New Phytologist

Research

Plant domestication and the assembly of bacterial and fungal communities associated with strains of the common sunflower, *Helianthus annuus*

Jonathan W. Leff[1,2], Ryan C. Lynch[2,3], Nolan C. Kane[2] and Noah Fierer[1,2]

[1]Cooperative Institute for Research in Environmental Sciences, University of Colorado, Boulder, CO 80309-0216, USA; [2]Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309, USA; [3]Medicinal Genomics, 12 Gill St, Woburn, MA 01801, USA

Author for correspondence:
Noah Fierer
Tel: +1 303 492 5615
Email: Noah.Fierer@colorado.edu

Received: *14 September 2016*
Accepted: *9 October 2016*

New Phytologist (2016)
doi: 10.1111/nph.14323

Key words: bacteria, domestication, fungi, *Helianthus annuus* (common sunflower), rhizosphere, root, seed, vertical transmission.

Summary

- Root and rhizosphere microbial communities can affect plant health, but it remains undetermined how plant domestication may influence these bacterial and fungal communities.
- We grew 33 sunflower (*Helianthus annuus*) strains ($n = 5$) that varied in their extent of domestication and assessed rhizosphere and root endosphere bacterial and fungal communities. We also assessed fungal communities in the sunflower seeds to investigate the degree to which root and rhizosphere communities were influenced by vertical transmission of the microbiome through seeds.
- Neither root nor rhizosphere bacterial communities were affected by the extent of sunflower domestication, but domestication did affect the composition of rhizosphere fungal communities. In particular, more modern sunflower strains had lower relative abundances of putative fungal pathogens. Seed-associated fungal communities strongly differed across strains, but several lines of evidence suggest that there is minimal vertical transmission of fungi from seeds to the adult plants.
- Our results indicate that plant-associated fungal communities are more strongly influenced by host genetic factors and plant breeding than bacterial communities, a finding that could influence strategies for optimizing microbial communities to improve crop yields.

Introduction

Root and rhizosphere microbial communities play key roles in determining plant health and productivity (Berendsen *et al.*, 2012; Chaparro *et al.*, 2012; Bulgarelli *et al.*, 2013; Verbon & Liberman, 2016), yet our understanding of how these communities are assembled remains rudimentary. For example, although different strains of *Arabidopsis* are known to harbor distinct root microbiomes (Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012), we currently lack a predictive understanding of the biotic and abiotic factors responsible for these observed differences. A better understanding of root and rhizosphere microbial community assembly will improve our ability to predict the structure of plant-associated microbial communities and their effects on plant health. Ultimately, such knowledge could be used to directly or indirectly manipulate these microbial communities to enhance the health and productivity of agricultural crops (Berg, 2009; Compant *et al.*, 2010; Singh *et al.*, 2011; Turner *et al.*, 2013; Bender *et al.*, 2016).

Previous work has demonstrated that soil properties are the dominant factors structuring root and rhizosphere microbial communities (Bulgarelli *et al.*, 2012; Philippot *et al.*, 2013). However, other factors such as plant species identity or genotype can also have measurable influence on their composition (Hardoim *et al.*, 2011; Bulgarelli *et al.*, 2012; Philippot *et al.*, 2013; Coleman-Derr *et al.*, 2015). Therefore, a critical next step is to determine how and when differences in plant genotypes matter for root and rhizosphere microbial community assembly (Lareen *et al.*, 2016). For example, root and rhizosphere bacterial communities have been shown to differ across related strains of *Arabidopsis* (Lundberg *et al.*, 2012; Schlaeppi *et al.*, 2014), maize (Peiffer *et al.*, 2013), and rice (Hardoim *et al.*, 2011; Edwards *et al.*, 2015), but the specific nature of these interactions and the factors driving these host genotype–microbial associations remain largely undetermined.

In agriculture, knowing the factors that favor the assembly of beneficial bacterial and fungal associations with crop plants could be leveraged to enhance crop yields, given the potential importance of these below-ground microbial associations in mediating nutrient acquisition, environmental tolerances, and disease resistance (Rodriguez *et al.*, 2008; Mei & Flinn, 2010; Farrar *et al.*, 2014). In particular, we need to know whether crop-associated microbial communities have shifted as a result of plant domestication and whether potentially beneficial interactions between plants and their microbial symbionts have been lost during the domestication process. If so, it could be possible to modify

agricultural practices to account for this loss and improve crop yields. More generally, domestication of plants has resulted in a number of changes to their interactions with other organisms and their effects on agroecosystems – processes that are still poorly understood (García-Palacios *et al.*, 2013; Milla *et al.*, 2015; Turcotte *et al.*, 2015). Likewise, there has been some speculation that the selective breeding involved with domestication and the conditions in which domesticated plants are typically grown can influence their microbial communities (Pérez-Jaramillo *et al.*, 2015; Pieterse *et al.*, 2016; Schmidt *et al.*, 2016). For instance, differences in the necessity for stronger stress tolerance or the ability to grow under systematic disturbances such as plowing could promote differences between microbial communities associated with wild and domesticated plants. Previous work suggests that differences in rhizosphere bacterial communities associated with maize might be linked to domestication (Bouffaud *et al.*, 2014). Other studies have proposed that modern crops do not support beneficial microbes in their rhizosphere as readily as their wild ancestors as a result of modification of plant traits (Philippot *et al.*, 2013). For example, mutually beneficial associations between arbuscular mycorrhizal fungi (AMF) and wild relatives of crops could be less prevalent in modern crops as those crops might be less dependent on AMF in an agricultural setting (Sawers *et al.*, 2008). However, there is mixed empirical evidence for whether AMF more commonly form associations with wild plants compared with their domesticated relatives (Zhu *et al.*, 2001; An *et al.*, 2010; Lehmann *et al.*, 2012; Xing *et al.*, 2012; Leiser *et al.*, 2016; Turrini *et al.*, 2016). More broadly, relationships between crop evolutionary history and associated microbial communities are not always easily detectable (Bouffaud *et al.*, 2012), or the effects of domestication are sufficiently subtle that they can be difficult to quantify in highly diverse below-ground microbial communities (Bulgarelli *et al.*, 2015).

Differences in the microbes contained in seeds and the passage of these microbes to offspring (i.e. vertical transmission) is one possible mechanism that could promote genotype-specific differences in plant-associated microbial communities (Nelson, 2004; Truyens *et al.*, 2015). Plants could be under selective pressure to package microbes in seeds to ensure progeny are able to form associations with their most beneficial microbes, thus promoting plant genotype-specific seed communities (Ewald, 1987; Rudgers *et al.*, 2009; Truyens *et al.*, 2015). Previous work has shown that seeds could serve as an important vector for the transmission of microbes from one generation of plant to another for certain species (Kaga *et al.*, 2009; Johnston-Monje & Raizada, 2011; Hardoim *et al.*, 2012; Cope-Selby *et al.*, 2016; Pitzschke, 2016). Still, it is unclear whether vertical transmission of microbes through seeds can contribute to differences in microbial community composition across plant varieties and whether such vertical transmission can influence plant performance. Alternatively, given that the types of microbes found in soil appear to have a strong influence on what types of microbes associate with plant roots (Bulgarelli *et al.*, 2012), any potential influence of vertical transmission via seeds could be negligible in ultimately structuring the below-ground associations that adult plants form with microbes and have little effect on plant development and growth.

Here we sought to determine whether the structure of bacterial and fungal communities in roots and rhizosphere was predictable based on host genotype across 33 sunflower (*Helianthus annuus*) strains grown in the same soil type. We chose sunflower as a model species as it is a globally important crop, and we were able to obtain seeds from strains that spanned a wide gradient of wild, landrace (i.e. early domesticated strains), and modern domesticated cultivars. This enabled us to investigate whether domestication through selective breeding affected microbial community structure. We hypothesized that root and rhizosphere microbial community structure differs in consistent ways across sunflower strains and that these differences are related to the extent of plant domestication (i.e. whether the sunflower strain was classified as a wild, landrace, or modern strain). In addition, we characterized the bacterial and fungal communities in seeds from the same batches used to grow the sunflowers to assess the extent to which bacteria and fungi were transmitted from the seeds to the adult plant root and/or rhizosphere communities. Given that previous work has shown evidence in favor of vertical transmission in other plant species, we hypothesized that vertical transmission of seed-associated microbial communities may contribute to the observed strain-specific differences in the microbial communities associated with adult plants.

Materials and Methods

Plant material used

A total of 33 common sunflower (*Helianthus annuus* L.) strains were selected to span the full range of genetic variation in the species (Harter *et al.*, 2004), including 11 wild populations from across the native range in North America, 14 Native American landraces representing the diversity of premodern cultivated lineages that occurred following a single domestication event, *c.* 4000–5000 yr ago (Harter *et al.*, 2004; Blackman *et al.*, 2011; Kane *et al.*, 2013; Smith, 2014), and eight modern domestic varieties that are the product of the last few hundred years of more intensive breeding efforts (Supporting Information Table S1). This sample set thus allows us to evaluate the role of the original domestication event in North America, the result of thousands of years of selection by Native Americans in what is now the southeastern US, as well as the more recent, scientific breeding for modern agriculture. All seeds were obtained from the USDA National Plant Germplasm System (https://npgsweb.ars-grin.gov/gringlobal/; see Table S1 for seed accession information).

Plant growth and sample collection

Seeds of each strain ($n = 5$ per strain) were started in sterile Petri dishes containing moist paper towels and transferred to potting soil (type) once germinated. After 2 wk, seedlings were transplanted to a 350 m^2 field outside Boulder, CO, USA (40°02′24″ N 105°07′48″W). Plants were watered as needed. The soil in the field was a Mollisol classified as a Manter sandy loam. This soil type is characterized as being well drained and having a

circumneutral acidity (http://websoilsurvey.nrcs.usda.gov/). Individuals were planted in random locations across the field as small variations in soil and environmental conditions that might have existed in the field. There was minimal pre-existing plant cover in the experimental plot, and small weeds were removed before planting.

Plants were grown for 53 d and harvested when all plants were expected to be at or near peak height. Immediately before harvest, plant height, stem diameter, most recent fully expanded leaf length and width, the number of nodes, and the number of branches were recorded for each individual. Plants were manually uprooted from the soil, and roots were aggressively shaken in order to remove loose soil. The ends of multiple representative roots were cut from each plant and transferred to sterile 50 ml conical tubes (filling approximately half the volume of the tube). The samples in tubes were immediately transferred to the laboratory on ice. To remove rhizosphere soil, 10 ml of DNA-free water was added to each tube and vortexed for 10 s, with the rhizosphere soil collected after the slurry was allowed to settle for 24 h and the supernatant was decanted, following the general approach described previously (minus the centrifugation step; Lundberg *et al.*, 2012). The washed roots were then transferred to new tubes and the roots were further cleaned by adding 10 ml of DNA-free water to tubes, vortexing for 10 s, pouring out the water, and repeating. Subsamples (0.2 g) of the cleaned roots were then transferred to 1.7 ml tubes where they were processed with 100% ethanol, rinsed with water and then treated with propidium monoazide as in Nocker *et al.* (2007) to remove superficial, dead bacterial and fungal cells. Roots were macerated in their tubes with sterile pestles before DNA extraction.

Seeds that were from the same batch used to grow the plants were included in our bacterial and fungal community analyses ($n = 4$ per strain). Seeds were prepared by soaking the seeds in DNA-free water for 24 h, briefly submerging in 95% ethanol, and rinsing with water. This procedure was intended to soften seeds and remove superficial microbial cells, but microbial cells integrated in the seed coat were deliberately retained because they could influence the adult plant's microbial community. Each seed was then macerated separately with a sterile and DNA-free glass mortar and pestle.

Bacterial and fungal community analysis

Subsamples (100 µl) of rhizosphere slurries, macerated roots, and macerated seeds were transferred to 96-well plates for DNA extraction by mixing 150 µl DNA-free water with each sample and transferring 50 µl to an individual well. DNA extraction was performed using the PowerPlant Pro DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions, with appropriate negative control ('blanks') included in all steps of the process. The V4 region of the 16S rRNA gene and the ribosomal internal transcribed spacer 1 (ITS) were PCR-amplified to assess bacterial and fungal diversity, respectively. PCR protocols followed those used previously (Lundberg *et al.*, 2013; McGuire *et al.*, 2013) and included primers with barcodes unique to each sample to permit sample multiplexing. The primer set with linkers and adapters used for 16S rRNA gene amplification was 515-F (AATGATACGGCGACCACCGAG ACGTACGTACG GT GTGCCAGCMGCCGCGGTAA) and 806-R (CAAGCAG AAGACGGCATACGAGAT XXXXXXXXXXXX AGTCAGTC AG CC GGACTACHVGGGTWTCTAAT), where 'X' characters represent the 12 bp barcodes (Caporaso *et al.*, 2011; Fierer *et al.*, 2012). The primer sets with linkers and adapters used for fungal ITS amplification were ITS1-F (AATGATACGGCGA CCACCGAGATCTACAC GG CTTGGTCATTTAGAGGAAG TAA) and ITS2 (CAAGCAGAAGACGGCATACGAGAT XXXXXXXXXXXX AGTCAGTCAG AT GCTGCGTTCTT CATCGATGC; White *et al.*, 1990; McGuire *et al.*, 2013; Smith & Peay, 2014). Peptide nucleic acid PCR clamps were used when targeting 16S rRNA genes to inhibit the amplification of chloroplast and mitochondria genes as described in (Lundberg *et al.*, 2013). PCR products from triplicate reactions per sample were cleaned and pooled in equimolar concentrations using the SequalPrep kit (Thermo Fisher Scientific, Waltham, MA, USA). The cleaned amplicons were sequenced in three runs (two for bacteria and one for fungi) on an Illumina MiSeq instrument (San Diego, CA, USA) at the University of Colorado BioFrontiers Institute Next Generation Sequencing Facility using a paired-end 2 × 151 bp kit for the two bacterial sequencing runs and a 2 × 251 bp kit for the fungal sequencing run. All raw sequence data have been deposited in the National Center for Biotechnology Information Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra) under the accession number SRP075934.

Initial sequence processing was conducted similarly to that described by Prober *et al.* (2015) following the UPARSE pipeline implemented in USEARCH v.8 (Edgar, 2013). Briefly, sequencing adapters were removed from fungal ITS sequences using cutadapt (Martin, 2011), both bacterial and fungal sequences were assigned to individual samples (i.e. demultiplexed), and a *de novo* database was created by merging paired-end reads, quality filtering, dereplicating, removing unique (i.e. singleton) sequences, and clustering sequences into phylotypes at the 97% similarity threshold. Representative sequences from those phylotypes that were not ≥ 75% similar to any sequence in the Greengenes or UNITE databases were removed as they were assumed to be of low quality, chimeric, or a product of nonspecific amplification. Raw merged reads were then mapped to the *de novo* database in order to determine the number of sequences representing each phylotype for each sample. Taxonomy was determined for each phylotype using the RDP classifier (Wang *et al.*, 2007) trained on the Greengenes (McDonald *et al.*, 2012) and UNITE (Abarenkov *et al.*, 2010) databases for bacterial and fungal sequences. 16S rRNA sequences from chloroplasts, mitochondria, or archaea were removed before downstream processing as were bacterial or fungal sequences that were not classified to at least the phylum level of resolution. Owing to the high relative abundance of chloroplasts and sparsity of robust information on the seed bacterial communities, we did not use those samples for downstream analyses investigating the strain-specific differences in plant-associated bacterial communities. Bacterial data were rarefied to 2000 sequences per sample, and fungal data were rarefied to 1000 sequences per sample before all downstream analyses,

rarefaction depths that were chosen to balance the number of samples that could be included while maximizing the available number of sequences per sample. FunGuild (Nguyen *et al.*, 2015) was used to assign fungal phylotypes from the rarefied data to one of three trophic modes (saprotroph, symbiont, or pathogen) where possible.

Statistical analyses

We used the Shannon diversity metric to quantify bacterial and fungal diversity. Diversity across sample types was compared using linear mixed-effects models with sunflower strain as a random factor. Overall differences in bacterial or fungal community composition were assessed by calculating pairwise Bray–Curtis dissimilarities from square-root-transformed phylotype relative abundances. Differences in community composition across sample types were tested using permutational analysis of variance (PERMANOVA), and pairwise comparisons between sample types were tested by comparing two factor levels at a time using PERMANOVA and adjusting the resulting *P*-values for multiple comparisons with false discovery rate corrections. This was done using the function, 'CALC_PAIRWISE_PERMANOVAS' in MCTOOLSR (Leff, 2016), which implements the 'ADONIS' function in the VEGAN package (Oksanen *et al.*, 2016) in R (R Core Team, 2015). Differences in community composition across sunflower strains and across domestication levels were also tested using PERMANOVA. We verified that the data met the assumption of multivariate homogeneity of dispersions using the 'BETADISPER' function in the VEGAN package before running these tests. When testing for differences in community composition across domestication levels, dissimilarities were first averaged across replicates of each sunflower strain to avoid pseudoreplication. Differences in the relative abundances of fungal pathogens among domestication levels were compared using a linear mixed-effects model with sample type and domestication level as fixed effects and sunflower strain as a random effect. ANOVA was used to test for differences in the proportion of samples in each strain that had detectable fungal symbionts across domestication levels. Only sunflower strains with data from at least three replicate samples were used when making comparisons across strains or levels of domestication. Relationships between plant characteristics and microbial diversity were assessed with Spearman correlations, and relationships with microbial community composition were assessed with permutational Mantel tests. Differences in the relative abundance of fungal pathogens across sample types were tested using a linear mixed model with sample type as a fixed effect and plant strain as a random effect. We used R (R Core Team, 2015) for all statistical analysis.

Results

Microbial communities differ across plant compartments

Across all samples, fungal and bacterial community structure differed strongly among root, rhizosphere, and seed samples, regardless of sunflower strain (Fig. 1). Fungal and bacterial diversity differed significantly across compartments ($P < 0.001$ in both cases), and fungal and bacterial diversity in seeds was lower than in root and rhizosphere communities (Fig. 1a). Root bacterial communities were significantly more diverse than rhizosphere communities ($P < 0.001$), but fungal rhizosphere and root communities had equivalent degrees of diversity ($P > 0.1$; Fig. 1a). The higher bacterial diversity in root endosphere than in rhizosphere samples was related to the dominance of *Pseudomonas* in the rhizosphere samples.

For both bacteria and fungi, the rhizosphere, root, and seed communities were each significantly different in composition from one another ($P < 0.001$ in all pairwise comparisons; Fig. 1b). Root and rhizosphere bacterial and fungal communities were generally more closely related to one another than to seed communities. Seeds tended to have high relative abundances of the bacterial families, *Nocardiopsaceae*, *Enterobacteriaceae*, and *Sphingomonadaceae* compared with root and rhizosphere samples, which harbored high relative abundances of a number of families not commonly observed in seeds (Fig. S1). The composition of fungal communities in seeds were distinguished from those in roots and rhizospheres as a result of high relative abundances of *Pleosporaceae*, while root and rhizosphere communities had high relative abundances of *Nectriaceae*, *Olpidiaceae* and *Mortierellaceae* (Fig. S2).

Differences in microbial communities across sunflower strains

Neither bacterial nor fungal community diversity significantly differed across the root or rhizosphere communities from different sunflower strains ($P > 0.05$ in all cases). Bacterial root and rhizosphere and fungal root community composition also did not differ significantly across the strains ($P > 0.05$). However, fungal rhizosphere community composition did differ across the strains, albeit somewhat weakly ($R^2 = 0.25$, $P = 0.01$). No single sunflower strain appeared to drive the overall differences; instead the degree of dissimilarity in fungal community composition was similar across all sunflower strains (Fig. S3).

Differences in microbial communities across degrees of domestication

Neither bacterial nor fungal diversity differed across domestication levels in rhizospheres or roots ($P > 0.5$). Domestication level did significantly affect fungal rhizosphere community composition ($R^2 = 0.08$, $P = 0.03$). Within rhizospheres, unclassified *Pleosporales*, *Preussia* spp., unclassified *Thelebolaceae*, *Fusarium* spp., and *Conocybe* spp. tended to have higher relative abundances in modern strains, while unclassified *Chaetomiaceae* and *Mortierella* spp. had higher relative abundances in wild strains, and the *Chytridiomycota* genus *Olpidium* had higher relative abundances in Native American and wild strains than in modern strains (Fig. S4).

In addition to individual taxa, putative fungal pathogens had a lower relative abundance in the root and rhizosphere microbial communities of modern strains when compared with wild or

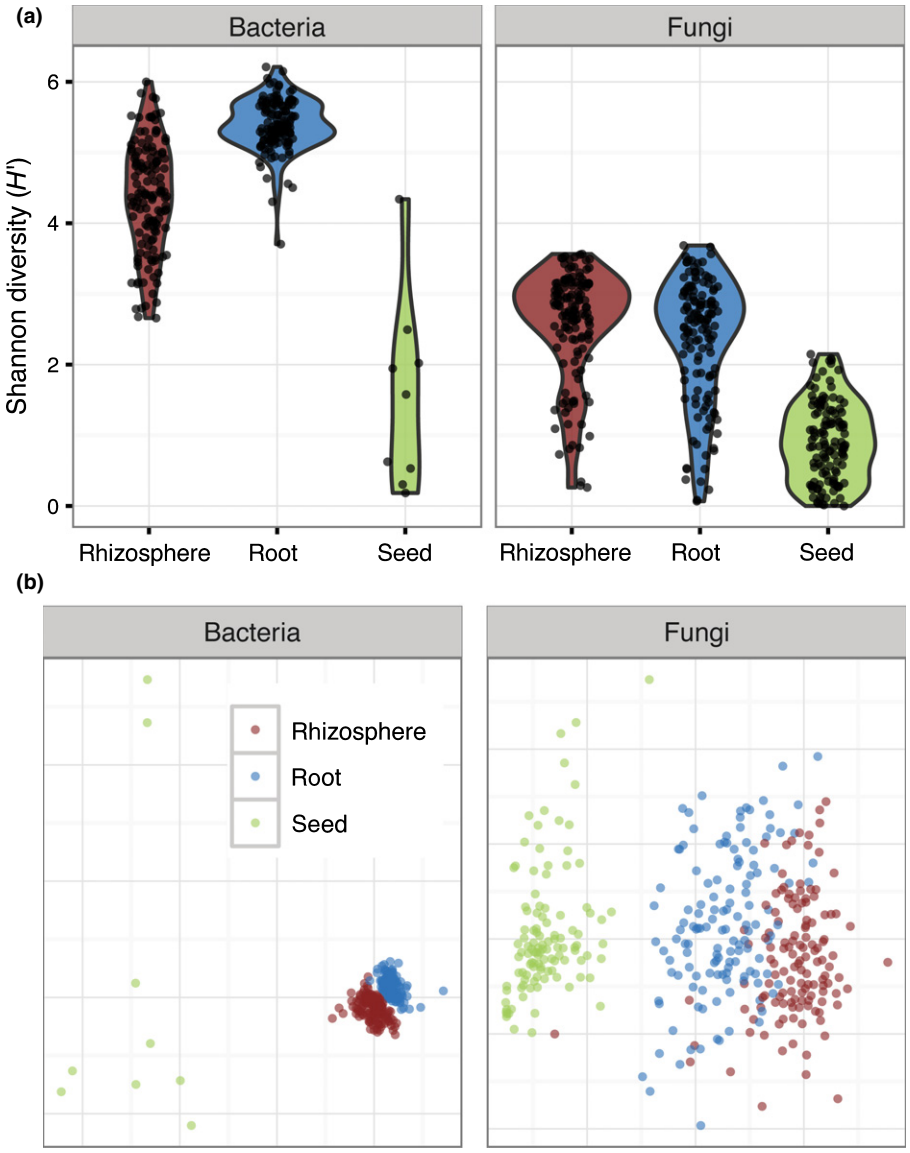

Fig. 1 Shannon diversity distributions (a) and nonmetric multidimensional scaling ordinations showing differences in community composition (b) of bacterial and fungal communities across rhizosphere, root, and seed samples collected from sunflowers (*Helianthus annuus*). The ordinations were based on Bray–Curtis dissimilarities calculated from square-root-transformed data.

native American strains ($P = 0.04$; Fig. 2a). Conversely, fungal symbionts (primarily *Glomeromycota*; Fig. S5) were only detected in a subset of the samples, and while not statistically significant, they were detected in a greater proportion of the roots of modern strains compared with wild strains ($P > 0.1$; Fig. 2b).

Are differences in microbial communities explained by plant attributes?

More domesticated strains tended to be taller, have broader leaves, wider stems and fewer branches after growing for the same length of time (Fig. 3). However, differences in root and rhizosphere bacterial and fungal communities were not strongly related to differences in plant attributes (Tables S2, S3). The diversity of rhizosphere bacterial communities and root fungal communities was weakly and inversely related to measured plant characteristics indicative of growth rate (larger plants tended to have lower bacterial and fungal diversity; Table S2). Neither bacterial nor fungal community composition were strongly related to any of the measured phenotypic characteristics (Table S3).

Seed fungal communities and their relationship with root and rhizosphere communities

The various sunflower strains harbored different seed-associated fungal communities (Fig. 4). On average, 7% of the fungal phylotypes in a given root sample and 5% of the phylotypes in a given rhizosphere sample were also observed in a given seed. These proportions were the same (7% and 5% for root and rhizosphere samples, respectively) whether calculated within individual strains or across all strains. There was no significant relationship between pairwise dissimilarities across seed communities and pairwise dissimilarities across root or rhizosphere communities ($P > 0.4$ in both cases).

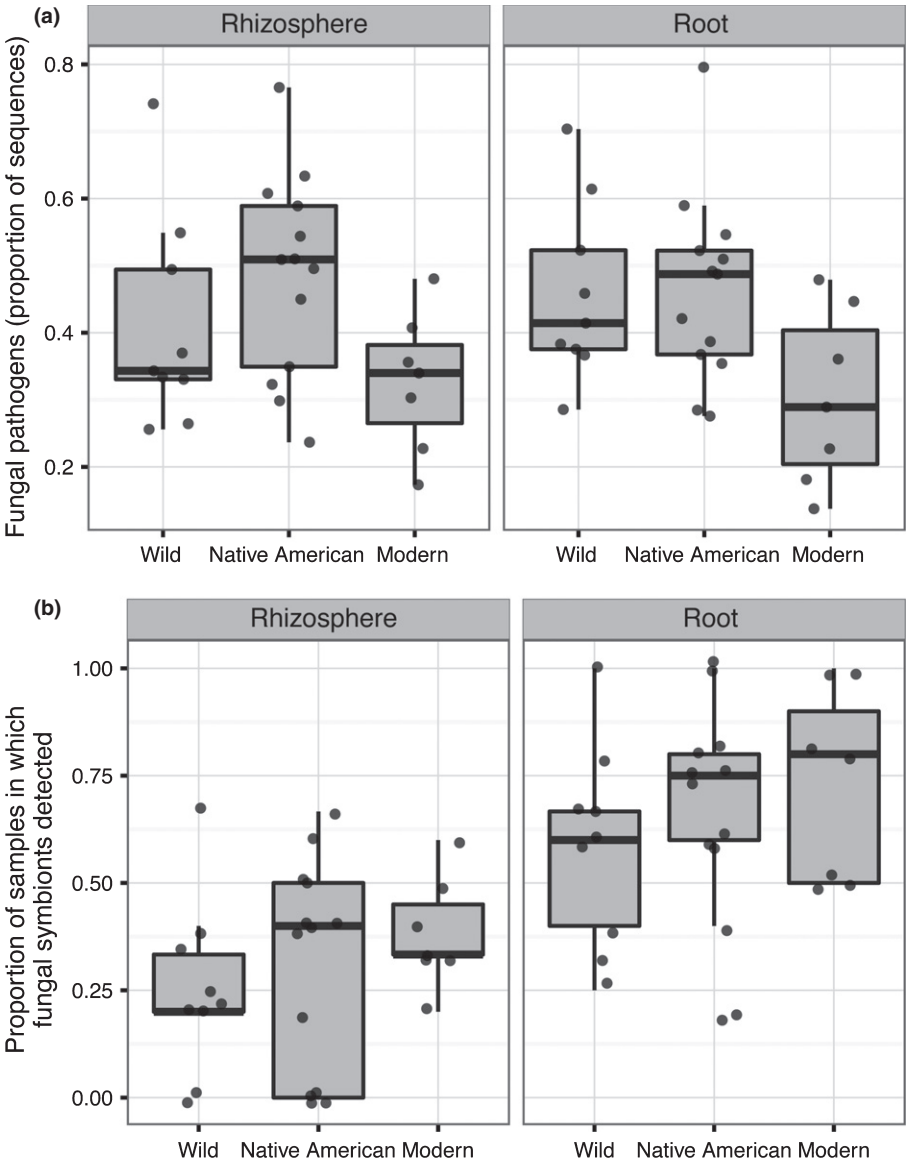

Fig. 2 The relative abundance of putative fungal pathogens (a) and the presence of putative fungal symbionts (b) in root and rhizosphere communities across wild, Native American, and modern sunflower (*Helianthus annuus*) strains. Each point represents the mean value for each strain, and individual points are presented over boxplots, where boxes and center lines represent first quartiles, medians, and third quartiles.

Most seed, root, and rhizosphere communities contained relatively few putatively symbiotic taxa, but we found that roots (63%) and rhizospheres (37%) had the largest proportion of samples that contained detectable fungal symbionts (primarily members of the phylum, *Glomeromycota*; Fig. S5) while seeds had the lowest proportion (3.3%; Fig. 5a). Although seeds rarely contained detectable amounts of known symbiotic fungi, 55% of fungal sequences from seeds were from putative pathogens. In comparison, 43% of fungal sequences from both root and rhizosphere samples were from putative pathogens, a significant difference ($P = 0.005$; Fig. 5b). There were multiple taxa identified as being potentially pathogenic, including *Alternaria* spp. and *Acremonium* spp., which had high relative abundances in seeds and were nearly absent in roots and rhizospheres (Fig. 5c). Relative abundances of putative pathogens in seeds also did not correspond to relative abundances in roots ($P = 0.3$), and they had a weak inverse relationship with relative abundances in rhizospheres ($r = -0.46$, $P = 0.02$).

Discussion

The root and rhizosphere bacterial and fungal communities were more similar to one another than to seed communities (Fig. 1b), which suggests that many root endophytes are derived from the rhizosphere, a finding in line with results from other plant species (Rosenblueth & Martínez-Romero, 2006; Bulgarelli *et al.*, 2012; Bai *et al.*, 2015). Likewise, our results corroborate previous work showing that taxa found in rhizospheres (Philippot *et al.*, 2013) tend to be distinct from those found in seeds (Truyens *et al.*, 2015). As the different plant compartments clearly have distinct bacterial and fungal communities, we investigated the factors influencing community assembly separately for each of these habitats.

One of the central goals of this study was to assess whether root and rhizosphere microbial communities differ in consistent ways across closely related strains of *H. annuus*. While previous work has shown that different plant hosts can harbor distinct

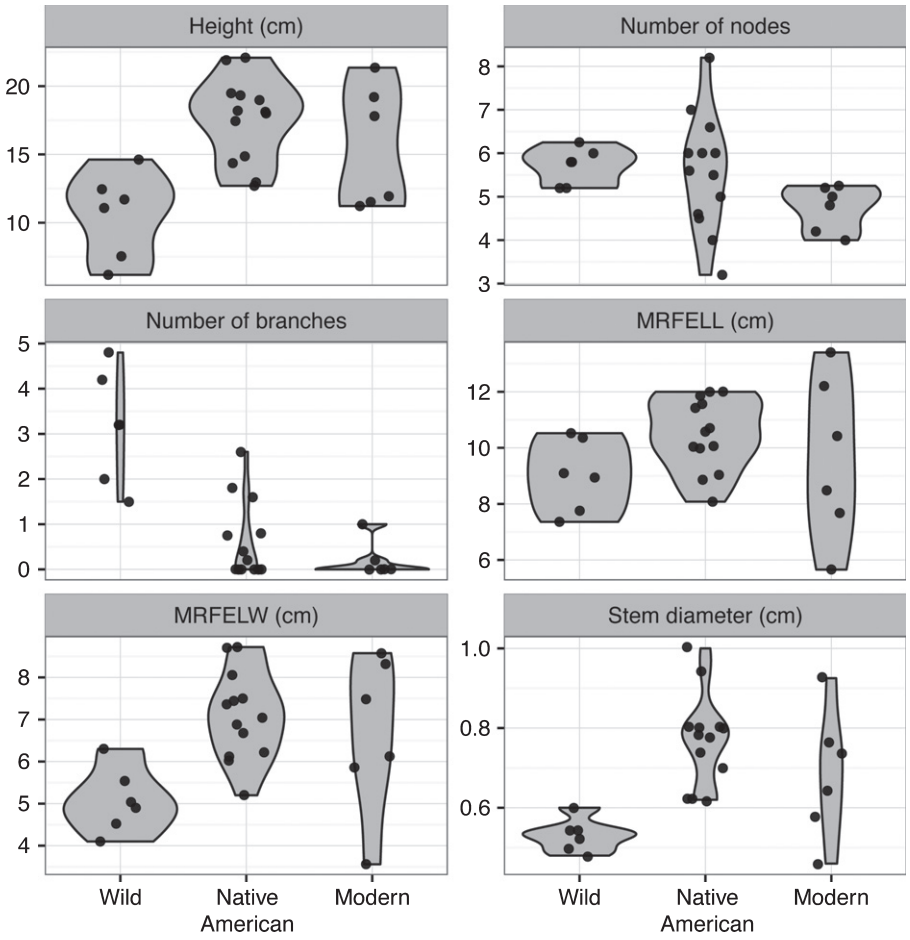

Fig. 3 Violin plots showing differences in phenotypes across wild, Native American, and modern sunflower (*Helianthus annuus*) strains at the time of sample collection. Each overlying point represents the mean value for sunflower strain. MRFELL, most recent fully expanded leaf length; MRFELW, most recent fully expanded leaf width.

rhizosphere and root bacterial communities (Wieland *et al.*, 2001; Ofek *et al.*, 2014), the magnitude of genotype effects on bacterial communities within individual host plant species is typically quite small (Inceoglu *et al.*, 2010; Weinert *et al.*, 2011; Lundberg *et al.*, 2012; Peiffer *et al.*, 2013; Marques *et al.*, 2014; Schlaeppi *et al.*, 2014; Wagner *et al.*, 2016). Fewer studies have investigated the effect of host genotype on overall fungal communities in rhizosphere or on root endophyte microbial communities (Bacilio-Jiménez *et al.*, 2003). Our observation that bacterial communities were not structured by sunflower genotype does not necessarily conflict with previous studies that have shown subtle genotype effects on bacterial communities in other plant species, as it is possible that below-ground bacterial communities respond more strongly to differences in certain host traits (e.g., starch content; Marques *et al.*, 2014) that may not differ across the sunflower strains examined here. It is noteworthy that we found a significant relationship between sunflower strain identity and fungal community composition as this suggests that fungal taxa are more sensitive to host traits and are more likely to exhibit strain specificity than bacteria (Cassman *et al.*, 2016). At a more basic level, the significant association between host genotype and fungal community suggests an important effect of host genotype on the rhizosphere community.

Although there was generally a weak effect of sunflower strain identity on the microbial communities found in roots and rhizospheres, we investigated whether there were broad effects of domestication on these communities across all strains. Our results indicated that the degree of domestication has little effect on overall bacterial communities in the rhizosphere and root, but there are potentially important effects of domestication on fungal communities. Domesticated crops probably interact with rhizosphere microbial communities in different ways from those of their wild counterparts (Wissuwa *et al.*, 2009; Pérez-Jaramillo *et al.*, 2015). The fact that the sunflower rhizosphere fungal community composition was related to the degree of domestication could be driven by indirect effects of domestication on soil characteristics moderated by differences in root traits. Domesticated strains have been bred to grow more quickly and develop at different rates, and they probably exude different quantities and types of organic compounds in their roots, which could have important effects on below-ground microbial communities (Haichar *et al.*, 2008; Pérez-Jaramillo *et al.*, 2015). Differences in organic compound production across degrees of domestication may be a result of known tradeoffs between growth rates and defense against biotic and abiotic stressors (Mayrose *et al.*, 2011). Some of these interactions are mediated by important secondary metabolites or defense compounds such as sesquiterpene lactones (Dempewolf *et al.*, 2008; Prasifka *et al.*, 2015), which could stimulate or hinder growth of different fungal taxa. Additionally, the conditions under which crops have been selectively bred may have contributed to the proliferation and demise of particular crop-associated microbial taxa (Wissuwa *et al.*, 2009). Few other

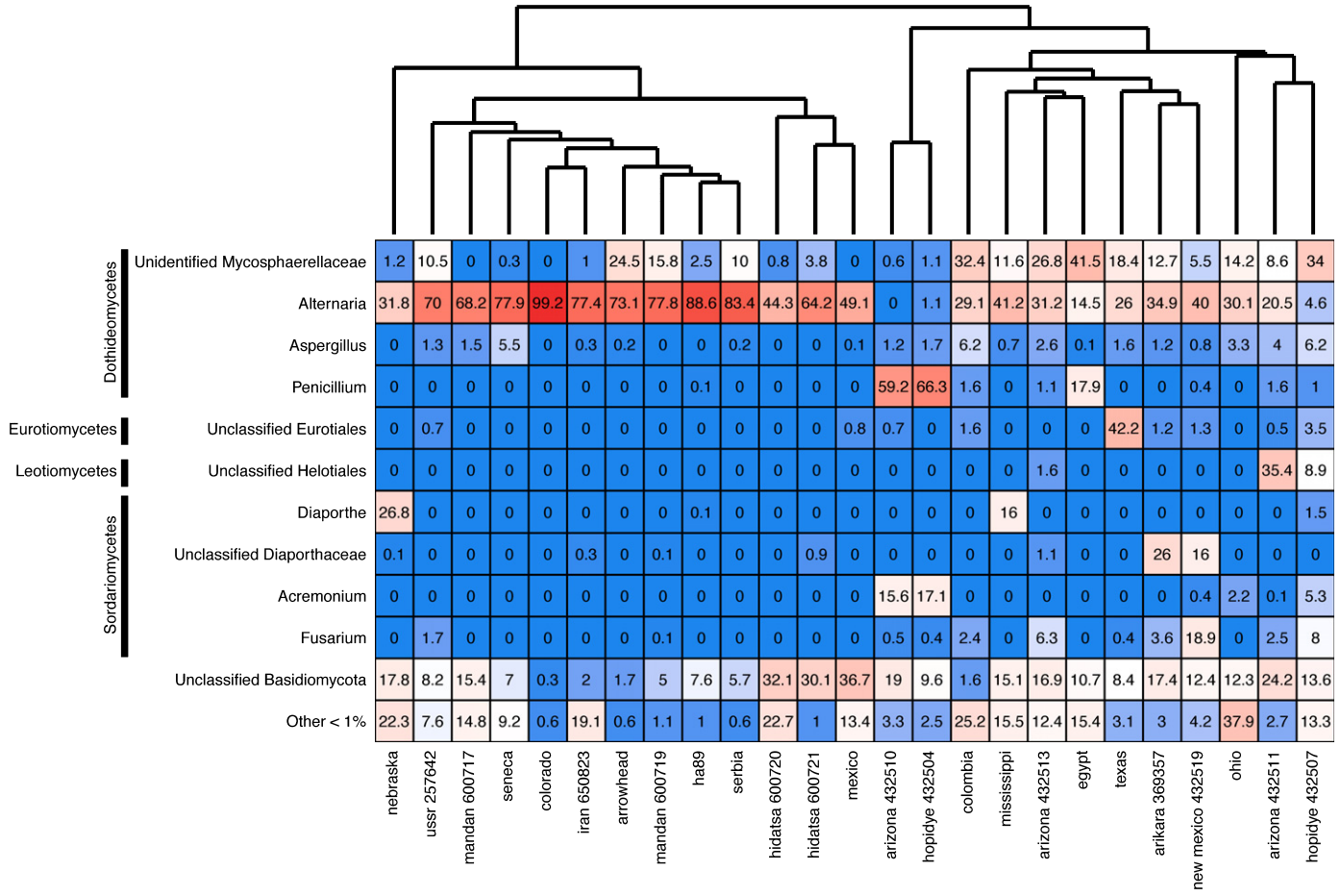

Fig. 4 Composition of fungal communities in seeds across sunflower (*Helianthus annuus*) strains. The heat map represents the relative abundance of genera ≥ 1%. Values represent mean relative abundances within individual strains (%), and colors indicate lower relative abundances (blue) and higher relative abundances (red). The cluster diagram shows clustering of sunflower strains by fungal community composition based on Bray–Curtis dissimilarities of square-root-transformed phylotypes relative abundances. See Supporting Information Table S1 for details on all the sunflower strains shown in this plot.

studies have directly assessed effects of domestication on microbial communities, but there is some evidence that bacterial communities from the roots and rhizosphere of landrace wheat cultivars are more diverse than their modern counterparts (Germida & Siciliano, 2001). Likewise, Szoboszlay *et al.* (2015) demonstrated that rhizosphere microbial community structure varies between a domesticated maize cultivar and more ancestral relatives. More generally, our results demonstrate that the effects of domestication on plant–fungal associations are in line with the numerous described effects of domestication on plant interactions with insects (Chen & Welter, 2005; Chen *et al.*, 2015).

It has been hypothesized that the domestication of crops might affect the prevalence of pathogens and symbionts naturally occurring with those crops (Pérez-Jaramillo *et al.*, 2015). Our results suggest that domestication of sunflowers actually decreased the prevalence of pathogens associated with the plants and might have even increased the prevalence of symbionts, but these patterns need to be investigated further as it is difficult to infer whether fungi (aside from *Glomeromycota*) are symbiotic or pathogenic from taxonomy alone (Nguyen *et al.*, 2015).

As expected, domesticated strains had phenotypic differences compared with more ancestral relatives (Fig. 3; Purugganan & Fuller, 2009), but these differences were largely unrelated to differences in root and rhizosphere bacterial and fungal communities. The one exception was that faster growing plants tended to have lower bacterial and fungal diversity, suggesting that plant growth rate can directly or indirectly control root and rhizosphere microbial diversity or that diverse microbial associations hinder plant growth. It is possible that slower and faster growing plants alter soil conditions in particular ways that promote more or less microbial diversity. For instance, faster-growing plants could excrete compounds into the rhizosphere that promote certain taxa and thus lower diversity (Grayston *et al.*, 1998; Oger *et al.*, 2004; Haichar *et al.*, 2008; Shi *et al.*, 2011). Variation in community composition across the root and rhizosphere samples was unrelated to the measured phenotypes, and thus the observed patterns were probably driven by other unmeasured traits that may have varied across the sunflower strains (e.g., root exudates, root physiology, or nutrient concentrations). We only measured above-ground plant traits in this study, and it is possible that microbial community composition is more strongly related to unmeasured below-ground plant traits (e.g. root exudate production). Additionally, it is possible that relationships between microbial community composition and plant traits were

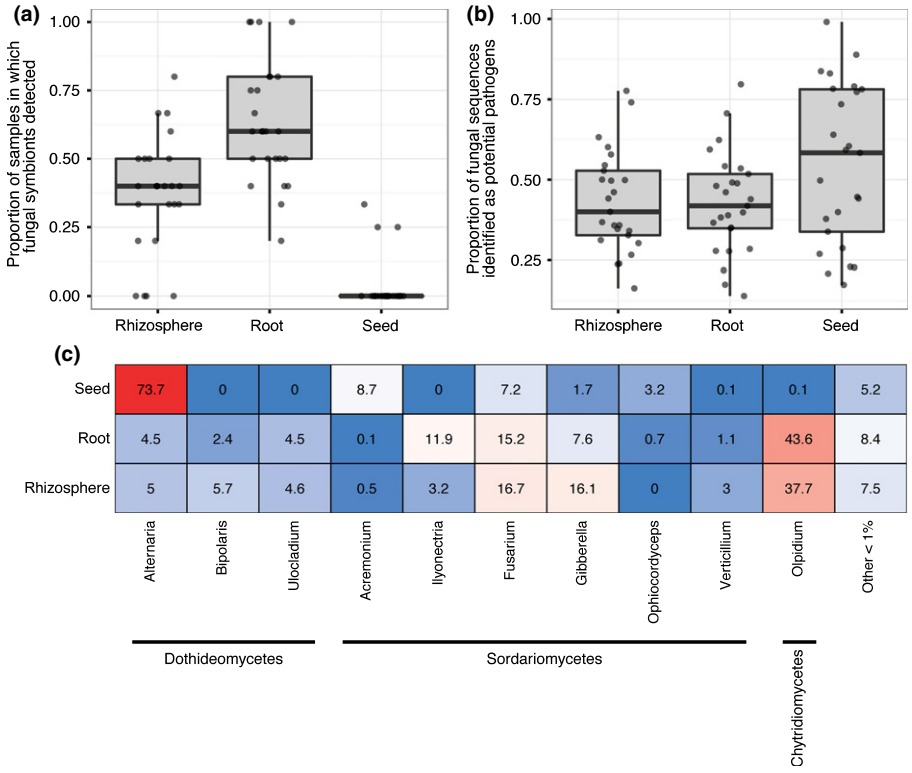

Fig. 5 The presence of putative fungal symbionts (a) and mean relative abundance of putative fungal pathogens (b) across rhizosphere, root, and seed samples. Each point represents the value for each sunflower (*Helianthus annuus*) strain, and individual points are presented over boxplots, where boxes and center lines represent first quartiles, medians, and third quartiles. (c) Genus-level differences in putative pathogen fungal community composition across rhizosphere, root, and seed samples are shown as a heat map. Values represent mean relative abundances within individual strains (%), and colors indicate lower relative abundances (blue) and higher relative abundances (red).

obscured by variation in soil properties across the field. Future glasshouse experiments could help to elucidate subtler relationships by more carefully controlling variation in soil and environmental factors.

We next sought to determine if seed microbial communities were important in structuring the microbial communities found in the roots and in the rhizosphere of the adult plants (i.e. if there was evidence for vertical transmission via seeds). We did observe strong differences in seed fungal community composition across sunflower strains, which could have been responsible for the observed differences in adult plant fungal communities. Our results are consistent with previous work showing differences in microbial communities across seeds from different plants (Barret *et al.*, 2015; Truyens *et al.*, 2015). However, contrary to expectations, the seed communities were not very similar to root and rhizosphere communities, and the diversity in seeds was much lower than in root and rhizosphere communities (Fig. 1). Moreover, only a small proportion of the root or rhizosphere fungal phylotypes were represented in the seeds. It is possible that this small fraction was derived from the seed, but it is equally likely that those phylotypes could have come from other environmental sources, such as the surrounding soil. If those seed-associated microbes served as the inocula for root and rhizosphere communities, we would expect the proportion of phylotypes observed in adult plants that were also observed in seeds to be greater within individual sunflower strains than between strains. However, we found that the proportion was very consistent (5–7%, on average across all strains) regardless of whether it was calculated within or between strains, suggesting that root and rhizosphere fungal communities are not predictable based on differences in seed communities across sunflower strains.

We also investigated whether those sunflower strains with more distinct root and rhizosphere communities had more distinct seed communities. If seed-associated microbes served as important inocula to root and rhizosphere communities, we would expect seeds with more dissimilar communities also to have more dissimilar root and/or rhizosphere communities. Yet there was no significant relationship in either case, again highlighting that the strain-specific differences in seed-associated fungal communities were probably not responsible for the observed differences in root and rhizosphere communities across the sunflower strains.

Although we found minimal evidence that seed fungi contribute significantly to the assembly of root and rhizosphere fungal communities, certain fungal taxa might be transmitted from seeds to adult plants. For instance, there could be a selective pressure for plants to have symbiotic fungal taxa residing in their seeds (Ewald, 1987; Rudgers *et al.*, 2009). While there were relatively few putatively symbiotic taxa, probably as a result of symbiotic fungi being rare and/or not well represented in the database we used, our results suggest that symbiotic fungi are much more readily promoted in roots and rhizosphere than in seeds. In addition, our results indicate lower fungal pathogen prevalence in root and rhizosphere samples than in seeds (Fig. 5), suggesting that the sunflower seeds may in fact be more susceptible to fungal pathogens than roots or rhizosphere and that those pathogens are filtered out of roots as the plant grows (Afzal *et al.*, 2010).

Given our findings and previous work showing that soil is an important inoculum governing the assembly of the plant

microbiome (Turner *et al.*, 2013; de Souza *et al.*, 2016), microorganisms external to seeds and seedlings are likely to be the principal inocula for newly developing plants. If plant-associated microbial communities are largely assembled from external sources, it may be possible to manipulate these sources, and thus the plant microbiome. Together, these results suggest that this may be done by altering the environment or potentially by selecting for plant varieties with different effects on microbial communities (e.g. Panke-buisse *et al.*, 2014). This could provide important opportunities for agricultural improvements where specific plant-associated microbial communities can increase crop yield, improve crop characteristics, and potentially decrease the reliance on irrigation and fertilizer inputs.

Conclusions

As plants and their associated microbial communities depend on one another (Vandenkoornhuyse *et al.*, 2015), a more comprehensive understanding of these relationships is critical for efforts to manage or manipulate the plant microbiome to improve crop yields (Farrar *et al.*, 2014). Our results demonstrate that intraspecific differences in bacterial communities across sunflower strains spanning different degrees of domestication are relatively minor, but that there is probably an important effect of plant genotype on the assembly of rhizosphere fungal communities. Moreover, intrinsic differences in microbial communities across the seeds of different strains are unlikely to be important contributors to root and rhizosphere community assembly in healthy adult plants. This information helps us better understand plant–microbe relationships and could be used to improve crop yields.

Acknowledgements

We thank Xavier Rojas for assistance with laboratory work. We are grateful to Indigo Agriculture for funding this study.

Author contributions

All authors designed the research. J.W.L. and R.C.L. performed the research. J.W.L. performed the data analysis. J.W.L. and N.F. wrote the manuscript with assistance from R.C.L. and N.C.K.

References

Abarenkov K, Henrik Nilsson R, Larsson K-H, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E, Pennanen T *et al.* 2010. The UNITE database for molecular identification of fungi – recent updates and future perspectives. *New Phytologist* **186**: 281–285.

Afzal R, Mughal SM, Munir M, Sultana K, Qureshi R, Arshad M, Laghari MK. 2010. Mycoflora associated with seeds of different sunflower cultivars and its management. *Pakistan Journal of Botany* **42**: 435–445.

An GH, Kobayashi S, Enoki H, Sonobe K, Muraki M, Karasawa T, Ezawa T. 2010. How does arbuscular mycorrhizal colonization vary with host plant genotype? An example based on maize (*Zea mays*) germplasms. *Plant and Soil* **327**: 441–453.

Bacilio-Jiménez M, Aguilar-Flores S, Ventura-Zapata E, Eduardo P-C, Bouquelet S, Zenteno E. 2003. Chemical characterization of root exudates from rice (*Oryza sativa*) and their effects on the chematactic response of endophytic bacteria. *Plant and Soil* **249**: 271–277.

Bai Y, Müller DB, Srinivas G, Garrido-Oter R, Potthoff E, Rott M, Dombrowski N, Münch PC, Spaepen S, Remus-Emsermann M *et al.* 2015. Functional overlap of the Arabidopsis leaf and root microbiota. *Nature* **528**: 364–369.

Barret M, Briand M, Bonneau S, Préveaux A, Valière S, Bouchez O, Hunault G, Simoneau P, Jacques M-A. 2015. Emergence shapes the structure of the seed microbiota. *Applied and Environmental Microbiology* **81**: 1257–1266.

Bender SF, Wagg C, van der Heijden MGA. 2016. An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. *Trends in Ecology and Evolution* **31**: 440–452.

Berendsen RL, Pieterse CMJ, Bakker PAHM. 2012. The rhizosphere microbiome and plant health. *Trends in Plant Science* **17**: 478–486.

Berg G. 2009. Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Applied Microbiology and Biotechnology* **84**: 11–18.

Blackman BK, Scascitelli M, Kane NC, Luton HH, Rasmussen DA, Bye RA, Lentz DL, Rieseberg LH. 2011. Sunflower domestication alleles support single domestication center in eastern North America. *Proceedings of the National Academy of Sciences, USA* **108**: 14360–14365.

Bouffaud ML, Kyselková M, Gouesnard B, Grundmann G, Muller D, Moënne-Loccoz Y. 2012. Is diversification history of maize influencing selection of soil bacteria by roots? *Molecular Ecology* **21**: 195–206.

Bouffaud ML, Poirier MA, Muller D, Moënne-Loccoz Y. 2014. Root microbiome relates to plant host evolution in maize and other Poaceae. *Environmental Microbiology* **16**: 2804–2814.

Bulgarelli D, Garrido-Oter R, Münch PC, Weiman A, Dröge J, Pan Y, McHardy AC, Schulze-Lefert P. 2015. Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell Host & Microbe* **17**: 1–12.

Bulgarelli D, Rott M, Schlaeppi K, Ver Loren van Themaat E, Ahmadinejad N, Assenza F, Rauf P, Huettel B, Reinhardt R, Schmelzer E *et al.* 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature* **488**: 91–95.

Bulgarelli D, Schlaeppi K, Spaepen S, Ver Loren van Themaat E, Schulze-Lefert P. 2013. Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology* **64**: 807–838.

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences, USA* **108**(Suppl): 4516–4522.

Cassman NA, Leite MFA, Pan Y, de Hollander M, van Veen JA, Kuramae EE. 2016. Plant and soil fungal but not soil bacterial communities are linked in long-term fertilized grassland. *Scientific Reports* **6**: 23680.

Chaparro JM, Sheflin AM, Manter DK, Vivanco JM. 2012. Manipulating the soil microbiome to increase soil health and plant fertility. *Biology and Fertility of Soils* **48**: 489–499.

Chen YH, Gols R, Benrey B. 2015. Crop domestication and naturally selected species trophic interactions. *Annual Review of Entomology* **60**: 35–58.

Chen YH, Welter SC. 2005. Crop domestication disrupts a native tritrophic interaction associated with the sunflower, *Helianthus annuus* (Asterales: Asteraceae). *Ecological Entomology* **30**: 673–683.

Coleman-Derr D, Desgarennes D, Fonseca-Garcia C, Gross S, Clingenpeel S, Woyke T, North G, Visel A, Partida-Martinez LP, Tringe S. 2015. Plant compartment and biogeography affect microbiome composition in cultivated and native Agave species. *New Phytologist* **209**: 798–811.

Compant S, Clément C, Sessitsch A. 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry* **42**: 669–678.

Cope-Selby N, Cookson A, Squance M, Donnison I, Flavell R, Farrar K. 2016. Endophytic bacteria in *Miscanthus* seed: implications for germination, vertical inheritance of endophytes, plant evolution and breeding. *GCB Bioenergy*. doi:10.1111/gcbb.12364.

Dempewolf H, Rieseberg LH, Cronk QC. 2008. Crop domestication in the Compositae: a family-wide trait assessment. *Genetic Resources and Crop Evolution* **55**: 1141–1157.

Edgar RC. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods* **10**: 996–998.

Edwards J, Johnson C, Santos-Medellín C, Lurie E, Podishetty NK, Bhatnagar S, Eisen JA, Sundaresan V. 2015. Structure, variation, and assembly of the root-associated microbiomes of rice. *Proceedings of the National Academy of Sciences, USA* **112**: E911–E920.

Ewald PW. 1987. Transmission modes and evolution of the parasitism–mutualism continuum. *Annals of the New York Academy of Sciences* **503**: 295–306.

Farrar K, Bryant D, Cope-Selby N. 2014. Understanding and engineering beneficial plant–microbe interactions: plant growth promotion in energy crops. *Plant Biotechnology Journal* **12**: 1193–1206.

Fierer N, Leff JW, Adams BJ, Nielsen UN, Bates ST, Lauber CL, Owens S, Gilbert JA, Wall DH, Caporaso JG. 2012. Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proceedings of the National Academy of Sciences* **109**: 21390–21395.

García-Palacios P, Milla R, Delgado-Baquerizo M, Martín-Robles N, Álvaro-Sánchez M, Wall DH. 2013. Side-effects of plant domestication: ecosystem impacts of changes in litter quality. *New Phytologist* **198**: 504–513.

Germida JJ, Siciliano SD. 2001. Taxonomic diversity of bacteria associated with the roots of modern, recent and ancient wheat cultivars. *Biology and Fertility of Soils* **33**: 410–415.

Grayston SJ, Wang S, Campbell CD, Edwards AC. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biology and Biochemistry* **30**: 369–378.

Haichar FEZ, Marol C, Berge O, Rangel-Castro JI, Prosser JI, Balesdent J, Heulin T, Achouak W. 2008. Plant host habitat and root exudates shape soil bacterial community structure. *ISME Journal* **2**: 1221–1230.

Hardoim PR, Andreote FD, Reinhold-Hurek B, Sessitsch A, van Overbeek LS, van Elsas JD. 2011. Rice root-associated bacteria: Insights into community structures across 10 cultivars. *FEMS Microbiology Ecology* **77**: 154–164.

Hardoim PR, Hardoim CCP, van Overbeek LS, van Elsas JD. 2012. Dynamics of seed-borne rice endophytes on early plant growth stages. *PLoS One* **7**: e30438.

Harter AV, Gardner KA, Falush D, Lentz DL, Bye RA, Rieseberg LH. 2004. Origin of extant domesticated sunflowers in eastern North America. *Nature* **430**: 201–205.

Inceoglu Ö, Salles JF, Van Overbeek L, Van Elsas JD. 2010. Effects of plant genotype and growth stage on the betaproteobacterial communities associated with different potato cultivars in two fields. *Applied and Environmental Microbiology* **76**: 3675–3684.

Johnston-Monje D, Raizada MN. 2011. Conservation and diversity of seed associated endophytes in *Zea* across boundaries of evolution, ethnography and ecology. *PLoS One* **6**: e20396.

Kaga H, Mano H, Tanaka F, Watanabe A, Kaneko S, Morisaki H. 2009. Rice seeds as sources of endophytic bacteria. *Microbes and Environments* **24**: 154–162.

Kane NC, Burke JM, Marek L, Seiler G, Vear F, Baute G, Knapp SJ, Vincourt P, Rieseberg LH. 2013. Sunflower genetic, genomic and ecological resources. *Molecular Ecology Resources* **13**: 10–20.

Lareen A, Burton F, Schäfer P. 2016. Plant root-microbe communication in shaping root microbiomes. *Plant Molecular Biology* **90**: 575–587.

Leff JW. 2016. *mctoolsr: microbial community data analysis tools. R package version 0.1.0.12.* [WWW document] URL https://github.com/leffj/mctoolsr [accessed June 2016].

Lehmann A, Barto EK, Powell JR, Rillig MC. 2012. Mycorrhizal responsiveness trends in annual crop plants and their wild relatives-a meta-analysis on studies from 1981 to 2010. *Plant and Soil* **355**: 231–250.

Leiser WL, Olatoye MO, Rattunde HFW, Neumann G, Weltzien E, Haussmann BIG. 2016. No need to breed for enhanced colonization by arbuscular mycorrhizal fungi to improve low-P adaptation of West African sorghums. *Plant and Soil* **401**: 51–64.

Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, Tremblay J, Engelbrektson A, Kunin V, Del Rio TG *et al.* 2012. Defining the core *Arabidopsis thaliana* root microbiome. *Nature* **488**: 86–90.

Lundberg DS, Yourstone S, Mieczkowski P, Jones CD, Dangl JL. 2013. Practical innovations for high-throughput amplicon sequencing. *Nature Methods* **10**: 999–1002.

Marques JM, da Silva TF, Vollu RE, Blank AF, Ding GC, Seldin L, Smalla K. 2014. Plant age and genotype affect the bacterial community composition in the tuber rhizosphere of field-grown sweet potato plants. *FEMS Microbiology Ecology* **88**: 424–435.

Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet Journal* **17**: 10.

Mayrose M, Kane NC, Mayrose I, Dlugosch KM, Rieseberg LH. 2011. Increased growth in sunflower correlates with reduced defences and altered gene expression in response to biotic and abiotic stress. *Molecular Ecology* **20**: 4683–4694.

McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, Andersen GL, Knight R, Hugenholtz P. 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME Journal* **6**: 610–618.

McGuire KL, Payne SG, Palmer MI, Gillikin CM, Keefe D, Kim SJ, Gedallovich SM, Discenza J, Rangamannar R, Koshner JA *et al.* 2013. Digging the New York City Skyline: soil fungal communities in green roofs and city parks. *PLoS One* **8**: e58020.

Mei C, Flinn BS. 2010. The use of beneficial microbial endophytes for plant biomass and stress tolerance improvement. *Recent Patents on Biotechnology* **4**: 81–95.

Milla R, Osborne CP, Turcotte MM, Violle C. 2015. Plant domestication through an ecological lens. *Trends in Ecology and Evolution* **30**: 463–469.

Nelson EB. 2004. Microbial dynamics and interactions in the spermosphere. *Annual Review of Phytopathology* **42**: 271–309.

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG. 2015. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology* **20**: 241–248.

Nocker A, Sossa-Fernandez P, Burr MD, Camper AK. 2007. Use of propidium monoazide for live/dead distinction in microbial ecology. *Applied and Environmental Microbiology* **73**: 5111–5117.

Ofek M, Voronov-Goldman M, Hadar Y, Minz D. 2014. Host signature effect on plant root-associated microbiomes revealed through analyses of resident vs. active communities. *Environmental Microbiology* **16**: 2157–2167.

Oger PM, Mansouri H, Nesme X, Dessaux Y. 2004. Engineering root exudation of *Lotus* toward the production of two novel carbon compounds leads to the selection of distinct microbial populations in the rhizosphere. *Microbial Ecology* **47**: 96–103.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P *et al.* 2016. *vegan: community ecology package. R package version 2.4-1.* [WWW document] URL https://CRAN.R-project.org/package=vegan [accessed June 2016].

Panke-buisse K, Poole AC, Goodrich JK, Ley RE, Kao-kniffin J. 2014. Selection on soil microbiomes reveals reproducible impacts on plant function. *ISME Journal* **9**: 980–989.

Peiffer JA, Spor A, Koren O, Jin Z, Tringe SG, Dangl JL, Buckler ES, Ley RE. 2013. Diversity and heritability of the maize rhizosphere microbiome under field conditions. *Proceedings of the National Academy of Sciences, USA* **110**: 6548–6553.

Pérez-Jaramillo JE, Mendes R, Raaijmakers JM. 2015. Impact of plant domestication on rhizosphere microbiome assembly and functions. *Plant Molecular Biology* **90**: 635–644.

Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH. 2013. Going back to the roots: the microbial ecology of the rhizosphere. *Nature Reviews Microbiology* **11**: 789–799.

Pieterse CMJ, de Jonge R, Berendsen RL. 2016. The soil-borne supremacy. *Trends in Plant Science* **21**: 171–173.

Pitzschke A. 2016. Developmental peculiarities and seed-borne endophytes in Quinoa: omnipresent, robust bacilli contribute to plant fitness. *Frontiers in Microbiology* **7**: 1–15.

Prasifka JR, Spring O, Conrad J, Cook LW, Palmquist DE, Foley ME. 2015. Sesquiterpene lactone composition of wild and cultivated sunflowers and biological activity against an insect pest. *Journal of Agricultural and Food Chemistry* **63**: 4042–4049.

Prober SM, Leff JW, Bates ST, Borer ET, Firn J, Harpole WS, Lind EM, Seabloom EW, Adler PB, Bakker JD *et al.* 2015. Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. *Ecology Letters* **18**: 85–95.

Purugganan MD, Fuller DQ. 2009. The nature of selection during plant domestication. *Nature* **457**: 843–848.

R Core Team. 2015. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

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

Rosenblueth M, Martínez-Romero E. 2006. Bacterial endophytes and their interactions with hosts. *Molecular Plant–Microbe Interactions* **19**: 827–837.

Rudgers JA, Afkhami ME, Rúa MA, Davitt AJ, Hammer S, Huguet VM. 2009. A fungus among us: broad patterns of endophyte distribution in the grasses. *Ecology* **90**: 1531–1539.

Sawers RJH, Gutjahr C, Paszkowski U. 2008. Cereal mycorrhiza: an ancient symbiosis in modern agriculture. *Trends in Plant Science* **13**: 93–97.

Schlaeppi K, Dombrowski N, Oter RG, Ver Loren van Themaat E, Schulze-Lefert P. 2014. Quantitative divergence of the bacterial root microbiota in *Arabidopsis thaliana* relatives. *Proceedings of the National Academy of Sciences, USA* **111**: 585–592.

Schmidt JE, Bowles TM, Gaudin ACM. 2016. Using ancient traits to convert soil health into crop yield: impact of selection on maize root and rhizosphere function. *Frontiers in Plant Science* **7**: 1–11.

Shi S, Richardson AE, O'Callaghan M, Deangelis KM, Jones EE, Stewart A, Firestone MK, Condron LM. 2011. Effects of selected root exudate components on soil bacterial communities. *FEMS Microbiology Ecology* **77**: 600–610.

Singh LP, Gill SS, Tuteja N. 2011. Unraveling the role of fungal symbionts in plant abiotic stress tolerance. *Plant Signaling & Behavior* **6**: 175–191.

Smith BD. 2014. The domestication of *Helianthus annuus* L. (sunflower). *Vegetation History and Archaeobotany* **23**: 57–74.

Smith DP, Peay KG. 2014. Sequence depth, not PCR replication, improves ecological inference from next generation DNA sequencing. *PLoS One* **9**: e90234.

de Souza RSC, Okura VK, Armanhi JSL, Jorrín B, Lozano N, da Silva MJ, González-Guerrero M, de Araújo LM, Ferreira NV, Bagheri HC *et al.* 2016. Unlocking the bacterial and fungal communities assemblages of sugarcane microbiome. *Scientific Reports* **6**: 28774.

Szoboszlay M, Lambers J, Chappell J, Kupper JV, Moe LA, McNear DH. 2015. Comparison of root system architecture and rhizosphere microbial communities of Balsas teosinte and domesticated corn cultivars. *Soil Biology and Biochemistry* **80**: 34–44.

Truyens S, Weyens N, Cuypers A, Vangronsveld J. 2015. Bacterial seed endophytes: genera, vertical transmission and interaction with plants. *Environmental Microbiology Reports* **7**: 40–50.

Turcotte MM, Lochab AK, Turley NE, Johnson MTJ. 2015. Plant domestication slows pest evolution. *Ecology Letters* **18**: 907–915.

Turner TR, James EK, Poole PS. 2013. The plant microbiome. *Genome Biology* **14**: 209.

Turrini A, Giordani T, Avio L, Natali L, Giovannetti M, Cavallini A. 2016. Large variation in mycorrhizal colonization among wild accessions, cultivars, and inbreds of sunflower (*Helianthus annuus* L.). *Euphytica* **207**: 331–342.

Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A. 2015. The importance of the microbiome of the plant holobiont. *New Phytologist* **206**: 1196–1206.

Verbon EH, Liberman LM. 2016. Beneficial microbes affect endogenous mechanisms controlling root development. *Trends in Plant Science* **21**: 218–229.

Wagner MR, Lundberg DS, del Rio TG, Tringe SG, Dangl JL, Mitchell-Olds T. 2016. Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. *Nature Communications* **7**: 1–15.

Wang Q, Garrity GM, Tiedje JM, Cole JR. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* **73**: 5261–5267.

Weinert N, Piceno Y, Ding GC, Meincke R, Heuer H, Berg G, Schloter M, Andersen G, Smalla K. 2011. PhyloChip hybridization uncovered an enormous bacterial diversity in the rhizosphere of different potato cultivars: many common and few cultivar-dependent taxa. *FEMS Microbiology Ecology* **75**: 497–506.

White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR protocols: a guide to methods and applications*. San Diego, CA, USA: Academic Press, 315–322.

Wieland G, Neumann R, Backhaus H. 2001. Variation of microbial communities in soil, rhizosphere, and rhizoplane in response to crop species, soil type, and crop development. *Applied and Environmental Microbiolgy* **67**: 5849–5854.

Wissuwa M, Mazzola M, Picard C. 2009. Novel approaches in plant breeding for rhizosphere-related traits. *Plant and Soil* **321**: 409–430.

Xing X, Koch AM, Jones AMP, Ragone D, Murch S, Hart MM. 2012. Mutualism breakdown in breadfruit domestication. *Proceedings of the Royal Society B: Biological Sciences* **279**: 1122–1130.

Zhu YG, Smith SE, Barritt AR, Smith FA. 2001. Phosphorus (P) efficiencies and mycorrhizal responsiveness of old and modern wheat cultivars. *Plant and Soil* **237**: 249–255.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 Heat map showing bacterial family relative abundances across sample types.

Fig. S2 Heat map showing fungal family relative abundances across sample types.

Fig. S3 Cluster diagram showing differences in rhizosphere fungal community composition across sunflower (*Helianthus annuus*) strains.

Fig. S4 Heat map showing fungal genus relative abundances of rhizosphere communities across domestication levels.

Fig. S5 The composition of fungi identified as symbiotic in root and rhizosphere samples.

Table S1 The sunflower (*Helianthus annuus*) strains used in this study and characteristics (mean values) at the time of sample collection

Table S2 Spearman correlations between bacterial and fungal Shannon diversity in rhizosphere and root samples and plant characteristics measured at the time of sample collection

Table S3 Spearman correlations and Mantel tests assessing relationships between dissimilarity in bacterial and fungal community composition in rhizosphere and root samples and Euclidian distances between plant characteristics from different samples

Please note: Wiley Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.