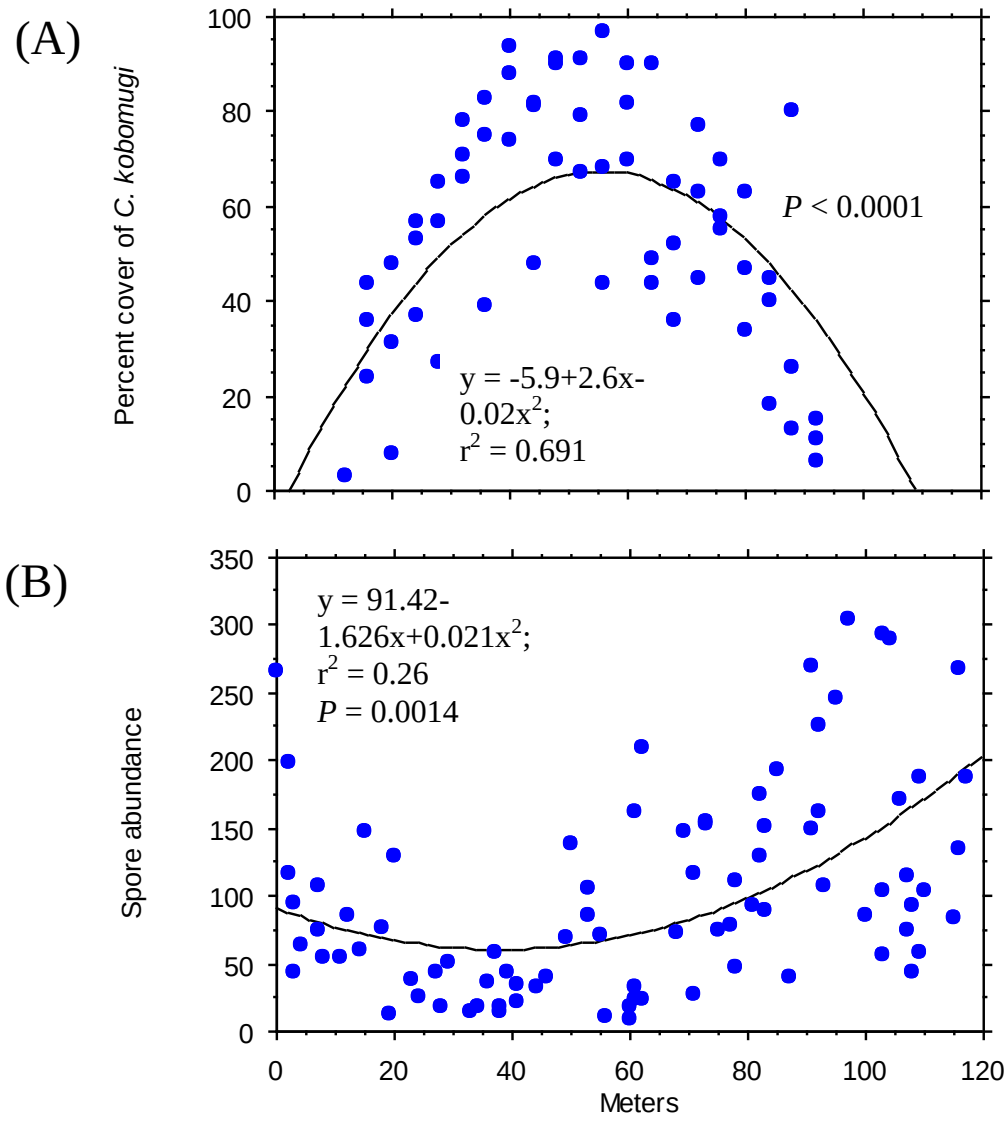


Figure 17. (A) Percent cover of *C. kobomugi* in comparison to meters across all transects. (B) Spore abundance of AMF in comparison to meters across all transects (Low, Mid and High). Spore abundance is the number of spores per 200 ml soil. Data are from assessments in 2010.

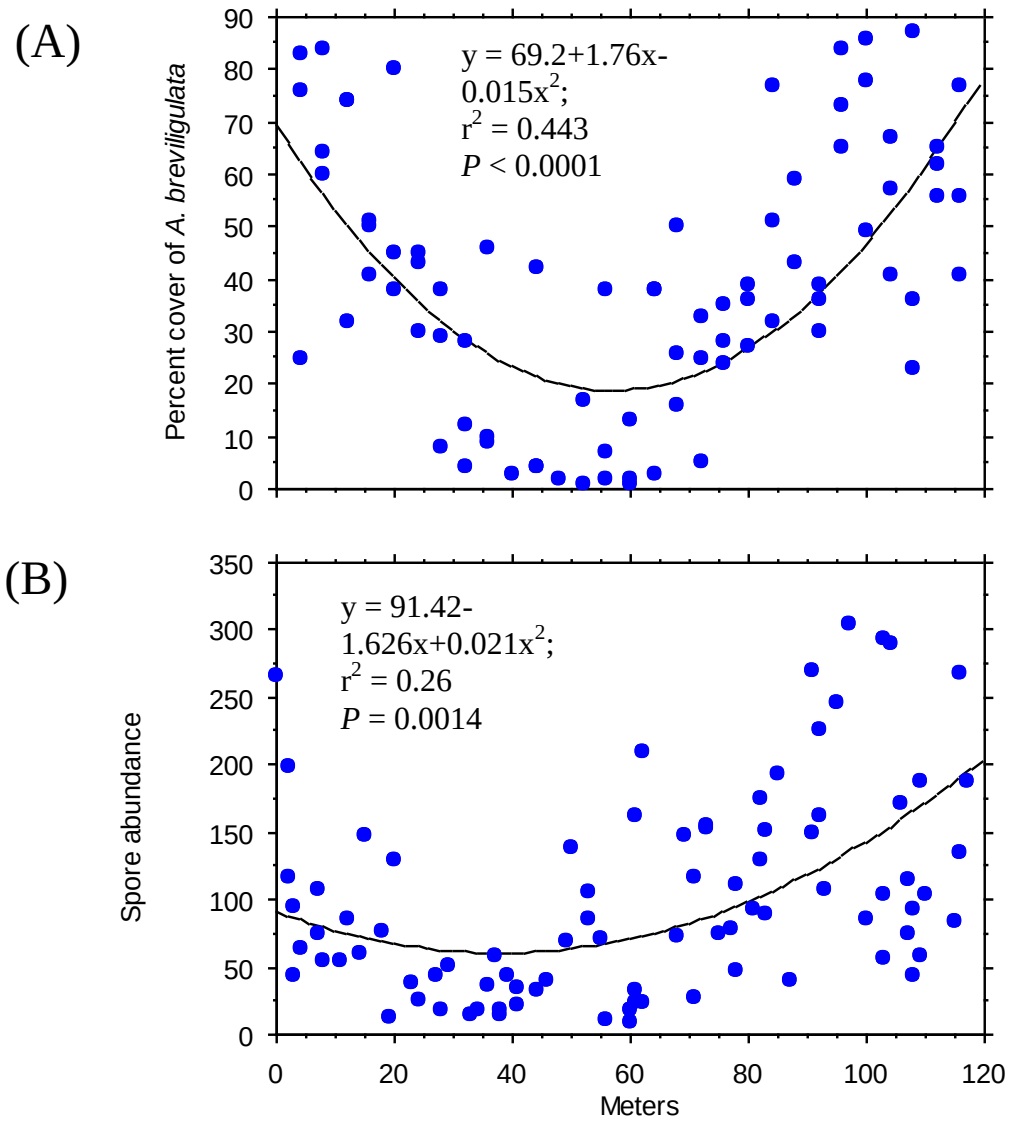


Figure 18. (A) Percent cover of A. breviligulata in comparison to meters across all transects. (B) Spore abundance of AMF in comparison to meters across all transects (Low, Mid and High). Spore abundance is the number of spores per 200 ml soil. Data are from assessments in 2010.

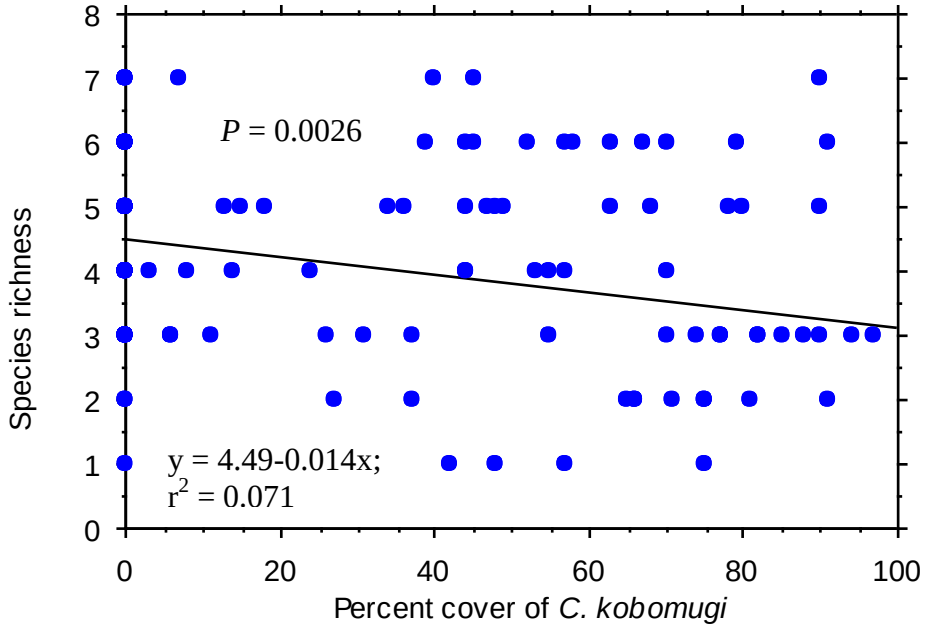


Figure 19. Mean species richness of AMF and percent cover of *C. kobomugi*. Data are from assessments in 2009 and 2010.

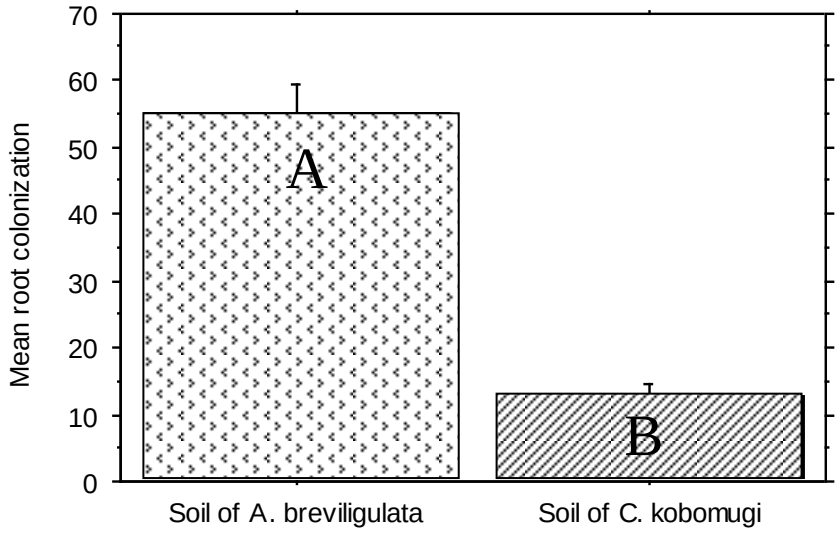


Figure 20. Mean root colonization by AMF (%) of MIP #2 plants in comparison to soil treatments, with standard error of mean [SEM]. Plants grown in *A. breviligulata* field soil had significantly higher root colonization in comparison to *C. kobomugi* field soil ($P < 0.0001$).

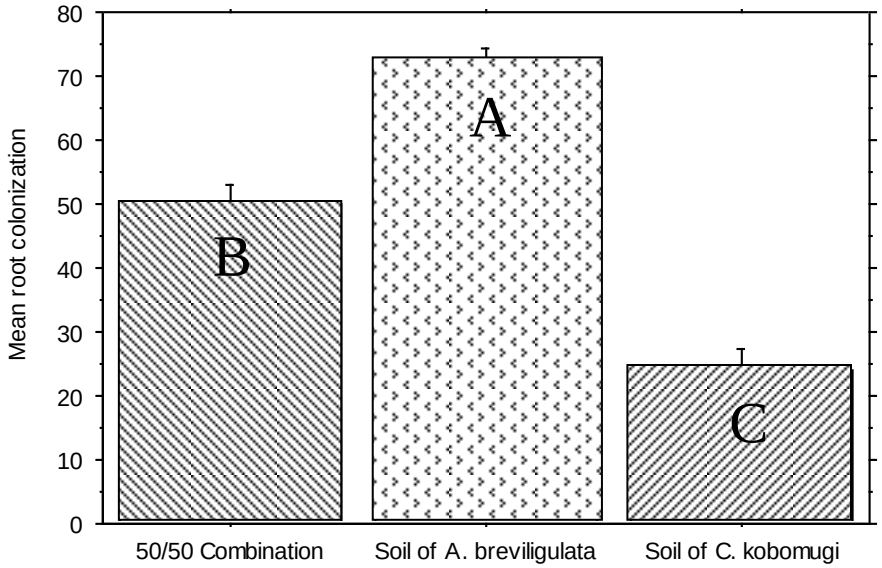


Figure 21. Mean root colonization by AMF (%) of MIP #3 plants in comparison to soil treatments, with standard error of mean [SEM]. Plants grown in field soil of *A. breviligulata* had significantly higher root colonization in comparison to those in field soil of *C. kobomugi* ($P < 0.0001$) and the 50% C. kobomugi / 50% A. breviligulata soil combination ($P = 0.0009$). Plants grown in soil of *C. kobomugi* had less mean root colonization than the 50% C. kobomugi / 50% A. breviligulata soil combination ($P = 0.0004$).

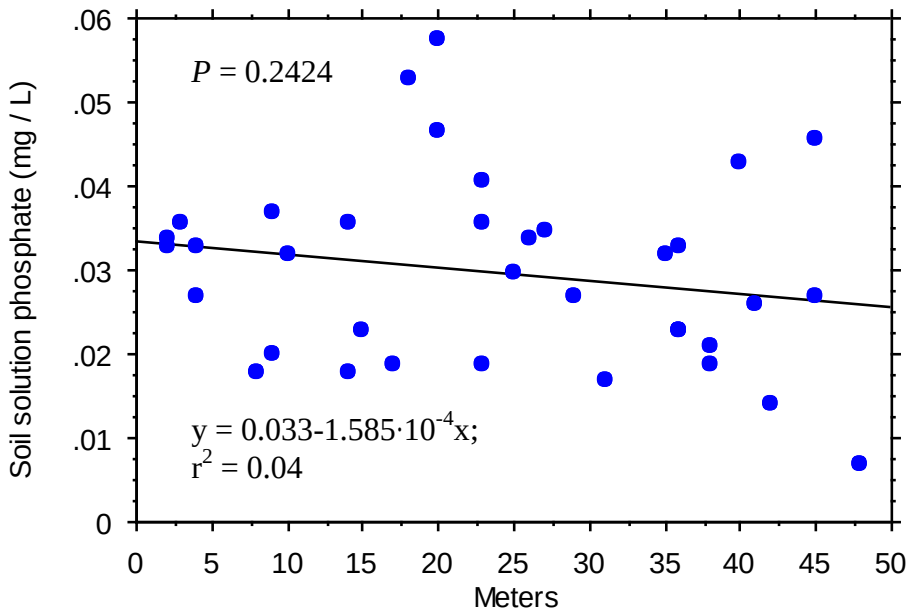


Figure 22. Soil solution phosphate as compared to meters a long a transect following spore collection points in 2009. Changes in soil solution phosphate across transect meters were not significant. Data are from assessments in 2009.

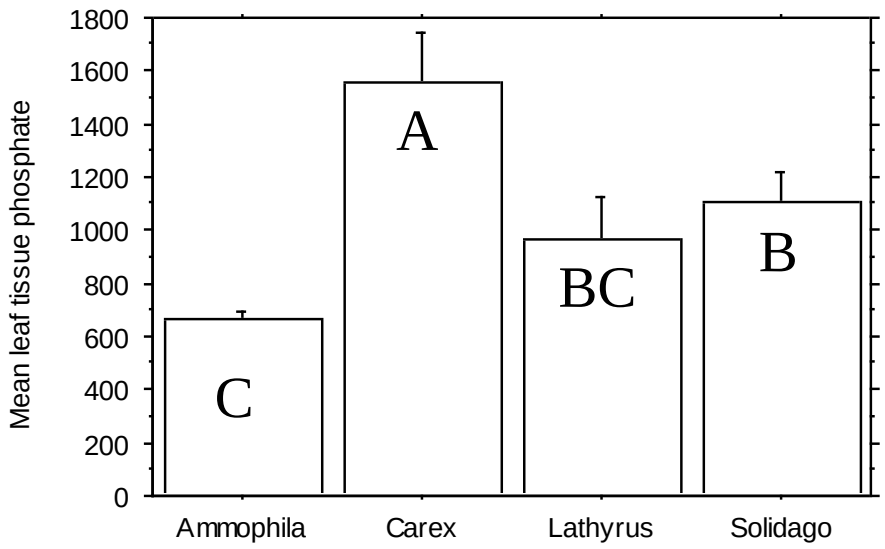


Figure 23. Mean field plant tissue leaf phosphate (µg P/g) of species sampled, with standard error of the mean [SEM]. *C. kobomugi* had significantly higher leaf tissue phosphate than *A. breviligulata* ($P < 0.0001$), *L. japonicus* ($P = 0.0019$), and *S. sempervirens* ($P = 0.0268$). Data are from assessments in 2010.

(A)

(B)

Figure 24. Photographs of holes dug to a depth of approximately 30 cm in areas of both (A) dense *C. kobomugi* and (B) dense *A. breviligulata.* These photographs were meant to demonstrate differences in the density of rhizome networks between the two study species. The high density of fine rhizomes of *C. kobomugi* may contribute to its superior dune-stabilization ability.

Figure 25. Photograph of an uprooted individual of *C. kobomugi* with a high proportion of bound sand grains. This photograph (taken in 2008) depicts the ability of the fine root system of *C. kobomugi* to bind belowground sand. Photograph courtesy of Richard Koske, 2011.

(A)

(B)

Figure 26. Photographs showing (A) an individual *C. kobomugi* located more than 200 m away from foredune patches populations and (B) a population of *C. kobomugi* located more than 80 m from dune crest in backdune areas of East Beach, Rhode Island.

(A)

(B)

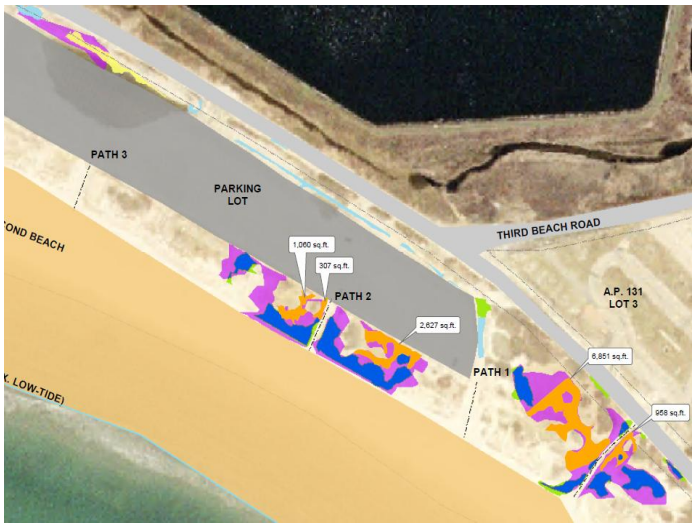
Figure 27. Photographs showing *C. kobomugi* in areas of high beach traffic (A) along a backdune road and (B) in a cleared open area under conifers at East Beach, Rhode Island.

(A)

(B)

Figure 28. Photographs showing (A) typical beach vehicle paths at East Beach and (B) vehicle paths straying into a dense area of *C. kobomugi.* These events not only contribute to the range of the invasive by creating new open-niche space (disturbance) and also have the potential to transport seeds and vegetative fragments to other locations along the dune or backdune.

(A)



(B)

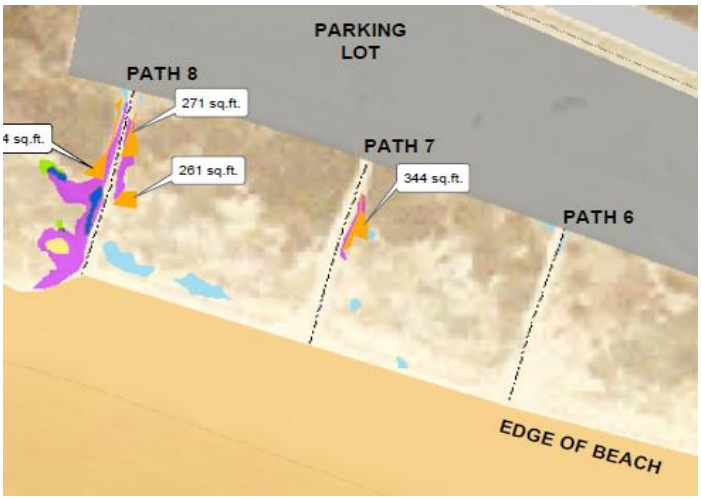


Figure 29. Details from a vegetation treatment map showing that areas of dense *C. kobomugi* occur around paths and roads on both the (A) western and (B) eastern portions of Sachuest Beach, in Middletown RI. High disturbance or traffic may contribute to the expansion of the invasive. Shaded regions represent *C. kobomugi* growth. Map obtained from Warren Hall and the Town of Middletown, Rhode Island.

Figure 30. Photograph showing *A. breviligulata* that has been disturbed by vehicles at East Beach, Rhode Island. Straying of vehicles into areas of the foredune creates disturbances that threaten to destabilize entire regions of the dune. These disturbance events could also make available open niche space for secondary invasions of *C. kobomugi*.