esa ECOSPHERE

Beachgrass invasion in coastal dunes is mediated by soil microbes and lack of disturbance dependence

Aaron S. David,[1,3,]† Georgiana May,[1,2] Derek Schmidt,[1] and Eric W. Seabloom[1]

[1]*Department of Ecology, Evolution, and Behavior, University of Minnesota, Saint Paul, Minnesota 55108 USA*
[2]*Department of Plant Biology, University of Minnesota, Saint Paul, Minnesota 55108 USA*

Citation: David, A. S., G. May, D. Schmidt, and E. W. Seabloom. 2016. Beachgrass invasion in coastal dunes is mediated by soil microbes and lack of disturbance dependence. Ecosphere 7(11):e01527. 10.1002/ecs2.1527

Abstract. Biological invasions are a threat to ecological communities and ecosystems, yet understanding the processes that mediate invasion and the environmental contexts in which these processes are important remains a challenge. In this study, we investigated how disturbance and biotic interactions with pathogens and microbial symbionts mediate invasion of American beachgrass (*Ammophila breviligulata*) in early- and late-successional coastal dune habitats. First, we tested the effect of disturbance on biomasses of two exotic beachgrass species (the invading *A. breviligulata* and the established *A. arenaria*) and a native beachgrass (*Elymus mollis*) by growing plants with and without a disturbance manipulation in early- and late-successional habitats. We quantified root colonization by fungal symbionts and infection by plant-parasitic nematodes to determine the effect of potential mutualists and pathogens on plant biomass. Second, we tested whether soil microbes associated with the established *A. arenaria* mediated *A. breviligulata* invasion by inoculating potted plants with microbes from early- and late-successional habitat. We found no effect of disturbance on biomass of the invading *A. breviligulata* or native *E. mollis*, whereas *A. arenaria* biomass increased when grown with disturbance. Colonization by arbuscular mycorrhizal fungi and by fungal endophytes were poor predictors of plant biomass, and plant-parasitic nematodes were infrequently observed. Microbe addition increased belowground biomass of *A. breviligulata*, but not *A. arenaria*, compared to control treatments. Overall, our results suggest that *A. breviligulata* invasion in both the early- and late-successional habitat is mediated by its lack of disturbance dependence and positive effects of soil microbes. These results have important implications for other primary successional systems and contribute to our understanding of how ecological contexts influence invasion processes.

Key words: *Ammophila;* arbuscular mycorrhizal fungi; disturbance; endophyte; succession.

Received 1 August 2016; accepted 15 August 2016. Corresponding Editor: Debra P. C. Peters.

[3] Present address: Department of Biology, University of Miami, Coral Gables, Florida 33146 USA.
† **E-mail:** asdavid@bio.miami.edu

INTRODUCTION

Biological invasions can radically transform ecological communities and ecosystem processes (e.g., D'Antonio and Vitousek 1992, Lodge 1993, Prach and Walker 2011, Seabloom et al. 2013). The processes that mediate the establishment and persistence of invasive plants play a critical role in the interactions among invading and established species and for the consequences of invasion to ecological communities and ecosystem processes. Two processes—response to disturbance (e.g., D'Antonio and Vitousek 1992, Seabloom et al. 2003, MacDougall and Turkington 2005) and facilitative and antagonistic interactions with biota (Maron and Vila 2001, Mitchell et al. 2006, Bever et al. 2010)—are commonly evoked to explain large-scale invasions. Disturbance and biotic interactions are not mutually exclusive, and elucidating their relative importance and the environmental conditions under which they are important remains a challenge.

The extent to which a species depends on disturbance to invade has implications for the scale of the invasion and consequences for established species. Here, we define "invading species" or "invader" as a species rapidly expanding its range after introduction to a given habitat and "established species" as those native or exotic species present prior to the arrival of the invading species. Invading species are often limited to frequently or recently disturbed environments, resulting in transient invasions and short-term negative effects on ecological communities (Seabloom et al. 2003, MacDougall and Turkington 2005). However, invading species that lack such disturbance dependence could invade established communities in the absence of disturbance. For instance, invading species that utilize unused resources by established species (Tilman 2004) or that obtain resources through interference competition with established species (e.g., Amarasekare 2002, Hart and Marshall 2012) might be predicted to successfully invade undisturbed environments. The effects of less disturbance dependent, invading species on established species can be long term (Prach and Walker 2011, David et al. 2015).

Biotic interactions with pathogens and mutualists can also mediate invasion, and these interactions are context-dependent (Mitchell et al. 2006, Kardol et al. 2007, Blumenthal et al. 2009, Pringle et al. 2009). Invading species can benefit from biotic interactions by, for example, avoiding natural enemies of the established species (Maron and Vila 2001), disrupting mutualisms of established species (Stinson et al. 2006), or arriving with their own mutualists from their native habitat (Dickie et al. 2010). Because pathogenic and mutualistic species accumulate during ecological succession, their effects on hosts increase over time (van der Putten et al. 2007, Koziol and Bever 2015). For instance, accumulation of plant-parasitic nematodes has been implicated in the decline of the beachgrass *Ammophila arenaria* in late-successional dunes in its native range (van der Putten et al. 1993). Likewise, late-successional plant species have been found to associate more often with arbuscular mycorrhizal fungi than early-successional species (Koziol and Bever 2015).

Dunes along the USA Pacific Northwest Coast represent an ecosystem particularly well suited for investigating the relative importance of disturbance and biotic interactions on plant invasion. Dunes are formed via sand deposition to create a chronosequence extending from early-successional shoreline habitat to late-successional inland habitat (Hacker et al. 2012, David et al. 2015). The dune ecosystem, particularly the early-successional habitat, is prone to disturbance caused by storm surges that wash away vegetation (Ruggiero et al. 2001). Two successive invasions of congenic plant species have occurred in this coastal dune ecosystem over the past century (Schwendiman 1977, Seabloom and Wiedemann 1994). The established species, European beachgrass *A. arenaria* (L.) Link, was introduced in the early 1900s and grows in near monoculture along the coastline. The invading species, American beachgrass *A. breviligulata* Fernald, was introduced in the 1930–1940s and has spread and displaced *A. arenaria* throughout the northwest in both early- and late-successional dune habitat (Schwendiman 1977, Seabloom and Wiedemann 1994, David et al. 2015). Both *Ammophila* species, as well as the native beachgrass *Elymus mollis* (Trin.), spread via clonal growth and are important ecosystem engineers that stabilize dunes and protect inland development from coastal flooding (Zarnetske et al. 2012, Seabloom et al. 2013).

Here, we used the *A. breviligulata* invasion to evaluate how disturbance and biotic interactions mediate the beachgrass' initial establishment in early- and late-successional habitat dominated by the established *A. arenaria*. First, we conducted a field experiment to examine the effects of physical disturbance on the performances of beachgrass species in early- and late-successional dune habitat. We also characterized the plant-parasitic nematode and root-associated fungal communities in the experimental plants to evaluate the roles of natural enemies and mutualists on invasion. Second, in a growth chamber experiment, we evaluated the role of *A. arenaria*-associated soil microbes on the performance of the two *Ammophila* species.

MATERIALS AND METHODS

Study system

The coastal dunes of Oregon are a dynamic ecosystem important for the protection of coastal development (Cooper 1958, Seabloom et al.

2013). Early-successional habitat in foredunes (sand ridges adjacent to the shoreline) is characterized by lower plant species richness, lower nutrient soils, and higher salinity than late-successional, backdune habitat further inland (David et al. 2015). In the early 1900s, the European beachgrass *A. arenaria* was introduced for dune stabilization and rapidly transformed dunes from shifting sand masses with sparse vegetation to stabilized, near-monocultures of beachgrass (Schwendiman 1977). Later, in the 1930–1940s, the American beachgrass *A. breviligulata* was introduced from the Eastern United States to the Southern Washington coast and, in a rarely documented instance of nearly complete regional competitive exclusion, has rapidly displaced *A. arenaria* on most of the dunes (Seabloom and Wiedemann 1994, Hacker et al. 2012, David et al. 2015). Additionally, the *A. breviligulata* invasion has decreased plant diversity and dune height, leading to reduced coastal protection from flooding (Seabloom and Wiedemann 1994, Hacker et al. 2012, Seabloom et al. 2013). Beachgrass species are dispersed onto new beaches as tillers (defined here as a stem, rhizome, and root) via ocean transport and, while seed germination has been observed, these species primarily rely on rhizomatous growth for propagation (Wiedemann and Pickart 1996). Therefore, we used harvested tillers rather than seedlings in the following experiments.

Experiment 1: disturbance study

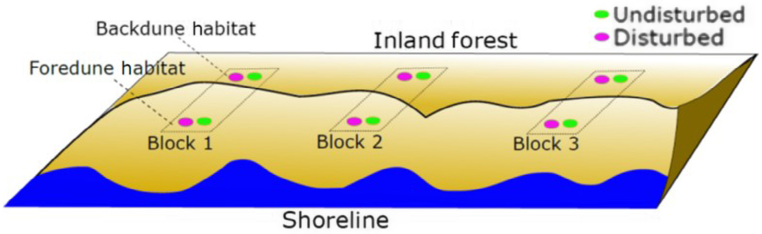
To quantify the disturbance dependence of each beachgrass species, we grew individuals with and without a disturbance manipulation in the early-successional foredune and late-successional backdune habitats. The study was conducted at three sites along the Oregon coast where *A. arenaria* was dominant: Pacific City (Bob Straub State Park, Pacific City, Oregon, USA, 45°10′ N, 123°58′ W), Sand Lake (Sand Lake Recreation Area, Cloverdale, Oregon, USA, 45°17′ N, 123°57′ W), and Cape Meares (Bayocean Peninsula County Park, Tillamook, Oregon, USA, 45°30′ N, 123°57′ W). At each site, we haphazardly selected locations for three experimental blocks (Fig. 1). Each block consisted of two pairs of adjacent, circular plots (0.6 m diameter), of which one pair was established in the early-successional habitat and the other pair in the late-successional habitat. We randomly assigned each plot within a pair to either the "disturbed" or "undisturbed" treatment. Within plots, we planted two individual tillers of each plant species collected from one of two sources, the foredune or backdune (3 plant species × 2 tiller sources = 6 plants per plot). In total, there were 216 experimental tillers at the start of the experiment (3 sites × 3 blocks per site × 4 plots per block × 6 plants per plot). The experiment ran for 11 weeks from May to August 2012.

The early-successional foredune habitat plots were established on the shoreside slopes of the dunes, and the late-successional backdune habitat plots were established behind dunes at the edge of herbaceous dune habitat (Fig. 1). Soils in foredune plots were characterized by higher pH, higher sodium concentrations, and lower total carbon and nitrogen concentrations than those in backdune plots (A. S. David, E. W. Seabloom, and G. May, *unpublished manuscript*). Backdune plots at the beginning of the experiment were characterized by higher plant species richness than foredune plots (A. S. David, E. W. Seabloom, and G. May, *unpublished manuscript*).

To manipulate disturbance, we manually dug out all soil to a depth of 0.6 m and sieved the soil back into the plot using a 0.635-cm screen to remove plant roots and debris. We did not observe colonization from naturally occurring plants in any of the disturbed plots throughout the duration of the experiment. Plots assigned to the undisturbed treatment were not modified.

We transplanted tillers of each beachgrass species collected in the field to the experimental plots. Tillers of *E. mollis*, *A. arenaria*, and *A. breviligulata* were collected in May 2012 from the Pacific City site (see details below) where all three plant species co-occurred. Because tillers have preexisting microbial symbionts that differ between the foredune and backdune (David et al. 2016*b*), we used tillers collected from both habitats. Following collection, tillers were soaked in tap water for 10 d to stimulate root growth and increase survival in the field. Tillers were soaked in individual containers to avoid potential dispersal of microbes among different individuals and were trimmed to a single stem and two leaves to standardize aboveground size. Pretreatment wet weight, tiller height, and rhizome length were then measured to estimate pretreatment dry weight (Appendix S1).

(A) Dune cross section



(B) Example planting within plot

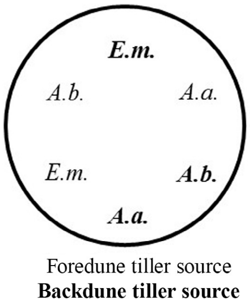


Fig. 1. Schematic of experimental design. The experiment was replicated at three sites, with three experimental blocks established within each site. (A) Dune cross section showing experimental plots in relation to foredune (near shoreline) and backdune (near inland forest) at each site. Each block contained one pair of plots in the foredune and another pair of plots in the backdune. Plots were randomly assigned to "disturbed" or "undisturbed." (B) Example of planting within plot. Six individual grass tillers were planted in each plot at randomly assigned planting positions. Tillers originated from a foredune source or a backdune source. Three beachgrass species were used as follows: *Elymus mollis* (E.m.), *Ammophila arenaria* (A.a.), and *A. breviligulata* (A.b.). Graphics credit: Monica Watson.

Pretreatment characterization of endophyte communities showed that fore- and backdune sources tillers significantly differed in community composition and that foredune tillers had higher incidences of the fungal endophyte *Microdochium bolleyi* (A. S. David, E. W. Seabloom, and G. May, *unpublished manuscript*).

At the conclusion of the experiment, we harvested all above- and belowground biomass of the experimental tillers. Approximately 30 cm of root tissue from each surviving plant was collected for microscopy and culturing of microbial symbionts (see below). For the subset of plants used for root nematode extraction (see below), we removed all remaining roots, and these roots were weighed after the extraction and included in biomass estimates. The remaining plant material was dried at 60°C for 3 d, and above- and belowground biomass was recorded.

We characterized fungal symbiont communities using microscopy and culture-based approaches. We quantified colonization by arbuscular mycorrhizal fungi and fungal endophytes using microscopy. Roots were washed in tap water and stored in 50% ethanol until staining. We used the ink and vinegar method (Vierheilig et al. 1998) to clear and stain fungal structures. Roots were soaked in 10% KOH solution and stained using Parker blue ink (5% ink: acetic acid). We used the line-intersect method (McGonigle et al. 1990) to record the presence of arbuscular mycorrhizae hyphae, dark septate endophyte hyphae, and microsclerotia (fungal resting structures) in 40 fields of view (200×) using a compound light microscope.

We used the culture-based approach to identify the most common fungal endophytes associating with beachgrass species. To culture fungal root endophytes, we used a modified approach described in David et al. (2016*b*) (Appendix S1). Briefly, root tissue was surface sterilized with successive rinses of ethanol and bleach. We placed 20 segments (~1.5 mm^2 each) of root tissue on 2% malt extract agar. We extracted DNA from emergent fungal colonies, amplified the internal transcribed spacer (ITS) region using polymerase chain reaction (PCR), and sequenced the resulting amplicons. Sequence reads were clustered into operational taxonomic units (OTUs) at 97% similarity and assigned taxonomy based on a Bayes classifier. Similar to previous findings (David et al. 2016*b*), the most common fungal endophyte was *M. bolleyi*, and we include this endophyte in our analyses (Appendix S2).

To evaluate the role of pathogens, we surveyed plant-parasitic nematodes found in the soil and roots. Nematodes are an important group of pathogens for beachgrass and contribute to their population decline in late-successional habitat (van der Putten et al. 1993). To survey ectoparasitic nematodes residing in the soil, we used the Baermann funnel method. Soil from outside each pair of disturbed and undisturbed plots was first passed through a 0.635-cm mesh screen to remove plant roots and debris in the field. In the laboratory, we extracted nematodes from a total of 200 g of soil for each sample over the course of 5 d. To survey endoparasitic nematodes residing within roots, we placed roots in a mist chamber for 7 d and collected nematodes that emerged from a subset of 32 plants in the Sand Lake site undisturbed plots. For both protocols, we counted all plant-parasitic nematodes (defined as those with a stylet) in the sample and identified these to genus when possible.

All analyses were conducted using R version 3.2.3 (R Core Team 2015), the lme4 package (Bates et al. 2015), and the MuMIn package (Barton 2014). We analyzed plant responses to experimental factors using separate mixed-effects models for each plant species and analyzed log-transformed total biomass, aboveground biomass, and belowground biomass as response variables. We modeled response variables as a function of the log-transformed pretreatment dry weight, source of the individual, the presence or absence of AMF, dark septate endophyte hyphae, microsclerotia, and *M. bolleyi*, disturbance treatment, habitat, and the two-way interactions between habitat, disturbance treatment, and the source. We used plot, plot pairings, block, and site as nested random effects. Using the *dredge()* function in the MuMIn package (Barton 2014), we identified and averaged all models within four AIC units of the best model. We report the conditional model-averaged coefficients and the importance values (weighted percentage of models within 4 AIC units in which the term appears).

We analyzed responses of the fungal symbionts to the experimental treatments. We analyzed the effects of the plant species, tiller source, disturbance treatment, dune habitat, and the two-way interactions between tiller source, disturbance, and dune habitat on each symbiont's incidence (when the symbiont was present on an individual tiller) using generalized mixed-effects models with binomial error with random variables of plot nested within block nested within site. We used the model-averaging approach described above to evaluate the significance and importance of model terms.

Ectoparasitic nematode abundance in soil was analyzed using a generalized linear mixed-effects model with Poisson error using block nested within site as a random effect and habitat as a fixed effect. Endoparasitic nematodes in roots were too infrequent to formally analyze due to lack of statistical power, and we instead report the observed count data.

Experiment 2: microbe addition study

The purpose of the *Microbe Addition Study* was to quantify the effects of soil microbes associated with the established *A. arenaria* on the performance of *A. arenaria* and the invading *A. breviligulata*. We collected soil from the Pacific City site because it occurred at the edge of the southern invasion front of *A. breviligulata* and was therefore most relevant for testing the effects of *A. arenaria*-associated microbes on invasion. We made experimental filtrates representative of the foredune and backdune microbial communities associated with *A. arenaria* and a control filtrate of deionized (DI) water. To make the soil filtrates, we first passed the soil through a 0.635-cm mesh screen in the field. In the laboratory, we mixed 18.2 kg of soil with 5 L of DI water and let the mixture soak for 4 h. The liquid filtrate was poured off and passed through successive screens of 250-μm, 63-μm, and 38-μm mesh to remove plant debris, nematodes, and sand particles. The three filtrates contained negligible differences in total dissolved nitrogen, and different fungal and bacterial taxa, suggesting that any effects caused by the filtrates could be attributed to the microbes present and not to differences in nutrient content (Appendix S2).

We inoculated the filtrates onto experimental tillers in a growth chamber. As in Experiment 1, we collected and treated tillers from the fore- and backdune source habitats. Tillers were potted in 25.2 cm depth cone-tainers (Stuewe and Sons, Tangent, Oregon, USA) in a 50:50 mix of Nurserymen's Preferred Playsand (TCC Materials, Mendota Heights, Minnesota, USA) and autoclaved Sunshine Professional Growing Mix potting mix (Sun Gro Horticulture, Agawam, Massachusetts, USA). Pots were placed under controlled conditions (23°C, 16:8 h, day: night, 400-W high-pressure sodium bulbs) in a walk-in growth chamber (Jordon Model HWT-9219; Jordon Group Refrigeration, Oldham, UK). Within the growth chamber, two blocks were established on different shelves under separate light sources. Individual plants were randomly assigned to one of two blocks and one soil filtrate treatment, to give a total of three replicates for each species × source × soil filtrate combination in each block. Beginning one day after planting, plants were inoculated with 50 mL of their assigned soil filtrate for three consecutive days. To measure plant performance, plants were harvested after 16 weeks, and separated into belowground, live aboveground, and dead aboveground tissues, dried at 60°, and weighed. We characterized fungal symbionts using the culturing and microscopy techniques described for Experiment 1.

We analyzed the effect of the soil filtrate on plant performance using linear models with two independent contrasts. The first contrast tested for the overall effect of microbes on the plants by comparing the fore- and backdune filtrates versus the control. The second contrast tested for differences between the fore- and backdune filtrates. We also included block, log-transformed pretreatment dry weight, source of the tiller, plant species, and the interaction between plant species and filtrate contrasts in the model. We performed *F* tests to test for overall treatment differences. We considered the response variables of log-transformed live aboveground biomass, belowground biomass, root: shoot ratio, and total biomass.

All data sets are available for download through the Data Repository for the University of Minnesota (David et al. 2016*a*).

RESULTS

Experiment 1: disturbance study

We found evidence that *A. arenaria* performance was disturbance-dependent and limited by dune habitat, whereas *A. breviligulata* and *E. mollis* performance did not depend on these factors. *A. arenaria* had 23% greater total biomass and 34% greater aboveground biomass (conditional model-averaged coefficients) with disturbance (Fig. 2; Appendix S2). In contrast, neither total biomass nor aboveground biomass of either *E. mollis* or *A. breviligulata* showed significant differences between disturbed and undisturbed treatments (Fig. 2; Appendix S2). Furthermore, *A. arenaria* had 47% lower belowground biomass in the backdune habitat compared with the foredune habitat, and neither *A. breviligulata* nor *E. mollis* showed differences in biomass between habitats (Fig. 2; Appendix S2). We found no significant interactions between the disturbance treatment and habitat for any of the three beachgrass species (Appendix S2), indicating that there were no differences in the plant species' responses to disturbance between the early-successional foredune and the late-successional backdune habitats. The source of the tiller influenced total biomass for *E. mollis* and *A. arenaria*. The *E. mollis* tillers from the backdune source achieved 24% greater total biomass and 28% greater aboveground biomass than tillers from the foredune source (Appendix S2). *A. arenaria* tillers from the backdune source had 61% lower belowground biomass compared to those from the foredune source (Appendix S2).

The presence of the four symbionts (arbuscular mycorrhizal fungi, dark septate endophyte hyphae, and microsclerotia structures observed from microscopy, and *M. bolleyi* observed from culturing) was not significant predictors of biomass for any of the three beachgrass species. *E. mollis* aboveground biomass was 29% less when AMF were present, but this result had a low importance value (importance = 0.58) and was only marginally significant (Appendix S2).

We found that plant species, tiller source, disturbance, and dune habitat predicted symbiont incidence and colonization to varying degrees (Appendix S2). None of the interactions in any model had high importance values (importance <0.62 for all interaction terms in all

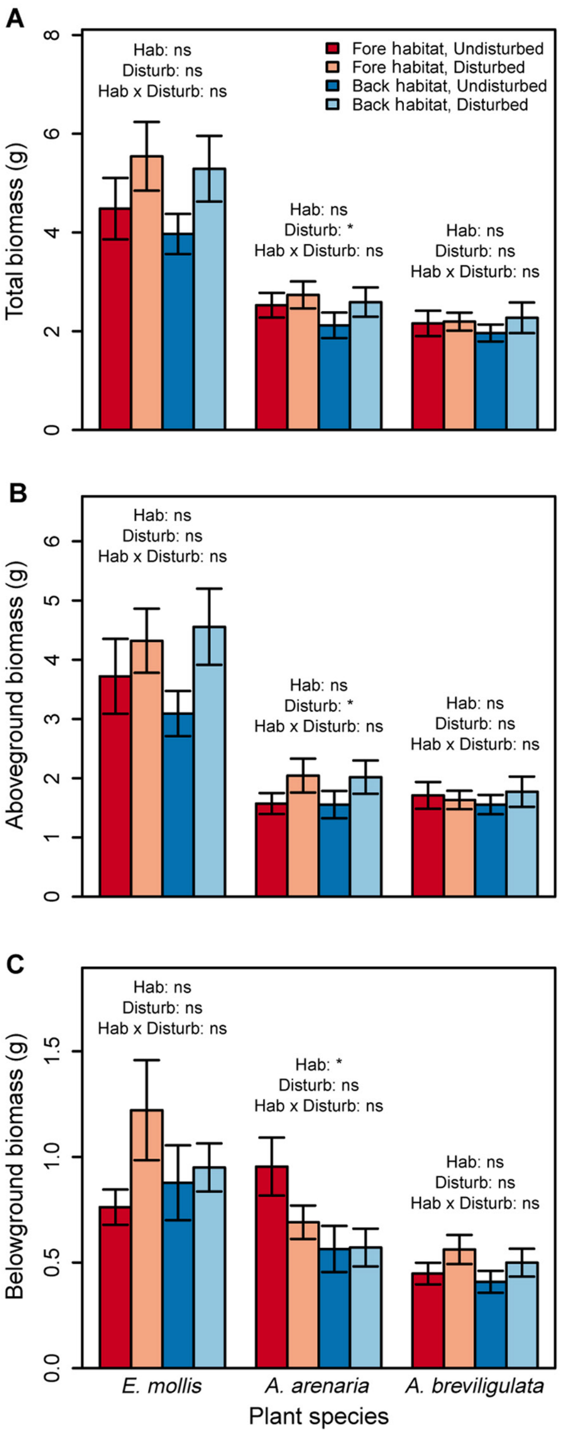


Fig. 2. Mean biomass of the three plant species in the habitat (Hab) and disturbance (Disturb) treatments. (A) Total biomass, (B) aboveground biomass, and (C) belowground biomass. Tillers of each species were grown in factorial combination in foredune/backdune habitats and disturbed/undisturbed plots. Error bars show 1 SEM. Significance determined by model-averaged coefficients shown in Appendix S2. The * denotes significance at the $P < 0.05$ level, and "ns" denotes not significant.

models). Plant species was the primary factor influencing the incidence of arbuscular mycorrhizal fungi (74.5% incidence with *E. mollis*, 46.8% with *A. arenaria*, 41.5% with *A. breviligulata*) and was included in all averaged models (importance = 1.0; Fig. 3). Plant species was also important for microsclerotia incidence (importance = 0.73; Fig. 3). The odds of microsclerotia incidence with *A. arenaria* were 45.1% lower than with *A. breviligulata*, but did not differ between *E. mollis* and *Ammophila* species (Appendix S2). Plant species was not important for incidences of either dark septate endophyte hyphae or *M. bolleyi* (Fig. 3; Appendix S2). Tiller source was associated with microsclerotia (importance = 1.0) and *M. bolleyi* incidence

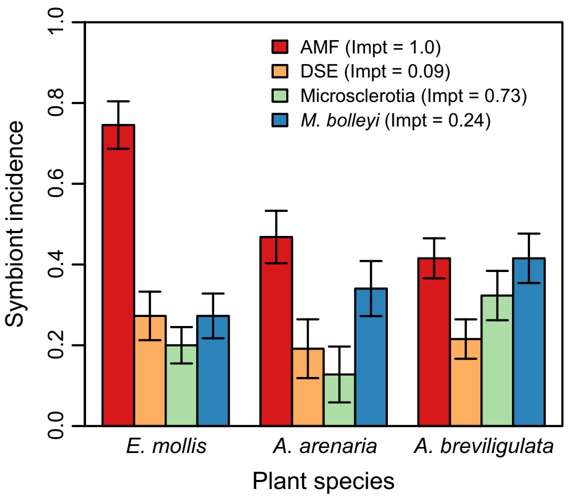


Fig. 3. Incidences of symbionts in the Disturbance Study. Figure shows the mean incidence ±1 binomial SEM of arbuscular mycorrhizal fungi (AMF), dark septate endophyte hyphae (DSE), microsclerotia, and cultured *Microdochium bolleyi* in *Elymus mollis*, *Ammophila arenaria*, and *A. breviligulata*. Legend shows the importance value for "plant species" in the model for each symbiont. Importance values were calculated as the weighted proportion of the number of models in which the term appeared. See Appendix S2 for full results.

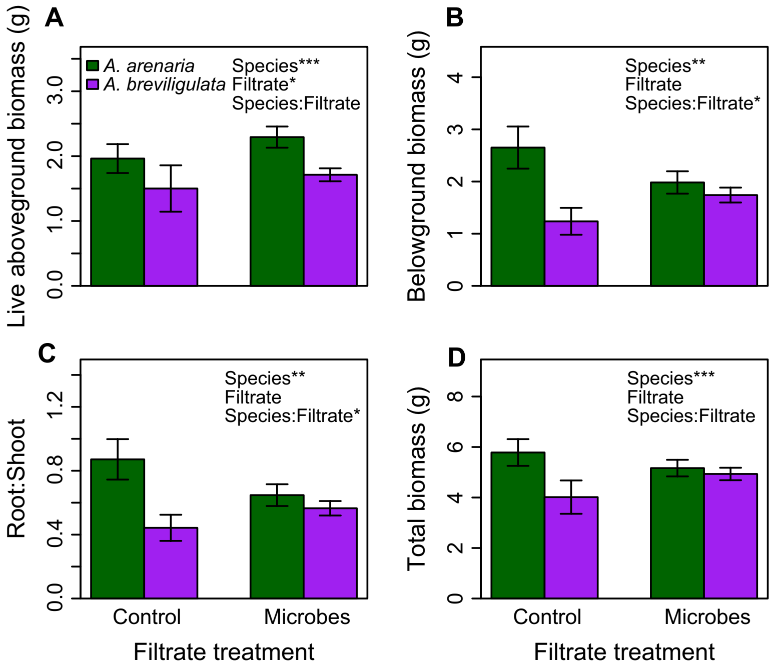


Fig. 4. Results from growth chamber experiment. (A) Aboveground biomass, (B) belowground biomass, (C) root: shoot ratio, and (D) total biomass. Plots show the effect of microbes (foredune and backdune filtrates) compared with the control filtrate. Points show mean ± 1 SEM. Significance of F tests of species, microbial filtrate, and species × filtrate interaction shown, * denotes $P < 0.05$, ** denotes $P < 0.01$, and *** denotes $P < 0.001$. See Appendix S2 for detailed results of statistical tests.

(importance = 1.0), and foredune sourced tillers had higher incidences of both symbionts than did backdune sourced tillers (Appendix S2). Disturbance increased the odds of microsclerotia incidence by 72.3%. Disturbance (importance 0.97) and dune habitat (importance = 0.81) were included in the models for *M. bolleyi*, but neither factor significantly influenced *M. bolleyi* incidence (Appendix S2).

Plant-parasitic nematode abundance in soils and roots was generally low, and the genera included *Helicotylenchus*, *Pratylenchus*, and *Paratylenchus*. Ectoparasitic nematodes in soil were more abundant in backdunes (mean 11.6 ± 3.7 SEM per 200 g soil) than in foredunes (mean 2.67 ± 0.90 SEM per 200 g soil) (generalized linear mixed-model $P < 0.001$). Endoparasitic nematodes in roots were also rare (0–3 per sample), although we did find two infestations (1184 and 57 endoparasitic nematodes, respectively) that we were unable to identify to genus among the nine *E. mollis* root samples surveyed.

Experiment 2: microbe addition study

Soil microbes increased aboveground biomass for both *Ammophila* species, but equalized species differences in belowground biomass. The microbial filtrates increased live aboveground biomass by 7.6% in *A. arenaria* and by 9.5% in *A. breviligulata* relative to the control filtrate, although there were no significant differences between the fore- and backdune filtrates or between *Ammophila* species responses to the filtrate (Fig. 4A; Appendix S2). The microbial filtrates did differentially affect belowground biomass in the two *Ammophila* species by increasing belowground biomass by 15.5% in *A. breviligulata* relative to the control filtrate and decreasing belowground biomass in *A. arenaria* by 8.1%, although this decrease was not statistically significant (Fig. 4B; Appendix S2). We found no differences between fore- and backdune filtrates on belowground biomass (Appendix S2). Similarly, the microbial filtrates also increased the root: shoot ratio of *A. breviligulata* by 9.8% relative to the control filtrate indicating greater belowground investment, and marginally significantly decreased root: shoot ratio in *A. arenaria* by 9.7% (Fig. 4C; Appendix S2). Finally, there were no significant effects of the filtrate treatments on total biomass of either species (Fig. 4D; Appendix S2); however, *A. breviligulata* biomass was 11% greater in microbial treatments than in the controls, and this effect was marginally significant (Appendix S2). The incidence of AMF (7.0% of individuals), dark septate endophyte hyphae (4.2%), microsclerotia (2.8%), and *M. bolleyi* (20%) was generally low.

Discussion

In this study, we evaluated the roles of disturbance dependence and biotic interactions in mediating invasion of the Eastern United States beachgrass *A. breviligulata* into primary successional dunes along the USA Pacific Northwest coast. Our results suggest that growth of the invading *A. breviligulata*, compared with the established European beachgrass *A. arenaria* and native *Elymus mollis*, is less dependent on disturbance. Furthermore, biotic interactions between beachgrasses and soil microbes increased belowground growth of *A. breviligulat*a while slightly decreasing belowground growth of the *A. arenaria*. We found little evidence that escape from pathogenic nematodes or increased associations with potentially mutualistic fungi played a role in the invasion. *E. mollis* had infestations of nematodes and a higher likelihood of colonization by arbuscular mycorrhizal fungi than did either *Ammophila* species, but there were no differences in nematode abundance of AMF colonization between *A. arenaria* and *A. breviligulata*. Importantly, we found no evidence suggesting that the importance of disturbance dependence or biotic interactions differed between the early-successional foredune and late-successional backdune. Overall, our findings contribute to a broader understanding of how ecological context influences the mechanisms driving biological invasions.

Our results show that *A. breviligulata* does not require disturbance to invade established *A. arenaria* stands and implicate *A. breviligulata* as a direct driver of change in plant communities of coastal dunes. The *A. breviligulata* invasion, which has resulted in the decline of native plant species diversity and increased risk of coastal flooding (Hacker et al. 2012, Seabloom et al. 2013), is not simply the result of frequent disturbance. Previous work has shown that positive interactions among beachgrasses may even facilitate *A. breviligulata* invasion (Zarnetske et al. 2013). Species not limited to disturbed habitat may invade a broad range of habitats and harm native species diversity and ecosystem function (D'Antonio 1993, Lodge 1993). Our results therefore contribute to the idea that *A. breviligulata* is a "driver" of change in coastal dunes, and not solely a "passenger" that capitalizes on and is limited by disturbance (sensu MacDougall and Turkington 2005).

We also found support that biotic interactions between beachgrass and soil microbes mediate *A. breviligulata* invasion. The microbial filtrates in the *Microbe Addition Study* increased *A. breviligulata* belowground biomass but decreased that of *A. arenaria*. While our study only used soil microbes collected from a single site and cannot account for variability in soil microbial communities among sites, the site from which we collected microbes was located at the invasion front and therefore appropriate for understanding the initial stages of invasion. We suggest that the species' differential responses to microbes associated with *A. arenaria* soil could mediate competitive interactions between the two *Ammophila* species in the early stages of invasion. Microbial effects generated by plant–soil feedbacks allow for both stabilizing mechanisms (increased intraspecific competition relative to interspecific competition) and equalizing mechanisms (minimized fitness difference between species) for coexistence (Adler et al. 2007, Bever et al. 2010). Our results suggest microbes associated with the established *A. arenaria* could serve as a fitness-equalizing mechanism for belowground growth between the two species by reducing belowground growth of *A. arenaria* and increasing that of *A. breviligulata*. Yet, we caution that because our study was limited to the initial stage of *A. breviligulata* invasion, we cannot make long-term predictions about the role of soil microbes for coexistence among beachgrass species.

Our results contribute to a broader understanding of how soil pathogens affect beachgrass performance. Other studies of *A. arenaria* in its invasive and native ranges have similarly found negative effects of associated soil microbes on *A. arenaria* biomass (Beckstead and Parker 2003, Brinkman et al. 2005). In one study conducted in California south of our study sites, soil microbes reduced both belowground and aboveground biomass of *A. arenaria* seedlings suggestive of soil pathogens (Beckstead and Parker 2003). While we observed a positive effect of microbes on *A. arenaria* aboveground biomass, this discrepancy with Beckstead and Parker's (2003) results could be attributable to several differences in experimental designs, including inoculating tillers versus seedlings. Furthermore, modeling work shows that accumulation of microbes in the soil by *A. arenaria* reduces abundance of native species through spillover effects (Eppinga et al. 2006). It is possible that *A. breviligulata*, which is associated with lower native plant abundance than is *A. arenaria* (Hacker et al. 2012), could similarly accumulate microbes in the soil that suppress native plant abundance, and this may be an area for future work. In contrast, plant-pathogenic nematodes, despite strong negative effects on *A. arenaria* in its native European range, are rare in the introduced range and likely do not contribute to beachgrass invasion. Our findings of low nematode abundances match those of Beckstead and Parker (2003), who reported low abundances of nematodes in coastal dunes of California south of our study system. In a global study of nematodes associated with *A. arenaria*, van der Putten et al. (2005) showed *A. arenaria* in Oregon harbored a similar diversity of nematode taxa as did neighboring native grasses, further suggesting little effect of nematodes on beachgrass invasion.

Mutualists such as arbuscular mycorrhizal fungi and fungal endophytes are often implicated for their roles in plant invasion (Pringle et al. 2009, Aschehoug et al. 2012), but we found no evidence for this in our study. The incidence of AMF was highest in the native *E. mollis* compared to the two *Ammophila* species. However, we found that plant biomass was not significantly associated with AMF infection. Nevertheless, if plant species associate with or respond uniquely to different AMF species, this could suggest a more complex role for AMF in mediating plant invasion (Richardson et al. 2000, Klironomos 2003, Pringle et al. 2009). Similarly, we found little evidence that fungal endophytes play a role in invasion. We found no significant associations between incidence of dark septate endophyte hyphae, microsclerotia, or *M. bolleyi* on plant biomass. It is possible that other symbionts that were not measured could mediate invasion. For instance, nitrogen-fixing bacteria (*Burkholderia* spp.) that are found on beachgrass roots can provide nitrogen to plant species found in our study region (Dalton et al. 2004). In other dune systems, aboveground fungal endophytes such as *Fusarium culmorum* and *Epichloë* species can also provide benefits such as tolerance to salt stress (Rodriguez et al. 2008) or drought (Emery et al. 2010), respectively, yet our previous work showed that these particular endophyte taxa were not present in our system (David et al. 2016*b*). Our results do suggest further investigation into the role of root endophytes producing microsclerotia on plant fitness. While presence of microsclerotia was not predictive of plant biomass, microsclerotia were found most frequently in roots of the invading *A. breviligulata*. Dark septate endophytes, which can produce microsclerotia, are known to provide mutualistic benefits to their hosts, but their effects widely vary across host species or genotypes and endophyte strain (Newsham 2011, Mandyam et al. 2013).

Biological invasions may occur via several mechanisms, and determining the ecological contexts in which different mechanisms operate is critical to our understanding of the invasion process. We have shown that the invasion of *A. breviligulata* into early and late-successional USA Pacific Northwest dunes is likely mediated by the species' lack of disturbance dependence and by *A. arenaria*-associated microbes. We found little evidence that soil pathogens or mutualists mediate the invasion. Disentangling the many hypothesized mechanisms for invasion is essential for determining the underlying causes and future consequences of invasion and informing management decisions.

ACKNOWLEDGMENTS

We thank S. Hacker, J. Spatafora, and R. Ingham for providing laboratory facilities. R. Ingham and N. Wade assisted with nematode identification. This manuscript was greatly improved by comments and suggestions from P. Kennedy, L. Kinkel, and D. Tilman, as well as B. Grewell and two anonymous reviewers. We thank Oregon Parks and Recreation Department and

Tillamook County Parks Department for granting us permits to conduct this research. Funding was provided by the NSF Integrative Graduate Education and Research Traineeship Introduced Species and Genotypes program (DGE-0653827), NSF Graduate Research Fellowship program (NSF 00039202), and University of Minnesota Rothman Fellowship to ASD, NSF Dimensions of Biodiversity (DEB 1045608) to GM, the University of Minnesota Undergraduate Research Assistantship to DS, and the Environmental Protection Agency (EPA/NCER R833836) to EWS.

Literature Cited

Adler, P. B., J. Hillerislambers, and J. M. Levine. 2007. A niche for neutrality. Ecology Letters 10:95–104.

Amarasekare, P. 2002. Interference competition and species coexistence. Proceedings of the Royal Society B: Biological Sciences 269:2541–2550.

Aschehoug, E., K. Metlen, R. Callaway, and G. Newcombe. 2012. Fungal endophytes directly increase the competitive effects of an invasive forb. Ecology 93:3–8.

Barton, K. 2014. MuMIn: multi-model inference. R package version. 1.12.1. http://CRAN.R-project.org/package=MuMIn

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48.

Beckstead, J., and I. Parker. 2003. Invasiveness of *Ammophila arenaria*: Release from soil-borne pathogens? Ecology 84:2824–2831.

Bever, J. D., I. A. Dickie, E. Facelli, J. M. Facelli, J. Klironomos, M. Moora, M. C. Rillig, W. D. Stock, M. Tibbett, and M. Zobel. 2010. Rooting theories of plant community ecology in microbial interactions. Trends in Ecology and Evolution 25:468–478.

Blumenthal, D., C. E. Mitchell, P. Pysek, and V. Jarosík. 2009. Synergy between pathogen release and resource availability in plant invasion. Proceedings of the National Academy of Sciences USA 106:7899–7904.

Brinkman, E. P., S. R. Troelstra, and W. H. van der Putten. 2005. Soil feedback effects to the foredune grass *Ammophila arenaria* by endoparasitic root-feeding nematodes and whole soil communities. Soil Biology and Biochemistry 37:2077–2087.

Cooper, W. S. 1958. Coastal sand dunes. Geological Society of America, Memoir 72, Boulder, Colorado, USA.

Dalton, D. A., S. Kramer, N. Azios, S. Fusaro, E. Cahill, and C. Kennedy. 2004. Endophytic nitrogen fixation in dune grasses (*Ammophila arenaria* and *Elymus mollis*) from Oregon. FEMS Microbiology Ecology 49:469–479.

D'Antonio, C. M. 1993. Mechanisms controlling invasion of coastal plant communities by the alien succulent *Carpobrotus edulis*. Ecology 74:83–95.

D'Antonio, C. M., and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual Review of Ecology and Systematics 23:63–87.

David, A. S., E. W. Seabloom, and G. May. 2016*a*. Data files for beachgrass invasion and root-associated fungi studies. Data Repository for University of Minnesota. http://conservancy.umn.edu/handle/11299/181235

David, A. S., E. W. Seabloom, and G. May. 2016*b*. Plant host species and geographic distance affect the structure of aboveground fungal symbiont communities, and environmental filtering affects belowground communities in a coastal dune ecosystem. Microbial Ecology 71:912–926.

David, A. S., P. L. Zarnetske, S. D. Hacker, P. Ruggiero, R. G. Biel, and E. W. Seabloom. 2015. Invasive congeners differ in successional impacts across space and time. PLoS One 10:e0117283.

Dickie, I. A., N. Bolstridge, J. A. Cooper, and D. A. Peltzer. 2010. Co-invasion by Pinus and its mycorrhizal fungi. New Phytologist 187:475–484.

Emery, S. M., D. Thompson, and J. A. Rudgers. 2010. Variation in endophyte symbiosis, herbivory and drought tolerance of *Ammophila breviligulata* populations in the Great Lakes Region. American Midland Naturalist 163:186–196.

Eppinga, M. B., M. Rietkerk, S. C. Dekker, P. C. De Ruiter, and W. H. Van Der Putten. 2006. Accumulation of local pathogens: a new hypothesis to explain exotic plant invasions. Oikos 114:168–176.

Hacker, S. D., P. Zarnetske, E. Seabloom, P. Ruggiero, J. Mull, S. Gerrity, and C. Jones. 2012. Subtle differences in two non-native congeneric beach grasses significantly affect their colonization, spread, and impact. Oikos 121:138–148.

Hart, S. P., and D. J. Marshall. 2012. Advantages and disadvantages of interference-competitive ability and resource-use efficiency when invading established communities. Oikos 121:396–402.

Kardol, P., N. J. Cornips, M. M. L. van Kempen, J. M. T. Bakx-Schotman, and W. H. van der Putten. 2007. Microbe-mediated plant-soil feedback causes historical contingency effects in plant community assembly. Ecological Monographs 77:147–162.

Klironomos, J. N. 2003. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology 84:2292–2301.

Koziol, L., and J. D. Bever. 2015. Mycorrhizal response trades off with plant growth rate and increases with plant successional status. Ecology 96:1768–1774.

Lodge, D. M. 1993. Biological invasions: lessons for ecology. Trends in Ecology and Evolution 8:133–137.

MacDougall, A., and R. Turkington. 2005. Are invasive species the drivers or passengers of change in degraded ecosystems? Ecology 86:42–55.

Mandyam, K. G., J. Roe, and A. Jumpponen. 2013. *Arabidopsis thaliana* model system reveals a continuum of responses to root endophyte colonization. Fungal Biology 117:250–260.

Maron, J. L., and M. Vila. 2001. When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. Oikos 95:361–373.

McGonigle, T. P., M. H. Miller, D. G. Evans, G. L. Fairchild, and J. A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytologist 115:495–501.

Mitchell, C. E., et al. 2006. Biotic interactions and plant invasions. Ecology Letters 9:726–740.

Newsham, K. K. 2011. A meta-analysis of plant responses to dark septate root endophytes. New Phytologist 190:783–793.

Prach, K., and L. R. Walker. 2011. Four opportunities for studies of ecological succession. Trends in Ecology and Evolution 26:119–123.

Pringle, A., J. D. Bever, M. Gardes, J. L. Parrent, M. C. Rillig, and J. N. Klironomos. 2009. Mycorrhizal symbioses and plant invasions. Annual Review of Ecology, Evolution, and Systematics 40:699–715.

R Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org

Richardson, D. M., N. Allsopp, C. M. D'Antonio, S. J. Milton, and M. Rejmánek. 2000. Plant invasions–the role of mutualisms. Biological Reviews of the Cambridge Philosophical Society 75:65–93.

Rodriguez, R. J., J. Henson, E. Van Volkenburgh, M. Hoy, L. Wright, F. Beckwith, Y.-O. Kim, and R. S. Redman. 2008. Stress tolerance in plants via habitat-adapted symbiosis. ISME Journal 2:404–416.

Ruggiero, P., P. D. Komar, W. G. Mcdougal, J. J. Marra, and A. Reggie. 2001. Wave runup, extreme water levels and the erosion of properties backing beaches. Journal of Coastal Research 17:407–419.

Schwendiman, J. L. 1977. Coastal sand dune stabilization in the Pacific Northwest. International Journal of Biometeorology 21:281–289.

Seabloom, E. W., W. S. Harpole, O. J. Reichman, and D. Tilman. 2003. Invasion, competitive dominance, and resource use by exotic and native California grassland species. Proceedings of the National Academy of Sciences USA 100:13384–13389.

Seabloom, E. W., P. Ruggiero, S. D. Hacker, J. Mull, and P. Zarnetske. 2013. Invasive grasses, climate change, and exposure to storm-wave overtopping in coastal dune ecosystems. Global Change Biology 19:824–832.

Seabloom, E. W., and A. M. Wiedemann. 1994. Foredunes of the Washington distribution and effects of *Ammophila breviligulata* Fern. (American Beach Grass) on the foredunes of the Washington coast. Journal of Coastal Research 10:178–188.

Stinson, K. A., S. A. Campbell, J. R. Powell, B. E. Wolfe, R. M. Callaway, G. C. Thelen, S. G. Hallett, D. Prati, and J. N. Klironomos. 2006. Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. PLoS Biology 4:e140.

Tilman, D. 2004. Niche tradeoffs, neutrality, and community structure: a stochastic theory of resource competition, invasion, and community assembly. Proceedings of the National Academy of Sciences USA 101:10854–10861.

van der Putten, W. H., J. N. Klironomos, and D. A. Wardle. 2007. Microbial ecology of biological invasions. ISME Journal 1:28–37.

van der Putten, W. H., C. Van Dijk, and B. A. M. Peters. 1993. Plant-specific soil-borne diseases contribute to succession in foredune vegetation. Nature 362:53–56.

van der Putten, W. H., G. W. Yeates, H. Duyts, C. S. Reis, and G. Karssen. 2005. Invasive plants and their escape from root herbivory: a worldwide comparison of the root-feeding nematode communities of the dune grass *Ammophila arenaria* in natural and introduced ranges. Biological Invasions 7:733–746.

Vierheilig, H., A. P. Coughlan, U. R. S. Wyss, and C. De Recherche. 1998. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Applied and Environmental Microbiology 64:5004–5007.

Wiedemann, A. M., and A. Pickart. 1996. The Ammophila problem on the Northwest coast of North America. Landscape and Urban Planning 34:287–299.

Zarnetske, P. L., T. C. Gouhier, S. D. Hacker, E. W. Seabloom, and V. A. Bokil. 2013. Indirect effects and facilitation among native and non-native species promote invasion success along an environmental stress gradient. Journal of Ecology 101:905–915.

Zarnetske, P. L., S. D. Hacker, E. W. Seabloom, P. Ruggiero, J. R. Killian, T. B. Maddux, and D. Cox. 2012. Biophysical feedback mediates effects of invasive grasses on coastal dune shape. Ecology 93:1439–1450.

SUPPORTING INFORMATION

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.1527/full