Microb Ecol
DOI 10.1007/s00248-015-0712-6

PLANT MICROBE INTERACTIONS

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

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Received: 26 June 2015 / Accepted: 16 November 2015

Abstract Microbial symbionts inhabit tissues of all plants and animals. Their community composition depends largely on two ecological processes: (1) filtering by abiotic conditions and host species determining the environments that symbionts are able to colonize and (2) dispersal-limitation determining the pool of symbionts available to colonize a given host and community spatial structure. In plants, the above- and belowground tissues represent such distinct habitats for symbionts that we expect different effects of filtering and spatial structuring on their symbiont communities. In this study, we characterized above- and belowground communities of fungal endophytes—fungi living asymptomatically within plants—to understand the contributions of filtering and spatial structure to endophyte community composition. We used a culture-based approach to characterize endophytes growing in leaves and roots of three species of coastal beachgrasses in dunes of the USA Pacific Northwest. For leaves, endophyte isolation frequency and OTU richness depended primarily on plant host species. In comparison, for roots, both isolation frequency and OTU richness increased from the nutrient-poor front of the dune to the higher-nutrient backdune. Endophyte community composition in leaves exhibited a distance-decay relationship across the region. In a laboratory assay, faster growth rates and lower spore production were more often associated with leaf- than root-inhabiting endophytes. Overall, our results reveal a greater importance of biotic filtering by host species and dispersal-limitation over regional geographic distances for aboveground leaf endophyte communities and stronger effects of abiotic environmental filtering and locally patchy distributions for belowground root endophyte communities.

Keywords Endophyte · Community assembly · Environmental drivers · Dunes · Ammophila · Spatial structure

Introduction

Microbial symbionts inhabit all plants and animals, with their effects on hosts spanning from mutualism to parasitism [1–3]. Because interactions between hosts and their symbionts have important implications for host fitness, it is critical to understand the ecological factors that underlie the assembly of symbiont communities [e.g., 3, 4]. Symbiont communities are influenced by several abiotic and biotic factors that may limit symbiont species' abilities to colonize, persist, and disperse [5–9]. Furthermore, ecological communities, and symbiont communities in particular, are hierarchically structured by processes occurring at regional, local, and within-host scales [6]. Understanding how abiotic and biotic factors can influence communities across scales may provide insights into the processes governing assembly of symbiont communities, and ultimately how they may affect host growth and reproduction.

Ecological filters can determine which individual species are capable of colonizing and persisting in the host, and, in the case of symbionts, these filters can broadly be subdivided into the environment external to the host (hereafter "environment") and host species [4, 8]. The environment includes abiotic

Electronic supplementary material The online version of this article (doi:10.1007/s00248-015-0712-6) contains supplementary material, which is available to authorized users.

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Published online: 01 December 2015

factors such as climate (temperature, humidity, precipitation, etc.) and soil properties (soil type, pH, nutrient availability, etc.) that may influence a symbiont species' ability to persist in that environment [10–13]. For instance, desert soil provides a filter for thermophilic fungi that are able to grow at high temperatures [14]. The biotic filter of the host may include factors such as host defense and host tissue chemistry that influence the symbiont species' ability to infect, grow, and reproduce within a host [8, 9]. While both the environment and host species contribute to symbiont community composition [e.g., 15], the relative importance of each for symbiont community assembly is not yet clear. Many symbiont species have an apparently broad host range, and such communities may be completely shaped by the environment [e.g., 8]. Other symbiont species have a more narrow host range and their communities are likely more strongly influenced by host species or genotype than by environment [e.g., 16, 17].

Furthermore, the importance of dispersal-limitation, or the inability of propagules of species to arrive at a given site [18], has become more widely recognized in microbial communities [19–21]. Microbial dispersal-limitations have been long overlooked due in part to the pervasive and influential Baas-Becking hypothesis [22] that "everything is everywhere." While microbes may indeed be dispersal-limited [e.g., 20, 21], the role of dispersal-limitation in structuring microbial communities, particularly symbionts, is not well understood and may vary for different microbial and host species and across different spatial scales. While dispersal itself is notoriously difficult to measure for any organism, dispersal-limitation is inferred from assessments of spatial structure [19]. At the regional scale, if symbiont species vary in their dispersal, then communities will be more dissimilar from one another with increasing geographic distance and exhibit spatial structure assessed as a distance-decay relationship [20, 21, 23, 24]. However, the lack of a distance-decay relationship could have several causes, such as strong, local filtering by the environment or hosts, or communities composed of highly mobile and less dispersal-limited species.

While the relative strengths of filtering and dispersal-limitation in structuring symbiont communities may vary across host species and environments, there is also variation in communities within a host individual [6]. Symbiont communities are often heterogeneous in composition across host tissues [9, 25–27], and some component species may provide tissue-specific functions [28, 29]. For symbionts of plants, the air mediates symbiont community assembly in aboveground tissue yet provides a relatively weak environmental filter, while soil mediates belowground tissue communities and provides a relatively strong filter [sensu 30]. Moreover, above- and belowground plant tissues differ greatly as habitats for microbial growth and reproduction, and if fungal endophyte species have adapted differently to these environments, relative investment in vegetative growth versus spore production may differ among symbionts. For instance, aboveground symbionts might be expected to produce more spores and thus travel greater distances between plants than do belowground symbionts [e.g., 31, 32]. Conversely, if belowground symbionts colonize hosts via a hyphal network growing through the soil, their hyphae may grow faster than that of aboveground symbionts.

Here, we considered how the assembly of above- and belowground symbiont communities differ with respect to (1) the importance of filtering by environment and host species, (2) filtering-independent spatial structure at the regional scale, and (3) the growth and sporulation of individual symbionts. Our general expectations were that belowground symbiont communities should exhibit greater filtering by the environment and greater spatial structure than aboveground symbiont communities, and that the belowground symbionts should grow faster and produce fewer spores than those isolated from aboveground communities. A priori, we expected that filtering by host species should not differ between above- and belowground symbiont communities. To investigate these questions, we characterized communities of fungal endophytes for each of three species of beachgrasses along the USA Pacific Northwest coast by culturing and sequencing fungi from asymptomatic plant tissues and delimiting operational taxonomic units (OTUs) based on sequence similarity. Fungal endophytes are a diverse group of fungi primarily belonging to the phylum Ascomycota and inhabiting tissues of all plants without causing disease symptoms [9, 26]. While some endophytes grow systemically throughout the plant and are vertically transmitted, most cause small, local infections and are horizontally transmitted [9]. We evaluated the importance of abiotic environment (physical location along the dune and soil properties) and biotic environment (host species), in affecting endophyte isolation frequency, OTU richness, and community composition at both local (within sites) and regional (across sites) levels. For both above- and belowground communities, spatial structure was evaluated by determining relationships between community similarity and geographic distance (distance-decay) and differences in turnover in composition across sites (i.e., beta diversity). Finally, we quantified differences in life-history traits of individual symbionts by measuring colony growth rate and asexual spore production of the 25 most commonly occurring endophyte OTUs.

Methods

System

We studied culturable fungal endophyte species in beachgrasses that occurred in dunes along the Pacific Coast of Oregon and Washington, USA. These dunes provide habitat for endemic and federally listed plant and bird species, and the three dominant common grass species studied here—the native *Elymus mollis*, and the exotic *Ammophila arenaria* and *Ammophila breviligulata* introduced to stabilize dunes and

protect inland developments from flooding [33–35]. Dunes contain a strong environmental gradient from the shore to inland areas in which the backsides of dunes tend to be shielded from wind and are composed of more stable soil with higher C, N, and micronutrients than are soils at the front of the dune [36, 37]. We categorized local environments as the front, crest, and backdunes (hereafter "dune locations") constituting, respectively, the front slope of the dune, the top of the dune, and the area beyond the back slope of the dune.

Sampling

We sampled from five sites in which all three grass species co-occurred along the Oregon and Washington coast in late August to early September 2011. The five sites included four in Oregon (Pacific City, Sand Lake, Seaside, and Fort Stevens) and one in Washington (Grays Harbor). Distances between sites ranged from 8 to 180 km. Within sites, we sampled along one to four transects, each containing a single 3 × 3 m quadrat in the front, crest, and back of the dune (mean transect length= 48.64 m±6.64 S.E.). We selected locations for transects to represent a broad range of relative amount of cover by the three grass species, which we visually estimated for each quadrat. We collected roots and leaves from three individual plants (defined as one or more tillers attached to a single rhizome) and placed them in Ziploc bags until processing.

One soil core (10-cm deep) was taken from each quadrat. Soils were air-dried and analyzed for percent C and percent N at the University of Nebraska Ecosystem Analysis Laboratory (Lincoln, NE, USA) and pH, cation exchange capacity, organic matter, and concentrations of Ca, K, P, Na, S by A&L Analytical Laboratories (Memphis, TN, USA).

Endophyte Culturing and Identification

Under sterile laboratory conditions, we surface sterilized plant tissue using a rinse in sterile DI water followed by successive baths of 70 % ethanol (1 min), 70 % bleach (3.675 % NaOCl, 2 min), 70 % ethanol (1 min), and a final rinse in sterile DI water. Tissue was cut into ~1.5 mm^2 segments and placed onto 2 % malt extract agar (MEA). For each individual plant, 20 sterilized tissue segments were placed in MEA, 10 from leaves and 10 from roots. Cultures were allowed to grow for 4 months before sub-culturing onto separate Petri dishes. Control plates were made by pressing surface-sterilized tissue to the media and checked for any subsequent fungal growth that would indicate the presence of surface fungi.

We extracted total genomic DNA and PCR amplified the ITS rDNA region from each isolate using one of two methods. In the first method, we used SDS buffer and phenol:chloroform extraction [38]. The ITS-LSU gene was amplified as a single amplicon using the ITS1F [39] and LR3 primers [40] and Takara Taq (Takara Bio Inc. Otsu, Shiga, Japan) (40 μL reaction consisting of 5 μL Takara 10× buffer, 4 μL dNTP (10 μM), 1 μL (10 μM) each of forward and reverse primer, 35 cycles of 95 °C for 1 min, 52 °C for 1 min, 72 °C for 1.30 min). In the second method, we used the Extract N' Amp ReadyMix kit (Sigma Aldrich Corp., St. Louis, MO, USA) to extract and amplify the DNA (20 μL reaction consisting of 5 μL REDExtract N' Amp buffer, 0.8 μL (10 μM) each of forward and reverse primer, 35 cycles of 95 °C for 30 s, 54 °C for 30 s, 72 °C for 1 min). Sanger sequencing of amplicons was conducted at Beckman Coulter (Brea, CA, USA). Sequences were edited using Geneious [41] and clustered into OTUs at the 97 % similarity level using the workflow developed by Monacell and Carbone [42] that uses ITS extractor [43] to extract the ITS1 and ITS2 regions, MOTHUR [44] to check for chimeras, and ESPRIT [45] to cluster sequences based on alignment-free similarity.

Growth Assays

We measured colony growth rates and asexual spore production of the 25 most commonly isolated OTUs (Table 1). Three isolates representing each OTU were randomly selected and plated onto starter cultures on MEA. From the starter cultures, two plugs (0.5-cm diameter) were each plated onto separate MEA culture plates. We measured the diameter of the cultures every 2 days for 14 days when the fastest growing cultures neared the edge of their plates. At the conclusion of the study, we collected the asexual spores by flooding the Petri plates with 10 mL DI water, scraping the spores off the culture, and counting the number of spores in a hemacytometer.

Data Analysis

We used R Version 3.0.2 [46] to conduct all analyses and to generate figures. Data, GenBank accession numbers, and additional sample location information were deposited in the publically available Data Repository for the University of Minnesota [47].

Filtering

To evaluate the importance of filtering, we examined the relationship of abiotic environmental factors and host species on the frequency at which we obtained fungal endophyte cultures (isolation frequency), OTU richness, and endophyte community composition at spatial scales of quadrat and site. Because several soil variables were highly correlated, we clustered variables based on Ward's minimum variance method to determine collinearity (Online Resource 1). Based on this analysis, we included the effects of %N, organic matter, and soil pH as environmental

Table 1 OTU assignments based on alignment-free clustering at the 97 % similarity level.

OTU	Total isolations (leaf isolations)	Phylum	Class	Order	Closest BLAST match (GenBank accession no.)	Query length (coverage)	Similarity %
154	2 (0)	Ascomycota	Dothideomycetes	Botryosphaeriales	*Microdiplodia hawaiiensis* (GU361956)	428 (89.64)	90.5
13[a]	7 (6)	Ascomycota	Dothideomycetes	Capnodiales	*Cladosporium cladosporioides* (JQ724382)	466 (100)	100
157[a]	6 (1)	Ascomycota	Dothideomycetes	Capnodiales	*Cladosporium sphaerospermum* (AB572897)	467 (100)	100
145[a]	10 (10)	Ascomycota	Dothideomycetes	Capnodiales	*Penidiella strumelloidea* (EU019277)	434 (95.11)	91.9
148	3 (3)	Ascomycota	Dothideomycetes	Capnodiales	*Penidiella strumelloidea* (EU019277)	443 (97.33)	92.6
170	1 (1)	Ascomycota	Dothideomycetes	Capnodiales	*Penidiella strumelloidea* (EU019277)	468 (98.73)	95.7
169	1 (1)	Ascomycota	Dothideomycetes	Pleosporales	*Drechslera erythrospila* (EU552124)	554 (100)	100
41	13 (0)	Ascomycota	Dothideomycetes	Pleosporales	*Drechslera nobleae* (AY004792)	474 (100)	91.2
151	1 (1)	Ascomycota	Dothideomycetes	Pleosporales	*Fusicladium sicilianum* (FN549914)	283 (50.25)	85.3
141[a]	23 (21)	Ascomycota	Dothideomycetes	Pleosporales	*Lewia infectoria* (EF104194)	513 (99.61)	95.1
158	2 (1)	Ascomycota	Dothideomycetes	Pleosporales	*Lewia infectoria* (GU584953)	498 (97.46)	100
88[a]	13 (9)	Ascomycota	Dothideomycetes	Pleosporales	*Lewia infectoria* (JX421701)	501 (97.46)	98.4
168	1 (0)	Ascomycota	Dothideomycetes	Pleosporales	*Ophiosphaerella agrostis* (AF191550)	506 (100)	89.8
149[a]	7 (7)	Ascomycota	Dothideomycetes	Pleosporales	*Phaeosphaeria insignis* (AF439485)	503 (99.6)	98.6
166[a]	4 (4)	Ascomycota	Dothideomycetes	Pleosporales	*Phaeosphaeria nigrans* (AF439492)	485 (97.59)	97.7
161	1 (1)	Ascomycota	Dothideomycetes	Pleosporales	*Phaeosphaeria triglochinicola* (AF439507)	493 (99.2)	88.6
146[a]	5 (4)	Ascomycota	Dothideomycetes	Pleosporales	*Pleospora herbarum* (GU584954)	494 (100)	99.6
25	2 (0)	Ascomycota	Eurotiomycetes	Chaetothyriales	*Cladophialophora chaetospira* (EU137333)	528 (100)	100
97[a]	3 (0)	Ascomycota	Eurotiomycetes	Chaetothyriales	*Exophiala salmonis* (AF050274)	550 (97.68)	97.5
22[a]	16 (1)	Ascomycota	Eurotiomycetes	Chaetothyriales	*Exophiala salmonis* (AM176667)	535 (97.46)	95.9
10	4 (0)	Ascomycota	Eurotiomycetes	Chaetothyriales	*Exophiala salmonis* (GU586858)	561 (99.82)	99.8
94	1 (0)	Ascomycota	Eurotiomycetes	Eurotiales	*Aspergillus tubingensis* (JQ693399)	514 (100)	100
142	7 (4)	Ascomycota	Eurotiomycetes	Eurotiales	*Penicillium brevicompactum* (FJ004277)	493 (100)	100
60[a]	4 (0)	Ascomycota	Eurotiomycetes	Eurotiales	*Penicillium canescens* (JN585940)	380 (76.31)	100
164	1 (1)	Ascomycota	Leotiomycetes	Helotiales	*Cadophora hiberna* (AF530463)	459 (100)	92.8
124[a]	3 (0)	Ascomycota	Leotiomycetes	Helotiales	*Cryptosporiopsis rhizophila* (AY176753)	472 (100)	100
21	1 (0)	Ascomycota	Leotiomycetes	Helotiales	Dactylaria appendiculata (AY265339)	545 (100)	90.6
34	1 (1)	Ascomycota	Leotiomycetes	Helotiales	*Dactylaria appendiculata* (AY265339)	531 (100)	95
3[a]	8 (0)	Ascomycota	Leotiomycetes	Helotiales	*Leptodontidium orchidicola* (AF214577)	534 (97.8)	100
68	1 (0)	Ascomycota	Leotiomycetes	Helotiales	*Leptodontidium orchidicola* (FJ665276)	537 (99.08)	97.8
72	2 (0)	Ascomycota	Leotiomycetes	Helotiales	*Phialocephala fortinii* (EU314682)	479 (100)	99.4
155	2 (2)	Ascomycota	Leotiomycetes	Thelebolales	*Thelebolus microsporus* (DQ402525)	428 (84.2)	88.6
85	4 (0)	Ascomycota	Leotiomycetes	uncertain	*Leohumicola minima* (AY706329)	464 (100)	92.5
54[a]	4 (0)	Ascomycota	Leotiomycetes	uncertain	*Leohumicola verrucosa* (AY706325)	467 (100)	89.6
70	2 (0)	Ascomycota	Leotiomycetes	uncertain	*Meliniomyces bicolor* (AJ308340)	470 (100)	98.3
29	5 (0)	Ascomycota	Sordariomycetes	Diaporthales	*Phomopsis columnaris* (GU934561)	488 (100)	100
18	1 (0)	Ascomycota	Sordariomycetes	Hypocreales	*Acremonium cavaraeanum* (JF912333)	484 (96.71)	90.2
56	1 (1)	Ascomycota	Sordariomycetes	Hypocreales	*Acremonium nepalense* (GU586837)	475 (100)	96.2
65[a]	14 (0)	Ascomycota	Sordariomycetes	Hypocreales	*Acremonium strictum* (AM924152)	501 (100)	100
78[a]	5 (0)	Ascomycota	Sordariomycetes	Hypocreales	*Acremonium strictum* (HM016899)	482 (96.82)	95.1
51	3 (0)	Ascomycota	Sordariomycetes	Hypocreales	*Fusarium avenaceum* (JX074742)	478 (100)	100
128	1 (0)	Ascomycota	Sordariomycetes	Hypocreales	*Fusarium pseudograminearum* (JF739304)	468 (100)	100
66	2 (0)	Ascomycota	Sordariomycetes	Hypocreales	*Hirsutella rhossiliensis* (DQ345567)	501 (100)	100
16[a]	3 (0)	Ascomycota	Sordariomycetes	Hypocreales	*Neonectria ditissima* (JF735309)	447 (97.42)	83.2
33	2 (0)	Ascomycota	Sordariomycetes	Hypocreales	*Neonectria radicicola* (GU934547)	459 (100)	100
37	2 (0)	Ascomycota	Sordariomycetes	Hypocreales	*Trichoderma pubescens* (EU280121)	516 (100)	99.8
8	1 (0)	Ascomycota	Sordariomycetes	Hypocreales	*Trichoderma viride* (AF359255)	518 (100)	100
2[a]	4 (0)	Ascomycota	Sordariomycetes	Magnaporthaceae	*Gaeumannomyces cylindrosporus* (JF508361)	509 (100)	99.8

Table 1 (continued)

OTU	Total isolations (leaf isolations)	Phylum	Class	Order	Closest BLAST match (GenBank accession no.)	Query length (coverage)	Similarity %
103[a]	3 (0)	Ascomycota	Sordariomycetes	Magnaporthaceae	*Gaeumannomyces graminis* var tritici (FJ771005)	484 (100)	100
165	1 (0)	Ascomycota	Sordariomycetes	Microascales	*Corollospora maritima* (JN943388)	533 (96.17)	81
147	1 (0)	Ascomycota	Sordariomycetes	Microascales	*Microascus trigonosporus* var trigon (AM774156)	402 (82.29)	89.4
119[a]	4 (0)	Ascomycota	Sordariomycetes	Microascales	*Pseudallescheria boydii* (JN207435)	356 (67.49)	82
95	1 (0)	Ascomycota	Sordariomycetes	Microascales	*Scedosporium apiospermum* (JN872195)	250 (47.41)	90.1
62	1 (0)	Ascomycota	Sordariomycetes	Phyllachorales	*Plectosphaerella cucumerina* (JQ796755)	469 (100)	100
115	1 (0)	Ascomycota	Sordariomycetes	Sordariales	*Cercophora coprophila* (AY999136)	466 (96.82)	92.4
30	2 (0)	Ascomycota	Sordariomycetes	Sordariales	*Chaetomium funicola* (FN394680)	495 (100)	100
24	1 (1)	Ascomycota	Sordariomycetes	Sordariales	*Kylindria ellisii* (EF029190)	360 (74.27)	81.3
143	4 (0)	Ascomycota	Sordariomycetes	Sordariales	*Podospora glutinans* (AY615208)	496 (100)	98.8
6[a]	3 (0)	Ascomycota	Sordariomycetes	Sordariales	*Podospora minicauda* (GQ922539)	473 (100)	97.7
159	1 (1)	Ascomycota	Sordariomycetes	Sordariales	*Podospora pleiospora* (AY515364)	481 (100)	100
160	2 (2)	Ascomycota	Sordariomycetes	Trichosphaeriales	*Nigrospora oryzae* (JN211105)	479 (100)	97.9
162	1 (0)	Ascomycota	Sordariomycetes	uncertain	*Arthrinium phaeospermum* (AJ279447)	526 (100)	99.2
156	1 (0)	Ascomycota	Sordariomycetes	uncertain	*Myrmecridium schulzeri* (EU041772)	481 (99.17)	96.9
1[a]	66 (1)	Ascomycota	Sordariomycetes	Xylariales	*Microdochium bolleyi* (GU934539)	469 (100)	100
153	2 (0)	Ascomycota	Sordariomycetes	Xylariales	*Microdochium nivale* (AM502260)	468 (100)	100
79[a]	5 (1)	Ascomycota	Sordariomycetes	Xylariales	*Microdochium phragmitis* (AM502263)	462 (100)	99.6
144	1 (1)	Ascomycota	Sordariomycetes	Xylariales	*Rosellinia pepo* (AB017659)	480 (97.88)	80.4
102	2 (0)	Ascomycota	uncertain	uncertain	*Chalara piceaeabietis* (FR667230)	467 (100)	91.3
9[a]	19 (1)	Ascomycota	uncertain	uncertain	*Dokmaia monthadangii* (JN559405)	448 (100)	99.1
5[a]	18 (0)	Ascomycota	uncertain	uncertain	*Xenochalara juniperi* (DQ132827)	481 (100)	92.1
104	6 (0)	Basidiomycota	Agaricomycetes	Agaricales	*Marasmius tricolor* (JN943601)	618 (100)	98.5
150	2 (0)	Basidiomycota	Agaricomycetes	Agaricales	*Panaeolus acuminatus* (JF908518)	569 (100)	99.8
163	1 (0)	Basidiomycota	Agaricomycetes	Agaricales	*Tetrapyrgos subdendrophora* (EF175521)	650 (100)	99.4
152	1 (0)	Basidiomycota	Agaricomycetes	Cantharellales	*Tulasnella calospora* (AB369940)	552 (100)	98.9
39	2 (0)	Basidiomycota	Tremellomycetes	Tremellales	*Exidia uvapsassa* (DQ241776)	505 (100)	87.4
167	1 (0)	Zygomycota	uncertain	Mortierellales	*Mortierella globulifera* (JN943800)	584 (99.83)	99.7

Columns show the OTU name assigned in this study, the total number of times the OTU was isolated and the number of times it was isolated in leaves, the taxonomy based on the closest BLAST match, query length with coverage, and percent similarity of the query to the BLAST match

[a] Those OTUs used in the growth assay

covariates. We also included latitude as an environmental variable to account for regional changes in the relative abundances of the three grass species [33].

We determined the effect of host community by examining the relationship between the neighboring conspecific host cover endophyte abundance and OTU richness. A positive relationship could suggest that the neighboring conspecific hosts serve as source of endophyte inocula. Cover was scaled as the relative proportion of the percent covers of the three grass species in a quadrat (i.e., the relative covers of all three species in a quadrat summed to 1), and the conspecific cover was the cover value for a given host species. We visualized these results using the predicted values of a simplified generalized linear mixed-model with binomial error with site and transect as nested random effects and host species and conspecific host cover as fixed effects.

We estimated endophyte OTU richness, the number of different OTUs present in a sample, at the quadrat, site, and regional levels. At the quadrat level, OTU richness was calculated as the rarefied number of OTUs found within conspecific plant hosts within a quadrat. Richness was rarefied according to the lowest sampling effort (i.e., number of surface-sterilized tissue segments) of a host species within a quadrat. We used this quadrat-level approach to avoid pseudoreplication caused by sampling multiple individuals of the same plant species within a quadrat. At the site-level, we calculated a rarefied OTU richness for each dune location (front, crest, backdune) within sites and pooled across all three host species. At the regional level, we calculated richness using a similar rarefaction approach, but only estimated one value for each dune location pooled across all sites.

We used the lme4 package [48] in R to construct mixed-effects models to analyze the effects of environment and host species on isolation frequency and OTU richness. Transect was nested within site as random effects, and latitude, %N, organic matter, pH, conspecific host cover, dune location, host species, and the interactions conspecific host cover × host species and dune location × host species were fixed effects. All continuous predictor variables were scaled to a mean of 0 and standard deviation of 1 to allow for comparison of estimates [49]. Differences in the categorical variables dune location and host species were tested using independent contrasts. Dune location was analyzed first using a linear contrast that tested differences in the response variable between the front and back locations, and second with a quadratic contrast that tested whether the response variable for the crest location was higher or lower than expected along the linear transect. Host species was analyzed with the first contrast comparing *E. mollis* to both the *Ammophila* species, and the second contrast comparing *A. arenaria* to *A. breviligulata*.

For quadrat-level analyses, we used generalized mixed-effect models with binomial error structure to analyze endophyte abundance and a linear mixed-effect models to analyze rarefied OTU richness. Endophyte abundance in roots was quantified as the isolation frequency (i.e., proportion of sterilized tissue segments with an emergent fungus). Because we recovered endophytes from a low proportion of leaves, we analyzed endophyte abundance per leaf as the presence/absence of any endophytes in a leaf. For the richness analyses, only those plants with at least one OTU were used in the analysis to avoid effects of plants for which no endophytes were recovered and data were natural-log transformed to meet model assumptions. We used a model-averaging approach with the MuMIn package [50] in R to identify the important factors in each model. This approach first identified the best models within four AIC units, then averaged the coefficients in these models [49]. The importance value is a weighted average of the best models in which a term appears. For the site-level analyses, we used linear mixed-effects models to test how dune location and soil properties affected rarefied site-level richness. We used the model-averaging approach previously described and considered the effects of latitude, N, organic matter, pH, and dune location on rarefied species richness using site as a random effect.

To analyze sources of variation in endophyte community composition in leaves and roots, we used the *adonis*() function in the vegan package [51] to perform permutational multivariate analysis of variance (perMANOVA). We conducted a preliminary analysis to verify that endophyte community composition differed between leaves and roots ($F_{1,115}=7.48$, $p<0.001$) and, therefore, analyzed the two communities separately. We modeled endophyte community composition at the quadrat level (for quadrats in which at least one OTU was found) as a function of site, transect within a site, latitude, %nitrogen, organic matter, and pH dune location, host species, and dune location × host species. We then reduced models by sequentially eliminating non-significant terms.

Regional Spatial Structure

To assess distance-decay relationships in leaf and root endophyte community composition at the regional level, we performed multiple regression on distance matrices (MRM). Bray-Curtis community similarity matrices were calculated based on an average of 100 rarefactions of endophyte communities pooled across all individuals in each quadrat. Similarity matrices were regressed against a Euclidian distance matrix of physical distances among quadrats using the ecodist package [52]. We included the distance matrices for differences in %nitrogen, organic matter, and pH to control for effects of soil properties. We analyzed distance-decay for both above- and belowground community composition in two ways: first using a global analysis that included all quadrats, and second using separate analyses to analyze distance-decay patterns for each of the three dune locations. To visualize the error associated with isolation by distance estimates, we used a jackknife approach to calculate a standard error.

To analyze compositional turnover in endophyte communities across spatial scales, we compared the regional-level rarefied OTU richness to quadrat- and site-level richness. We also calculated a multivariate measure of turnover as the dispersion of quadrat-level community composition of each host species in multivariate space (i.e., average distance of points from the group centroid) and tested for differences across groups using the betadisper() function in the vegan package [51]. High compositional turnover is consistent with, but not proof of, dispersal-limitation.

Growth and Sporulation

We determined if endophytes found in leaves and roots differed in the rate of colony growth or sporulation by asking whether differences in these life-history traits predicted the tissue from which an OTU was isolated. For growth assays, we calculated the average growth rate for each individual culture plate as the slope of the linear regression of diameter colony growth against the number of days of growth (mean R^2 for all regressions =0.98, minimum $R^2=0.88$). We calculated a mean growth rate to characterize each OTU as an average of six slopes (three isolates per OTU × two replicate plates per isolate). Because the assay ended when the first isolates neared the edge of their Petri dishes, growth curves were linear and did not asymptote. We calculated spore production as the total spores estimated per culture divided by the final diameter length of the colony. This metric represented the number of spores produced per unit diameter of the colony. Analysis of total (unscaled) spore production yielded similar

results to those obtained using the spore production scaled by diameter. We used a GLM with quasibinomial error to account for overdispersion to analyze the effects of growth rate and spore production on the probability that a given OTU was found in a leaf. We tested the model for significance using an analysis of variance with type II sum of squares using the car package [53]. To assess model fit, we regressed the observed means as a function of the predicted means.

Results

We isolated a total of 607 fungal cultures out of 5538 surface sterilized plant tissues, 134 of which were isolated from leaves and 473 from roots. From these, we successfully extracted DNA, amplified, sequenced, and clustered 363 isolates into 76 OTUs at the 97 % level of sequence identity, 27 of which were found in leaves, 60 in roots, and 11 in both leaves and roots (Table 1). OTUs primarily belonged to the phylum Ascomycota (70 OTUs), but we also found members of the Basidiomycota (5 OTUs) and the Zygomycota (1 OTU). The most common classes included the Dothidiomycetes, Eurotiomycetes, Leotiomycetes, and Sordariomycetes. Several of the OTUs belonged to genera previously characterized as dark septate endophytes such as *Botryosphaeria*, *Cadophora* (*Phialophora*), *Exophiala*, *Leptodontidium*, *Leptosphaeria*, *Microascus*, *Microdochium*, *Periconia*, *Pleospora*, and *Xylaria* [54–56]. OTUs related to the genus *Lewia* were the most commonly isolated from leaves, and OTUs related to the genus *Microdochium* were most commonly isolated from roots (Table 1).

The OTUs associated with leaf communities were typically shared across hosts and locations (Fig. 1a). In total, *E. mollis* harbored 10 of 11 OTUs found more than once in leaves (non-singletons), while the *Ammophila* hosts tended to harbor somewhat smaller subsets of all non-singleton leaf endophytes (8/11 for *A. arenaria*, 6/11 for *A. breviligulata*; Fig. 1c). The OTUs associated with roots were more specific to dune locations than were those associated with leaves, as evidenced by the high number of OTUs found in only one dune location (Fig. 1b). Root endophyte OTUs exhibited a range of host species associations (Fig. 1d).

Evidence for Filtering

We first evaluated the effects of filtering on endophyte abundance in leaf and root tissues. Model averaging revealed that the abundance of endophytes in leaves was primarily influenced by host species (Table 2). The proportion of leaves from which we obtained at least one endophyte was higher for the native *E. mollis* than either of the *Ammophila* species (Table 2, Fig. 2). Isolation frequency in roots generally increased from

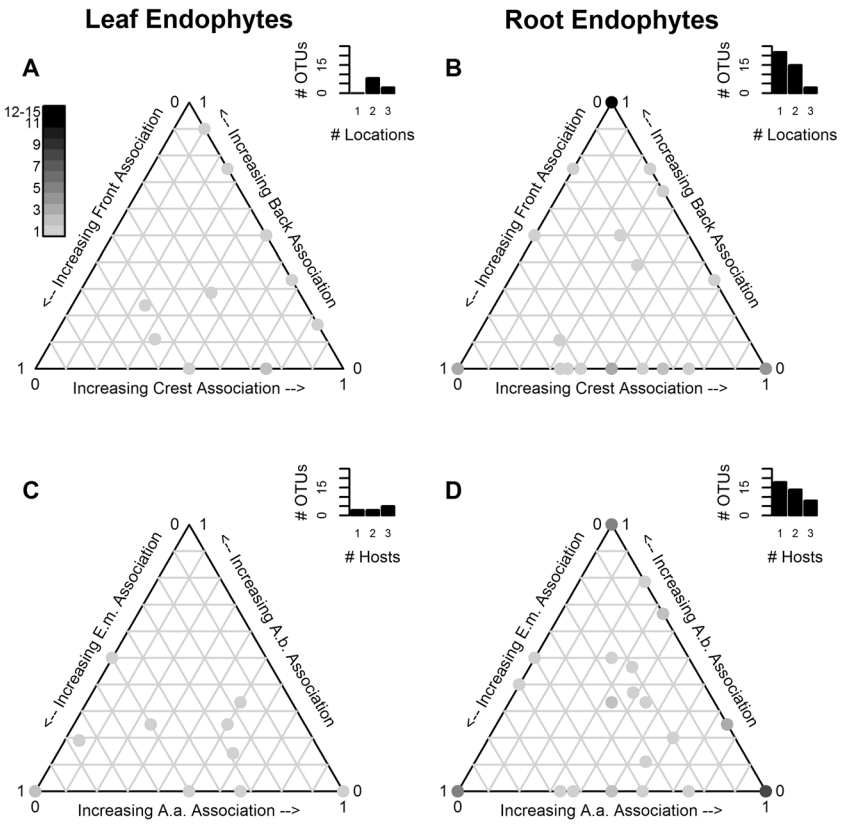

Fig. 1 Triangle plots showing endophyte OTUs shared across dune locations (**a**–**b**) and host species (**c**–**d**). Leaf endophytes are shown in (**a**) and (**c**), and root endophytes are shown in (**b**) and (**d**). Points are shaded based on the number of OTUs they represent. *Inset bar graphs* show the total number of OTUs shared across 1, 2, or 3 locations or host species. For host species, *E.m. Elymus mollis*, *A.a. Ammophila arenaria*, *A.b. A. breviligulata*

Table 2 Factors influencing quadrat-level endophyte isolation frequency and rarefied richness in leaves and roots based on model averaging

Term	Level	Leaves				Roots			
		Iso. freq.		Richness		Iso. freq.		Richness	
		Impt.	Est. (S.E.)	Impt.	Est. (S.E.)	Impt.	Est. (S.E.)	Impt.	Est. (S.E.)
(Intercept)			−1.10 (0.17)***		0.76 (0.04)***		−1.82 (0.27)***		1.00 (0.04)***
Latitude		0.12	0.07 (0.16)	0.20	0.04 (0.04)	0.25	−0.28 (0.27)	0.14	0.02 (0.04)
%Nitrogen		0.15	0.13 (0.23)	0.17	0.03 (0.04)	0.45	0.18 (0.12)	0.12	0.01 (0.04)
Organic matter		0.35	−0.24 (0.21)	0.26	−0.04 (0.04)	1.00	−0.29 (0.09)***	0.12	0.00 (0.04)
pH		0.15	−0.07 (0.16)	1.00	−0.12 (0.04)**	0.88	−0.38 (0.14)**	1.00	−0.18 (0.04)***
Conspecific host cover		0.84	0.40 (0.19)*	0.11	−0.02 (0.03)	0.16	0.05 (0.07)	0.14	−0.02 (0.04)
Conspecific host cover × host species	*E. mollis* vs. *Ammophila*	0.05	−0.17 (0.13)	0.00		0.00		0.00	
	A. arenaria vs. *A. breviligulata*		0.01 (0.23)						
Dune location	Linear	0.05	0.02 (0.27)	0.00		1.00	0.32 (0.18)	0.00	
	Quadratic		−0.35 (0.26)				0.46 (0.11)***		
Host species	*E. mollis* vs. *Ammophila*.	1.00	0.69 (0.13)***	0.00		1.00	0.00 (0.05)	0.07	0.01 (0.03)
	A. arenaria vs. *A. breviligulata*		0.27 (0.21)				0.01 (0.07)		0.06 (0.05)
Dune location × host species	Linear: (*E. mollis* vs. *Ammophila*)	0.00		0.00		1.00	−0.29 (0.07)***	0.00	
	Quadratic: (*E. mollis* vs. *Ammophila*)						0.28 (0.08)***		
	Linear: (*A. arenaria* vs. *A. breviligulata*)						0.02 (0.12)		
	Quadratic: (*A. arenaria* vs. *A. breviligulata*)						−0.19 (0.11)		
	# models within δ=4	13		8		7		6	

Importance values (*Impt.*) give the weighted proportion of models that a term appeared in with a cutoff of δ=4 AIC units. Estimates (*Est.*) are the model averaged estimates with the adjusted standard errors shown in parentheses. Only those terms that appeared in models within the δ=4 have estimates. All continuous variables were scaled prior to analyses to allow for comparison of estimates. Isolation frequency was analyzed with generalized mixed-effects models with binomial error structure. Richness was rarefied and natural-log transformed, and was analyzed using linear mixed-effects models

*$p<0.05$, **$p<0.01$, ***$p<0.001$

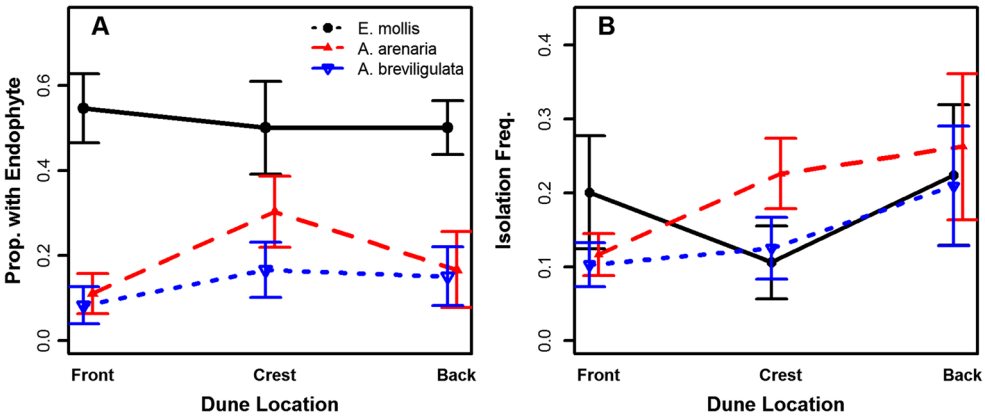

Fig. 2 Endophyte abundance in **a** leaves and **b** roots. In leaves, abundance is quantified as the proportion of individuals in a quadrat in which at least one endophyte was found. For roots, abundance is quantified as the isolation frequency (emergent fungi divided by total sterilized tissue segments) of endophytes in a quadrat. Means±1 S.E.M. are shown

the front to the back of the dune, though isolation frequency in *E. mollis* was lower in the crest relative to the front and backdunes (Table 1, Fig 2). Isolation frequency in roots was significantly negatively associated with organic matter and soil pH (Table 2). We found that conspecific host cover was positively associated with the proportion of hosts from which at least one leaf endophyte was isolated, and this effect did not differ across host species (Table 2, Fig. 3).

OTU richness at the quadrat-level increased with soil pH in both leaves and roots, but there were no significant effects of dune location or host species (Table 2, Fig. 4). At the site-level, we found no influences of any predictor variables on OTU richness of leaves, but higher pH did have a negative effect on OTU richness in roots (Table 3, Fig. 4).

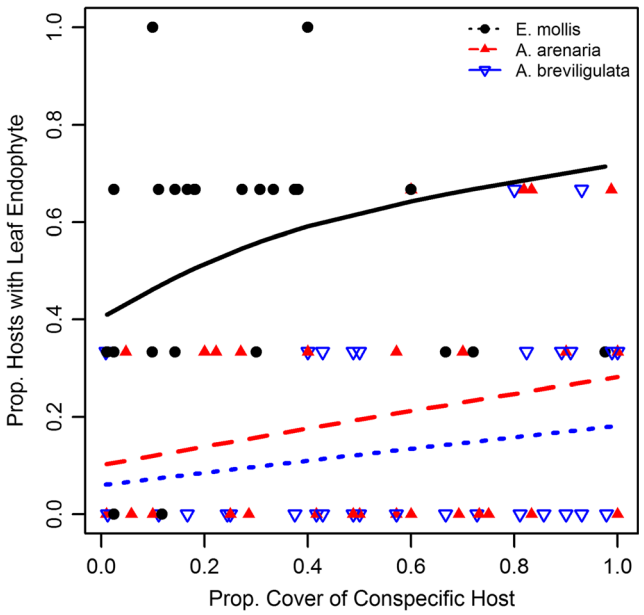

Fig. 3 Proportion of hosts with at least one leaf endophyte increases with proportional cover of the conspecific host species. *Trend lines* show predicted values from generalized mixed-effects model with binomial error. *Y-axis* represents the proportion of hosts in a quadrat with at least one leaf endophyte. No significant interactions were found between host species and conspecific cover

Our perMANOVA analyses revealed that endophyte community composition in leaves was significantly structured by an interaction between dune location and host species ($F_{4,26}$= 1.50, R^2=0.110, p=0.040), and all other environmental covariates were dropped from the model (Fig. 5a). In contrast, endophyte community composition in roots was significantly structured by dune location ($F_{2,52}$=1.53, R^2=0.041, p=0.003) and marginally affected by soil pH ($F_{1,52}$=1.53, R^2=0.021, p= 0.058) (Fig. 5b). For community composition in both leaves ($F_{4,26}$=1.95, R^2=0.143, p=0.004) and roots ($F_{4,52}$=2.32, R^2= 0.122, p<0.001), we found a strong effect of site (Fig. 5c, d).

Evidence for Regional Spatial Structure

We found that root endophyte communities were structured at the local spatial scale whereas the leaf communities showed evidence of regional spatial structure. Endophyte communities in leaves exhibited a significant distance-decay relationship, as quadrats closer to one another tended to harbor more similar endophyte communities (Table 4, Fig. 6). When separately tested for each dune location, we found that this pattern held for front dunes, but not for the crest or backdune locations (Table 4). In contrast, we did not find evidence of a distance-decay relationship in root communities across all quadrats or within quadrats (Table 4).

We found higher compositional turnover in endophyte communities of roots than those of leaves. Regional OTU richness in leaves increased from the front to the crest, but plateaued from the crest to the back (solid line in Fig. 4a), while richness in roots increased from the front to the back (solid line in Fig. 4b). This result, in conjunction with the previously stated result that OTU richness at the quadrat- and site-levels did not change with dune location (dashed and dotted lines in Fig. 4), suggests that turnover across sites accounts for the increases in OTU richness from the front to the back of dunes, particularly for communities in roots. Indeed, dispersion (multivariate measure of compositional turnover) was higher in root than leaf communities (permuted $F_{1,173}$=4.893, p=0.021). For

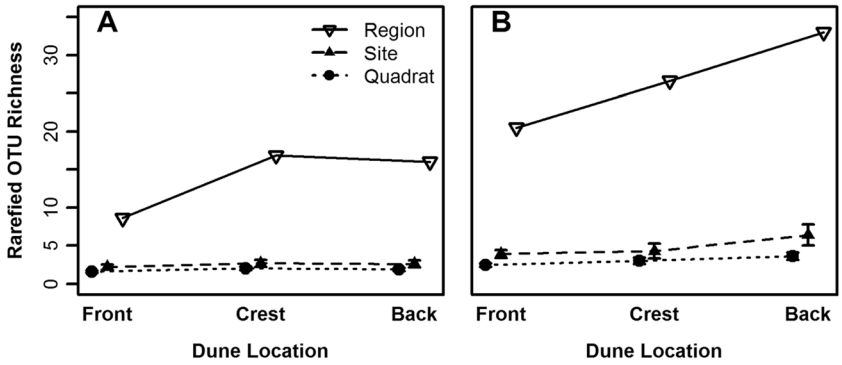

Fig. 4 Endophyte OTU richness across **a** leaves and **b** roots. Quadrat, site, and regional rarefied richness are shown on each figure panel. Note that these three richness metrics were rarefied to different levels, so richness is not directly comparable among the three levels. No significant differences in quadrat or site richness were found among dune locations (mean±1 S.E.M. is shown). Regionally, richness increased from the front to the back of dunes

endophyte communities in leaves, there were no differences in dispersion among dune locations pooled across host species (permuted $F_{8,53}=1.300$, $p=0.257$) or within-host species ($p>0.05$ for all three host species). Similarly, endophyte communities in roots showed no differences in dispersion among dune locations (permuted $F_{8,104}=1.441$, $p=0.190$), although there was a significant increase in dispersion for communities in *A. breviligulata* from the front to the back of the dune (permuted $F_{2,35}=3.276$, $p=0.044$).

Growth and Sporulation

The mean hyphal growth rates and spore production of isolates representing OTUs varied greatly (Online Resource 2). Contrary to our expectations, we found that OTUs with faster growth rates were more likely to be isolated from leaves ($df=1$, $\chi^2=10.7$, $p=0.001$), while OTUs with greater spore production were less likely to be found in leaves ($df=1$, $\chi^2=31.7$, $p<0.001$; Fig. 7). When we regressed the observed against the expected results, we found the model slightly underestimated the probability that an OTU was found in a leaf (slope=0.89; adjusted $R^2=0.41$). Interestingly, OTU1 (*Microdochium bolleyi*), the most common endophyte (34 % of all sequenced isolates) and typically found in roots, exhibited the fastest growth rate among OTUs predominantly found in roots and was among the highest spore producers (Online Resource 2).

Discussion

In this study, we investigated the structure of above- and belowground fungal endophyte communities in beachgrasses along the USA Pacific Northwest Coast. We report three key findings. First, host species provided the strongest filter for leaf endophyte communities, whereas the abiotic environment provided the strongest filter for root endophyte communities. Isolation frequency and OTU richness was greatest for endophytes from *E. mollis* leaves than for leaves of *A. arenaria* or *A. breviligulata*, the leaves of which contained subsets of the OTUs found in *E. mollis*. Endophyte isolation frequency in leaves was greater for hosts neighbored by high abundances of conspecific hosts. These results suggest that leaf endophytes have a degree of host association specificity, and thus, their distribution could be limited by the abundance of neighboring

Table 3 Factors influencing site-level endophyte richness in leaves and roots based on model averaging

Term	Level	Site OTU richness in leaves		Site OTU richness in roots	
		Impt.	Est. (S.E.)	Impt.	Est. (S.E.)
(Intercept)			2.48 (0.27)***		4.85 (0.41)***
Latitude		0.10	0.18 (0.28)	0.12	−0.38 (0.44)
%Nitrogen		0.10	0.14 (0.21)	0.00	
Organic matter		0.08	0.06 (0.21)	0.00	
pH		0.18	−0.26 (0.20)	1.00	−1.82 (0.38)***
Dune location	Linear	0.00		0.00	
	Quadratic				
	# models within δ=4	4		2	

Sampled plants were pooled by dune location in each site and analyzed using a linear mixed-effects model. See Table 2 for details on model averaging

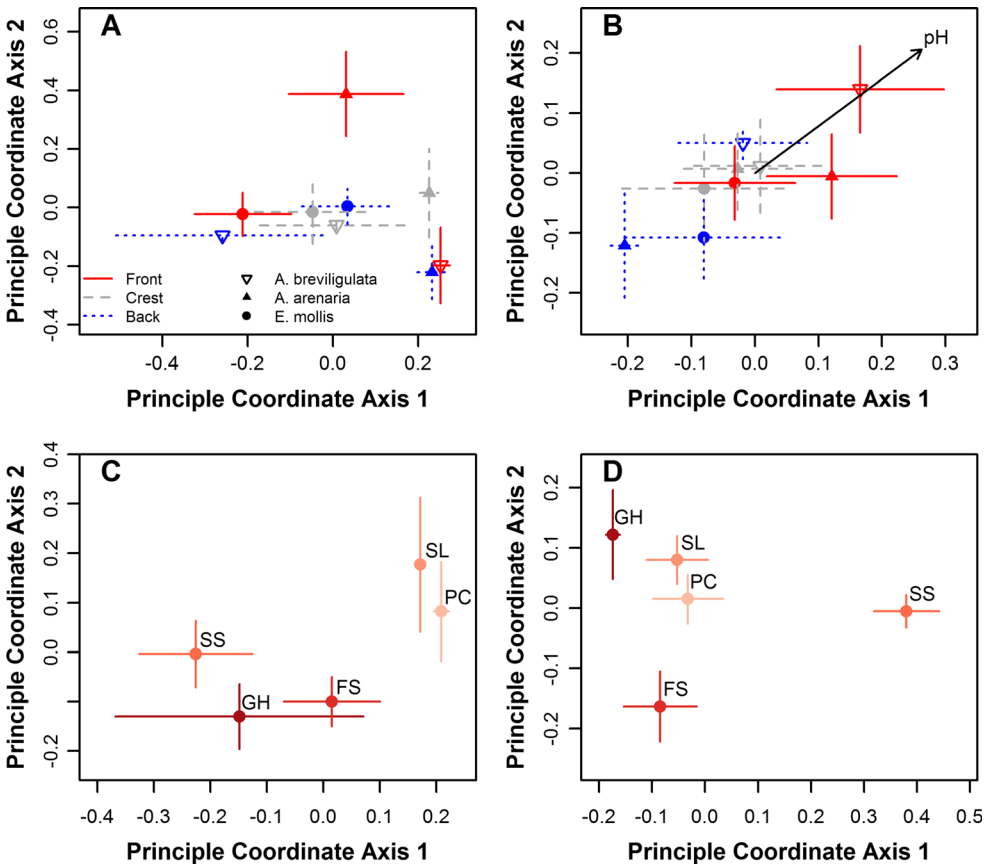

Fig. 5 Community analyses for quadrat-level endophyte communities. **a**, **b** Principle coordinates analyses for leaves and roots, respectively, along with significant environmental vectors. Leaf endophyte communities showed a significant interaction between dune location and host species, while root endophyte communities were structured by dune location and pH. Means± 1 SEM >of axes 1 and 2 are shown. **c**, **d** Site means from the same principle coordinates analyses. Sites are shaded according to latitude, with *lighter red* signifying southern sites and *darker red* signifying northern sites. *PC* Pacific City, *SL* Sand Lake, *SS* Seaside, *FS* Fort Stevens, *GH* Grays Harbor. Means±1 S.E.M. are shown

hosts as sources of inocula. In contrast, endophyte abundance, richness, and community composition in roots depended more highly on abiotic environment (dune location and pH) than did leaf endophyte communities. Second, evidence for regional spatial structure differed for endophytes in leaves and roots. Community similarity decreased with geographic distance in leaf but not in root endophyte communities, and endophyte turnover in roots had greater turnover across sites than those in leaves. These results suggest that leaf endophyte species are dispersing more widely than are species of the root endophyte communities, which are more locally structured. Third, although we expected that endophytes most often found in leaves should produce more spores and grow more slowly than those found in roots, we instead found the reverse, a result that could suggest different modes of dispersal between above- and belowground endophytes. Taken together, leaf endophyte communities were more strongly structured by host species and geographic distance, and root communities were more strongly structured by filters of the local environment.

Environmental Versus Host Filtering

Our findings contribute to our general understanding of the effects of filtering on microbial symbiont communities. Filtering by the environment and host species both play important roles in the assembly of symbiont communities in many systems [e.g., 1, 12, 57]. Our results suggest that the relative importance of the environment and host species for structuring endophyte communities depends on the plant tissue. The soil environment, particularly soil pH, provided a strong filter for endophyte communities in roots, a result that is similar to those of other recent studies demonstrating strong environmental filters and patchy, local distributions of root-associated fungi [12, 58, 59]. Organic matter was also a predictor of isolation frequency in roots, but surprisingly, the two were negatively associated. Organic matter generally increases with dune succession, particularly in northern dunes dominated by *A. breviligulata* [36] and is a potential resource for species of facultative endophytes that also live saprobically in the soil [60]. Yet, previous work with the root endophyte *Phialocephala fortinii* showed no change in root colonization when organic matter was added to the soil [61]. The utilization of organic matter therefore may not necessarily lead to increased root colonization by endophytes. In contrast to the endophyte communities in roots, our results showed that those in leaves demonstrated weak effects of environmental filtering, and this could be attributable to colonization by airborne inocula. However, we did find that increasing soil pH decreased OTU richness in leaves at the quadrat level, suggesting an indirect effect of the soil environment in shaping these communities.

Table 4 Results from multiple regression on distance matrices (MRM) analyses for endophyte community composition in leaves and roots as a function of soil properties and geographic distance

	Leaves		Roots	
Term	Slope	*p*	Slope	*p*
Across dune locations				
(Intercept)	0.363	0.003	0.146	0.131
N	−0.032	0.237	−0.020	0.378
Organic matter	0.022	0.535	0.004	0.879
pH	0.000	0.976	−0.007	0.649
Distance	−0.051	0.001	−0.007	0.270
Regression	R^2=0.064	0.005	R^2=0.016	0.538
Within dune locations				
Front				
(Intercept)	1.025	0.003	0.007	0.978
N	0.003	0.978	−0.085	0.161
Organic matter	0.034	0.581	0.065	0.154
pH	−0.052	0.424	0.087	0.106
Distance	−0.181	0.003	0.023	0.454
Regression	R^2=0.291	0.008	R^2=0.131	0.151
Crest				
(Intercept)	0.088	0.561	−0.014	0.892
N	0.019	0.603	−0.046	0.016
Organic matter	−0.075	0.059	0.035	0.061
pH	0.030	0.48	0.003	0.879
Distance	0.011	0.747	0.022	0.134
Regression	R^2=0.064	0.391	R^2=0.111	0.075
Back				
(Intercept)	0.172	0.332	0.103	0.396
N	−0.016	0.797	−0.030	0.289
Organic matter	0.032	0.557	0.022	0.543
pH	0.078	0.111	0.021	0.424
Distance	−0.042	0.298	−0.009	0.664
Regression	R^2=0.121	0.466	R^2=0.042	0.614

Analyses were conducted across all dune locations and within dune locations

Implications for Symbiont Dispersal-Limitation

While the classic Baas-Becking hypothesis [22] has been rejected by several studies showing evidence for dispersal-limitation in microbial communities [e.g., 20, 21, 62], the extent to which plant-associated symbionts are dispersal-limited remains unclear. The results of our study show that above- and belowground symbionts display different regional spatial structure. Aboveground symbiont propagules are likely aerially dispersed over relatively longer distances, resulting in the observation that more similar communities are found in closer physical locations (i.e., distance-decay relationship). Similar patterns of spatial structuring according to geographic distance have also been found among horizontally transmitted leaf endophytes of tropical grasses [62] and temperate forests [15]. In contrast, belowground symbiont taxa are likely highly limited in their dispersal among sites and experience strong effects of environmental filtering, resulting in the observation of high turnover in belowground communities among sites and the absence of a distance-decay relationship. However, we caution that our results, like those of other studies that have sought to identify dispersal-limitation based on spatial structure, could still have been driven by some unmeasured environmental variable not accounted for in our model. For instance, it is plausible that slight differences in precipitation or temperature along our latitudinal gradient could have accounted for the effect of geographic distance we found in the aboveground endophytes.

Conspecific host species may serve as sources of inoculum for dispersal of endophytes. We found that the probability of endophyte isolation in leaves increased with conspecific host abundance, suggesting that these neighboring hosts supply propagules of endophytes to new, conspecific hosts. Similar effects have been observed in pathogen systems, in which the transmission rate is dependent on the local abundance of compatible hosts [63, 64]. This result may be caused by specific endophyte × host species interactions in leaves, or by the presence of favorable microclimate conditions (e.g., wind flow [65]) for endophytes in leaves of a particular host species.

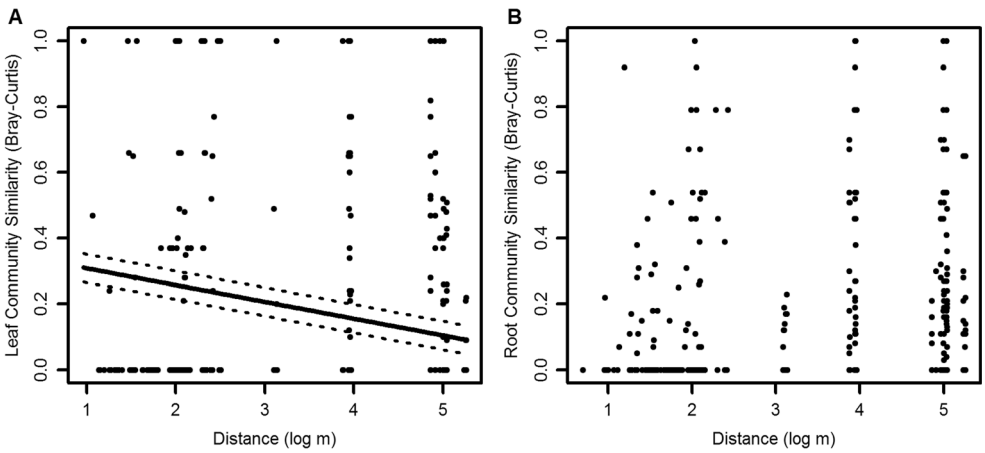

Fig. 6 Regional Bray-Curtis community similarity as a function of distance in **a** leaves and **b** roots. *Points* represent pairwise similarities between quadrats (across all hosts within the quadrat). *Line* shows significant effect of distance (log10 scale) in endophyte communities of leaves, and *dashed lines* show standard error around the slope estimate using a jackknife approach

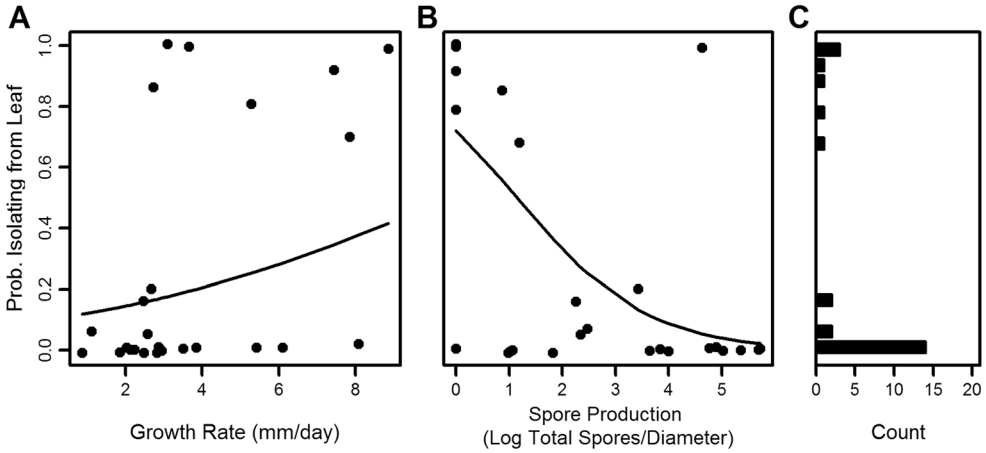

Fig. 7 Probability that a given OTU is isolated from a leaf as a function of **a** growth rate and **b** spore production. Each data point represents an individual OTU. Higher probabilities suggest OTUs are more commonly found in leaves, while lower probabilities suggest OTUs are more commonly found in roots. **a** Growth rate for each culture was calculated as the slope of the diameter as a function of the number of days cultures were growing, and the OTU growth rate was calculated as an average of these slopes. **b** Total spore production per plate was scaled by the diameter of the culture when the spores were sampled and averaged for each OTU on a log10 scale. Y-axis values were jittered to more easily visualize overlapping data points. **c** A histogram of the probabilities in (**a**) and (**b**)

In contrast, belowground symbionts, whose communities were less influenced by the host species, were subsequently not affected by conspecific neighboring host abundance.

Finally, our results suggest that the mode of dispersal may account for differences in the spatial structure of above- and belowground endophyte communities. Growth rates and spore production reasonably predicted the tissue from which an OTU was isolated, but surprisingly, fast growth and low spore production were associated with leaf infection, not root infection. Although the endophyte OTUs we identified here clearly exhibit a wide range of growth and sporulation rates, we speculate that growth and sporulation may have different roles in above- and belowground plant symbionts. For instance, the faster colony growth rates of leaf symbionts may correlate with competitive ability. In dune systems, wind-blown sand causes abrasions on leaves [66] as the host plants capture sand [65]. It is plausible that sand could deliver hyphae directly to the leaf, and the hyphae subsequently enter the leaf through these abrasions. Within plants, faster growth may be more beneficial in leaves than in roots, especially if leaves are relatively short-lived compared to roots. In contrast, endophyte species belowground might rely on spores to colonize new hosts, particularly if they are poor competitors in the soil against saprobic fungi or if their hyphal networks are frequently disturbed. Spores may also be important survival structures for symbiont species in belowground communities [67].

Endophyte Diversity in Dune Plants

Dune systems provide an environmentally stressful habitat of strong winds, salt spray, and low nutrient levels for both plants and fungi [36, 37] and, therefore, represent an opportunity to add to our understanding of fungal diversity in these habitats. In one such study, Rodriguez et al. [68] found that an endophyte, *Fusarium culmorum*, conferred tolerance to salt stress in *E. mollis*. Though *F. culmorum* was isolated from *E. mollis* individuals growing north of our field sites on islands in the Puget Sound, WA, USA, we did not detect this endophyte in any of our samples. It is also worth noting that we found no instances of *Epichloë* spp., a genus that grows systemically in grasses and may confer resistance to herbivores or drought [17]. Research in the Great Lakes region of the USA found that *Epichloë* infects some populations of *A. breviligulata* and is present in commercial varieties used in plantings [69]. We suspect that *A. breviligulata* was either introduced from a different, non-infected commercial variety, or has since lost its association with *Epichloë*. We did find a high abundance of *M. bolleyi* (OTU1) in all three grass hosts. *Microdochium* has previously been reported to colonize *A. arenaria* [70] and is a common root endophyte of grasses [71]. The effects of *Microdochium* on its host may be positive or negative depending on the genotypes of the fungus and the plant [71]. It is possible that *M. bolleyi* is an opportunistic colonizer in beachgrass, as it exhibited both relatively fast hyphal growth and high spore production.

Conclusions

Our study shows the varying degrees of filtering and regional spatial structure and differences in symbiont growth of above- and belowground symbiont communities. We found that communities of aboveground endophytes were structured according to host species and geographic distance, while communities belowground were structured by environmental filtering. As we attempt to understand the drivers of the microbial

symbiont communities associated with plants and animals, it is important to consider how the host tissue exposes symbiont species to different environmental or host-specific filters and could favor species with particular dispersal processes.

Acknowledgments We thank D. Asson, S. Gerrity, Y. Kim, P. Lenz, and A. Pradeep for field and laboratory assistance and S. Hacker and J. Spatafora for providing laboratory resources and advice at Oregon State University. We also thank P. Kennedy, L. Kinkel, and D. Tilman for their feedback on this manuscript. We thank the U.S. Fish and Wildlife Service, Oregon Parks and Recreation Department, and Washington State Parks and Recreation Commission for granting us permits to conduct this research. This work was funded by the United States Environmental Protection Agency (EPA/NCER R833836) to EWS, NSF Dimensions of Biodiversity (1045608) to GM, National Science Foundation Integrative Graduate Education and Research Traineeship (NSF-IGERT) Introduced Species and Genotypes program (DGE-0653827), NSF Graduate Research Fellowship program (NSF 00039202), and University of Minnesota Rothman Fellowship to ASD.

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