Disentangling environmental and host sources of fungal endophyte communities in an experimental beachgrass study

Aaron S. David1 | Eric W. Seabloom1 | Georgiana May1,2

1Department of Ecology, Evolution, and Behavior, University of Minnesota, Saint Paul, MN, USA
2Department of Plant Biology, University of Minnesota, Saint Paul, MN, USA

Correspondence
Aaron S. David, Department of Biology, University of Miami, Coral Gables, FL, USA.
Email: asdavid@bio.miami.edu

Funding information
University of Minnesota; US Environmental Protection Agency, Grant/Award Number: EPA/NCER R833836; National Science Foundation, Grant/Award Number: DEB 1045608, Graduate Research Fellowship Program, NSF 00039202; Puget Sound Mycological Society, Grant/Award Number: Ben Woo Fellowship; NSF Integrative Graduate Education and Research Traineeship Introduced Species and Genotypes program, (DGE-0653827)

Abstract
Disentangling the ecological factors that contribute to the assembly of the microbial symbiont communities within eukaryotic hosts is an ongoing challenge. Broadly speaking, symbiont propagules arrive either from external sources in the environment or from internal sources within the same host individual. To understand the relative importance of these propagule sources to symbiont community assembly, we characterized symbiotic fungal endophyte communities within the roots of three species of beachgrass in a field experiment. We manipulated two aspects of the external environment, successional habitat and physical disturbance. To determine the role of internal sources of propagules for endophyte community assembly, we used beachgrass individuals with different pre-existing endophyte communities. Endophyte species richness and community composition were characterized using culture-based and next-generation sequencing approaches. Our results showed that external propagule sources associated with successional habitat, but not disturbance, were particularly important for colonization of most endophytic taxa. In contrast, internal propagule sources played a minor role for most endophytic taxa but were important for colonization by the dominant taxon Microdochium bolleyi. Our findings highlight the power of manipulative field experiments to link symbiont community assembly to its underlying ecological processes, and to ultimately improve predictions of symbiont community assembly across environments.

KEYWORDS
community assembly, culture-based sequencing, disturbance, endophyte, Illumina MiSeq, priority effects

1 | INTRODUCTION

Disentangling the ecological factors that contribute to the assembly of the microbial symbiont communities within eukaryotic hosts is an ongoing challenge (Arnold, 2007; Borre, Kinkel, May, & Seabloom, 2013; Saunders, Glenn, & Kohn, 2010). Communities of host-associated microbial symbionts are structured by biotic interactions with host species and other microbial symbionts and by abiotic factors (e.g., Arnold, 2007; Blaalid et al., 2014; Busby, Peay, & Newcombe, 2016; Costello et al., 2009; David, Seabloom, & May, 2016b; Pan, Baumgarten, & May, 2008). While we have gained insight into the structure and diversity of symbiont communities associated with various ecological factors, the processes by which these communities are assembled and the sources of propagules necessary for establishing symbiotic communities are still poorly understood. Broadly speaking, propagules must either arrive to a host from the environment external to the host or from internal tissues of the same host individual (Christian, Whitaker, & Clay, 2015). Here, we use an experimental field approach to partition the roles of these propagule sources in the establishment of root-inhabiting fungal endophyte communities, symbionts that associate with all plant lineages without apparent symptoms (Carroll, 1988; Mandyam & Jumpponen, 2005; Rodriguez, White, Arnold, & Redman, 2009).
We focus on two important ecological processes that may alter the external pool of endophytic taxa available in the environment: successional changes in a community through time, and disturbances that may interrupt or change the trajectory of succession. Succession affects both the abiotic conditions and the biotic community of host plants (e.g., Connell & Slatyer, 1977) in which endophytes are harboured. For example, changes in soil properties (e.g., pH) during succession correspond with changes in fungal endophyte species richness and community composition (Blaalid et al., 2014; David et al., 2016b; Yao, Vik, Brysting, Carlsen, & Halvorsen, 2013). Disturbance, often associated with but not limited to early successional habitats, might affect plant-associated fungal communities independently of the impacts on plant and soil communities. For instance, physical disturbance, even at small spatial scales within the rhizosphere, can disrupt hyphal networks of mycorrhizal fungi, thereby reducing root colonization and altering community composition (Bruns, 1995; Jasper, Abbott, & Robson, 1991; Lindahl, de Boer, & Finlay, 2010). Furthermore, disturbance could shift community composition by favouring opportunistic, ruderal taxa over slow-growing, competitive taxa (Bruns, 1995; Connell & Slatyer, 1977; Grime, 1977). Presently, the impacts of successional habitat and disturbance on the environmental pool of endophytic propagules, and on their association with different plant species, are not well understood and limit prediction of endophyte community assembly.

Internal sources of endophytic taxa from within the host could bypass the barriers to infection posed by the dense medium of the soil environment and potentially limit the acquisition of new endophytic taxa from the environment. For instance, some endophytic taxa colonize host tissue from infected tissue of the same host individual (Rodriguez et al., 2009), a process common to many fungal plant pathogens that spread via hyphal growth or spores (Agrios, 2005). However, most endophytic taxa form small, localized infections and rarely, if ever, produce spores on their hosts (Rodriguez et al., 2009), suggesting that internal sources of endophytes may be of little consequence for newly established communities. Still, for endophytic taxa associated with seeds or vegetative tissues such as rhizomes, plant dispersal will also disperse associated endophytes (Arnold, 2007; Fischer & Rodriguez, 2013) and could circumvent the dispersal limitations of many of these taxa (e.g., David et al., 2016b; Higgins, Arnold, Coley, & Kursar, 2014). Early colonization by these taxa might limit the colonization by other fungal species, leading to priority effects (Vannette & Fukami, 2014). Currently, we lack a basic understanding of the relative importance of external and internal sources of inoculum in the establishment and assembly of root endophyte communities, nor do we understand transmission patterns for most endophytic taxa.

The coastal dunes of Oregon, USA, represent a dynamic ecosystem well suited for determining ecological factors affecting the assembly of endophyte communities. A successional gradient is created perpendicularly across the dunes by sand deposition and by more frequent storm disturbance events in the early successional foredune habitats (sand ridges adjacent to the shoreline) compared to the late successional backdune habitats (the inland area beyond the foredune) (Ruggiero, Komar, McDougal, Marra, & Reggie, 2001). The foredune habitats are characterized by lower soil fertility and lower plant diversity than are backdune habitats (David et al., 2015; Hacker et al., 2012). In previous work, we showed that root endophyte communities differed between these fore- and backdune successional habitats with greater endophyte richness occurring in the backdune habitats (David et al., 2016b). Because these very different successional habitats and endophyte communities occur in close physical proximity along the entire northwest coastline, the system allows regionally replicated experimental treatments at different sites along the coast.

Here, we experimentally determined the relative importance of external and internal sources of propagules for endophyte community composition in three beachgrass species. To estimate the contributions of external sources of endophyte propagules due to successional habitat and disturbance within these habitats, we established experimental plots that were either manually disturbed or left undisturbed in both the foredune and backdune habitats. To estimate the contribution of internal propagule sources, we reciprocally transplanted individual tillers with pre-existing endophyte communities of the three beachgrass species between fore- and backdune habitats. At the end of the growing season, we characterized fungal endophyte species richness and community composition in new roots of experimental plants using both culture-based and next-generation sequencing-based approaches. Based on previous findings (David et al., 2016b), we predicted that external sources would more strongly affect endophyte richness and community composition than would internal sources. If so, then tillers transplanted to the late successional backdunes should harbour greater endophyte richness than those planted into the early successional foredune, regardless of the communities they harboured at the start of the experiment. Finally, we expected that the disturbance treatment would more strongly decrease endophyte richness and alter community composition in the backdunes, where natural disturbances occur less frequently than in foredunes.

2 METHODS

2.1 Study system

The coastal dunes of Oregon are an ecologically and geomorphologically dynamic ecosystem critical for protection of coastline development (Cooper, 1958; Seabloom, Ruggiero, Hacker, Mull, & Zarnetske, 2013). Within individual dunes in this region, the foredune and the backdune represent distinct habitats along a successional gradient (David et al., 2015); younger, foredune habitats are characterized by lower plant species richness, lower soil C and N concentrations, and higher salinity than older, backdune habitats.

Dune vegetation in the Pacific Northwest is dominated by three species of beachgrass species (Hacker et al., 2012). The native Elymus mollis (American dunegrass) is found throughout the west coast dune regions and grows in low density (Hacker et al., 2012). The European beachgrass Ammophila arenaria was introduced in the early
1900s (Hacker et al., 2012; Schwendiman, 1977) and occurred in near monoculture at the study sites used here. Finally, the congeneric American beargrass species, *A. breviligulata* (native to Eastern USA), was more recently introduced in the mid-20th century and is currently displacing *A. arenaria* as it spreads into the Oregon dunes from Washington (Hacker et al., 2012; Schwendiman, 1977; Seabloom & Wiedemann, 1994). In our study, *A. breviligulata* only occurred at one of the study sites (Pacific City) (David, May, Schmidt, & Seabloom, 2016; Figure S1). All three beargrass species primarily spread by clonal growth of rhizomes rather than seeds (Hacker et al., 2012) and are dispersed widely by storms and ocean currents as rhizomes and tillers (stems attached to a rhizome) (Wiedemann & Pickart, 1996).

### 2.2 Experimental design

The experimental design was previously described in David, May, et al. (2016), and we provide a brief summary here. We conducted the experiment at three sites in northwestern Oregon—Pacific City (Bob Straub State Park, Pacific City, OR, USA, 45°10’N, 123°58’W), Sand Lake (Sand Lake Recreation Area, Cloverdale, OR, USA, 45°17’N, 123°57’W) and Cape Meares (Bayocean Peninsula County Park, Tillamook, OR, USA, 45°30’N, 123°57’W). At each of the three sites, we established three experimental blocks. Within each block, a pair of plots was established in the early successional foredune habitat and a pair of plots in the late successional backdune habitat (Figure S2). Each plot within a pair was randomly assigned to the “disturbed” or “undisturbed” treatment, thus providing three replicate disturbed and undisturbed plots within each fore- and backdune environment at each site. We planted tillers of each beargrass species collected from the fore- and backdune source habitat into each plot (3 species × 2 tiller sources = 6 tillers per plot; 3 sites × 3 blocks per site × 4 plots per block × 6 plants per plot = 216 tillers total). We harvested all tillers after 11 weeks, at which point we collected approximately 30 cm of healthy, new root tissue from surviving plants for characterization of endophyte communities.

### 2.3 Experimental setup

To examine the importance of external propague sources, we established plots in early and late successional habitat and manipulated physical disturbance. The early successional “foredune habitat” plots were located on the seaward-facing slope of the foredune, and the late successional “backdune habitat” plots were located at the most inland spot before the tree line behind the backdune. In preliminary assessments (Supporting Information; Additional Methods), we verified that soils in foredune plots were characterized by higher pH, higher sodium concentrations, and lower total carbon and nitrogen concentrations than backdune plots. Backdune plots were characterized by higher plant species richness than were foredune plots. We implemented the disturbance treatment by removing all above- and below-ground vegetation and debris for those plots assigned to the disturbance treatment. In undisturbed plots, we left all vegetation intact.

To examine the importance of internal propague sources, we transplanted individual plant tillers collected from foredune and the backdune habitats at the Pacific City site into our experimental plots at all sites. Because previous work had shown that root endophyte communities differed between plants growing in the foredunes and backdunes (David et al., 2016b), we assumed that transplanted tillers would carry with them pre-existing differences in endophyte communities. We verified that assumption (see Results) and subsequently used the statistical effect of tiller source on endophyte richness and community composition at the end of the experiment as a proxy measure of internal propague source. By collecting fore- and backdune-sourced tillers from a single site, we sought to minimize variation in pretreatment endophyte communities. Each experimental tiller was soaked in tap water for 10 days in an individual water-filled plastic bag to stimulate root growth and improve transplant success while avoiding cross-contamination among plants. In preliminary work, we used the culture-based approach to evaluate the effect of soaking on recovery of fungal colonies for a subset of 30 tillers, and we found no significant difference in the number of recovered fungal colonies before and after soaking (paired t test, df = 29, t = 1.39, p = .175).

### 2.4 Culture-based approach

Using a culture-based approach previously described, we obtained fungal cultures from pretreatment roots of individual tillers to complement the existing data set for post-treatment cultured fungal endophytes obtained in the previous study (David, May, et al., 2016). Briefly, we surface-sterilized root samples from each tiller using successive rinses of ethanol (70%, 1 min), bleach (0.5% NaOCl, 2 min) and ethanol (70%, 1 min). Surface-sterilized roots were cut into 20 segments (~1.5 mm²) and plated on 2% malt extract agar. Approximately 4 months after plating, we extracted DNA from all fungal isolates, amplified the internal transcribed spacer (ITS) region using PCR and sequenced the resulting amplicons to assign taxonomy to fungal isolates.

### 2.5 Next-generation sequencing approach

We used a next-generation sequencing (NGS) approach to characterize root endophyte communities at the conclusion of the experiment (post-treatment). We harvested and surface-sterilized root tissue from individual tillers as described in David, May, et al. (2016) and froze a subset of the tissues at −80°C until DNA processing. We ground 10 mg of fresh weight roots with a mortar and pestle and extracted total DNA using a CTAB-chloroform:isoamyl extraction protocol (Arnold, Henk, Eells, Lutzoni, & Vilgalys, 2007). DNA was further purified using the MO BIO PowerClean DNA Clean-Up kit (MO BIO, Carlsbad, CA, US). The ITS1 region was PCR amplified with ITS1F forward and ITS2 reverse primers modified with Illumina adapters and unique barcode sequences that allowed for
identification of individual samples in multiplexed runs (Smith & Peay, 2014). PCR was accomplished using 20 µl reactions with the REDExtract-N-Amp kit (Sigma-Aldrich Co., St Louis, MO, USA) and PCR protocol as described in David, May, et al. (2016) but with 30 rounds of amplification. To evaluate whether the nonproofreading Taq polymerase used in the REDExtract-N-Amp kit might result in sequence errors that inflate the number of OTUs estimated (Oliver, Brown, Callaham, & Jumpkomen, 2015), we also amplified a subset of 20 samples with a proofreading Qiagen HotStart Taq (Qiagen, Valencia, CA, USA) (20 µl reaction, 0.7 µl (10 µM) each of forward and reverse primer, 30 cycles of 95°C for 30 s, 56°C for 30 s, 72°C for 1 min). To account for any contamination during the DNA extraction, PCR or sequencing steps, we conducted two mock DNA extractions with no plant material and amplified any extracted DNA using the PCR protocol above. We quantified the concentration of PCR products using Qubit HS DNA kit (Invitrogen, Carlsbad, CA, USA).

We pooled 7.0 ng from each barcoded PCR product for experimental samples and all of the PCR product for the two poorly amplifying negative controls (4.0 ng and 6.5 ng) into one library. The library was purified using the Qiagen QIAquick PCR Purification Kit and concentrated to 10 ng/µl. The library was sequenced under conditions for 250 bp paired-end sequencing on a single Illumina MiSeq run (Illumina, Inc. San Diego, CA, USA) at the University of Minnesota Genomic Center.

Following sequencing, forward sequence reads were sorted by sample barcodes (de-multiplexing). For each sequenced sample, we used CUTADAPT (Martin, 2011) to remove the distal primer and barcode sample barcodes (de-multiplexing). For each sequenced sample, we run (Illumina, Inc. San Diego, CA, USA). The library was sequenced under conditions for 250 bp paired-end sequencing on a single Illumina MiSeq run (Illumina, Inc. San Diego, CA, USA) at the University of Minnesota Genomic Center.

Following sequencing, forward sequence reads were sorted by sample barcodes (de-multiplexing). For each sequenced sample, we used CUTADAPT (Martin, 2011) to remove the distal primer and barcode sequences and TRIMMOMATIC (Bolger, Lohse, & Usadel, 2014) to trim low-quality regions from the ends of reads. Next, we discarded reads less than 220 base pairs and those with ambiguous, low-quality bases using MOTHUR (Schloss et al., 2009). Using USEARCH (Edgar, 2010), we de-replicated sequences (identified identical sequences and chose representatives) and removed unique reads that only appeared in the data set once. We then clustered OTUs defined at the 97% sequence identity level using USEARCH (Edgar, 2010). Representative OTU sequences were screened against a combination of the UNITE (Abarenkov et al., 2010) and EMERENCIA databases (Ryberg, Kristiansson, Sjökvist, & Nilsson, 2009), and those OTUs that shared <60% similarity with any known fungal sequence were removed. We assigned taxonomy using the Bayes classifier in MOTHUR implemented through QIIME (Caporaso et al., 2010). Finally, sequence reads were mapped to the final OTU data set using USEARCH (Edgar, 2010). We used rarefaction curves to verify that the sampling effort was sufficient to recover OTUs for both culture-based and NGS data sets (Figure S3).

We determined that the presence of sequences representing OTUs in the NGS-negative controls were likely due to contaminating DNA in PCR reactions. Two OTUs in particular, identified as Fusarium sp. and Microdochium bolleyi, accounted for 59% of the sequences in the negative control samples (256 total OTUs ranging from 1 to 4,831 sequence reads) and were confirmed as two of the most common endophytic genera in the culture-based approach (see Results). We concluded that subtracting reads of contaminant OTUs in the NGS data set, rather than removing OTUs altogether, was the most appropriate approach to account for inflation of counts due to contamination during sample processing (Nguyen, Smith, Peay, & Kennedy, 2015). Therefore, we subtracted the number of sequence reads of each OTU present in negative control samples from the number of sequences representing the corresponding OTU in each experimental sample.

### 2.6 | Data analysis

Pretreatment and post-treatment endophyte community data sets were analysed separately. We used analyses of the pretreatment culture-based data set to verify our assumptions that pretreatment fore- and backdune-sourced tillers differed in OTU richness and community composition. Once verified, we could then use a significant effect of tiller source on endophyte richness and community composition in sampled roots as a proxy measure of the importance of internal propagule sources as explained above. We used post-treatment data sets (culture-based and NGS-based data sets) to evaluate the effects of the experimental treatments on OTU richness and community composition.

#### 2.6.1 | Endophyte OTU richness

We tested the effect of our experimental treatments on endophyte OTU richness in the culture-based (separate pretreatment and post-treatment analyses) and NGS-based (post-treatment analysis only) data sets. For the culture-based data set, OTU richness was defined as the number of different OTUs per tiller sample (20 surface-sterilized tissue segments).

For the NGS-based data set, we estimated OTU richness per sample while accounting for unequal sequencing depth among samples by generating rarefied community matrices. Rarefaction was accomplished by resampling 1,700 reads (lowest number of reads for any one of our samples after processing) 100 times. We then averaged the counts per OTU for each tiller across resampled matrices and summed the number of OTUs per tiller as richness. In addition to total OTU richness, we also investigated the distribution of OTUs in the fungal phyla most abundant in our samples, Ascomycota and Basidiomycota. To allow better comparison of NGS results with culture-based results, we also determined OTU richness within the Pezizomycotina, as nearly all of the culture-based OTUs belonged to this subphylum of the Ascomycota. In preliminary analyses, we evaluated whether rarefying might bias our results (McMurdie & Holmes, 2014). Following Nguyen et al. (2016) and Tedersoo et al. (2016), we compared results of rarified richness to those of species richness after accounting for the number of sequence reads using a regression approach. We obtained similar results, suggesting that rarefying our data did not bias the results (Table S1).

For both culture-based and NGS-based data sets, we tested the effects of the experimental treatments on OTU richness using linear mixed-effects models with the nlme package in R (Pinheiro, Bates, DebRoy, Sarkar, & Team, 2017; R Development Core Team 2017). We used the nested random effects of site, block within site, paired disturbed/
undisturbed plots within block and plot within pairs. We tested the fixed effects of dune habitat, disturbance treatment, host species, tiller source and all interactions in the models. For the post-treatment culture-based analyses, we also included the culture-based OTU richness estimate from the pretreatment samples as a covariate. We tested for significance using analysis of deviance implemented with the CAR package (Fox & Weisberg, 2011). We investigated significant terms using Tukey contrasts for multiple comparisons implemented with the Multcomp package (Hothorn, Bretz, Westfall, & Heiberger, 2008).

### 2.6.2 Endophyte community composition

To analyse effects of the experimental treatments on endophyte OTU community composition for both the culture-based and NGS-based data sets, we conducted a permutational analysis of variance using the adonis() function in the vegan R package (Oksanen et al., 2016). In these analyses, community composition was analysed by partitioning sources of variation in Bray–Curtis pairwise distances calculated from square-root transformed, abundance-weighted community matrices (Oksanen et al., 2016). We used “block” as strata, such that permutations were only conducted among experimental units within the same experimental block. We used the same fixed effects as described above for the richness models.

Next, we used the NGS data set, which better represented the diversity of taxa present in sampled roots than the culture-based data set, to evaluate how the abundances of taxa representing fungal phyla, classes and genera differed in response to experimental treatments. For each of these taxonomic levels, we used multivariate linear models implemented with the mvabund R package (Wang et al., 2012) to test the effects of site, dune habitat, disturbance treatment, host species and tiller source on the endophyte community composition for each individual plant. We fit separate multivariate models for each taxonomic level and tested the significance of predictor variables at the community level or the taxon level using resampling based hypothesis testing (Wang et al., 2012). Because most of the common phyla and classes were present in all samples, we evaluated their relative abundances using a logit transformation of the rarified matrices with the manyglm() function (Wang et al., 2012). Because the incidence of common genera was relatively low across samples, abundance results would be strongly zero-inflated and instead, we evaluated their presence or absence in each sample with the manyglm() function using a binomial error structure (Wang et al., 2012). We present both uncorrected p-values and p-values corrected for multiple comparisons for results of significance tests.

### 3 RESULTS

Of the 216 tillers used in the experiment, 167 (77.3%) individuals survived to the end of the experiment. We were able to harvest sufficient newly grown root tissue from the 167 surviving individuals for the culture-based approach, and from 142 individuals for the NGS-based approach.

### 3.1 Description of endophyte communities

The culture-based and NGS-based approaches both revealed that fungal root endophyte communities were dominated by taxa in the Ascomycota phylum (Table 1). Members of the Pezizomycotina within Ascomycota dominated results for both data sets with 93% of all individual fungal cultures (combined pre- and post-treatment data sets) and 83% of reads in the NGS data set belonging to this subphylum. The culture-based and NGS-based approaches differed in their ability to detect taxa from a diversity of phylogenetic lineages. The culture-based approach retrieved a much narrower diversity of OTUs than did the NGS-based approach, as 94% of all culture-based OTUs belonged to Pezizomycotina whereas only 51% of OTUs were in this subphylum for the NGS-based data set (chi-squared test, df = 1, $\chi^2 = 75.9, p < .001$). The NGS-based approach produced sequences with taxonomies assigned to all of the Ascomycota classes found with the culture-based approach (Dothideomycetes, Eurotiomycetes, Leotiomycetes and Sordariomycetes) and, in addition, detected the classes Lecanoromycetes, Orbiliomycetes and Pezizomycotina of the subphylum Pezizomycotina, as well as the yeast-like subphyla Saccharomycotina and the Taphrinomycotina. In both data sets, Microdochium bolleyi was the identity of the most abundant OTU (Table 1).

The pretreatment and post-treatment culture-based results recovered similar taxa at the phylum and class ranks (Table 1). OTUs identified as M. bolleyi, Exophiala opportunista and E. salmonis were among the most common taxa in both culture-based data sets.

In the NGS-based approach, the nonproofreading Taq polymerase did not lead to inflated numbers of estimated OTUs, as we found no significant difference in the number of OTUs estimated for samples amplified with proofreading and nonproofreading Taq polymerases (paired t test, df = 19, $t = 1.69, p = .108$).

### 3.2 Pretreatment endophyte richness and community composition

Analysis of the pretreatment endophyte communities using the culture-based approach validated a crucial assumption that endophyte communities differed in tillers obtained from fore- and backdune sources. OTU richness across the three beachgrass species was greater in backdune-sourced tillers (1.6 ± 0.2 OTUs per 20 tissue segments, back-transformed least-squares mean ± 1 SEM) than foredune sourced tillers (0.7 ± 0.1 OTUs), although the difference in richness between tiller sources was <1 OTU on average (Table S1; Figure 1a). OTU richness was also significantly affected by host species and was highest in E. mollis tillers (2.0 ± 0.3 OTUs), followed by A. arenaria (1.1 ± 0.2 OTUs), and then A. breviligulata (0.5 ± 0.1 OTUs) (Tukey post hoc tests; Table S1; Figure S4). We assessed whether there were unknown biases in tillers deployed to different experimental treatments, and we determined that none of these treatment factors (dune habitat, disturbance and interactions terms) was predictive of OTU richness in pretreatment samples (Table S1). Furthermore, results from multivariate analyses of the pretreatment
**TABLE 1** Summary of fungal, endophytic taxa found in culture-based pretreatment, culture-based post-treatment and next-generation sequence based post-treatment studies

<table>
<thead>
<tr>
<th></th>
<th>Culture-based pretreatment</th>
<th>Culture-based post-treatment</th>
<th>NGS-based post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total isolations/sequence reads analysed</td>
<td>721</td>
<td>833</td>
<td>5,265,036 (11,765,722 before processing)</td>
</tr>
<tr>
<td>Average # isolates/sequence reads per tiller ± 1 SEM</td>
<td>5.9 ± 0.5 (per 20 tissue segments)</td>
<td>6.8 ± 0.5 (per 20 tissue segments)</td>
<td>37,190 ± 1,384</td>
</tr>
<tr>
<td>Min.-Max. # isolates/sequence reads Per Tiller</td>
<td>0-20</td>
<td>0-20</td>
<td>1,704-95,340</td>
</tr>
<tr>
<td>Total OTUs</td>
<td>84</td>
<td>71</td>
<td>1,132</td>
</tr>
<tr>
<td>Phylum</td>
<td># OTUs (% of all isolations/sequence reads)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascomycota</td>
<td>81 (98%)</td>
<td>68 (99%)</td>
<td>783 (86%)</td>
</tr>
<tr>
<td>Basidiomycota</td>
<td>3 (2%)</td>
<td>2 (&lt;1%)</td>
<td>307 (14%)</td>
</tr>
<tr>
<td>Chytridiomycota</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>3 (&lt;1%)</td>
</tr>
<tr>
<td>Glomeromycota</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>18 (&lt;1%)</td>
</tr>
<tr>
<td>Zygomycota</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>21 (&lt;1%)</td>
</tr>
<tr>
<td>Unclassified</td>
<td>0 (0%)</td>
<td>1 (&lt;1%)</td>
<td>—</td>
</tr>
<tr>
<td>Class</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dothideomycetes</td>
<td>15 (11%)</td>
<td>8 (11%)</td>
<td>163 (5%)</td>
</tr>
<tr>
<td>Eurotiomycetes</td>
<td>15 (20%)</td>
<td>15 (21%)</td>
<td>70 (4%)</td>
</tr>
<tr>
<td>Leotiomycetes</td>
<td>12 (11%)</td>
<td>11 (7%)</td>
<td>113 (21%)</td>
</tr>
<tr>
<td>Sordariomycetes</td>
<td>37 (55%)</td>
<td>32 (56%)</td>
<td>228 (53%)</td>
</tr>
<tr>
<td>Identities of most common OTUs</td>
<td>Taxa (% of all isolations/sequence reads)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microdochium bolleyi</td>
<td>(32%)</td>
<td>Microdochium bolleyi</td>
<td>(37%)</td>
</tr>
<tr>
<td>Exophiala opportunistica</td>
<td>(8%)</td>
<td>Exophiala salmonis</td>
<td>(6%)</td>
</tr>
<tr>
<td>Ilyanectria mars-panacis</td>
<td>(6%)</td>
<td>Fusarium commune</td>
<td>(5%)</td>
</tr>
<tr>
<td>Exophiala salmonis</td>
<td>(6%)</td>
<td>Exophiala opportunistica</td>
<td>(5%)</td>
</tr>
<tr>
<td>Pyrenochaetopsis leptospora</td>
<td>(5%)</td>
<td>Penicillium sp.</td>
<td>(4%)</td>
</tr>
</tbody>
</table>

**FIGURE 1** Fungal operational taxonomic unit (OTU) richness per individual plant for the (a) pretreatment culture-based data set, (b) post-treatment culture-based data set and (c) next-generation sequencing-based (NGS) data sets. Back-transformed least-squares means ± 1 SEM shown in each panel. In (a) and (b), mean OTU richness is shown per 20 segments of surface-sterilized root tissue. In (c), mean OTU richness was obtained from rarefied data sets (resampling 1,700 sequence reads). The disturbance treatment did not cause significant effects for either culture-based or NGS data sets. Letters denote significant differences between groups using Tukey contrasts for multiple comparisons. See Table S1 for full model results.
endophyte composition found significant effects of host species, tiller source and host species × tiller source (Figure 2a; Table S2). As with pretreatment OTU richness, we determined that there were no apparent biases for endophyte composition in the tillers deployed to the different dune habitat and disturbance treatments (Table S2). We concluded that fore- and backdune tillers harboured sufficiently different pre-existing fungal communities that we could use effects of tiller source as a proxy to evaluate the importance of internal propagule sources in different experimental treatments.

3.3 | Post-treatment endophyte OTU richness

For the culture-based results, we found that OTU richness was significantly affected by the dune habitat × tiller source interaction but not by the disturbance treatment (Table S1). Tillers transplanted to the backdune contained higher OTU richness than those transplanted to the foredune, but this was only found for foredune sourced tillers and not backdune-sourced tillers (Tukey contrasts; Figure 1b). Host species also significantly affected OTU richness in culture-based results, yet surprisingly, A. arenaria (1.4 ± 0.2 OTUs) and A. breviligulata (1.3 ± 0.2 OTUs) had higher OTU richness than E. mollis (0.8 ± 0.2 OTUs), a reversal from the pretreatment findings (Table S1; Figure S4). There were no significant interactions between host species and any experimental treatment (Table S1), indicating that the responses of endophyte OTU richness to the experimental treatments did not differ among host species.

In contrast, the NGS-based results showed dune habitat alone, and not disturbance or tiller source, had a significant effect on OTU richness (Table S1). OTU richness was greater in the backdune habitat (23.0 ± 2.1 OTUs per 1,704 sequences reads) than the foredune habitat (16.5 ± 1.4 OTUs) (Figure 1c; Table S1). We found a significant effect of host species on OTU richness (Table S1), although none of the comparisons among species were significant in Tukey contrasts (Figure S4). Similar to the culture-based results, a lack of significant interactions between host species and the experimental treatments in the NGS-based results suggested that host species did not uniquely respond to these treatments (Table S1). To determine whether the OTU richness effect was driven by taxa in the phyta Ascomycota or Basidiomycota, or the Ascomycete subphylum Pezizomycotina, we conducted additional OTU richness analyses that were restricted to the OTUs classified to each taxonomic group. For analyses restricted to Ascomycota and Pezizomycotina, we obtained similar results as when considering all phyla (Table S1). For the analysis restricted to Basidiomycota, we found a significant host species × dune habitat interaction (Table S1), although Tukey contrasts revealed that within-Basidiomycota OTU richness did not differ between dune habitats for any of the three host species. These results suggest that the differences in OTU richness of fore- and backdune tillers are largely due to higher richness of taxa within the Pezizomycotina.

3.4 | Post-treatment endophyte community composition

In analyses of the culture-based data set, community composition was significantly affected by tiller source, but none of the other model terms (dune habitat, disturbance, host species or any interaction terms; Figure 2a; Table S1). Interestingly, when we reanalysed these data after removing the most common OTU (assigned to M. bolleyi), we instead found a significant effect of dune habitat but not of tiller source (Table S2). Together, these results indicate that the significant effect of tiller source on community composition is likely due to the greater abundance of M. bolleyi in tillers sourced from the foredune habitat and suggests an internal source for
M. bolleyi propagules. For all other taxa, endophyte community composition was more strongly affected by dune habitat, suggesting an external source of these propagules.

The results for the NGS-based data set showed that OTU community composition was significantly affected by tiller source, dune habitat and host species (Figure 2b; Table S2). The disturbance treatment had marginally significant effects, and none of the interaction terms was significant (Table S2). Comparing the culture-based and NGS-based results for community composition, both showed an effect of tiller source and thus suggest a role for internal sources of propagules. However, the NGS-based results also revealed a strong effect of dune habitat suggestive of external sources of inoculum for most taxa, a result that may be masked by the prevalence of the M. bolleyi in the culture-based data set.

We further investigated the relative importance of external and internal sources to colonization by specific fungal taxa at the levels of phylum, class and genus. The phylum-level analysis compared the responses of evolutionarily distant lineages of fungi, the phyla Ascomycota, Basidiomycota, Chytridiomycota, Zygomycota and Glomeromycota. We found significant differences in phylum-level composition associated with dune habitat (Table S3). The phylum-level effect was attributable to higher relative abundance of the Glomeromycota (arbuscular mycorrhizal fungi) in backdunes (Table 2). Disturbed plots had greater relative abundance of Ascomycota and lower relative abundance of Basidiomycota compared to undisturbed plots, but the effect was only significant in the test using p-values uncorrected for multiple comparisons (Table 2; Table S3). Tiller source was not associated with compositional differences at the phylum level (Table S3). Host species was significantly associated with compositional differences (Table S3), and this was attributable to higher Chytridiomycota abundance in E. mollis individuals compared to Ammophila individuals (Table 2).

Analysis at the class level evaluated responses of the four classes of fungi to which most endophytic taxa belong: Dothideomycetes, Eurotiomycetes, Leotiomycetes and Sordariomycetes, all of which are Ascomycota (Rodriguez et al., 2009). We found a significant difference in class level composition associated with dune habitat but not with disturbance treatment, tiller source or host species (Table S3). The relative abundance of Sordariomycetes was higher in the foredune (71% of reads) than in the backdune (45% of reads), and Leotiomycetes abundance was higher in the backdunes (7.3% of reads) than the foredunes (0.003% of reads). The other two classes did not differ significantly in abundance across these experimental variables (Table S4).

The analysis at the genus level allowed for a detailed evaluation of the response of the most abundant genera in our study to experimental treatments. We included the 12 most abundant genera (Microdochium, Exophiala, Fusarium, Leptodontidium, Cladophialophora, Dactylaria, Penicillium, Pyrenochaetopsis, Colletotrichum, Myrmecridium, Alternaria and Apodus) in the analysis. These genera were present in at least 25 individual plants and accounted for 78.7% of all sequence reads in the NGS-based data set. Dune habitat and tiller source, but not disturbance or host species had significant effects on the community composition at the genus level (Table S3). The genera Fusarium and Apodus occurred more frequently in foredune habitats, while Exophiala, Leptodontium, Cladophialophora, Dactylaria occurred more frequently in backdune habitats (Table 3). Tiller source significantly affected the abundance of two genera, Microdochium (higher

<table>
<thead>
<tr>
<th>Terms</th>
<th>Ascomycota</th>
<th>Basidiomycota</th>
<th>Chytridiomycota</th>
<th>Zygomycota</th>
<th>Glomeromycota</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>9.91e−01</td>
<td>8.05e−03</td>
<td>7.44e−06</td>
<td>2.01e−05</td>
<td>6.35e−06</td>
</tr>
<tr>
<td>Site</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>SL vs. PC</td>
<td>3.55e−05</td>
<td>−3.50e−04</td>
<td>−1.68e−06</td>
<td>−1.29e−05</td>
<td>2.93e−06</td>
</tr>
<tr>
<td>CM vs. PC</td>
<td>1.31e−03</td>
<td>−2.35e−03</td>
<td>−2.77e−06</td>
<td>−1.17e−05</td>
<td>−3.05e−06</td>
</tr>
<tr>
<td>Dune Habitat</td>
<td></td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Back vs. Fore</td>
<td>1.65e−03</td>
<td>−1.16e−03</td>
<td>−2.65e−06</td>
<td>1.57e−05</td>
<td>2.87e−05</td>
</tr>
<tr>
<td>Disturbance</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Disturbed vs. Undisturbed</td>
<td>2.08e−02</td>
<td>−1.92e−02</td>
<td>1.87e−06</td>
<td>−1.46e−05</td>
<td>−2.41e−07</td>
</tr>
<tr>
<td>Host species</td>
<td></td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>E. mollis vs. Ammophila</td>
<td>−3.41e−03</td>
<td>3.07e−03</td>
<td>2.38e−06</td>
<td>5.92e−06</td>
<td>6.39e−07</td>
</tr>
<tr>
<td>A. arenaria vs. A. breviligulata</td>
<td>3.69e−04</td>
<td>−1.17e−03</td>
<td>3.94e−07</td>
<td>−3.37e−06</td>
<td>−6.77e−07</td>
</tr>
<tr>
<td>Tiller source</td>
<td></td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Back vs. Fore</td>
<td>−1.07e−03</td>
<td>2.03e−04</td>
<td>2.81e−06</td>
<td>7.18e−06</td>
<td>−1.03e−06</td>
</tr>
</tbody>
</table>

*Significance of model terms (p < .05) in the ANOVA with p-values corrected for multiple comparison, and *significance of model terms (p < .05) uncorrected for multiple tests. Sites are Pacific City (PC), Sand Lake (SL) and Cape Meares (CM).
### Table 3

Effects of experimental factors on presence or absence of 12 most common genera from the next-generation sequencing-based data set. Table shows the model coefficients for the probability that a genus will be present within an individual plant sample (multivariate generalized linear model).

<table>
<thead>
<tr>
<th>Terms</th>
<th>Microdochium</th>
<th>Exophiala</th>
<th>Fusarium</th>
<th>Leptodontidium</th>
<th>Cladophialophora</th>
<th>Dactylaria</th>
<th>Penicillium</th>
<th>Pyrenochaetopsis</th>
<th>Colletotrichum</th>
<th>Myrmecridium</th>
<th>Alternaria</th>
<th>Apodus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>4.49e-01</td>
<td>2.66e-01</td>
<td>6.29e-01</td>
<td>1.37e-01</td>
<td>2.45e-01</td>
<td>1.47e-01</td>
<td>3.04e-01</td>
<td>2.08e-01</td>
<td>1.39e-01</td>
<td>2.32e-01</td>
<td>2.47e-01</td>
<td>1.97e-01</td>
</tr>
<tr>
<td>Site</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SL vs. PC</td>
<td>2.05e-01</td>
<td>1.97e-01</td>
<td>-9.80e-02</td>
<td>-6.70e-02</td>
<td>-7.00e-03</td>
<td>-4.00e-02</td>
<td>-6.90e-02</td>
<td>2.40e-02</td>
<td>9.40e-02</td>
<td>6.60e-02</td>
<td>-5.20e-02</td>
<td>3.30e-01</td>
</tr>
<tr>
<td>CM vs. PC</td>
<td>2.71e-01</td>
<td>7.60e-02</td>
<td>-9.80e-02</td>
<td>-3.60e-02</td>
<td>-3.30e-02</td>
<td>-4.00e-03</td>
<td>-5.80e-02</td>
<td>-2.30e-02</td>
<td>2.00e-01</td>
<td>-1.15e-01</td>
<td>5.90e-02</td>
<td>3.30e-02</td>
</tr>
<tr>
<td>Dune Habitat</td>
<td>*</td>
<td>**</td>
<td></td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Back vs. Fore</td>
<td>1.00e-01</td>
<td>2.37e-01</td>
<td>-2.26e-01</td>
<td>4.17e-01</td>
<td>3.43e-01</td>
<td>1.84e-01</td>
<td>2.30e-02</td>
<td>6.30e-02</td>
<td>-7.20e-02</td>
<td>-7.90e-02</td>
<td>-6.00e-02</td>
<td>-1.91e-01</td>
</tr>
<tr>
<td>Disturbance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disturbed vs. Undisturbed</td>
<td>-7.70e-02</td>
<td>-3.40e-02</td>
<td>8.50e-02</td>
<td>-6.80e-02</td>
<td>4.00e-02</td>
<td>-4.50e-02</td>
<td>2.70e-02</td>
<td>-6.60e-02</td>
<td>-3.90e-02</td>
<td>-6.40e-02</td>
<td>1.20e-01</td>
<td>-1.80e-02</td>
</tr>
<tr>
<td>Host species</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. mollis vs. A. arenaria</td>
<td>-4.80e-02</td>
<td>3.30e-02</td>
<td>0.00e+00</td>
<td>3.60e-02</td>
<td>2.60e-02</td>
<td>7.00e-03</td>
<td>-3.00e-02</td>
<td>2.20e-02</td>
<td>2.40e-02</td>
<td>1.40e-02</td>
<td>-1.70e-02</td>
<td>-1.00e-02</td>
</tr>
<tr>
<td>A. arenaria vs. A. breviligulata</td>
<td>-7.40e-02</td>
<td>1.40e-02</td>
<td>4.00e-02</td>
<td>3.40e-02</td>
<td>-8.00e-02</td>
<td>-2.80e-02</td>
<td>-4.80e-02</td>
<td>-3.50e-02</td>
<td>1.00e-02</td>
<td>-3.90e-02</td>
<td>-4.80e-02</td>
<td>8.80e-02</td>
</tr>
<tr>
<td>Tiller source</td>
<td>*</td>
<td>**</td>
<td></td>
<td>*</td>
<td>*</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Back vs. Fore</td>
<td>-2.95e-01</td>
<td>3.00e-02</td>
<td>9.50e-02</td>
<td>2.04e-01</td>
<td>1.60e-02</td>
<td>4.90e-02</td>
<td>6.50e-02</td>
<td>-3.80e-02</td>
<td>3.80e-02</td>
<td>-1.40e-02</td>
<td>2.00e-02</td>
<td>2.50e-02</td>
</tr>
</tbody>
</table>

*Significance of model terms ($p < .05$) in the ANOVA with $p$-values corrected for multiple comparison, and *significance of model terms ($p < .05$) uncorrected for multiple tests. The sites are Pacific City (PC), Sand Lake (SL) and Cape Meares (CM).
abundance in foredune sourced tillers) and Leptodontium (higher abundance in backdune-sourced tillers) regardless of dune habitat. None of the genera was significantly affected by disturbance or host species (Table 3).

4 | DISCUSSION

In this study, we examined the relative importance of two sources of propagules for the assembly of fungal endophyte communities: the environment external to the host and the internal sources from within the host. We found that external sources of propagules were generally more important to endophyte richness and community composition than were internal sources, although internal sources were important for colonization of the dominant endophytic taxon, Microdochium bolleyi. Beachgrass tillers transplanted to the late successional backdune habitat had higher root endophyte richness and distinct endophyte community composition compared to those in early successional foredune habitat regardless of disturbance. Overall, our findings were consistent across three host plant species and largely supported by both the culture-based and NGS-based approaches. To our knowledge, ours is the first experimental study to investigate the relative importance of external and internal sources of propagules on establishment of endophytic fungal communities. The results further our understanding of the ecological processes underlying the patterns of microbial symbiont diversity and advance our ability to predict symbiont community assembly.

Our results demonstrate that external sources of endophyte propagules associated with successional habitat mediate endophyte community assembly regardless of whether those habitats are disturbed or intact. These results are consistent with previous findings that soil properties, particularly soil pH, are strong predictors of root endophyte community composition in beachgrasses (David et al., 2016b; Johansen et al., 2017) and suggest that edaphic properties may differentially affect the persistence of fungal propagules such as spores, hyphal fragments and sclerotia (Currah, Tsuneda, & Mukarami, 1993). The distinct environmental conditions of the foredune and backdune habitats likely act as habitat filters that limit the diversity of endophytic taxa available to colonize roots. Indeed, the taxonomic composition of endophyte communities of plants in fore- and backdune habitats differed, as we found that the Ascomycota class Sordariomycetes (particularly the genera Fusarium and Apodus) were more often found in early successional forested habitat, while the phylum Glomeromycota and the Ascomycota classes Eurotiomycetes and Leotiomyentes were found more often in late successional backdune habitats. Because most endophytic taxa were apparently drawn from external sources in the environment, and we observed few instances of host species × habitat effects, the structure of endophyte communities may be more strongly related to the survival and reproduction outside hosts than to specific interactions with plant hosts.

The disturbance treatment did not significantly affect endophyte richness or community composition in either the frequently disturbed foredune or the relatively stable backdune. Given the importance of disturbance for structuring communities of other symbionts such as arbuscular and ectomycorrhizal fungi (Bruns, 1995; Jasper et al., 1991; Lindahl et al., 2010), it is surprising that beachgrass root endophyte communities are not similarly affected, especially in the backdune where the Glomeromycota (arbuscular mycorrhizal fungi) are more common. These results suggest that many of the taxa we observe are not recruited from hyphal networks in the soil. Instead, we infer that endophytic asexual spores, hyphal fragments and sclerotia are important propagule sources that are unaffected by disturbance. While different types of disturbance such as fire or foliar herbivory may affect endophyte communities (Christian, Sullivan, Visser, & Clay, 2016; David, Quiram, et al., 2016; Huang, Devan, U'Ren, Furr, & Arnold, 2015), soil disturbance and the removal of vegetation appears to have little effect on root endophyte communities in beachgrass.

Internal sources of propagules played an important role in the establishment of the dominant endophyte genus, Microdochium. Microdochium was more common in tillers collected from the foredune than from the backdune both before and after the experiment, suggesting that internal sources of Microdochium propagules are important for colonization of new roots. A critical next step in endophyte ecology is understanding the mechanisms by which internal sources of endophyte propagules colonize new tissue. Previous results suggest that Microdochium likely colonizes new roots from infected roots of the same individual as field collected tillers grown in sterile soils with no other source of inoculum were colonized by Microdochium (David, May, et al., 2016). Furthermore, while most endophytic taxa are thought to form small, local infections within host tissue, dark septate endophytes like Microdochium extensively colonize the root, and can form loose hyphal networks on the root surface (Rodriguez et al., 2009). Due to the close physical proximity of older, infected roots and new roots, Microdochium could be among the first taxa to colonize new roots and therefore cause a strong priority effect on the resulting fungal endophyte community (Vannette & Fukami, 2014). Yet, because we found significant effects of tiller source on abundance for only one other endophyte, the dark septate endophyte Leptodontidium sp., internal propagule sources may be the exception rather than the rule for most endophytic taxa of roots. Nonetheless, dark septate root endophytes such as Microdochium may well play critical roles in the establishment of new endophyte communities.

Results of our study are robust to the different biases that either culture-based and NGS-based approaches alone might cause (Arnold et al., 2007; Nguyen et al., 2015). Similar to the results of Arnold et al. (2007), the NGS-based data set generated OTUs belonging to a wider diversity of fungal phyla whereas culture-based results were apparently biased towards recovery of members of the Pezizomycotina subphylum of Ascomycota. Nonetheless, the results using the culture-based and NGS-based data sets agreed for most analyses, likely because the Pezizomycotina in fact are the dominant taxa in beachgrass root endophyte communities. In cases where results from the two approaches seemingly disagreed, we could attribute the discrepancy to the limited number of taxa recovered by the culture-based approach. For example, the endophyte community composition
analysis showed an effect of dune habitat in the NGS-based but not the culture-based approach (Figure 2), a difference we attributed to the biased recovery of *Microdochium bolleyi* in the culture-based data set. Finally, while NGS-based studies have become increasingly common, culture-based studies provide critical verification that the organisms are present within the host that is necessary for appropriate interpretation of sequences in negative control samples.

Our study, along with other recent studies (Leff et al., 2015; Rudgers et al., 2014), demonstrates the power of manipulative field experiments for understanding the ecological processes contributing to the assembly of symbiotic microbial communities within hosts. Importantly, our results show that endophyte communities are drawn largely from external sources of propagules associated with specific habitats. Moreover, the ability of fungal taxa to persist and reproduce outside the host may be as important as their interactions with the host itself in determining these communities, and future work might profitably be directed towards understanding the ecology of endophytes, including life-history phases outside of the host (e.g., Thomas, Vandegrift, Ludden, Carroll, & Roy, 2016; U’Ren & Arnold, 2016). Our study advances our understanding of the ecological processes underlying the patterns of microbial symbiont community composition and ultimately contributes to our ability to predict microbial symbiont community assembly.

**ACKNOWLEDGEMENTS**

We thank D. Schmidt for field and laboratory assistance; S. Hacker and J. Spatafora for providing laboratory facilities; D. Gohl and the Univ. of Minnesota Genomic Center for sequencing assistance; and Z. Song, B. Condon, N. Nguyen and R. Johansen for assistance with bioinformatics. We also thank P. Kennedy, L. Cline, M. Afkhami and C. Searcy for their thoughtful feedback on this manuscript. We acknowledge Oregon Parks and Recreation Department and Tillamook County Parks Department for granting permits to conduct this research.

**DATA ACCESSIBILITY**

All successfully sequenced cultured isolates and potential contaminants were deposited in GenBank under Accession nos. KU837877–KU839620. Cultures were deposited in the May Lab culture collection at the University of Minnesota. For the NGS study, all raw sequence files were deposited in the NCBI Sequence Read Archive SRP070824. All data files, including taxonomic assignments for culture-based and NGS OTUs, are available at http://conservancy.umn.edu/handle/11299/181235 (David, Seabloom, & May, 2016a). Additional methods, including bioinformatics processing scripts, are provided in the Supplemental Information.

**AUTHOR CONTRIBUTIONS**

A.S.D., E.W.S. and G.M. designed the study. A.S.D. conducted the study, analysed the data and wrote the manuscript with input from E.W.S. and G.M.

**REFERENCES**


Cooper, W. S. (1958). *Coastal sand dunes*. Geological Society of America, Memoir 72, Boulder, CO.


SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.