

Local environment drives rapid shifts in composition and phylogenetic clustering of seagrass microbiomes

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Abstract

Plant microbiomes depend on environmental conditions, stochasticity, species, and genotype identity. Eelgrass (*Zostera marina*) is a unique system for plant-microbe interactions as a physiologically-challenged, marine angiosperm growing in anoxic sediment, periodic exposure to air at low tide, and gradients in water clarity and flow. We tested the influence of host origin versus environment on microbiome composition by transplanting 768 plants among four sites within Bodega Harbor, CA. Over three months following transplantation, we sampled microbial communities monthly on leaves and roots and sequenced the V4-V5 region of the 16S rRNA gene to assess community composition. The main driver of leaf and root microbiome composition was destination site; more modest effects of origin site did not last over a month. Community phylogenetic analyses suggested that environmental filtering structures these communities, but the strength and nature of this filtering varies among sites and over time. We demonstrate that local environmental differences create rapid shifts in associated microbial community composition with potential functional implications for hosts under shifting environmental conditions.

Introduction

Host-associated microbial communities are increasingly identified to have important effects on their host^{1,2}. The composition and structure of host-associated microbial communities vary by geographic region^{3,4}, host health⁵, successional stage⁶⁻⁹ and environmental conditions^{10,11}, but the extent to which the host is a cause of, or faces consequences from, this variation is often unclear¹². Making progress on this question requires an understanding of the forces structuring these communities. Such an understanding will be key toward manipulating the microbes in the interest of enhancing functionality and predicting microbiome composition and function under environmental change. This requires experimental and observational work to disentangle the roles of temporal and environmental variation in driving community structure at different scales.

Microbial communities, like many ecological communities, vary predictably with seasonal and interannual variation in environmental drivers and host characteristics^{7,13,14}. Microbial communities often shift composition as environmental conditions change, but whether these changes are due to direct environmental changes or changes in the host is often unclear. For example, coral bacterial communities resemble their new sites when hosts were transplanted among sites for 21 months¹⁵. Similarly, temporal/seasonal variation was the dominant driver of sponge microbiome composition among nearby sites, despite differences in tidal depth¹³. Some terrestrial plant-microbe common garden studies that have run for at least two years also find a dominance of local environmental over host characteristics in driving microbial assemblages¹⁶. However, the coarse temporal resolution of these studies limits the ability to assess how quickly a change in local environment changes microbiomes. Thus, the speed of microbiomes matching novel environments is often unknown, yet is critical for assessing the capacity of the microbiome to buffer the host against a changing environment.

Community composition is determined by the source pool of potential inhabitants in conjunction with numerous abiotic and biotic filters restricting certain members^{17,18}. In order to assess the various roles of different filters, community ecologists have used functional similarity to describe likely niche overlap between members of a guild: using the observed patterns in communities to infer the process of assembly¹⁹. When good functional data is not available, as is the case for many uncultured members of metagenomic sequenced communities, phylogenetic similarity can be used, with the assumption that conservation of key traits is held within clades^{20–23}. Given this assumption, if microbial communities are phylogenetically clustered (i.e., more phylogenetically similar than expected by chance), local environmental filtering may exert a dominant influence on microbiomes. Alternatively, if microbiomes are phylogenetically overdispersed (less similar than expected by chance) resource partitioning or inter-taxon facilitation (i.e., cross-feeding, environmental buffering) could be the dominant driver of assembly²⁰. Furthermore, comparing phylogenetic clustering (or overdispersion) among sites can provide insights into the contingent nature of community assembly. For example, variation among sites in clustering vs. dispersion suggest different environmental drivers or variation in strength of biotic interactions in driving assembly.

Microbial communities associated with the marine angiosperm eelgrass (*Zostera marina*) are of increasing interest for their role in host ecology and ecosystem functioning. Eelgrass is a foundation species that provides food and habitat for a diversity of animals²⁴, stabilizes sediment^{25,26}, and mediates nutrient cycling²⁷. The potential role of the microbiome in mediating these effects will clarify how to best conserve and restore the important functions of eelgrass. Both leaves and roots differ in composition from source pools in water and sediments, but roots are more differentiated from sediment than leaves are from water²⁸. Additionally, there seems to be an enhanced abundance of potentially functionally important sulfide oxidizing bacteria in communities collected from roots²⁹. Leaf microbial communities are locally and regionally variable^{28,30} and may reflect other environmental patterns and guild structures³⁰, whereas root microbiomes seem to vary less geographically²⁸. No work in eelgrass to date has tested the role of host versus environment in microbial community structure but we know that there are strong differences in host genotypic composition, genetically-based traits, and environment on spatial scales less than 1 km^{31–34} and that the plant microbiome varies on global scales²⁸.

To understand the assembly processes of seagrass microbiomes, we performed a reciprocal transplant experiment among four sites within a single 5 km² embayment in Bodega Harbor, California, USA, along a gradient from the mouth of the harbor (most oceanic) to the head (most estuarine). This gradient (see Fig. 1) consists of increasing temperature, decreasing flow, and changing sediment grain size and organic content, all of which potentially influence the root and leaf microbiomes; local adaptation of plants to these differences could also moderate microbiome assembly. We tracked changes in the bacterial assemblages associated with eelgrass roots and leaves across a time course of three months (July to September) to examine the relative importance of host factors vs environment in determining microbiome composition. We then applied community phylogenetic approaches to assess the role of environmental filtering vs. other processes in structuring bacterial assemblages.

Results

On both roots and leaves, plants strongly resembled destination and not origin site after one month

Leaf bacterial community composition based on phylogenetic isometric log-ratio transformed data from samples transplanted among our four sites quickly resembled destination site (the site to which shoots were transplanted) and not origin site (the site of collection) across our four sites (Figure 2, Table 1), suggesting that eelgrass microbiomes rapidly change as a function of local conditions. After 1 month, there was also an effect of origin site and its interaction with destination site, though these effects were weaker than the effect of destination site (Figure 2A, PERMANOVA results in Table 1). After two and three months, there was still a strong effect of destination site, but no effect of origin site or its interaction with destination site (2 months: Figure 2B, Table 1; 3 months: Figure 2C, Table 1). There was strong differentiation by destination site across all three time points (Figure 2A-C, Table 1). This suggests that destination environment, and/or plant changes that happen as a result of the destination environment, were the primary drivers of leaf microbiome and these overwhelmed any residual effect of origin site within 2 months at most. These differences were associated with differences in variance among sites within time points (betadisper, ANOVA $p_1 = 0.0016$, $p_2 = 0.0003$, $p_3 = 0.05127$): after one month, Mason's Marina (MM) had higher within-site variance than all other sites, and after two months was still greater than Doran Beach (DB) and Westside Park (WP) but not Campbell Cove (CC).

As there was a significant interaction between destination and origin sites after one month, we compared all possible combinations of origin and destination sites. Regardless of origin site, bacterial communities differed between all pairwise destination sites after one month (Supplemental Table S1 & S2 for p and R^2 values). After one month, leaves of plants transplanted to DB from MM were distinct from those transplanted from CC or DB. Leaf bacterial communities of plants transplanted to MM from CC and MM were also still distinct from each other (Supplemental Table S3 & S4 for pairwise p and R^2 values). All pairwise combinations of leaf bacterial communities after two and three months were distinct from each other by destination site only.

Alpha diversity also varied as a function of destination site, although these differences only emerged after 2 months. After one month, there were no significant differences in amplicon sequence variant (ASV) richness among destination or origin sites (negative binomial GLM, $p > 0.05$, Figure 2D). After two months, plants at CC had a higher number of ASVs than those at DB and plants at WP had a higher number of ASVs observed than MM and DB (negative binomial GLM, $p < 0.001$, all significant pairwise comparisons also $p < 0.001$; Figure 2E). Finally after three months, WP had a higher number of ASVs than DB and MM ($p_{vsDB} < 0.001$, $p_{vsMM} < 0.001$) and CC had more ASVs than DB ($p < 0.001$, Figure 2F). After two months, there was also a small effect of origin site on richness due to differences between plants from CC and MM ($p = 0.040$). Considering all time points, fewer ASVs were present on leaves when at DB as destination site and more bacterial ASVs on leaves when at WP.

Root bacterial communities followed similar patterns as leaves. After one month, destination site had the strongest effect on microbiome composition, but there was also an effect of origin site and an interaction between origin and destination site (Figure 3A, PERMANOVA statistics in Table 2), but this was absent after two months (Figure 3B, Table 2) and three months (Figure 3C, Table 2). After two and three months, microbiomes still showed differences among all destination sites (Figure 3). There was no difference in within-site variance among sites at any time point (betadisper ANOVA, $p_1 = 0.0912$, $p_2 = 0.6264$, $p_3 = 0.3975$).

We explored the interaction between destination and origin site on root microbiome after one month, by comparing microbiomes in all possible combinations of origin and destination site at this time point. Regardless of origin site, bacterial communities differed between all destination sites after one month (all pairwise results (p-values and R^2) can be found in Supplemental Tables S5 and S6). However, origin site only influenced community composition for some origins at some destinations and only after one month. On roots of plants transplanted to MM, bacterial communities varied when comparing plants from MM vs. any other site while communities on roots of other sites did not differ from each other. The same pattern occurred at WP, where all plants transplanted from WP were distinct from all other sites and all other sites could not be distinguished from each other. At CC, bacterial communities roughly formed two groups with root bacterial communities – plants from CC and DB hosted similar communities and plants from MM and WP hosted a different distinct community. At DB, we saw no effect of origin site (all pairwise results p-values and R^2 s can be found in Supplemental Tables S7 and S8). There were no differences in alpha diversity among roots by destination after one or three months (negative binomial GLM, $p_1, p_3 > 0.05$), but after two months plants planted at WP had more ASVs (Figure 3D-F; negative binomial GLM, $p_{\text{destination}} = 0.007$, $p_{\text{origin}} = 0.023$, $p_{\text{destination:origin}} = 0.447$; significant pairwise differences: $p_{\text{destination CCvsWP}} = 0.011$, $p_{\text{destination MMvsWP}}$, $p_{\text{origin MMvsWP}} = 0.023$).

Most sites show phylogenetic clustering, degree of clustering varies by destination site

Given that all sites are within a few kilometers, and the high degree of tidal exchange within Bodega Harbor, all sites are likely exposed to a very similar pool of colonizing microbes; therefore we expect that the compositional differences among sites we observed (Figure 2) are driven by local factors rather than dispersal limitation. To assess whether these differences were due to variation in the strength of environmental filtering vs. biotic interactions among sites, we compared the phylogenetic composition of microbiomes among sites using Nearest Relative Index (NRI), the average phylogenetic distance among all pairs of taxa. This measure gives a sense of whether, on average, community members are more or less related to each other than expected by chance.

When we examined leaf and root bacterial communities across sites, we found that these communities were largely phylogenetically clustered; no community showed evidence of phylogenetic overdispersion in NRI (Figure 4, Supplemental Table S9). Leaf microbiomes varied in the degree of clustering by site, with communities at the most oceanic sites being least clustered and those at sites that were warmer or with less water flow were most clustered (Figure 4A-C). After one month, leaf bacterial communities were more

clustered at DB than CC or WP; DB is the site furthest from the mouth of the harbor (Figure 4A). After two months, we observed that communities at MM were the most clustered followed by DB and WP followed by CC which was neither clustered nor overdispersed; MM is the site with the least flow and CC is the site closest to the mouth of the harbor and the coolest site (Figure 4B). There was no evidence of differences in phylogenetic clustering at any site after three months (Figure 4C). These changes in clustering across months suggests temporal variation in ecological filtering, perhaps due to low tides being less extreme and more nocturnal during September than earlier in the summer. This could have reduced environmental differences due to exposure differences among sites.

Root bacterial communities were always clustered relative to null expectations and also showed differences in degree of phylogenetic clustering among sites via NRI (Figure 4D-F) that were more consistent over time than we observed for leaves. Bacterial communities on roots at MM were less clustered than CC or DB sites after one month, after two and three months WP was more clustered than DB or MM. These differences may be associated with consistent water temperature differences across sites (DB and MM are warmer than CC and WP) or by sediment grain size (MM has a finer grain size than other sites).

Individual phylogenetic balances reveal that the clades driving differences vary among sites; more in roots than leaves

To determine which ASVs drove the differences among destination sites and identify where these differences might occur, we employed a phylogenetic balance approach to identify where in a phylogenetic tree there are differences among samples. This means we identify which nodes in a phylogenetic tree of ASV 16S sequences are more abundant on one branch of the node at some sites compared to others. Especially as clustering was a common observation, this allows us to identify the clades that varied among sites rather than just listing variable ASVs, including if differences are at a higher taxonomic level.

These balances ranged in phylogenetic position from the tips of trees containing only 2 ASVs (~0.02% of the ASVs in the pool), to near the base of the tree containing over 96% of ASVs in the case of a leaf node after both one and two months. However, the median balance across samples was relatively small containing 3-4 ASVs within a node for leaf samples (depending on time point) and 5 ASVs per node in root samples (across time points). When we examined placement of nodes that defined differential balances, the phylogenetic placement of nodes was not different from a null distribution except for root bacterial communities after two and three months where nodes that differed were less basal (closer to tips) than expected (Supplemental Table S10). Overall, we found between 5 and 35 balances that identified each site from others within a time point. See Supplemental Figures S2-S7 for specific balances that distinguished specific sites from others within a plant compartment and time point, and Supplemental Figure S1 for interpretive guidance.

As most of these nodes were at the level of differentiating among or within families (>97% did not contain a node at the level of family or above), we examined which differentiating families were in each

plant compartment. We found the families that differed in leaves were different than those that varied in roots (Fisher's Exact Test $p < 0.001$, Figure 5). The families most representative of differences in root microbiomes among sites include those likely involved in sulfate reduction (including *Desulfocapsaceae*), sulfur oxidation (*Sulfuraceae*) and nitrogen cycling (*Prolixbacteraceae*); but there were no consistent patterns of certain ASVs distinguishing certain sites across timepoints. In leaves, we did not identify families indicating processes that might differ among sites as we did in roots for sulfur and nitrogen cycling. We suggest that these families are important in root microbial communities and individual taxa may vary between environments and therefore functions – however further efforts in culturing members of these communities to allow for better differentiation among members would be required.

Transplant effect present in roots, but not leaves

Finally, we assessed the extent to which the experimental procedure: the process of uprooting, handling, transport and lab processing affected root and leaf microbiomes by comparing the bacterial communities from plants transplanted back to their origin site with undisturbed plants. This allowed us to examine the effect of transplantation as well as investigate differences among sites.

We found that leaf microbiomes were indistinguishable between transplant controls and undisturbed plants at any time point (Table 3, Figure 6A-C), and retained the among-site differences described in Figure 2. There were no differences in alpha diversity or variance among transplanted plant leaf bacterial communities and untransplanted controls at any timepoint, except after 3 months, when transplanted plants had slightly higher bacterial richness than controls (ANOVA negative binomial GLM, $p_1 = 0.942$, $p_2 = 0.942$, $p_3 = 0.036$; Supplemental Figure S8 A-C).

In contrast, we found persistent differences in composition between root bacterial communities from transplanted and control plants across all three months we sampled (PERMANOVA, Table 4, Figure 6D-F). However, there were no differences in ASV richness among control and transplanted roots (ANOVA negative binomial GLM, $p_1 = 0.071$, $p_2 = 0.572$, $p_3 = 0.984$) (Supplemental Figures S8 D-E), and variance within transplanted plants was typically the same as for the untransplanted control plants, except at three months (ANOVA, $p = 0.164$, $p_2 = 0.108$, $p_3 = 0.0009$). Despite this transplant effect, transplanted plant root microbiomes still more closely resemble control root microbiomes than leaf microbiomes (Supplemental Figure S9, PERMANOVA, $p_{\text{rootvsleaf}} = 0.001$).

Discussion

Seagrass beds within a harbor varied in their leaf and root microbiomes, and plants transplanted among these beds rapidly assumed the microbiome of the destination site, usually within a month. Root and leaf microbiomes were phylogenetically clustered within a site, but the degree of clustering varied, with sites more stressful for seagrass (warmer, lower water flow, farther from the open ocean) showing a greater degree of clustering. Our balance analysis allowed us to further identify that many of the differences in communities among sites were at shallow nodes indicating fine taxonomic scale differences in

communities. In conjunction with the shifts to match new environments and assuming that traits relevant to environmental tolerances are conserved, this suggests that microbiomes shift among functionally similar taxa that differ in environmental tolerances. Thus, we suggest that direct effects of environmental differences among sites drive these differences in microbial community assembly, though we cannot, as yet, conclusively identify the specific factors responsible. Furthermore, we highlight the differences in microbial community composition that can occur on small scales among seagrass beds within the same 5 km² embayment.

Terrestrial leaf phyllosphere microbial communities are more phylogenetically clustered on faster growing trees, potentially due to stronger ecological filters or decreased time for microbial succession to play out²³. With this in mind, our evidence of clustering in leaves is not surprising in eelgrass given the very quick turnover of leaf tissue (new leaves produced roughly every 14 days³⁵) which would reduce the amount of time that competition would have to play out on leaf surfaces before the leaf senesces. However, this indicates that there are potentially limited interactions among bacteria on the eelgrass leaf surface and suggests that the environment of the leaf surface and is critical. Recent work in terrestrial phyllospheres emphasizes establishing the patterns of assembly in phyllosphere, but provides little insight into what the dominant mechanisms are which requires more experimental work³⁶. Bacterial competition has been suggested as the major driver of community assembly given the low nutrient environments and likely competition for shared resources³⁷, though low nutrient environments often produce clustering for traits³⁸. While seagrass leaves may be more nutrient rich environments than terrestrial plants due to the abundance of epiphytic algae growing on surfaces that exude excess DOC, the rapid turnover likely reduces the influence of competitive interactions. We suggest that processes of assembly need to take into account the ephemeral nature of these environments and that leaf microbiomes likely adapt rapidly to seasonal and among site variation in environmental conditions. This also could partially explain lower levels of clustering we observed at CC – more random assembly could be driven by increased exposure time due to slower growth related to cooler temperatures at CC compared to other sites (in July and August DB and MM were more clustered than CC and WP and also both have warmer water temperatures). Unpublished growth data indicates that plants from CC grew slower than plants at DB (Kardish and Stachowicz unpublished data).

Additionally, there seem to be seasonal differences in leaf bacterial community assembly mechanisms. Our samples were taken at a standard position along the blade. Leaves grew more slowly and turning over slower in September compared to growth in July and August (Kardish and Stachowicz unpublished data), the standardized-sized leaf tissue we sampled would have been exposed for a slightly longer time and provide a more allowing more time for recruitment and biotic interactions to play out resulting in environmental filtering being less important, and biotic interactions among bacteria to becoming more important (and therefore NRI indicate more random rather than clustered assembly in September). However, this could also be driven by shallower, shorter, more nocturnal tides in September decreasing variability within environments among these intertidal beds, speeding succession in these communities. Further work would be needed to disentangle which seasonal effects are changing community assembly

mechanisms. Seasonal differences are critical to understanding differences in community assembled differences across seasons are understudied in community phylogenetics even in non-microbial systems (see ³⁹ for an example in tropical fish communities) and should be particularly prominent in systems like this where changing plant environment changes the microbiome in less than a month—a similar time scale to that of seasonal environmental change.

Studies of rhizospheric microbial communities are dominated by comparisons to bulk soil communities or across different environments^{40–42}. However, there is some evidence of predictable seasonal changes⁸, deterministic processes in root development⁴², and differences based on diversity of plant host species⁴³. We have similarly established that seagrasses host distinct communities from their surrounding sediments^{28,44}. We found different amounts of phylogenetic clustering at DB and MM than at WP or CC (the opposite pattern as seen in the first couple months in leaves where DB and MM were more clustered). Unlike in leaves, we were unsure of the duration of environmental exposure and turnover rate in roots which limits the conclusions we can draw.

Finally, we considered which phylogenetic balances drove differences in community structures in both leaves and roots. While we identified many balances that we used to identify different sites within time points, they varied by time point and site (i.e., we did not consistently identify the same balances differentiating certain sites). We found that the nodes that distinguished sites were not consistently shallow or deep within the tree (and generally were not differently placed than expected by chance). When we examined the families that distinguished leaf and root bacterial communities we found several differences, but perhaps most notably were differences within the family *Desulfocapsaceae* in roots. *Desulfocapsaceae* is a family containing known sulfate reducers associated with the top layers of marine sediments was found almost exclusively as an identifying balance in root bacterial community samples⁴⁵. This bacterial family distinguished roots at CC, MM, and DB from other sites after one month and multiple balances distinguished DB from other sites at time points two and three. This potentially indicates that sulfur cycling at DB requires different members of *Desulfocapsaceae* or a difference in tolerance to an environmental parameter (e.g., the warmer temperatures at DB). This could potentially be explored through further metagenomic work exploring different roles and abilities of members of *Desulfocapsaceae* at these different sites. Our previous work has identified many bacteria related to sulfur cycling enriched on seagrass roots, and especially noted the presence of sulfur oxidizers enhanced compared to sediments²⁸. The presence of differences among sites in which members of this family are present may indicate differences in sulfide reduction rates immediately near sites or simply community structuring of different members of the community. More direct experimental work with different members of this family would be necessary to tease apart any different functions or differences in sulfur reducing abilities, they might have to determine if these are functionally redundant or if they result in differences to functional potential as well.

While previous studies have not considered the effects of transplantation itself on changes in microbial communities, we show a significant effect of transplantation on root microbial communities highlighting

that comparing un-transplanted controls is an important consideration in future reciprocal transplant studies of microbiomes. In conjunction with the differences among destination sites and the quick acquisition of bacterial communities from a site, we concluded that these leaf bacterial communities are (1) relatively unaffected by transplantation stress and (2) likely acquire bacteria primarily from their local environment. Root communities, however, differed between control un-transplanted plants and plants transplanted back to their initial site. These differences were initially driven by more variable communities in transplanted vs control plants and higher alpha diversity in transplanted plants. However, these differences in richness and variance disappeared over time, while the effect of transplantation remained. We conclude that root surfaces also acquire bacteria from their local environment but are dramatically affected by transplantation stress in ways that do not vary by site. This could be caused by our methods of transplantation (which involved attachment to vexar to track shoots and a several day period in seawater (with roots out of sediment)) being more stressful to root bacterial communities than leaf communities resulting in selection for different microbial communities upon transplantation. Regardless, our work emphasizes the importance of measuring undisturbed controls when drawing conclusions about how microbiomes alter in new environments which has not been done in previous microbiome reciprocal transplant experiments, especially in communities that may select for unique communities. To keep these communities more intact, moving plants with their previously-associated sediments may be important to the success in transplantation efforts. However, eelgrass transplantation with or without sediment has shown similar trajectories and convergence of root and rhizosphere microbial communities within 4 weeks ⁶. As this did not include unmoved controls, further investigation would be needed to determine the overall role of transplantation stress on root microbial community structure. As transplanting individual plants is an important mechanism of eelgrass restoration^{46,47}, understanding the microbiome of transplanting plants, particularly among even similar environments is important, but is relevant to other studies examining microbial shifts under transplantation that also are involved in high levels of habitat restoration (e.g., corals).

Changes in microbiomes potentially buffer hosts against stressful environments^{48,49}, but understanding the pace of these shifts is critical for assessing whether the host can survive the period of mismatch. We found that eelgrass microbiomes rapidly shift (< 1 month) in response to novel environments, and that these microbiomes, especially in roots, are distinguishable among sites based on taxa associated with key functions of nitrogen and sulfur metabolism. This suggests the potential for the microbiome to buffer the host against a changing environment through rapid community re-assembly. Using manipulative experiments to assess microbiome functionality in field settings remains a challenging and important goal, and metagenomics studies, while powerful, can be limited in sample size due to costs. In this context, our approach of host transplantation combined with community phylogenetic and phylogenetic balance approaches has suggested some testable functional hypotheses and provides a viable approach for making progress on the functionality of microbiomes in non-model systems in field situations.

Methods

Reciprocal Transplant and Field Methods

We collected plants and reciprocally transplanted them among four seagrass beds within Bodega Harbor (California) at Campbell Cove ("CC", 38°18'36"N, 123°3'33"W), Mason's Marina ("MM", 38°20'10"N, 123°3'31"W), Westside Park ("WP", 38°19'7"N, 123°3'12"W), and Doran Beach ("DB", 38°19'21"N, 123°2'38"W). The sites represent discrete seagrass beds, but range in distance from each other from 0.9 km to 3.0 km. Plants at these sites have different phenotypic characteristics (e.g., nutrient uptake, morphological traits, growth traits, photosynthetic traits, and phenolics) when grown in common gardens³⁴ and show a small degree of genetic differentiation using microsatellite loci^{33,34}. MM is the most distinct genetically and environmentally, potentially driven by finer sediment with higher organics as well as reduced water flow. CC is closest to the harbor mouth with high flow and sandy sediment and (along with WP) has the coolest water temperature of these sites. DB is our warmest site about 2°C warmer in mean temperature than CC and WP and is also the site least impacted by human presence (all other sites are routinely visited by recreation clam fishers. In the summer of 2015, the mean temperature of these sites ranged from 15.9°C to 18.1°C (instantaneous temperature across sites temperature ranged from 12.4°C to 21.8°C by HOBO loggers) (See Supplemental Table S11 for temperature data by site).

We took collected plants to Bodega Marine lab, standardized shoot and rhizome length, and attached plants to vexar mesh blind to site of origin. Then we planted twelve vexar mesh screens with 16 plants at each of the four sites within an existing eelgrass bed with 1m in between screens (plants growing immediately below screens were removed). Two weeks before sampling for microbial samples, we marked plants to standardize the age of microbial samples.

We sampled plants destructively every month for three months during a pre-dawn low tide. When sampling, we randomly selected 3 screens at each site and measured and sampled from every (remaining, non-flowering) plant on those screens. Plants were rinsed in seawater on site before sampling to remove loose epiphytes and sediment. We collected approximately 10 roots along with a 2-3cm section of the oldest non-senescent leaf immediately distal to the marks made within the leaf sheaf two weeks prior. Because the meristem is within the sheath, this means that all leaf tissue sampled being approximately the same age and exposed outside the leaf sheath for approximately 2 weeks. This also means that the tissue sampled had not been exposed to the external environment from the origin site at the time of transplant. We immediately froze all microbe samples on dry ice and stored at -80°C. Additionally, from each plot we took parallel microbial samples from four control plants (leaves and roots) of similar size that had not been transplanted from around the perimeter of the experimental plots.

Molecular Methods

We extracted DNA with the MoBio PowerSoil DNA kit from the surface of the leaves and roots and from scaled samples of samples. To get the surface of the leaves and roots only, we vortexed each frozen sample with 500ul of MilliQ water and then added that liquid to the bead tubes and proceeded with the standard extraction protocol (full protocol available at github.com/mkardish/Transplants/Lab_Protocols). We lost DNA extractions for leaf samples

transplanted to CC from MM and WP and have therefore not been included in further analyses that follow. At the Integrated Microbiome Resource at Dalhousie University, we amplified and sequenced the V4-V5 region of the 16S rRNA gene on an Illumina MiSeq on an Illumina MiSeq to identify bacteria present with primers 515F and 926R^{50,51}.

Bioinformatic Analysis

We ran all bioinformatic and statistical analyses in R (version 3.6.1). We used a standard dada2 pipeline to error check our reads and to identify amplicon sequence variants⁵². We used only forward reads in our subsequent analyses (280 base pairs). We identified ASV taxonomy based on the SILVA database⁵³ and built a phylogeny of ASVs using alignments built with DECIPHER⁵⁴ then a tree built with FastTree2⁵⁵ then converted to ultrametric⁵⁶. We then rooted the bacterial tree with an archeal outgroup^{57,58}.

Sampling and sequencing success and results

We identified 43,118 bacterial ASVs across 681 leaf, root, sediment and water samples after quality filtering samples to 15,102,767 reads. Root samples contained between 239 and 1167 bacterial ASVs on their surface (we measured 330 root samples with read depth between 5,981 and 80,762 reads), and leaf samples between 118 and 1,010 bacterial ASVs (307 leaf samples with between 1,980 reads and 65,621 per sample).

Statistical analysis

We determined differences among groups of samples based on Euclidean distances after phylogenetic isometric log-transform implemented in the R-package “phlir” order to analyze the compositional changes in our dataset based on phylogenetic similarity⁵⁹. This analysis tests the differences in weighting of various regions of a phylogenetic tree rather than considering each ASV independently. We used PERMANOVA to determine differences among sample types (among sample types, sample timepoint, sample origin site and destination site) on ASVs present in at least 2 samples. We tested homogeneity of group dispersions with the betadisper function in ‘vegan’. To measure bacterial richness, we rarified all samples to 1980 reads, calculated the number of “Observed ASVs”, repeated this 200 times, and then used the average as our measure of bacterial richness in a sample⁶⁰. Pairwise-posthoc analyses were run to determine differences observed among groups.

We examined compositional differences among sites and treatments, by assessing phylogenetic clustering and overdispersion of groups relative to random draws from regional and local pools. We assume that many bacterial traits, especially those associated with major metabolic processes, are phylogenetically conserved, and thus conclude that phylogenetic clustering is associated with environmental filtering and dispersion with competitive forces, resource partitioning, or cross-feeding. We also examined the individual balances driving the differences in the communities we observed by identifying balances that made communities distinct from others.

We present data on the net relatedness index (NRI) of a community which is the negative of the standardized effect size of the calculated mean pairwise distance (MPD) compared to a null distribution. The NRI thus represents the average phylogenetic distance between all members of a community relative to an expected null value⁶¹. We did this through the R package MicEco which implements a parallelized version of picante^{62,63}. We used an independent swap null model in order to maintain sample richness and species occurrence frequency and compared MPD from our samples to 999 random draws. We only show data from abundance weighted models, but non-abundance weighted models show the same patterns. We tested for differences in clustering and dispersal among sites using ANOVAs or Kruskal Wallis tests when the residuals were not normal.

In addition to the clustering analysis, we also assessed which phylogenetic balances differed between groups of interest. This analysis uses multinomial logistic regression implemented in glmnet in order to identify which branches of the phylogeny differ in their representation among groups (Supplemental Figure S1)^{59,64}. To select significant balances, we repeated cross validation procedures selecting the minimum lambda 100 times for each comparison to establish balances that best represented sites. We report how many iterations we found any balance shown across these trials. Finally, we summarized these balances, examining which families differed in leaves and roots across all time points. We also used a t-test to examine whether the placement of distinguishing nodes on the phylogenetic tree were different than we would have expected due to chance alone.

Declarations

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Author Contributions

M.R.K. designed and ran experiment, performed analyses, and wrote the first draft of the manuscript. J.J.S advised on experimental design and reviewed and edited drafts extensively.

Data Availability Statement

All data and code can be found at <https://github.com/mkardish/Transplants> and sequences have been deposited under the NCBI BioProject ID PRJNA731931.

Competing interests

The authors declare no competing interests.

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Tables

Table 1: Results of PERMANOVA indicating differences among transplanted leaf microbial communities (see Figure 2).

	df	Sum Of Squares	R ²	F-Statistic	Pr(>F)
After one month					
Destination Site	3	9433.009	0.312	20.302	0.001
Origin Site	3	1050.953	0.035	2.262	0.009
Destination : Origin	7	1760.358	0.058	1.624	0.015
Residual	116	17966.060	0.595	NA	NA
Total	129	30210.379	1	NA	NA
After two months					
Destination Site	3	6100.417	0.371	14.782	0.001
Origin Site	3	511.728	0.031	1.240	0.174
Destination : Origin	9	1157.904	0.070	0.935	0.66
Residual	63	8666.808	0.527	NA	NA
Total	78	16436.857	1	NA	NA
After three months					
Destination Site	3	3759.551	0.289	6.242	0.001
Origin Site	3	652.923	0.050	1.084	0.301
Destination : Origin	8	1573.425	0.121	0.980	0.547
Residual	35	7026.486	0.540	NA	NA
Total	49	13012.385	1	NA	NA

Table 2: Results of PERMANOVA showing differences among transplanted root microbial communities (see Figure 3).

		df	Sum Of Squares	R ²	F-Statistic	Pr(>F)
After one month						
	Destination Site	3	12417.762	0.259	18.196	0.001
	Origin Site	3	1235.735	0.026	1.811	0.036
	Destination : Origin	9	3086.977	0.064	1.508	0.016
	Residual	137	31164.382	0.651	NA	NA
	Total	152	47904.856	1	NA	NA
After two months						
	Destination Site	3	11414.239	0.371	14.552	0.001
	Origin Site	3	600.808	0.020	0.766	0.704
	Destination : Origin	9	2295.097	0.075	0.975	0.49
	Residual	63	16472.277	0.535	NA	NA
	Total	78	30782.421	1	NA	NA
After three months						
	Destination Site	3	8991.433	0.360	8.760	0.001
	Origin Site	3	838.248	0.034	0.817	0.601
	Destination : Origin	8	2854.077	0.114	1.043	0.4
	Residual	36	12317.357	0.493	NA	NA
	Total	50	25001.115	1	NA	NA

Table 3: Results of PERMANOVA showing differences among based on variation in transplant status in leaves (Figure 3A-C).

		df	Sum Of Squares	R ²	F- Statistic	Pr(>F)
After one month						
	Site	3	10979.408	0.511	18.072	0.001
	Transplant Status	1	370.765	0.017	1.831	0.091
	Destination Site:Transplant Status	3	819.616	0.038	1.349	0.145
	Residual	46	9315.596	0.434	NA	NA
	Total	53	21485.385	1	NA	NA
After two months						
	Site	3	4093.618	0.349	6.359	0.001
	Transplant Status	1	282.124	0.024	1.315	0.158
	Site:Transplant Status	3	930.523	0.079	1.446	0.052
	Residual	30	6437.063	0.548	NA	NA
	Total	37	11743.328	1	NA	NA
After three months						
	Site	3	2938.888	0.279	3.559	0.001
	Transplant Status	1	413.074	0.039	1.501	0.08
	Site:Transplant Status	3	848.899	0.081	1.028	0.407
	Residual	23	6331.219	0.601	NA	NA
	Total	30	10532.080	1	NA	NA

Table 4: Results of PERMANOVA showing differences among based on variation in transplant status in roots (see Figure 6D-F).

		df	Sum Of Squares	R ²	F-Statistic	Pr(>F)
After one month						
	Site	3	7249.876	0.261	7.602	0.001
	Transplant Status	1	4336.789	0.156	13.643	0.001
	Site : Transplant Status	3	1605.829	0.058	1.684	0.056
	Residual	46	14622.473	0.526	NA	NA
	Total	53	27814.967	1	NA	NA
After two months						
	Site	3	4772.688	0.245	4.608	0.001
	Transplant Status	1	2723.638	0.140	7.888	0.001
	Site : Transplant Status	3	1596.102	0.082	1.541	0.081
	Residual	30	10358.056	0.533	NA	NA
	Total	37	19450.484	1	NA	NA
After three months						
	Site	3	3191.330	0.183	2.797	0.003
	Transplant Status	1	4062.769	0.233	10.682	0.001
	Site : Transplant Status	3	1788.470	0.103	1.567	0.08
	Residual	22	8367.592	0.481	NA	NA
	Total	29	17410.161	1	NA	NA

Figures



Figure 1

Map of reciprocal transplant sites in Bodega Harbor, Bodega Bay, CA. Campbell Cove (CC) is the site closest to the mouth of the harbor and a mean temperature of 15.8°C during our experiment. Doran Beach (DB) is the least impacted by human activities in the harbor (farther from clamming) and had a mean temperature of 15.9°C during our experiment. Mason's Marina (MM) has finer grained sediment than other sites and is a restored site with patchier seagrass growth; in other experiments its temperature profile has

been intermediate between the cooler sites and WP (HOBO logger at this site was lost). Westside Park (WP) had a mean temperature of 16.3°C during our experiment and is used for many seagrass experiments within Bodega Harbor.

Leaf bacterial communities by destination site

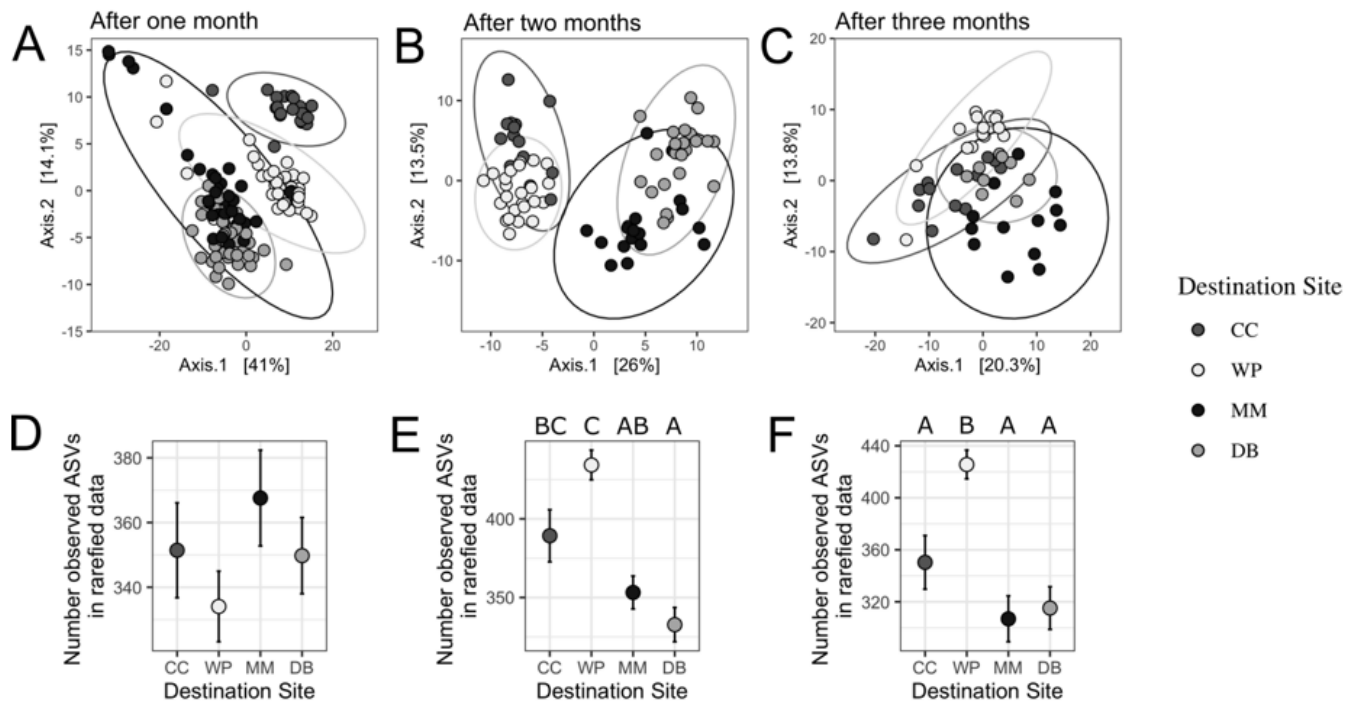


Figure 2

Differences in leaf microbial communities among destination sites. (A-C) Ordination of leaf bacterial community structure based on principal coordinate analysis of phylogenetic-isometric log-ratio transformed distances showing differences among sites by destination site (A) after one month, (B) after two months, and (C) after 3 months. All destination sites were distinct from others at all three timepoints (see Table 1). (D-F) Mean (+/- 1 SE) amplicon sequence variant (ASV) richness on leaves at each site after 1, 2, and 3 months. Different letters above data points indicate significantly different ASV richness among destination sites.

Root bacterial communities by destination site

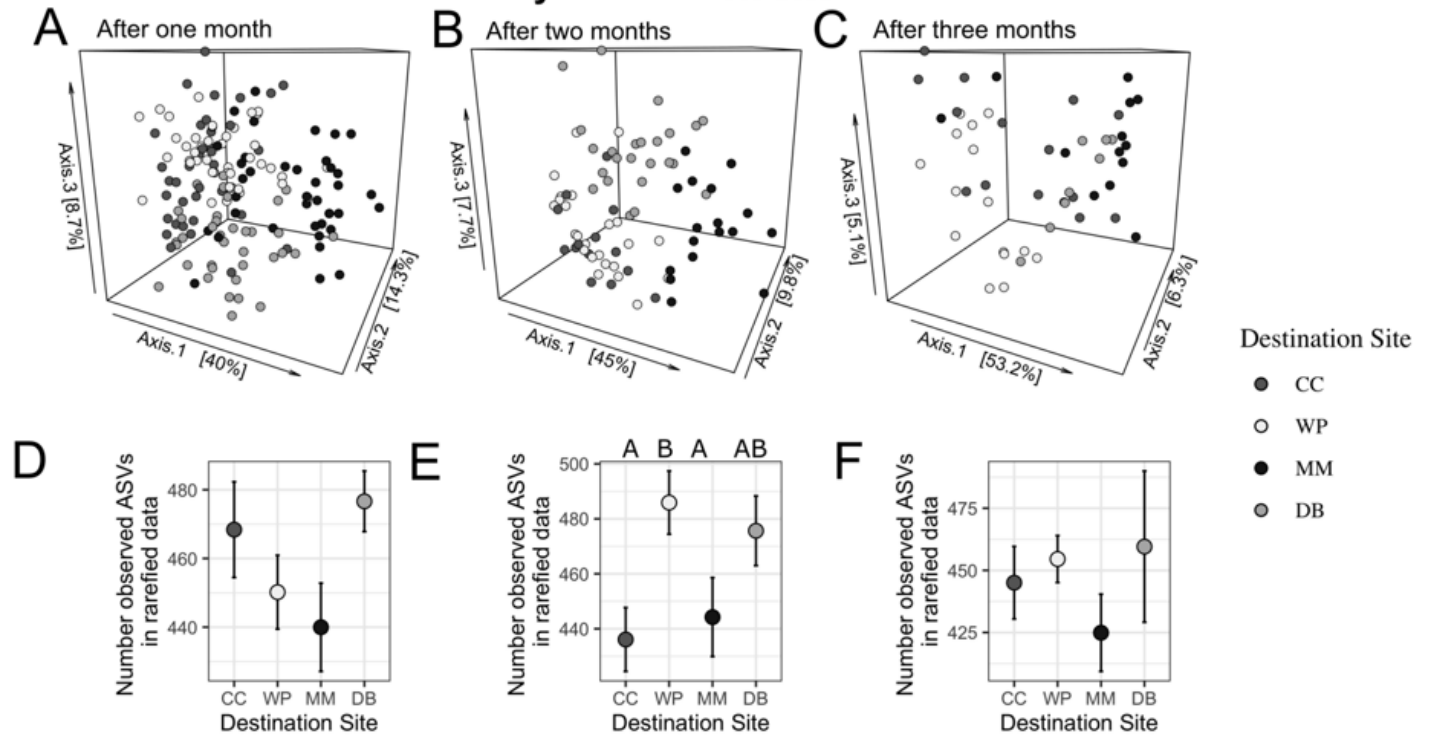
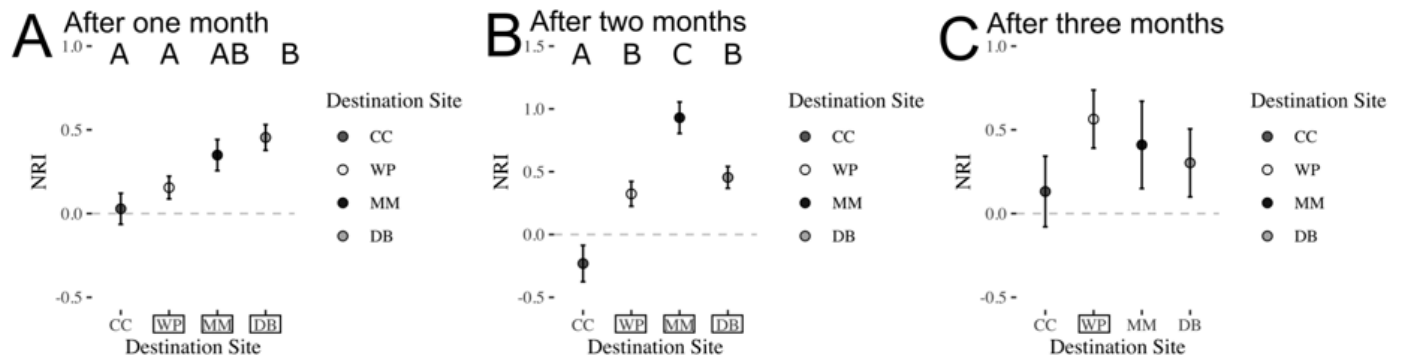


Figure 3

Differences in root microbial communities among destination sites. (A-C) 3-D ordination of root bacterial community structure based on principal coordinate analysis of phylogenetic-isometric log-ratio transformed distances. (A) shows differences among sites by destination site after one month, (B) after two months, and (C) after 3 months. All destination sites were distinct from others at all three timepoints (see Table 2). (D-F) Mean amplicon sequence variant (ASV) richness on roots at each site at 1, 2, and 3 months. Different letters above data points indicate significantly different ASV richness among destination sites.

Leaf bacterial community clustering



Root bacterial community clustering

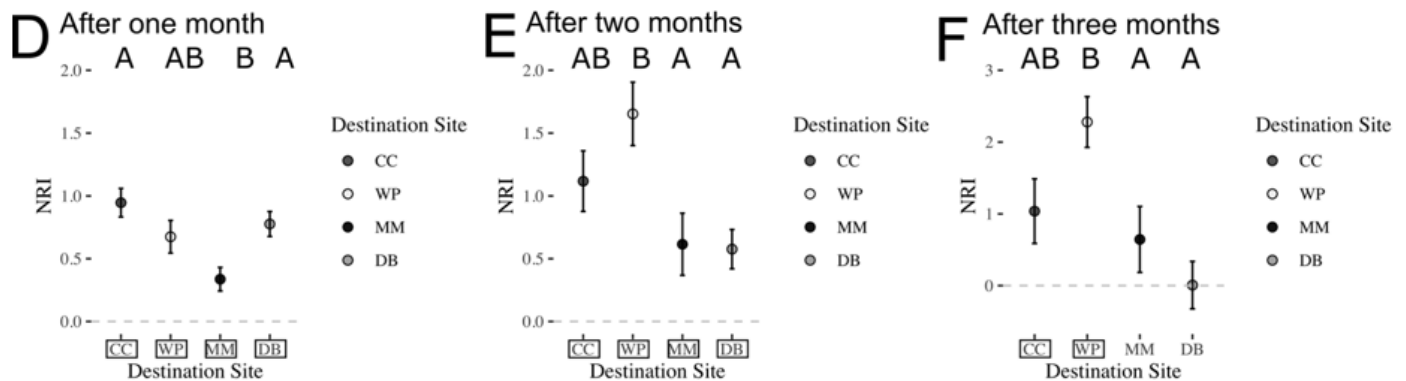


Figure 4

Leaf microbial communities were often phylogenetically clustered as measured by Net Relatedness Index (NRI) (mean $\pm$ SE) by destination site after one (A), two (B), and three (C) months. Destination sites are indicated by abbreviation and colors indicated on the figure. A positive NRI indicates that communities at a site are phylogenetically clustered, a negative NRI indicates that a community is phylogenetically overdispersed. If a community's NRI is significantly different from zero, we placed a box around the site name (see Supplemental Table S9 for more detailed statistics). Root microbial communities were consistently phylogenetically clustered as measured by Net Relatedness Index (NRI) (mean $\pm$ SE) is shown by destination site after one (D), two (E), and three (F) months.

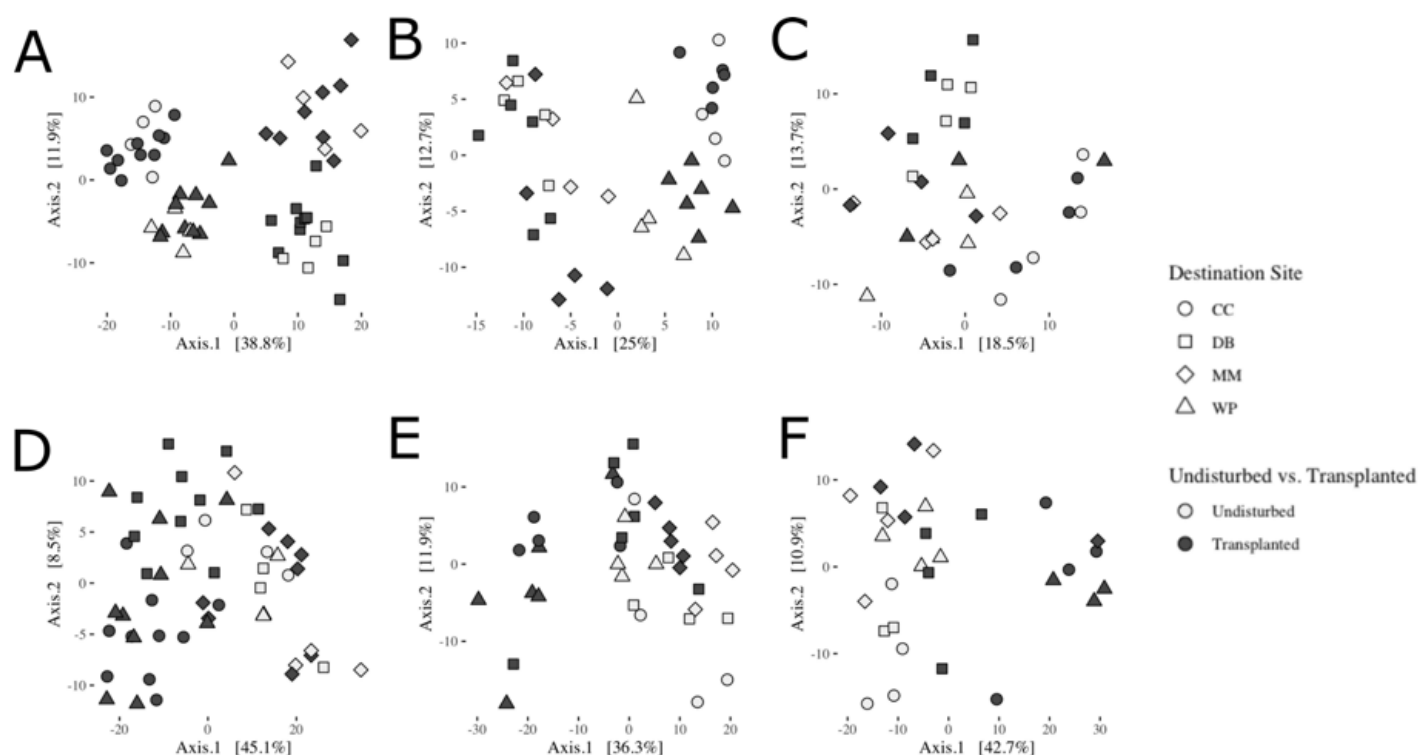


Figure 6

Differences in microbiome between home site transplants and transplant controls. Ordination of leaf bacterial community structure based on principal coordinate analysis of phylogenetic-isometric log-ratio transformed distances after (A) one month, (B) two months, and (C) three months. (D-F) Ordination of root bacterial community structure based on principal coordinate analysis of phylogenetic-isometric log-ratio transformed distances after (D) one month, (E) two months, and (F) three months. Leaf microbiomes were indistinguishable between transplant controls and undisturbed plants at any time point (A-C) and retained the among-site differences described in Figure 2. Root microbiomes were distinct between transplant controls and undisturbed plants at all time points (D-F), while both transplanted and undisturbed root microbiomes demonstrated differences among sites.

Supplementary Files

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