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Enriched root bacterial microbiome in invaded vs native ranges of the model grass allotetraploid *Brachypodium hybridum*

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Abstract Invasive species can shift the composition of key soil microbial groups, thus creating novel soil microbial communities. To better understand the biological drivers of invasion, we studied plant-microbial interactions in species of the *Brachypodium distachyon* complex, a model system for functional genomic studies of temperate grasses and bioenergy crops. While *Brachypodium hybridum* invasion in California is in an incipient stage, threatening natural and agricultural systems, its diploid progenitor species *B. distachyon* is not invasive in California. We investigated the root, soil, and rhizosphere bacterial composition of *Brachypodium hybridum* in both its native and invaded range, and of *B. distachyon* in the native range. We used high-throughput, amplicon sequencing to evaluate if the bacteria associated with these plants differ, and whether biotic controls may be

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driving *B. hybridum* invasion. Bacterial community composition of *B. hybridum* differed based on provenance (native or invaded range) for root, rhizosphere, and bulk soils, as did the abundance of dominant bacterial taxa. Bacteroidetes, Cyanobacteria and *Bacillus* spp. (species) were significantly more abundant in *B. hybridum* roots from the invaded range, whereas Proteobacteria, Firmicutes, *Erwinia* and *Pseudomonas* were more abundant in the native range roots. *Brachypodium hybridum* forms novel biotic interactions with a diverse suite of rhizosphere microbes from the invaded range, which may not exert a similar influence within its native range, ostensibly contributing to *B. hybridum's* invasiveness. These associated plant microbiomes could inform future management approaches for *B. hybridum* in its invaded range and could be key to understanding, predicting, and preventing future plant invasions.

Keywords Endophytic bacteria · *Brachypodium* · Invasive species · Rhizosphere · Root · Soil

Abbreviations

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spp	Species
QIIME	Quantitative insights into microbial ecology
PERMANOVA	Permutational multivariate analyses of variance
PCoA	Principal Coordinates Analysis
Hill 0	Hill number of 0
Hill 1	Hill number of 1
OTU	Operational taxonomic unit
N	Nitrogen
N_2	Dinitrogen

Background

Invasive species are eroding native biodiversity and ecosystem services in natural areas around the world, concerning resource conservation practitioners and land managers who are actively involved in reducing the spread of invasive species. While we are beginning to understand abiotic factors, such as resource availability, driving plant invasions (Corbin and D'Antonio 2012), we still have a limited understanding of the biological factors contributing to these invasions (Callaway et al. 2004; Dawson and Schrama 2016).

This is particularly true for relationships between plant invasion and microbial communities, despite ongoing research in this area (Egidi and Franks 2018; Le Roux et al. 2017; Lu et al. 2018; Ramirez et al. 2019).

Root-associated and rhizosphere microbes, which can differ based on geographic location and soil type, can directly and indirectly influence nutrient supplies, pathogen loads, and invasive host plants' stress tolerance. Although plant-soil feedbacks between invasive species and their associated microbes remain difficult to interpret, previous studies demonstrate that invasive species can shift the composition of key soil microbial groups, thus creating novel soil microbial communities (Busby et al. 2011; Hausmann and Hawkes 2009; Phillips et al. 2019; Zhang et al. 2010). Despite these insights, we have much to learn about the microbial dynamics of root and rhizosphere communities associated with invasive plants, and how they might differ across native and invaded ranges. Greater understanding of these dynamics could help us develop hypotheses about whether invasion may be partially controlled by biotic mechanisms, while providing important contextual information to better understand, predict, prevent and control plant invasions.

When investigating the plant-associated microbial community, it can be insightful to characterize different plant-associated microbiomes, such as endophytic root, rhizosphere, and bulk soil communities. The rhizosphere (i.e. plant-root interface) is a structured microhabitat driven by plant physiological processes and inhabited by numerous microbes (Hartmann et al. 2009; Hintner 1904). Previous studies show that rhizosphere microbial communities are influenced by site history and soil type, and may often illustrate greater similarities to bulk soil communities of the same site than to rhizosphere communities of other sites (Bakker et al. 2015; de Ridder-Duine et al. 2005; Singh et al. 2007). In invaded ecosystems, inputs from invasive plant species may differ from those of native plant species, and may influence root-associated and rhizosphere microbes (Reinhart et al. 2010; Wolfe and Klironomos 2005), invasive plant success (Inderjit and van der Putten 2010), and subsequent restoration outcomes (Richardson et al. 2011). Biotic interactions among invasive plants, rhizosphere soil, or root-associated microbes at the plant-root interface (i.e., rhizosphere mechanisms) could promote invasion, ostensibly due to the invasive plant producing foreign
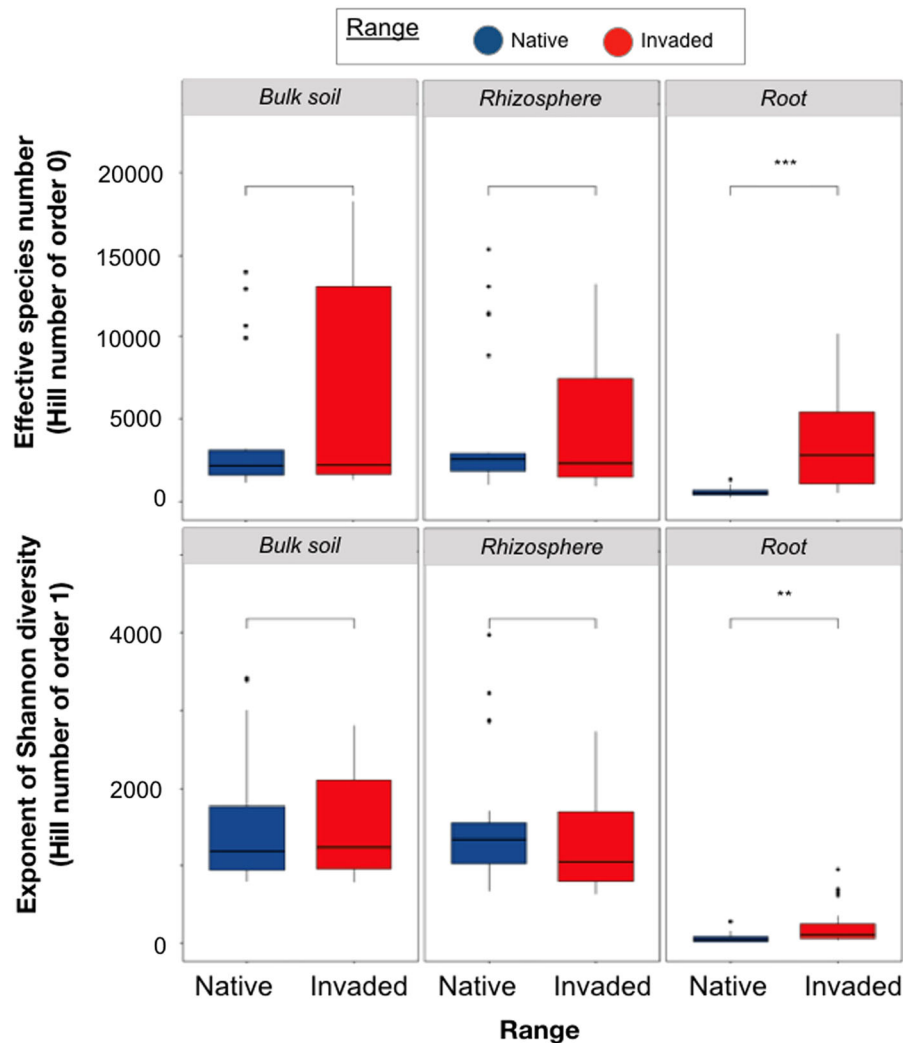
Fig. 1 Effective species count (Hill number of order 0) and Exponent of Shannon diversity (Hill number of order 1) for each microbiome pool between *B. hybridum* native and invaded range root, rhizosphere and bulk soil samples ("*", $p < 0.05$, "**", $p < 0.01$, "***", $p < 0.001$) (see Table 2)

substances (Bais et al. 2006) or directly manipulating local soil microbes. This could increase pathogen loads or disrupt root symbioses (Busby et al. 2013), which could indirectly alter bulk soil microbial communities and edaphic properties. Moreover, examining root, rhizosphere, and bulk soil microbial communities is important for fully characterizing the microbiome of an invasive plant.

To help us better understand the microbial dynamics of invasion, we studied species of the *Brachypodium distachyon* complex (Poaceae), which includes the diploid progenitor species *Brachypodium distachyon* (L.) P. Beauv. and its close congener *B.*

stacei (Catalán et al. 2012). The three species can be discriminated from each other based on their phenotypic, cytogenetic, molecular, and ecological traits, as well as via metabolomic analyses (Betekhtin et al. 2014; Catalán et al. 2016; López-Alvarez et al. 2012, 2015, 2017; Martínez et al. 2018). Overall, individuals of *B. distachyon* are small and distributed at high elevations and in mesic places, while *B. hybridum* are at least twice the size, and grow at a variety of altitudes and in mesic-to-aridic habitats (Catalán et al. 2012, 2016; López-Alvarez et al. 2015, 2017; Opanowicz et al. 2011).

The *B. distachyon* complex makes up one of the most important model systems for functional genomic studies of temperate cereals, forage grasses, and bioenergy crops (Brkljacic et al. 2011; International Brachypodium Initiative (IBI) 2010; Mur et al. 2011; Scholthof et al. 2018). Although there has been a spate of recent research on the genomic architecture and microbiome of *Brachypodium* (Kawasaki et al. 2016; Naylor et al. 2017, 2018; Sanchez-Canizares et al. 2017; Sasse et al. 2018; Tkacz et al. 2015), there remains a dearth of information on associated rhizosphere microorganisms. Recent research revealed that *Brachypodium* hosts a suite of root microorganisms, which are commonly found in other co-occurring grasses (Kawasaki et al. 2016). However, it is unknown whether the root microbiome of *Brachypodium* differs between the native and invaded range.

The grasses of the *Brachypodium distachyon* complex are native to the Mediterranean region, stretching from Spain and Portugal to Israel, and beyond. Spain (hereafter native range) was chosen for comparison sampling within this native range due to similarities in the climate and environmental conditions with coastal California (hereafter, the invaded range) (Catalán et al. 2016; López-Alvarez et al. 2015, 2017). Climate niche models constructed for *B. hybridum* in the Mediterranean basin based on 19 temperature and precipitation parameters (Lopez-Alvarez et al. 2015) could also recover a similar niche in coastal California with high probability. This makes a comparison of coastal California and Spain the best means of testing our hypotheses.

Current evidence suggests that invasive annual *Brachypodium* spp. are not only isolated from their native range by distance, but they are also highly differentiated and characterized by low genetic diversity values (Wilson et al. 2019). Moreover, several authors have demonstrated that all invasive *Brachypodium* accessions from California (Bakker et al. 2009), as well as invasive *Brachypodium* collected from other parts of the world (Catalán et al. 2016; Gordon et al. 2020), are tetraploid. Following on the heels of this revelation, a genome-wide association study (including thousands of *Brachypodium* spp. accessions collected globally) analyzed the distribution and genomic diversity of the *B. distachyon* complex (mostly *B. distachyon* and *B. hybridum*) (Wilson et al. 2019) to better understand its invasiveness. This study not only revealed that a majority of these worldwide accessions (56%) were actually *B. hybridum*, but also that *B. hybridum* essentially represents most invasive *Brachypodium* spp. Indeed, Wilson et al. (2019) identified every accession found outside of its native range (except one accession; Australia WLE2-2 *B. distachyon*) as *B. hybridum*.

Brachypodium hybridum was likely introduced to the invaded range as hay or alfalfa seed contaminants. The invasion in California is currently in an incipient stage, threatening natural and agricultural systems as it actively spreads across much of the western half of California. Herbarium specimens held at University of California, Berkeley, identify *B. distachyon* (most likely *B. hybridum* according to Bakker et al. 2009 and Wilson et al. 2019) as early as 1921 in Alameda County, CA, with specimens from Los Angeles County by 1931. According to a map generated by the California Invasive Plant Council (Cal-IPC) WeedMapper, *B. distachyon* (again, most likely *B. hybridum*) is currently invading most coastal counties of California, as well as the western slopes of California's Sierra Nevada mountains.

In this study, we sought to understand why allotetraploid *B. hybridum* was a better invader than diploid *B. distachyon*, with an emphasis on any root or rhizosphere associations specific to *B. hybridum* in California. To better understand the invasiveness of *B. hybridum*, we investigated the bacterial microbiome of *B. hybridum* and *B. distachyon* in their native range, and that of *B. hybridum* in the native and invaded range. We hypothesized that bacterial community composition associated with *B. hybridum* in the native range and the invaded range will differ, possibly explaining the degree of invasiveness within the invaded range. In addition, we hypothesized that root-associated bacteria will differ within the native range between *B. hybridum* and *B. distachyon*, as this may be a factor determining the greater invasiveness of *B. hybridum* vs. *B. distachyon*. Finally, among samples collected from the same range, we hypothesized that the root bacterial community would differ from both rhizosphere and bulk soil for all plants due to selective filtering of bacteria from surrounding soil. We also expected a large proportion of the microbial community members of the bulk soil to be represented in the rhizosphere soil.

Table 1 Site names, dates, and species for *Brachypodium* hybridum and B. distachyon sampling in Spain (native range) and California in the USA (invaded range, only for *B. hybridum*)

Brachypodium species	Population collection number	Location	Site name	Site code	Date of collection
B. hybridum	1	Spain, Jaen, Parador de Jaen	Parador	PA	5/11/16
B. hybridum	2	Spain, Alicante, Cabo de la Nao	Cabo de la Nao	CN	5/13/16
B. hybridum	3	Spain, Valencia, Cofrentes	Cofrentes	CF	5/14/16
B. hybridum	4	Spain, Balearic Islands, Mallorca	Cabo Blanc	CB	5/15/16
B. distachyon	5	Spain, Balearic Islands: Mallorca	San Servera	SS	5/16/16
B. hybridum	6	Spain, Girona, Roses, Castell de Trinitat	Roses,	RO	5/20/16
B. hybridum; B. distachyon	7	Spain, Huesca, Alquezar	Alquezar	AL	5/23/16
B. distachyon	8	Spain, Huesca, Benabarre	Benabarre	BN	5/23/16
B. hybridum	9	USA, CA, Crestridge Ecological Reserve	Thornmint Hill	TH	4/21/16
B. hybridum	10	USA, CA, Catalina Island	Catalina Island Site 1	CAT1	4/27/16
B. hybridum	11	USA, CA, Catalina Island	Catalina Island Site 2	CAT2	4/27/16
B. hybridum	12	USA, CA, Catalina Island	Catalina Island Site 3	CAT3	4/27/16
B. hybridum	13	USA, CA, Center for Natural Lands Management	Alicante	ALI	5/9/16
B. hybridum	14	USA, CA, Center for Natural Lands Management, Alganorte Park	Alganorte	ALG	5/9/16
B. hybridum	15	USA, CA, Rancho Jamul Ecological Reserve	Jamul	JAM	5/12/16
B. hybridum	16	USA, CA, Irvine Ranch Conservancy	Irvine RanchConservancy,	IRC	6/6/16
B. hybridum	17	USA, CA, UC Santa Barbara NRS. Rancho Marino	Marino	MAR	5/17/16

Methods

Focal plant species

The *Brachypodium distachyon* complex consists of two diploids, each with a different chromosome base number [*B. distachyon* (x = 5, 2n = 10); *B. stacei* (x = 10, 2n = 20)], and their derived allotetraploid *B. hybridum* (x = 5 + 10, 2n = 30). Within each site, in both the native and invaded ranges, a non-*Brachypodium* "other" species of annual grass was chosen for sampling. All "other" species were chosen due to their similarities with *Brachypodium*; they are all grasses that are widely invasive in California. Most of these collections consisted of co-occurring *Bromus* spp., a majority within the *B. rubens* complex. Of note, *Bromus* spp. have successfully invaded much of California since the 1800s (Barbour et al. 2007).

Additional "other" species included *Hordeum murinum* and *Avena* spp.

Field site information and sampling

Sites were sampled across Spain, within the native range of *B. hybridum* and *B. distachyon* (López-Alvarez et al. 2012, 2015), and in the invaded range, where the grass species are exotic/invasive. Site selection within the native range was based on previously published observations of *Brachypodium hybridum, B. distachyon*, or both grasses, primarily from López-Alvarez et al. (2012). We selected sites within the invaded range based on discussions with land managers across southern California to discover where *Brachypodium hybridum* was located. Within sites, samples were selected by surveying sites to determine the extent of *Brachypodium* growth, and

then randomly selecting samples at roughly 1 m spacing from any other samples collected that day. In a subset of sites within the native range, patchy distributions of target grass species led to collections within either a tighter or wider sampling arrangement. Site locations and dates of sampling are included in Table 1.

In any soil science study comparing invaded and native regions, it is normal to see differences in soil type between regions and even within regions (Callaway et al. 2004; Knevel et al. 2004; Reinhart et al. 2003). According to the internationally recognized (Teng et al. 2020) FAO–UNESCO Soil Map of the World Revised Legend (FAO/Unesco/ISRIC 1990), soils in the coastal invaded range sampling sites are classified as Luvisols, soils in the island invaded range (Catalina) are classified as Regosols, while soils in the native range sampling sites are classified as Cambisols.

Cambisols (native range) and Regosols (island invaded range) are characterized as soils with little or no profile differentiation, while Luvisols (invaded range) are characterized as soils with clay-enriched subsoil. More specifically, Cambisols are characterized by slight or moderate weathering of parent material and by the absence of clay, organic matter, aluminum and/or iron. Luvisols are characterized by lower clay content in the topsoil and higher clay content in the subsoil, loss of iron oxides and clay leads to a bleached eluviation horizon. Cambisols are sometimes found in conjunction with Luvisols (Świtoniak et al. 2016). Regosols are characterized as very weakly developed mineral soils made up of unconsolidated materials.

Due to these differences between the native and invaded range soils, we controlled for site-to-site variations as a random effect as described later in this manuscript.

Sampling occurred in 8 sites in the native range and 9 sites in the invaded range in 2016 (Table 1). Peak biomass for *Brachypodium hybridum* and *B. distachyon* occurs in April-June, therefore all samples were collected during these months. At each field site, samples from three replicate *Brachypodium* spp. and three replicate "other" plants were collected. From each plant, we collected a root, rhizosphere, and bulk soil sample to establish the microbiome of each. This gave us a grand total of 18 samples per site, 3 root, 3 rhizosphere, and 3 bulk for each of 2 species.

All tools were treated with 70% ethanol and 10% bleach solution, and then dried prior to sampling, with more extensive cleaning with detergent and bleach between sites. Grasses were carefully excavated using soil knives to avoid loss of root mass. After excavation, the plant was held by the shoot and shaken to dislodge loose soil. Clippers were used to separate the shoot from the root. The root, along with its associated rhizosphere soil, was placed in sterile plastic bags (Whirlpak by Nasco, Inc.). Bulk soil was collected from the excavation hole with a soil knife and placed into a separate Whirlpak bag. All belowground plant and soil samples were immediately placed on dry ice in an insulated cooler in the field and transferred to − 20 °C freezer at the University of California, Riverside, and the University of Zaragoza, Huesca, respectively, within 24 h.

DNA extraction, quantification, and barcoded amplicon sequencing

DNA extraction of soils in the native range were performed in the Catalán laboratory of the Universidad de Zaragoza, Huesca, Spain. DNA extraction of California plants and soils, and all further analysis of DNA extracts from both countries, were performed in the laboratory of the University of California, Riverside (UCR), CA. Rhizosphere soil was separated from roots (through shaking the root and the use of sterilized forceps and scalpels) in the laboratory prior to DNA extraction from either pool. As endophytes are the focus of our research, we chose to use bleach as a root-surface sterilizing agent prior to root DNA extraction (Richter-Heitmann et al. 2016). A 10% bleach solution was used to surface sterilize the roots in a petri dish for 30 s before washing with sterile deionized water.

The 16S ribosomal RNA gene (16S rRNA gene) V3 and V4 regions were analyzed to classify the diversity of bacteria and archaea in the soil (Klindworth et al. 2013). The use of 16S rRNA gene variable region analysis is the most common approach to identifying bacterial taxonomy (Ibal et al. 2019). While 16S rRNA gene analysis is well known for not being able to differentiate between the two species *Bacillus thuringiensis* and *Bacillus cereus*, the use of 16S rRNA gene is sufficient for identifying most bacterial species (Ibal et al. 2019). By comparing the 16S rRNA gene variable regions, we are able to identify many bacterial species with great certainty. As bacteria are

far more abundant in plant root and surface soil microbial communities than archaea, we generally here refer to the data generated from 16S rRNA gene sequencing as bacterial communities or just simply as the "microbiome" of a certain sample or sample type.

Microbial DNA was extracted from all samples using a MO BIO PowerSoil DNA Isolation kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) following the manufacturer's instructions. For samples from the invaded range, we used a PowerLyzer 24 bench top bead-based homogenizer (MO BIO Laboratories, Inc., Carlsbad, CA, USA), and for samples from the native range, we used a comparable Mini-Beadbeater-16 (Biospec Products, Bartlesville, OK, USA). The DNA extraction kits were shipped from MO BIO to Spain for their use in these extractions to ensure reproducibility in extractions between labs.

All downstream analyses were performed on DNA extracts shipped frozen to the UCR laboratory. A NanoDrop 2000/2000c UV–Vis spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) was used to quantify the DNA in soil extracts. PCR for bacteria was performed using primers that target the 16S rRNA gene V3 and V4 regions (S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21) (Klindworth et al. 2013) of the 16S rRNA gene. Microbial genomic DNA (2.5ul) was combined with forward and reverse primer (5ul each), and 2 × KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, Massachusetts, USA) (12.5ul). A Bio-Rad MJ Research PTC 200 Thermocycler was used to amplify 96 samples at a time with the following program, 95 °C for 3 min, 25 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 5 min, and hold at 4 °C. AMPure XP beads (Beckman Coulter Genomics, Danvers, Massachusetts, USA) were used to purify the 16S rRNA gene amplicon without primer and primer dimer sequences. Dual indices and Illumina sequencing adapters were attached to the amplicon using the Nextera XT Index Kit (Illumina, San Diego, California, USA). Amplicon DNA (5 ul) was combined with 2 × KAPA HiFi HotStart ReadyMix (25 ul), dual indexing primers (5 ul each), and PCR water (10 ul). The same thermocycler was used to amplify libraries with the following program, 95 °C for 3 min, eight cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, 72 °C for 5 min, and hold at 4 °C; a second bead cleanup was used to purify samples before quantification. The samples we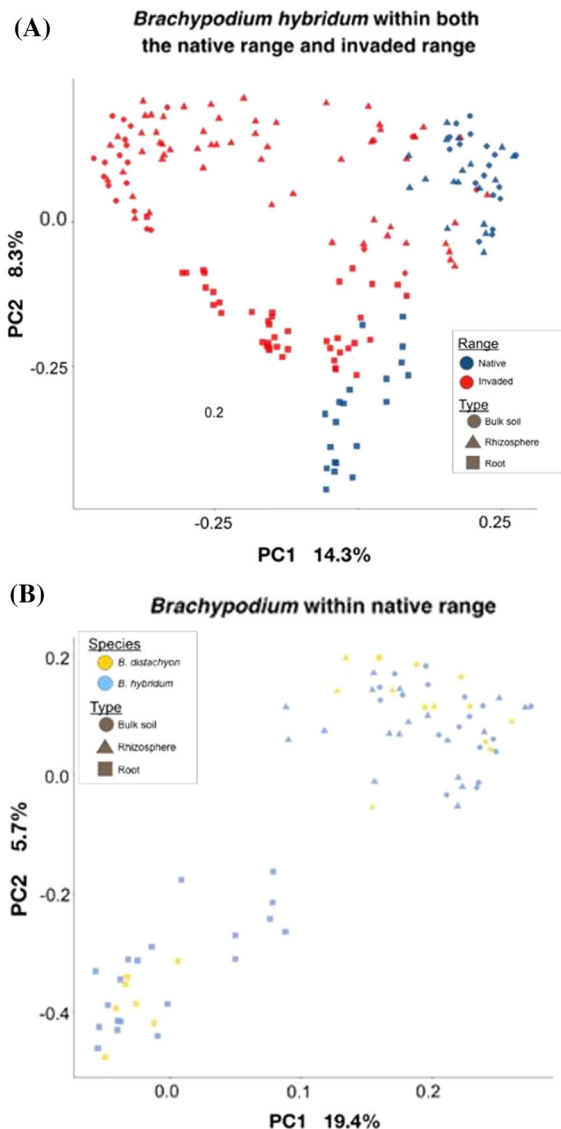re verified with gel electrophoresis after every step. The samples were quantified in duplicate using the Quant-iT PicoGreen dsDNA assay kit (Life Technologies, Grand Island, New York, USA). All samples were pooled together in equimolar concentrations then sequenced with an Illumina MiSeq instrument at UCR.

Data analysis

Quantitative Insights into Microbial Ecology (QIIME) (Kuczynski et al. 2012) was used to pre-process the sequences using default parameters (Caporaso et al. 2010). Paired-end reads were joined using QIIME default parameters, except that thresholds for maximum mismatch to reject alignments were 25%. Samples with fewer than 1000 reads after quality filtering were excluded from all further statistical and bioinformatic analysis. We used an open-reference Operational Taxonomic Unit (OTU) picking strategy against the Greengenes reference database using a high percentage of DNA similarity. Here we define an OTU as a group of bacterial organisms with a 97% DNA sequence similarity. The resulting OTU table was filtered to remove OTUs that could not be assigned to any taxonomic level. We then used QIIME open-reference operational taxonomic unit (OTU) picking strategies with uclust to match OTUs to the Greengenes database. OTUs observed only one or two times were removed. Further data analysis was conducted in R (R Core Team 2018), using vegan (Oksanen et al. 2018), ape (Paradis and Schliep 2019), ggpubr (Kassambara 2018), tidyr (Wickham and Henry 2018), and qiimer (Bittinger et al. 2015) packages.

Analysis of diversity and community composition

We characterized overall alpha diversity, beta diversity, and relative abundances of dominant taxa. Specifically, we summarized alpha diversity using the renyi function in the vegan R package to calculate effective species number (Hill number of order 0) and Exponent of Shannon Diversity (Hill number of order 1) (Jost 2006). Alpha diversity was compared between groups using a Wilcoxon rank-sum test. We computed distance matrices using the Bray–Curtis index, and used the adonis R function to perform Permutational Multivariate Analyses of Variance (PERMANOVAs) to compare overall community composition between

(A)

Brachypodium hybridum within both the native range and invaded range

(B)

Brachypodium within native range

Fig. 2 a Principal coordinates analysis (PCoA) of *Brachypodium hybridum*-associated 16S rRNA gene bacterial communities in the native vs the invaded ranges based on the Bray–Curtis dissimilarity metric. **b** PCoA of *Brachypodium hybridum* vs. *Brachypodium distachyon* associated 16S rRNA gene bacterial communities based on the Bray–Curtis dissimilarity matrix. Symbols for sample type and colors for native vs invaded ranges or for species type are indicated in the respective charts

groups ($\alpha = 0.05$) for the following factors, range (native or invaded), sample type (bulk soil, rhizosphere soil, root), plant type (*Brachypodium* or "other" grasses) and species (*B. distachyon* or *B. hybridum*). To control for site-to-site variation, as a random effect included in the PERMANOVA, we

used 'strata' in adonis to restrict permutations solely within site for plant or sample type comparisons.

With significant main effects of sample type (root, rhizosphere soil, bulk soil) for *Brachypodium* spp., we further probed drivers of microbial community structure with pairwise comparisons of sample types using separate PERMANOVA analyses. Stratification by site was only relevant for some measured variables and, therefore, we did not incorporate all factors into a single model. Principal Coordinates Analysis (PCoA) was used (within the ape package) to visualize dissimilarities between communities.

For the comparisons of sample types (root, rhizosphere soil, bulk soil), after finding that sample type was a significant factor, we ran separate pairwise comparisons, applying a Bonferroni correction to adjust our alpha for multiple hypothesis testing.

We further calculated, analyzed, and compared the relative abundance of dominant bacteria at the genus and phylum levels between (1) native range roots and invaded range roots, (2) bulk native range and bulk invaded range soils, (3) native range rhizosphere soil and invaded range rhizosphere soil, using Wilcoxon rank-sum tests.

Core microbiome

We determined the core microbiome of all native or invaded range root samples to better understand whether the root endophytic community was consistent across sites. Core communities were calculated in R with cutoffs at 95%, corresponding to OTUs found in 95% of all *Brachypodium* root samples within each geographic location or both locations combined.

Results

Measures of bacterial composition and alpha diversity

Overall, using PERMANOVA we detected differences in bacterial community composition and alpha-diversity between *B. hybridum* collected in the native range and the invaded range for some sample types. The effective species number (Hill number of order 0; "Hill 0") of *B. hybridum* roots in the native range was significantly lower than in the invaded range ($P < 0.001$), but not for rhizosphere or bulk soil

Table 2 Microbial community composition comparisons among native and invaded range samples

Sample type	*B. hybridum* vs. *B. distachyon* in the native range
Bulk soil	$p > 0.05$
Rhizosphere soil	$p > 0.05$
Roots	$p < 0.05$*

Pairwise comparisons of samples (bulk soil, rhizosphere soil, root) between B. hybridum and *B. distachyon* in the native range

Table 3 Separate pairwise comparisons between B. hybridum samples (bulk soil, rhizosphere soil, root) in the native range and in the invaded range

B. hybridum	Bulk vs. Rhizosphere	Bulk vs. roots	Roots vs. rhizosphere
Invaded range	$p < 0.001$***	$p < 0.001$***	$p < 0.001$***
Native range	n.s	$p < 0.001$***	$p < 0.001$***

Table 4 Pairwise comparisons of each of the B. hybridum samples (bulk soil, rhizosphere soil, root) between native and invaded range

Sample type	*B. hybridum* in the native vs. invaded range
Bulk Soil	$p < 0.001$***
Rhizosphere Soil	$p < 0.001$***
Roots	$p < 0.001$***

(Supplemental Table 1; Fig. 1). However, the Hill 0 for "other" plant species was significantly higher in the invaded range vs. the native range in every sample type (root, $p < 0.001$, bulk soil, $p < 0.01$, rhizosphere soil, $p < 0.05$) (Supplemental Table 1). The Hill 0 of *B. distachyon* compared to *B. hybridum* in the native range was not significantly different for any sample type (root, rhizosphere, or bulk soil; Supplemental Table 1) but the composition of roots differed between *B. distachyon* and *B. hybridum* ($p < 0.05$) in the native range (Fig. 2b; Table 2).

We found that Hill 0 of roots within the native range for both *Brachypodium* spp. and "other" species, was significantly less diverse than either rhizosphere or bulk soils (Supplemental Tabble 1). For the invaded range, "other" plants exhibited similar patterns of reduced richness in roots, as compared to bulk or rhizosphere richness. However, in *Brachypodium* spp. samples from the invaded range, *Brachypodium hybridum* roots were more diverse than the associated bulk soil (P < 0.05).

Exponent of Shannon Diversity (i.e., Hill number of order 1; Hill 1) for *B. hybridum* in the native range was significantly lower than the invaded range for roots ($p < 0.01$), but not for rhizosphere or bulk soil (Supplemental Table 2; Figure 1). Hill 1 for *B. distachyon,* as compared to *B. hybridum* in the native range, was not significantly different for any sample type (root, rhizosphere soil, bulk soil). Hill 1 for "other" plant species was significantly higher than Hill 1 for *Brachypodium* spp. in the invaded range for the rhizosphere soil ($p < 0.05$), but not for bulk soil or root samples. Hill 1 for "other" plant spp. was significantly higher in the invaded range compared to the native range for roots ($p < 0.001$), but not bulk or rhizosphere soils. Overall, pairwise comparisons between Hill 1 numbers of the sample types (root, rhizosphere, and bulk soil) revealed lower diversity for plant root microbial communities within both the native and invaded range for both *Brachypodium* spp. and "other" species, than their associated rhizosphere and bulk soils ($p < 0.001$) (Supplemental Table 2).

Fig. 3 Relative abundances of **a** the top five dominant bacterial phyla and **b** the top six bacteria genera that were significantly different in *B. hybridum* root samples between sites. Standard errors of the mean (SEs) are represented in the subfigures by the error bars attached to each column ("*", $p < 0.05$, "**", $p < 0.01$, "***", $p < 0.001$)
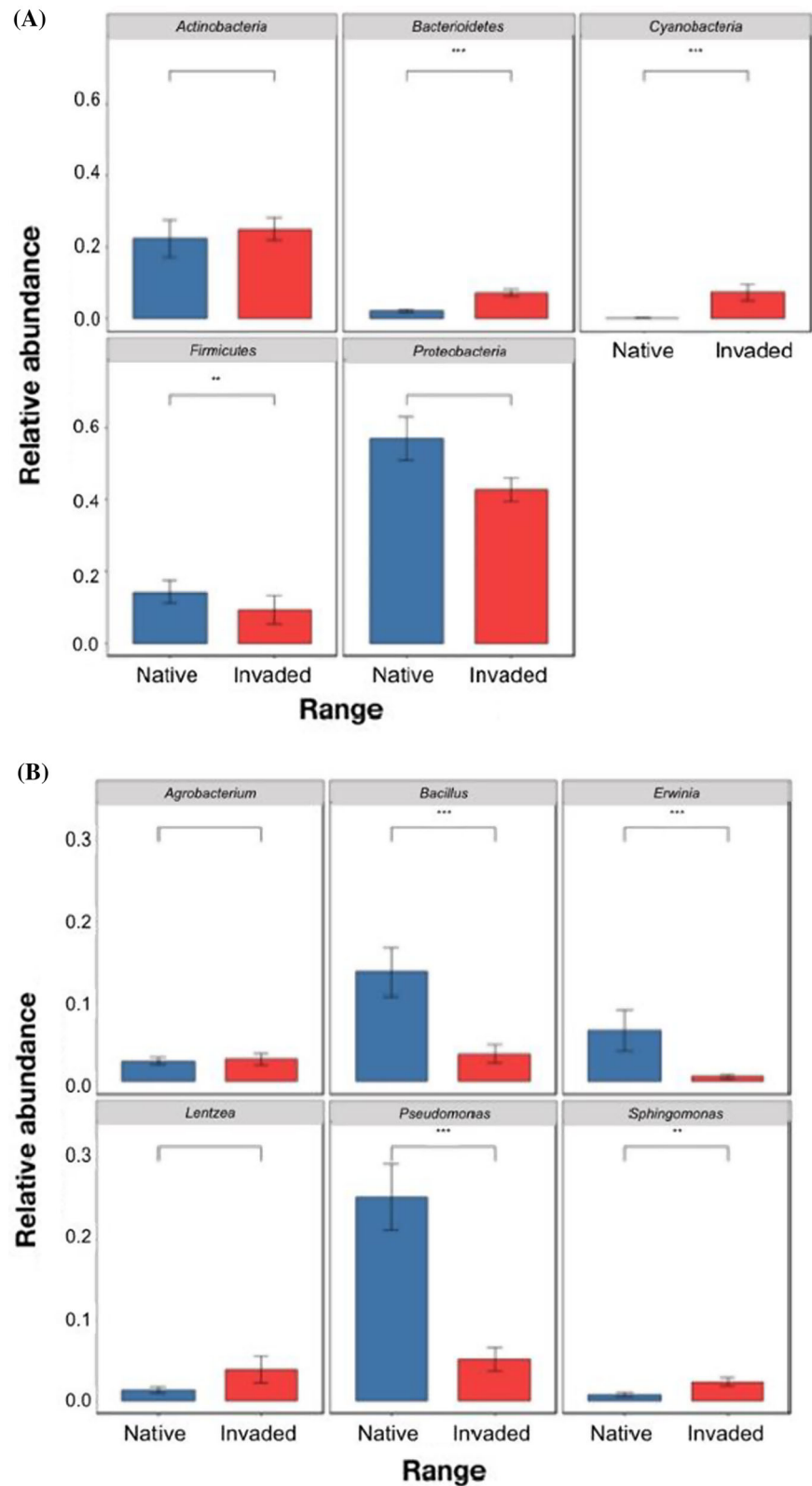
Community comparisons

Among samples collected in the native range and the invaded range, the root bacterial composition of *B. hybridum* differed from both the rhizosphere and bulk soil, respectively ($p < 0.001$; Table 3). Within the native range, the bacterial composition of bulk and rhizosphere soil did not differ (n.s.), but differed significantly within the invaded range ($p < 0.001$; Table 3). In addition, the bacterial composition for each sample type (bulk soil, rhizosphere soil, root) for *B. hybridum* in the native range compared to the invaded range was significantly different ($p < 0.001$; Table 4).

Further, the bulk and rhizosphere soil bacterial composition of *B. distachyon* and *B. hybridum* samples from the native range was equivalent (n.s.), while bacterial composition of plant roots differed significantly between species ($p < 0.05$; Table 2).

Relative abundance of dominant taxa in brachypodium hybridum roots, rhizosphere and bulk soil samples from native and invaded ranges

"Dominant Taxa" were defined as those taxa that accounted for more than half (50%) of the sequences in analyzed samples. We detected differences in the relative abundances of dominant bacterial taxa between the native and invaded range samples of *B. hybridum* roots (Fig. 3). Five phyla (Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Proteobacteria) dominated all invaded range root samples, together accounting for more than half the sequences in almost every sample ($\sim 91.7\%$). These same phyla, excluding Cyanobacteria, dominated all of the native range root samples, together accounting for $\sim 95.6\%$ of the sequences in nearly every sample. There were significant differences in the relative abundance of certain dominant phyla between *B. hybridum* roots in the native and invaded range, in native range roots, there were more Firmicutes ($p < 0.01$) and fewer Bacteroidetes ($p < 0.001$). Interestingly, Cyanobacteria were virtually undetectable within roots from the native range ($p < 0.001$; Fig. 3a).

Although no particular genus dominated in the observed sequences from the invaded range and native range root-associated microbiomes, six genera were the most abundant, *Lentzea* (phylum Actinobacteria),

Pseudomonas (phylum Proteobacteria), *Bacillus* (phylum Firmicutes), *Agrobacterium* (phylum Proteobacteria), *Erwinia* (phylum Proteobacteria), and *Sphingomonas* (phylum Proteobacteria). These accounted for 18.2% of invaded range sequences and 50% of the native range sequences. More specifically, *Lentzea* and *Pseudomonas* accounted for $\sim 3.9\%$ and $\sim 5.2\%$ of the observed sequences in the invaded range, while the native range root microbiomes were dominated by *Pseudomonas* and *Bacillus*, accounting for $\sim 25.3\%$ and $\sim 13.6\%$ of sequences, respectively. The three other genera (*Agrobacterium*, *Erwinia*, and *Sphingomonas*) were detected at $\sim 2\%$ relative abundance in either the native range or invaded range root samples. *Bacillus* ($p < 0.001$), *Erwinia* ($p < 0.001$), and *Pseudomonas* ($p < 0.001$) were more abundant in the native range, while *Sphingomonas* ($p < 0.01$) was more abundant in the invaded range (Fig. 3b).

Together, the dominant phyla within invaded and native range roots (described above; Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Proteobacteria) made up 63% of all sequences in invaded range bulk soil and 64.1% of the native range bulk soil. In native range bulk soil, there were significantly more Proteobacteria ($p < 0.001$) and Bacteroidetes ($p < 0.05$), and fewer Actinobacteria ($p < 0.01$) than in invaded range bulk soil. No significant differences in the relative abundance of dominant taxa were found between native range and invaded range *B. hybridum* rhizosphere soil.

Differences in Brachypodium hybridum bacteria between sample types in the invaded range and between B. hybridum and B. distachyon root bacteria in the native range

The dominant phyla within invaded range roots (described above) were not as dominant within bulk or rhizosphere soils. Together, these five phyla (Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Proteobacteria) made up 62.6% of all sequences. We detected differences in the relative abundances of these five phyla between the invaded range *B. hybridum* bulk and rhizosphere samples; in the rhizosphere soil there was more Bacteroidetes ($p < 0.01$). We detected some of the same genera in *B. hybridum's* invaded range bulk and rhizosphere soil, as were found to dominate the roots (dominant genera

described above); yet these genera were not as dominant in either bulk and rhizosphere soils. Together, *Lentzea, Pseudomonas, Bacillus, Agrobacterium, Erwinia*, and *Sphingomonas* accounted for only 1.4% of the observed sequences in bulk and rhizosphere soils. In bulk soil there was more *Bacillus* ($p < 0.05$), while in rhizosphere soil there was more *Pseudomonas* ($p < 0.01$).

The phyla that were dominant in the native range *B. hybridum* roots (described above; Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Proteobacteria) made up 96.5% of all combined *B. hybridum* and *B. distachyon* sequences We detected differences in the relative abundances of some of these taxa between the native range *B. hybridum* and *B. distachyon* roots; the *B. hybridum* roots had more Bacteroidetes ($p < 0.01$). The genera that were dominant in the native range *B. hybridum* roots (described above; *Lentzea, Pseudomonas, Agrobacterium, Bacillus, Sphingomonas*, and *Erwinia*) accounted for $\sim 56.4\%$ of the combined sequences in *B. hybridum* and *B. distachyon* roots. Only one dominant genus was significantly different between *B. hybridum* and *B. distachyon* roots, *B. distachyon* contained more *Bacillus* ($p < 0.01$). No differences were detected among dominant phyla and genera between *B. hybridum* and *B. distachyon* bulk and rhizosphere soil in the native range.

Core microbiome

There were 12 OTUs that comprised the core microbiome from the native range (i.e., 12 OTUs that were found in 95% of native range samples), and 28 OTUs that comprised the core microbiome from the invaded range (Supplemental Tabble 3). Most core OTUs were identified to genera, and 10 OTUs were in common between the native range and the invaded range.

Discussion

We examined microbial communities associated with *Brachypodium hybridum* and *B. distachyon* in the native range and *B. hybridum* in the invaded range, and found that (1) the bacterial community composition of *B. hybridum* sample types differed based on sample provenance, (2) the bacterial root composition of *Brachypodium distachyon* differed from that of *B.*

hybridum in the native range, despite the fact that rhizosphere and bulk soil bacterial composition did not differ, and (3) the root bacterial community differed from both rhizosphere and bulk soil for all plants.

It should be noted that naturally occuring differences between sites (e.g. pH, moisture, etc.) were controlled for in our study as site-to-site variation was controlled for as a random effect included in the PERMANOVA. In addition, while a common-garden experiment would have been an interesting addition to this study, our main focus does not depend on co-occuring native and invasive plants differing in their microbiomes, but rather the same plant within its native vs. invaded range, a key methodological difference. This project also relies on studying the microbime in situ, therefore, a common garden experiment would involve uprooting and analyzing the microbiome of sensitive native plants in the study area. Our results lend support to the three hypothetized scenarios:

Hypothesis 1   Brachypodium hybridum sample types differed by provenance

Our data support our first hypothesis, that bacterial community composition associated with *B. hybridum* in the native range differs from community composition within the invaded range (while accounting for site-to-site variation as a random effect). In particular, we found that the microbial community composition associated with roots, bulk soil, and rhizosphere soil differed by provenance. This finding suggests novel biotic interactions with *B. hybridum* in the invaded range, specifically among root endophyte and rhizosphere microbes, which are likely not exerting a similar influence on *B. hybridum* within its native range.

In the invaded range, *B. hybridum* root endophytic bacterial communities were richer and more diverse, compared to the native range. Furthermore, the root bacterial composition, compared to bulk and rhizosphere soil, was more diverse in the invaded range and less diverse in the native range. Only within the invaded range, "other" species' rhizosphere bacterial diversity was greater than *B. hybridum*. However, this is not unexpected, as the variation in species other than *Brachypodium* that were sampled would lead to a wider range of associated rhizosphere bacteria than those only associated with *Brachypodium*. Overall, in

the native range, roots harbored less microbial taxa than rhizosphere or bulk soils for both *Brachypodium* spp. and "other" plants, likely due to root filtering of taxa from the surrounding soil. Overall for *B. hybridum*, there was consistently more root microbiome diversity in the invaded range compared to the native range.

According to a metaanalysis performed in 2020 that synthesized the findings of $\sim$ 600 published studies, very few papers have compared the endophytic (inside roots, leaves, etc.) microbiomes of invasive plants in the native and invaded range (Harrison and Griffin, 2020). Two studies, one by Lu-Irving et al. (2019) and another by Ramirez et al. (2019), detected greater microbiome diversity for invasive plants within their native, or original, range than within their invaded range. However, these studies both focused on invading forbs, and neither study examined these patterns for invasive grasses. Our study found the opposite trend in root diversity for the invasive grass *B. hybridum*. Much more research is needed in this area before a general trend or pattern regarding root endophyte diversity in the invaded vs. native range becomes apparent.

It is important to note that our main goal was simply to use the "other" species as a control to check that the microbial community associated with *Brachypodim* spp. and "other" invasive grass species were similar. While we did find that "other" species' rhizosphere bacterial diversity was greater than *B. hybridum* in the invaded range, we did not find any other differences in the bacterial community composition between *Brachypodium* spp. and "other" species. As the focus of this study is *Brachypodium* spp. microbial composition in the native vs. invaded range, we did not investigate the taxonomic groups associated with the "other" species. However, as we did find that the Hill 0 for "other" plant species were significantly higher in the invaded range vs. the native range in every sample type, investigating the taxonomic differences for "other" species by provenance is a great area of future research with this dataset.

For *Brachypodium*-associated samples at both the genus and phylum levels, we detected differences in dominant taxa between the native and invaded range. Within soil sample types, these trends may be driven by soil nutrients, edaphic factors, or differences in habitat suitability across these environments, which could influence the soil microbiome, and filtering of root endophytes. In particular, Proteobacteria and Bacteroidetes were more abundant in the bulk soil of the native range as compared to the bulk soil of the invaded range. Furthermore, a higher abundance of Bacteroidetes in the native range bulk soil and the invaded range roots, but not in the invaded range bulk soil, could indicate that *B. hybridum* may have carried Bacteroidetes with it from the native range.

Although both Bacteroidetes and Proteobacteria are commonly found in a variety of soils, they may exhibit copiotrophic tendencies (i.e. found in environments rich in nutrients) (Fierer et al. 2007; Ramirez et al. 2012). The greater abundance of these phyla in the bulk soil could be due to higher concentrations of soil nutrients within the native range. *Brachypodium* spp. have been found to have relatively high nitrogen (N) contents when growing in high N media (David et al. 2019); therefore, *B. hybridum* growth may be expected to correlate with greater soil N concentrations in the native range soil. In addition, previous studies show that invasion of grasses with high N content increases the N pool of invaded soils, as compared to uninvaded soils (Liao et al. 2008). However, the increased presence of copiotrophic bacteria in the native range bulk soil could indicate that both the native and invaded range have high N content due to *B. hybridum* growth. While we did not investigate the nitrogen concentration of native and invaded range soils, this is an excellent avenue for future research. It is also worth noting that soil priming, where plant roots release organic acids and sugars into the rhizosphere region to encourage beneficial bacterial growth (Nunes et al. 2019), may have a greater influence on the rhizosphere microbiome than roots, and may play a facilitative role in *B. hybridum* invasion.

We detected differences in the abundance of Cyanobacteria, which significantly varied across *Brachypodium hybridum* roots in the native vs. invaded range, with far greater abundance in the invaded range. Cyanobacteria are photosynthetic, nitrogen-fixing bacteria, often found in damp soil and aquatic habitats (Vincent 2009), in arid environments as soil crusts (Isichei 1990), and in endolithic systems (de Los Rios et al. 2007). Cyanobacteria are also commonly found as endosymbionts in plants, lichens, and sponges. The high abundance of this phylum in the invaded range roots, and almost complete absence in the native range roots, shows

that invaded range plants hosted more endosymbiotic Cyanobacteria. Given that sampling occurred towards the end of an extended drought in the invaded range (~ 2013–2016 across California), the increased abundance of Cyanobacteria in the invaded range plant roots suggests that these microbes likely proliferated in drier conditions. Associations of Cyanobacteria with the roots of angiosperms other than those of the Gunneraceae family (species associated with humid and water-logged habitats) (Adams et al. 2006), have only ever been recorded at low abundances (Bulgarelli et al. 2015; Lundberg et al. 2012; Naylor et al. 2017; Xu et al. 2018) and associations with *Brachypodium* have never been recorded—making this finding quite interesting.

To ensure that our sequences belong to Cyanobacteria we chose a random subset of 20 representative sequences for Cyanobacteria generated during OTU picking. We then input those sequences into BLAST after limiting the search to Cyanobacteria. For the top 20 BLAST results, the average percent identity match was 86%, with the highest percent identity being 100% and the lowest being 81.67%. All E-values were less than 1e − 82. We can be confident that the Cyanobacteria found in the roots of our plant samples were real results and not plastid DNA.

Many Cyanobacteria are facultative heterotrophs, meaning they are capable of occupying regions of the host receiving little or no light, such as the roots of plants, where they provide fixed nitrogen to the host and receive fixed carbon in return (Adams et al. 2006). Due to the abundance of Cyanobacteria in the invaded range roots but not in native range roots, it is likely that members of this phyla are mutualists with *B. hybridum,* possibly improving this grass species' growth in the invaded range. While not addressed by our study, it may be useful to discover exactly what role Cyanobacteria plays in the roots of *B. hybridum*. The Cyanobacteria could be affecting *B. hybridum* growth in the invaded range through fixation of dinitrogen or through the substances it produces. A recent literature review by Santini et al. (2021), found that Cyanobacteria are rich in auxin and auxin-like compounds, which act as biostimulants in plants. These biostimulants result in increased root mass and elongation and increased plant nutrient uptake. For example, Mahado de Lima et al. (2020) found that biopriming arid plant seedlings with a mix of cyanobacteria resulted in three times larger roots in

hummock grasses (e.g. *Triodia epactia*). They found that the cyanobacterial inoculants produced substances chemically like plant hormones (i.e. indole-3-acetic acid) that may have increased the arid grass species' growth. This could be another explanation for the possible interaction of Cyanobacteria with *B. hybridium* in the invaded range.

We found a higher relative abundance of some potentially pathogenic bacteria (*Erwinia* and *Pseudomonas*) in the native range roots compared to the invaded range roots (Supplemental Tabble 4). These genera contain many putatively pathogenic bacterial species (van Baarlen et al. 2007). *Erwinia* is almost entirely composed of plant pathogenic species, which mainly infect woody plants, but have been found in grasses such as *Avena fatua* and *Bromus* spp, which are also invasive in the same region of California (McCarter-Zorner et al. 1985). High-profile examples include *E. amylovora*, which causes fire blight in fruit crops and *E. aphidicola*, which has annihilated ~ 50% of bean crops in the native range (Marín et al. 2011). *Pseudomonas* is composed of several plant-growth-promoting and plant-pathogenic species, many of which have been known to infect various grass species, and can act to protect plant diversity (Mishra et al. 2012). The relatively high abundance of these potentially pathogenic genera in the native range roots indicates that root-associated pathogen loads may be lower within the invaded range, suggesting that release from natural enemies may be contributing to *B. hybridum* invasiveness in the invaded range. Another bacterial genus detected in *Brachypodium* roots in the native range was *Sphingomonas,* which is ubiquitous and likely well adapted for living in semi-arid, exposed soils. It may be that these genera are indicative of a healthy soil community in the native soil compared to the invaded soil, but it is hard to assess its role in the root endophytic community.

We show differences in *B. hybridum* samples between native and invaded ranges. Future work should focus on understanding whether, and to what degree, these differences drive invasion and success of this invasive plant, compared to the scarce success of its diploid progenitor species, worldwide. The gene-for-gene hypothesis (Clay & Kover 1996; Pfender 2009), posits that in situ microbial communities are different for co-located invasive and native plants (Angeloni et al. 2006; Fitzpatrick et al. 2018; Knelman et al. 2012). Given that these plant–microbe

interactions may be highly specific, our study may contribute evidence for benefits provided by soil microbial communities to *B. hybridum* in the invaded range, as compared to the native range. While our comparison of the same grass within both its native and invasive range differs from those reported by others examining invasive forbs (Ramirez et al. 2019), our findings are explicit; *B. hybridum* root endophytic and soil microbiomes clearly differed between the native and invaded range.

Hypothesis 2 Brachypodium hybridum root microbiome differed from that of B. distachyon in the native range

In support of our second hypothesis, we found that root bacterial community composition differed between *B. hybridum* and *B. distachyon* in the native range, indicating Bray–Curtis distance matrices contained numerous differences; yet, we only detected significant differences within two dominant bacterial phyla and one dominant genus between *B. hybridum* and *B. distachyon*. *Brachypodium hybridum* roots had more Bacteroidetes and less Firmicutes. Bacteroidetes is a diverse phylum that includes many copiotrophs. The greater abundance of this taxon in *B. hybridum* (as compared to *B. distachyon*) roots indicates that *B. hybridum* may have carried Bacteroidetes along with it to its invaded range. This suggests that Bacteroidetes may be important for *B. hybridum* growth and invasion.

Although we found a greater abundance of *Bacillus* OTUs, within the phylum Firmicutes, in *B. distachyon* roots, as compared to *B. hybridum* roots, within its native range, *B. hybridum* was characterized by greater *Bacillus* abundance within its native than invaded range. *Bacillus* spp. are found in almost every environment, including those with extreme temperatures and high pH. Some *Bacillus* (e.g. *B. siamensis*) are able to produce antimicrobial compounds that inhibit plant pathogens and promote plant growth (Jeong et al. 2012). Unfortunately, many *Bacillus* OTUs in our dataset were not identified beyond genus, therefore observed differences in abundance may result from variation between one or more species.

We found no detectable differences among rhizosphere and bulk soil microbiomes between *B. hybridum* and *B. distachyon* within the native range. Furthermore, *B. distachyon* and *B. hybridum* microbiome richness and diversity did not differ in any sample type (root, rhizosphere soil, bulk soil) within the native range.

There are key physical and physiological characteristics that differ between these two close relatives that may explain differences in invasive abilities, including environmental range and plant size. In particular, genome duplication likely exerts a strong influence on *Brachypodium hybridum* physiology and ecology, such as changes in flowering-time and weediness (Bakker et al. 2009) and the development of a drought-escapist strategy (Manzaneda et al. 2015; Martínez et al. 2018). Thus, the allotetraploid *B. hybridum* may have had a heightened ability to persist and colonize dynamic environments found in the invaded range (te Beest et al. 2012). Although polyploidy could have both negative and positive impacts on plant growth, establishment, and survival (Comai 2005), *B. hybridum's* genomic architecture may facilitate the evolution of *B. hybridum's* invasiveness by either providing a fitness advantage (Ramsey 2011; Rey et al. 2017) or promoting subsequent adaptations to the novel environment via larger genetic diversity.

Our data show that endophytic microbial communities differ, which could have implications for plant stress-tolerance (Rodriguez et al. 2008). Although *B. hybridum* appears to thrive in its invaded range, future research would be required to link invasion success with any observed differences in endophytic microbes across regions, and how these may impact traits that allow some *Brachypodium* spp. to become invasive.

Hypothesis 3 Root microbiome differed from soil microbiome pools

We found root microbial communities differed from bulk and rhizosphere soils in both the native and invaded range, likely because plant-associated microbes will be present in the roots and largely absent from the surrounding bulk soil. Additionally, relationships among the microbiome composition of the root, rhizosphere, and bulk communities differed by source region. While native range rhizosphere and bulk soil communities were similar, invaded range rhizosphere and bulk soils differed. This differentiation in degree of filtering of the microbial community in surrounding bulk soil between the invaded range and the native range indicates that invaded range *B. hybridum* may be recruiting a different microbiome from its surrounding bulk soil than the native range *B.*

hybridum, which may contribute to its invasiveness in California. Across all sites, root microbiomes were less diverse than soil pools, which indicates that *B. hybridum* may selectively filter bacteria from surrounding soils in both the native and invaded ranges. Additionally, particular environmental cues may prime this differential filtering of surrounding bacteria by *B. hybridum* roots, resulting in different communities within the native vs. invaded ranges.

Previous studies show that root- and soil-associated microorganisms affect the naturalization of exotic plants in the introduced region, and the ability of these plants to outcompete native species (Coats & Rumpho 2014). Studies on grasses suggest that some endophytic microbes and rhizobacteria symbiotically conferred stress tolerance to their host when facing drought, heat, oxidative, and nitrosative stress (Park et al. 2017; Rodriguez et al. 2008; Vurukonda et al. 2016). In addition to facilitating invasive plant naturalization, interactions among invading plants and soil microbes in the rhizosphere, and shifts in rhizosphere microbes between the native and invasive ranges, may play a role in driving plant growth and resource acquisition. Some studies demonstrate invasive plants' capacity to bring along novel microbes from their native ranges, therefore altering dynamics within bulk soil and rhizosphere microbial communities in invaded ranges to favor invaders' growth and competitiveness (Trognitz et al. 2016). However, findings from our study indicate that *Brachypodium* spp. found in the invaded range likely recruited microbes from the surrounding soil and possibly carried Bacteroidetes with it to the invaded range. Our findings further suggest more complex plant-microbial interactions in native range rhizosphere soil with *B. hybridum* individuals, which have developed over longer time periods than within the invaded range.

Further investigations into these findings could elucidate corresponding hypotheses about whether invasion in the *Brachypodium* system may be controlled by biotic mechanisms such as enemy release hypothesis (Chun et al. 2010) or enhanced mutualism hypothesis (Sun & He 2010).

It is worth noting that bacterial communities represent only a portion of the soil and root microbiome, therefore, future work should investigate *Brachypodium* root-associated fungi within the context of ecological fitting or the enhanced mutualism hypothesis. Although examining fungal controls on *B.*

hybridum invasions was beyond the scope of our study, future research examining root-associated arbuscular mycorrhizal community composition would provide further insight into biotic drivers of *B. hybridum*'s invasion success.

Future research into biotic associations in this study system could evaluate the viability of using microbes as biocontrols for managing *B. hybridum* incipient invasion. As *Brachypodium distachyon* is a model grass for the genetic development of improved cereal crops, and part of the *B. distachyon-B. stacei-B. hybridum* complex, any knowledge of plant responses to microbial pathogens associated with *B. distachyon* and its close relatives, which may hinder *B. hybridum*'s performance, would be an asset for agricultural applications, as well as for future agricultural pest controls.

Conclusions

Overall, our study shows that in situ root and soil-associated microbial communities are different for *Brachypodium hybridum* between its invasive and native ranges. We detected differences in the root endophytic communities associated with *B. hybridum* and its diploid progenitor species *B. distachyon* within the native range. Our data indicate that microbiomes differed between roots and soils, but that the relationship between either of these sample types and the rhizosphere differed by source location. This research suggests that differences in microbial community composition between *B. hybridum* and *B. distachyon* may influence patterns of *B. hybridum* invasiveness in its invaded range.

Declarations

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