

# Spatial structuring of bacterial communities within individual *Ginkgo biloba* trees

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## Summary

**Plant-associated microorganisms affect the health of their hosts in diverse ways, yet the distribution of these organisms within individual plants remains poorly understood. To address this knowledge gap, we assessed the spatial variability in bacterial community diversity and composition found on and in aboveground tissues of individual *Ginkgo biloba* trees. We sampled bacterial communities from > 100 locations per tree, including leaf, branch and trunk samples and used high-throughput sequencing of the 16S rRNA gene to determine the diversity and composition of these communities. Bacterial community structure differed strongly between bark and leaf samples, with bark samples harbouring much greater bacterial diversity and a community composition distinct from leaves. Within sample types, we observed clear spatial patterns in bacterial diversity and community composition that corresponded to the samples' proximity to the exterior of the tree. The composition of the bacterial communities found on trees is highly variable, but this variability is predictable and dependent on sampling location. Moreover, this work highlights the importance of carefully considering plant spatial structure when characterizing the microbial communities associated with plants and their impacts on plant hosts.**

## Introduction

Large numbers of bacteria live inside plant tissues and on plant surfaces (Lindow and Brandl, 2003). These microbes have many potential effects on their host's growth and survival through a wide array of mechanisms (Lindow and Leveau, 2002; Gnanamanickam, 2006; Bulgarelli *et al.*, 2013). Still, we have a limited understanding of the diversity and spatial distribution of bacterial communities associated with plants, especially those taxa living in and on aboveground plant tissues. This knowledge gap persists in part because plants harbour a large diversity of bacteria that cannot be readily cultivated, and therefore, many of the bacterial taxa associated with plants have not been captured in the long history of culture-based surveys (Yang *et al.*, 2001; Yashiro *et al.*, 2011). Recent work on plant-associated bacteria has yielded a more comprehensive understanding of the diversity of bacterial communities and their spatial distributions while focusing on herbaceous model plants, such as *Arabidopsis thaliana*, using culture-independent techniques (Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012). In addition, a few studies have begun to apply similar techniques to characterize bacterial diversity associated with other plant species, including long-lived woody species (Redford *et al.*, 2010). Together, this work has highlighted that the microbial diversity associated with plants is vast and variable and that the identities and functional attributes of many of these taxa remain unknown (Chelius and Triplett, 2001; Rastogi *et al.*, 2013).

Because plants do not represent a homogeneous microbial habitat, understanding the spatial heterogeneity in microbial distributions across different anatomical tissues and morphological regions of plants is important for building a more complete picture of the bacterial communities associated with plants. For example, the biotic and abiotic characteristics of leaves differ from those of stems. Moreover, branch surfaces change over time from first-year epidermal primary tissues to phellogen-derived bark, which itself will vary significantly over the course of many years. Likewise, shade and sun leaves represent distinct microbial environments even though they exist in close proximity. Heterogeneous environmental conditions

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in tree canopies have been shown to affect the distribution of arboreal animals (Stork and Hammond, 2001; Scheffers *et al.*, 2013). Thus, the spatial distribution of arboreal microorganisms within a single canopy is also likely to covary with environmental characteristics. At the scale of an individual microbe, trees may represent heterogeneous environments where colonization, community assembly and succession patterns may shape microbial community structure.

A more detailed understanding of the spatial patterns exhibited by plant-associated bacteria will not only provide insight into the factors shaping the diversity and composition of plant-associated bacterial communities, but such studies can also yield important information on the natural history of these microorganisms, establishing a foundation for using plant-associated microbial communities as systems for testing ecological theories (Meyer and Leveau, 2012; Peñuelas and Terradas, 2014). Nutrient availability, for instance, may represent a particularly important broad-scale determinant of microbial communities across individual plants because there are ecological trade-offs between traits that contribute to an affinity for high nutrient conditions (copiotrophy) and low nutrient conditions (oligotrophy; Andrews and Harris, 2000). Similarly, other environmental conditions, including UV radiation and water availability, are likely important drivers of variation in microbial community composition both within and between plants (Lindow and Brandl, 2003; Whipps *et al.*, 2008; Vorholt, 2012). Taken together, these environmental factors should lead to predictable spatial patterns in microbial community structure within and between individual plants. Correspondingly, there is some evidence that this appears to be the case. For example, studies using culture-based techniques have linked microbial community differences in plants to microbial adaptations to specific environments (Kinkel, 1997; Andrews and Harris, 2000; Hirano and Upper, 2000). Likewise, culture-independent work has demonstrated that plant-interspecific variation is an important driver of differences in phyllosphere bacterial communities, patterns that are likely driven by a wide range of biotic and abiotic factors that differ among plant species (Baily *et al.*, 2006; Redford *et al.*, 2010; Kim *et al.*, 2012; Kembel *et al.*, 2014). One recent study found that different tree species living in close proximity harboured distinct communities, whereas different individuals of the same plant species growing thousands of kilometres apart harboured strikingly similar communities (Redford *et al.*, 2010). However, the majority of these studies have focused on bacterial community differences between plant species or between individual plants of the same species (Hunter *et al.*, 2010; Redford *et al.*, 2010; Finkel *et al.*, 2012; Rastogi *et al.*, 2013), with few studies examining the spatial variability in bacterial communities

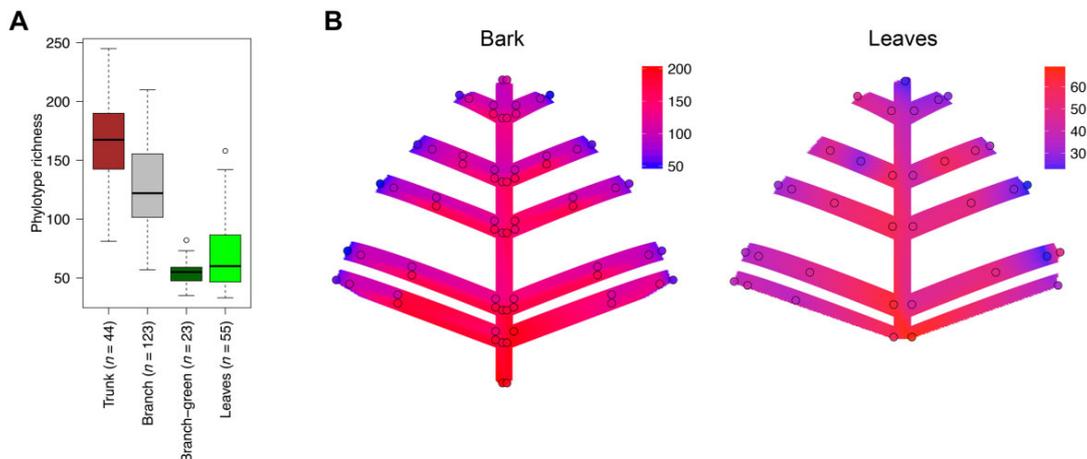
across different tissue types and locations within individual plants (Ottesen *et al.*, 2013; Lambais *et al.*, 2014). As a result, we do not know how the diversity and composition of bacterial communities vary as a function of plant anatomy, position within the architecture of the organism, and ontogeny (e.g. 1 year old bark versus 10 year old bark; young leaf surface versus older leaf surface), and whether there are predictable spatial patterns driven by heterogeneity in environmental conditions within plants. Given that plant-associated bacteria can have myriad impacts on plant health, documenting the spatial variability in these bacterial communities is critical to improving our understanding of plant–microbe interactions.

We analysed the bacterial communities found in about 100 individual aboveground locations, including trunk, branch and leaf-associated communities, on each of three *Ginkgo biloba* trees using high-throughput DNA sequencing of 16S rRNA genes. We focused on *Ginkgo biloba* because it is an exceedingly common tree in urban environments (Crane, 2013), it has a relatively simple morphology and architecture that made it feasible to design a sampling scheme that was consistent across replicate trees, and estimating the relative ages of various tree segments on *Ginkgo biloba* from its growth scars and its branching patterns is relatively straightforward. We hypothesized that bacterial community structure would exhibit predictable spatial heterogeneity across tissue types and across different locations within individual tissue types.

## Results

### *Variation in bacterial diversity within individuals*

In total, we collected 314 aboveground samples from the three trees in the Arnold Arboretum in Boston, MA, USA (42.297°N, 71.129°W; Figs S1 and S2) and assessed microbial community diversity and composition in the samples using amplicon sequencing of a region of the 16S rRNA gene (see Experimental procedures). Bacterial sequences were clustered into groups that share  $\geq 97\%$  sequence identity and are hereafter referred to as phylotypes. We observed 2345 unique bacterial phylotypes on average per tree at the rarefied sequencing depth of 575 sequences per sample. Bacterial phylotype richness was significantly different among the tissue types sampled, which included trunk bark, branch bark, new branch growth and leaves ( $P < 0.001$ ; Fig. 1A). This was also true for Shannon diversity, phylotype evenness and phylogenetic diversity, which displayed similar patterns (Fig. S3), and therefore, we use the term diversity to refer to phylotype richness hereafter. Trunk samples had the greatest diversity (156 unique phylotypes per sample on average), and new branch



**Fig. 1.** (A) Box plots showing the distribution of phylotype richness across the sampled ginkgo tissue types. Richness was estimated following rarefying to 575 sequences per sample. (B) Spatial variation of bacterial phylotype richness across the trees for bark and leaf samples. Shading is based on linear interpolation between mean sample values (represented by circles) with red indicating greater diversity and blue indicating lower diversity. Graphical representation of sample locations were adjusted to accommodate slight variations among the three replicate trees prior to computing mean richness values for a given location across the three trees. Branches on left and right extend approximately south and north respectively. Results based on samples rarefied to 575 and 200 sequences per sample for bark and leaves respectively.

growth and leaves had the lowest diversity (54 and 71 respectively), reflecting generally lower diversity on new plant tissue compared with branch and trunk tissue > 1 year old (Fig. 1A).

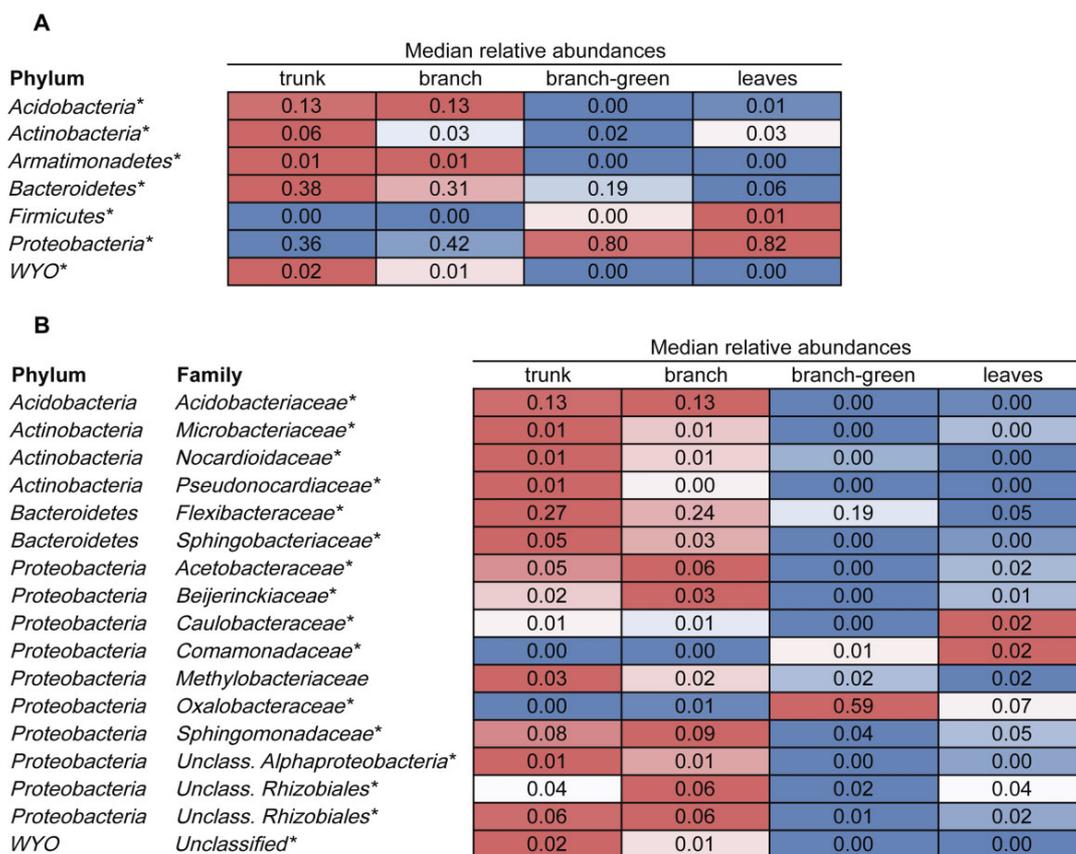
Bacterial diversity also varied spatially within tissue types. We categorized samples according to the age of the tissue they were collected from using bud scale scars resulting from annual terminal buds. Older branch and lower trunk surfaces and the undersides of branches tended to harbour more diverse bacterial communities than young branch and trunk surfaces further from the ground (Fig. 1B). Leaf-associated bacterial communities exhibited a similar pattern with leaves growing from older branch segments generally harbouring more diverse communities than leaves growing from younger branch segments (Fig. 1B).

#### Variation in bacterial community composition within individuals

Across all samples, the bacterial taxa observed were largely members of the *Acidobacteria*, *Actinobacteria*, *Bacteroidetes* and *Proteobacteria* phyla (Fig. 2A). However, bacterial community composition was significantly different across the samples taken from the different organ and tissue types ( $P = 0.001$ ; Fig. 3). Additionally, pairwise comparisons revealed that each sample type had a significantly distinct community composition from one another ( $P < 0.02$  in all cases). Differences among the three individual trees also contributed to variation among samples ( $P = 0.001$ ). However, the differ-

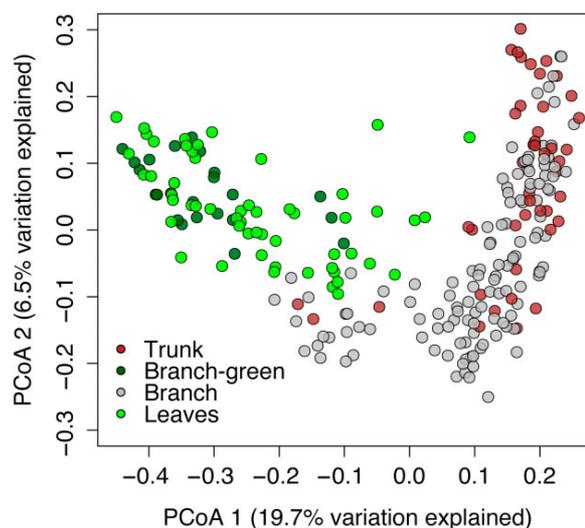
ences between the trees for a given tissue type were considerably less than the differences between tissue types (Fig. S4).

Even at a coarse level of taxonomic resolution, it is apparent that bacterial community composition varied across the different organ and tissue types (Fig. 2A). For example, *Acidobacteria* had a median relative abundance of 13% on trunk and branch samples > 1 year old but were < 1% on leaf samples and green branch samples. *Proteobacteria* exhibited the opposite pattern with a median relative abundance of 38% and 31% on trunk and branch samples, respectively, with mean relative abundances of approximately 80% on the leaf and green branch samples (Fig. 2A). Relative abundances of *Actinobacteria*, *Armatimonadetes* (formerly OP10), *Bacteroidetes*, *Firmicutes* and the WYO candidate phylum were also significantly different across the sample types (Fig. 2A). The organ and tissue types also significantly differed in their community compositions when viewed at the family level; younger plant tissues (leaves and green branches) were largely dominated by members of the *Flexibacteraceae*, *Oxalobacteraceae* and *Sphingomonadaceae*, whereas trunk and older branch communities were composed of several distinct family-level groups (Fig. 2B). The individual bacterial phylotypes with the greatest relative abundances across the sample types were identified as *Hymenobacter* sp. and one unclassified member of the *Rhizobiales* on the older branch and trunk samples, whereas an unclassified *Oxalobacteraceae* phylotype dominated on the green branches and leaves (Table S2).

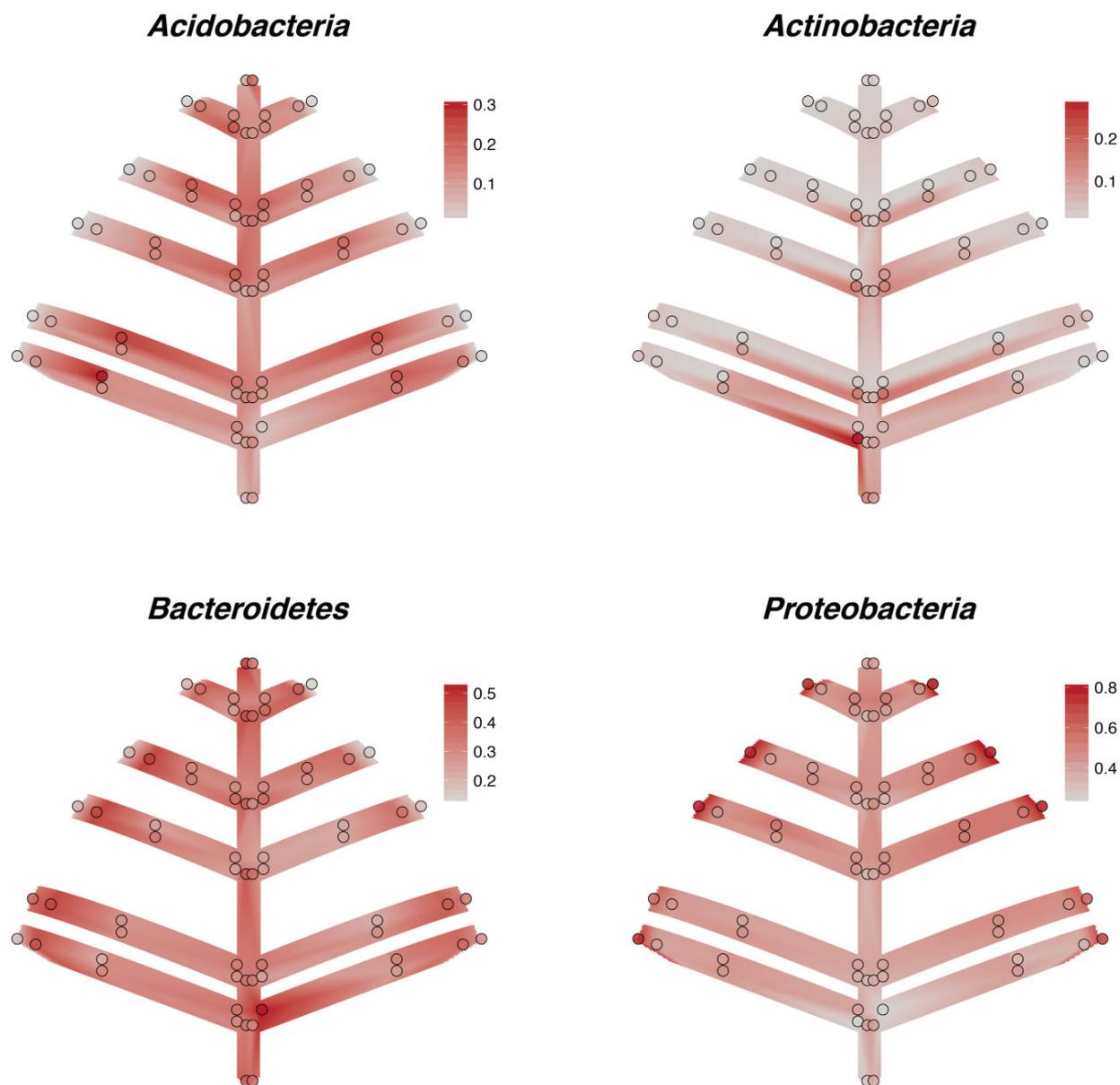


**Fig. 2.** Heat maps showing the median relative abundances of those taxa represented by  $\geq 1\%$  of the sequence reads in any sample type. Phylum level taxa (A), and family-level taxa (B) that were significantly different in relative abundances across sample types (Bonferroni-corrected  $P \leq 0.001$ ) are indicated with an asterisk. Colours are scaled to the range of values within each row.

To visualize spatial patterns in bacterial community composition for a given tissue type, we first investigated patterns within individual trees as the sampling locations differed slightly from tree to tree. These visualizations revealed that the general spatial patterns were similar across the trees (Fig. S5), a finding confirmed by the statistical analyses described above. Therefore, in order to simplify our analyses and visualizations, we averaged sample values that came from similar locations across the three individual trees that were sampled. These sampling locations included five vertical branch levels and seven vertical trunk levels that were shared across all trees. Similarly, four distances from the trunk were sampled along each branch (in most cases), which corresponded to four distinct branch ages as younger portions of the branch occur further from the tree. We found that the undersides of branches and lower parts of the trunk had bacterial communities that were relatively similar in composition and distinct from those found on the tops of branches and the upper parts of the trunk (Fig. S6). These differences were apparent when we compared the relative



**Fig. 3.** Principal coordinates analysis (PCoA) plot showing the relative similarities of samples taken from different tissue types. This ordination was created from unweighted UniFrac distances.



**Fig. 4.** Schematic of tree branch and trunk sampling sites with the shading indicating the relative abundances of the four dominant phyla on those surfaces. Branches on left and right extend approximately south and north respectively.

abundances of the four dominant phyla in these samples. *Acidobacteria* and *Proteobacteria* were relatively more abundant on the tops of branches and the upper trunk and lower in abundance on the undersides of branches and the lower trunk, with *Actinobacteria* exhibiting the opposite pattern. The relative abundance of *Bacteroidetes* was typically highest on branches of an intermediate age (Fig. 4).

Leaf-associated bacterial communities also exhibited predictable spatial structuring with leaves growing from younger branch segments harbouring communities that

were distinct from those found on older branch segments in the interior of the tree (Fig. S7). The *Acidobacteria* were relatively more abundant on the north inner leaves, with *Gammaproteobacteria* being relatively more abundant on the outer leaves (Fig. S8).

#### Discussion

The *Ginkgo* tissues harboured diverse bacterial communities containing representatives of 7 bacterial phyla and 17 families, confirming previous reports that plant leaves

can harbour a wide range of microbial taxa (Whipps *et al.*, 2008; Redford *et al.*, 2010; Vorholt, 2012). In addition, we found that this bacterial diversity was not evenly or randomly distributed across tissue and organ types (Fig. 1A). In particular, the trunk and branch bark (i.e. periderm) samples harboured more bacterial diversity than younger tissues such as leaves and new lateral stem segments, which potentially reflects broad differences between dead and living tissue. These differences in diversity were also accompanied by differences in the bacterial community composition among tissue and organ types (Figs 2 and 3).

Few studies have directly compared leaf microbial community structure with that of bark or characterized differences in bacterial community structure across various plant organs within the same species. However, one recent study showed differences in microbial communities across various organs within the tomato plant (Ottesen *et al.*, 2013), and another demonstrated differences in bacterial community structure across various grape vine tissues including bark and leaves (Martins *et al.*, 2013). Additionally, differences between rhizosphere and endosphere or root and aboveground bacterial communities have been reported (Garbeva *et al.*, 2001; Idris *et al.*, 2004; Gottel *et al.*, 2011; Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012; Bodenhausen *et al.*, 2013). Our finding that the diversity and composition of these bacterial communities found on trunk and branch surfaces were different across leaf and young branch tissues corroborates these previous findings and extends them to a tree species, supporting our hypothesis that different tree tissue and organ types support microbial communities that differ in their structure.

The leaf-associated bacterial taxa we observed were similar to those described by previous culture-based and culture-independent work in that these communities were largely comprised of members of the *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* phyla (Vorholt, 2012; Bulgarelli *et al.*, 2013; Turner *et al.*, 2013). At the family level, the common leaf-associated taxa we observed were generally similar to those found in previous studies. For example, *Sphingomonadaceae*, *Methylobacteriaceae* and *Flexibacteraceae* were relatively abundant across the leaves as has been observed previously with other plant species (Delmotte and Knief, 2009). We also observed a high relative abundance of *Oxalobacteraceae*, which were not particularly abundant in a previous survey of other tree leaf communities (Redford *et al.*, 2010) but may be more abundant on individual species of plants as they have been associated with the leaves of lettuce (Rastogi *et al.*, 2012; Leff and Fierer, 2013). The trunk and older branch surfaces had high relative abundances of taxa belonging to the phylum *Acidobacteria*, a phylum that was relatively rare on the

leaves. Since there are few cultured representatives in the *Acidobacteria* phylum, and there are few other studies using culture-independent techniques to study bark surface bacteria, it is difficult to compare this result with previous work. For example, Martins and colleagues (2013) did not observe members of this phylum on grape vine bark using a culture-based survey. *Acidobacteria* are dominant members of soil microbial communities (Lauber *et al.*, 2009), and these arboreal microbes may have arrived on the tree via dispersal from the soil beneath the tree.

Alternatively, *Acidobacteria* may be more specialized inhabitants of bark surfaces. They are thought to be largely comprised of slow-growing and oligotrophic taxa (Ward *et al.*, 2009), which could mean that they are adapted to inhabit older plant tissues such as bark rather than young tissues such as leaves or may take sufficiently long to colonize their plant substrate as to be detectable only on older surfaces. Besides the *Acidobacteria*, the trunk and branch samples contained members classified as belonging to the phylum *Armatimonadetes* and the candidate division, WYO. Representatives of *Armatimonadetes* [formerly candidate division OP10 (Tamaki *et al.*, 2011)] have been previously observed in plant-associated communities (Chelius and Triplett, 2001), but generally, members of these lineages are quite rare, and this finding suggests that tree bark can harbour many novel bacterial taxa that are far less abundant on more commonly studied plant tissues. The observed community differences between bark and leaf tissues could have been partially due to differences in the sampling methods (swabbing versus whole-leaf extractions). However, we highlight multiple reasons that it is unlikely for the sampling methods alone to account for the large differences in community composition observed here: (i) green branch and leaf tissue tended to have more similar communities (both in terms of diversity and community composition) than green branch tissue had with the other bark samples that were sampled in an identical manner; (ii) the taxa dominating the bark communities were quite rare in the leaf communities; and (iii) the quantities of tissue sampled for the DNA extractions were similar for the bark swabs and leaf samples.

In addition to differences across plant tissue types, we observed spatial structuring of bacterial diversity and community composition within tissue types. Overall, there were similar patterns with leaf and bark samples, but the patterns were stronger on bark surfaces than on leaves. Across leaf and bark samples, there was generally a greater diversity in samples from the inner, less exposed parts of the tree when compared with the more distal (outer) parts (Fig. 1B). Likewise, there were similar patterns with differences in community composition (Fig. S6). These patterns were expected given that bacterial

richness has been tied to differences in environmental conditions such as moisture (Turner *et al.*, 2013). Although we did not measure moisture availability within the trees we sampled, there are likely to be differences in humidity and exposure to precipitation between the samples taken from the central and distal portions of the tree where we saw the strongest differences in phylotype richness. Other factors such as the age of the tissue or the disturbance regime (including disturbances from UV radiation and precipitation) may have also contributed to this pattern. In addition to the observed differences in community structure between interior and exterior portions of the tree, we observed distinct differences in structure between the upper and lower sides of branches (Fig. 1B; Fig. S6). This pattern could be due to similar factors as the interior/exterior differences because the upper sides of the branches receive greater precipitation and UV radiation than the lower sides, which could lower diversity. However, the upper portions of the branches were less similar to the exterior portions of the tree with regard to their community composition (Fig. S6B), suggesting that characteristics of these tissues support bacterial taxa specific to their individual environments.

Differences in the relative abundance of *Acidobacteria* partially drove the overall differences in community composition within the bark samples. Members of this phylum tended to have greater relative abundances on interior portions of the tree and lower relative abundances on new branch growth (Fig. 4), a pattern that could be a product of the life history strategies of these taxa as discussed above, with these slower-growing taxa preferring older tissue types. In addition, *Acidobacteria* tended to be more abundant on northern interior leaves when compared with leaves found on other locations within a tree, which could suggest that greater UV radiation or lower moisture on southern leaves inhibits the growth of *Acidobacteria* taxa.

*Proteobacteria* generally displayed an opposite pattern to the *Acidobacteria* as *Proteobacteria* were relatively more abundant on the new branch growth. This pattern may be due to the tendency of many *Proteobacteria* to grow fast and proliferate in environments with fewer existing microorganisms or in environments where organic carbon resources are more available (Fierer *et al.*, 2007). The relative abundances of the other dominant phyla also showed spatial patterning, highlighting that there are often predictable patterns in the spatial distributions of a wide array of microbial taxa associated with different locations of a given tissue type. These patterns could have been driven by a number of mechanisms, which have been proposed in other studies to explain the occurrence of specific taxa in the phyllosphere: UV radiation, moisture, nutrient availability, and the amounts and types of available organic C (Andrews and Harris, 2000; Lindow and

Brandl, 2003; Vorholt, 2012) as well as other leaf characteristics or microbe–microbe interactions (Hunter *et al.*, 2010).

Along with previous work demonstrating variation in phyllosphere communities over time (Redford and Fierer, 2009), between plant species (Redford *et al.*, 2010; Kembel *et al.*, 2014) and with the geographic location of plants (Finkel *et al.*, 2012), this study demonstrates that plant-associated microbial communities also exhibit a high degree of variation within individual plants. For example, the differences we observed in the relative abundances of the dominant phyla across different locations within individual ginkgo trees were on par with the differences in phylum-level abundances observed on leaves from diverse tree species (Redford *et al.*, 2010). Our results also suggest that the distribution of these diverse bacterial taxa across individual trees is predictable, with community composition differing between plant tissues and exhibiting strong spatial patterns within individual trees that relate to their anatomical structure. Thus, just as single forehead skin sample would not be adequate to characterize the bacterial communities found on and in an individual human (Costello *et al.*, 2009), a single sample from a tree does not adequately characterize that entire tree's bacterial community. This predictable spatial variation should be considered when studying plant–microbe relationships and trying to understand the impacts of these plant-associated microbes on plant health.

## Experimental procedures

### Sample collection

Bacterial communities were sampled from three ginkgo (*Ginkgo biloba*) trees growing in a ~ 500 m<sup>2</sup> area at the Arnold Arboretum in Boston, MA, USA (42.297°N, 71.129°W; Figs S1 and S2). All trees were raised from cuttings collected in 1989 from separate individual trees growing wild on Tian Mu Mountain in Zhejiang Province, China. At the time of sampling, tree #1 (AA 1073-89-B, a female tree) was 7.3 m tall with an average spread of 5.4 m; tree #2 (AA 1223-89-A, a female tree) was 9.7 m tall with an average spread of 7.0 m; and tree #3 (AA 1072-89-F, male tree) was 9.2 m tall with an average spread of 6.2 m. All three trees were growing with supplemental irrigation and fertilization in the same general area and experienced the same growing conditions. All samples were collected between 26 and 28 of June 2012 just after a short period of light rain. Four types of samples were collected: trunk (bark tissue), branches (> 1 year old; bark tissue), new branch growth (< 1 year old epidermal primary tissue; green branches with intact epidermis) and leaves. Trunk and branch bacterial communities were collected by swabbing an approximately 5 cm<sup>2</sup> area on the surfaces with sterile swabs. Bacterial communities from leaves were collected by directly sampling four whole leaves collected in sterile plastic bags due to the inability to recover sufficient

microbial biomass by swabbing. In all cases, sampling was done aseptically while wearing nitrile gloves. Bacterial communities were sampled at sites in relation to branches at five vertical levels on each tree and separately on north and south sides of the trunk. When possible, samples were collected from branches at four distances away from the main trunk corresponding to distinct ages of the branch segments, and the tops and bottoms of branches were sampled separately on the older segments. The ages of the branch segments were calculated by identifying compact areas of bud scale scars left in the bark from annual terminal buds. The leaves from the youngest branch segments were associated with long shoots, whereas other leaves were associated with the short shoots characteristic of the ginkgo morphology in older branch segments.

Leaf samples were collected from each of the sampled branch segments. Swabs and leaves were stored at  $-20^{\circ}\text{C}$  prior to molecular analysis. Leaf samples were homogenized prior to DNA extraction by freezing at  $-80^{\circ}\text{C}$  and crushing them in the bags. DNA was extracted from swabs and the homogenized leaf material (each containing a similar amount and less than 50 mg of sample material) using the PowerPlant Pro kit (Mo Bio Laboratories, Carlsbad, CA, USA). Although only surface-associated bacteria were extracted from the trunk and branch samples, the leaf communities include those bacteria found on the leaf surfaces and those found inside the individual leaves.

#### *Determination of bacterial community composition and diversity*

To assess bacterial diversity and community composition in each of the 314 samples collected, a region of the 16S rRNA gene was amplified and analysed via barcoded sequencing following a procedure described in Redford and colleagues (2010). The DNA was polymerase chain reaction (PCR) amplified in triplicate using a primer pair (799f/1115r), which was designed to avoid amplification of chloroplast DNA (Chelius and Triplett, 2001; Redford *et al.*, 2010), and reduced the percentage of chloroplast sequences to minimal levels in this study (Table S2). To enable simultaneous sequencing of all samples, each sample was amplified with a primer set containing a unique 12 bp barcode. Following amplification, triplicate reactions were combined and visualized on an agarose gel along with positive and negative controls. Amplicons were combined in equimolar ratios, cleaned using the UltraClean PCR Clean-Up Kit (Mo Bio Laboratories, Carlsbad, CA, USA) and sequenced at the Engenore facility at the University of South Carolina on the Roche 454 sequencing platform.

Raw 16S rRNA gene sequences were processed using the QIIME v1.6.0 pipeline (Caporaso *et al.*, 2010) in order to perform initial steps of bacterial community characterization. These steps included: demultiplexing, quality filtering, phylotype clustering, taxonomy assignments, sequence alignments and computing pairwise community dissimilarities. Default parameters were used except for the following: only sequences between 270 and 370 bp were retained to remove poor quality sequences as the expected length was  $\sim 316$  bp, both forward and reverse primers were trimmed when contained in the sequence, phylotype clustering was

performed using the open reference (reference-based + de novo) implementation of UCLUST (Edgar, 2010) and relied on the GREENGENES October 2012 16S rRNA database clustered at 97% similarity (McDonald *et al.*, 2012) and taxonomic identities were assigned to phylotypes using the RDP classifier (Wang *et al.*, 2007) trained on the aforementioned GREENGENES database with a confidence threshold of 0.5. As an additional quality control measure, phylotypes classified as mitochondria or chloroplasts and those that could not be assigned to a specific phylum were removed prior to further analyses. Following initial processing, the sequence data were rarefied to 575 sequences per sample to account for the variable sequencing depth obtained (Table S2), which resulted in 191 of the 201 collected trunk and branch samples having sufficient sequencing depth to be retained for downstream analyses. For spatial (within sample type) analyses of leaf-associated bacterial communities, samples were rarefied to 200 sequences per sample in order to retain more samples (with 89 of the 113 collected leaf samples included in downstream analyses). Diversity metrics and unweighted UniFrac distances (Lozupone *et al.*, 2011) were calculated within QIIME. Although we could not assess the full extent of bacterial diversity with this sequencing depth, previous work demonstrates that this sequence depth is sufficient for accurate assessments of patterns in bacterial diversity and community composition on leaf surfaces (Redford *et al.*, 2010) and in other microbial habitats (Kuczynski *et al.*, 2010). Amplicon sequences were deposited in the European Nucleotide Archive (<http://www.ebi.ac.uk/ena/>) and can be accessed using the accession number, ERP005154.

#### *Statistical analyses and visualizations*

Differences in diversity among sample types were assessed using box plots and analysis of variance (ANOVA), whereas differences in community composition were assessed using principal coordinate analysis and permutational multivariate analysis of variance (PERMANOVA). In ANOVA and PERMANOVA models, sample type was included as a fixed factor and tree individual was included as a random effect. For pairwise comparisons among sample types, post-hoc tests were used within the PERMANOVA function. Two-dimensional linear interpolation was used to visualize spatial patterns across an individual model tree using the 'akima' package in R (R Core Team, 2013). Significant differences in the relative abundances of individual bacterial taxa across sample types were determined using Kruskal–Wallis tests and Bonferroni corrections. Univariate analyses and principal coordinate analysis were performed using R (R Core Team, 2013), and PERMANOVA was performed using PRIMER 6 (Clarke and Gorley, 2006).

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## Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Fig. S1.** Aerial photograph of the site at the Arnold Arboretum in Boston, MA, USA where ginkgo trees were sampled. Photograph from <http://google.com/maps>.

**Fig. S2.** Photograph of Ginkgo\_2.

**Fig. S3.** Box plots showing values for three different bacterial diversity metrics across the various ginkgo locations sampled. PD, phylogenetic diversity.

**Fig. S4.** Principal coordinate analysis plot showing all samples as points colored by individual tree. This ordination was created using unweighted UniFrac.

**Fig. S5.** Spatial heat maps on schematic diagrams of the three replicate ginkgo trees sampled showing overall levels of bacterial community similarity calculated using principal coordinate axes scores (PC1 and PC2). Different colours represent different principal coordinate axis 1 and axis 2 scores. Trees 1 and 3 were male and tree 2 was female.

**Fig. S6.** Spatial variation of bacterial community composition on bark. More dissimilar colours represent more dissimilar communities using principal coordinate analysis scores from (A) the first coordinate and (B) the second coordinate. Shading is based on linear interpolation between average sample values (indicated with circles). Representation of sample locations were adjusted to accommodate slight variations among the three replicate trees prior to computing average values for a given location, and the left represents south facing branches and the right represents north-facing branches. Distances based on unweighted UniFrac distances.

**Fig. S7.** Spatial variation of bacterial community composition on leaves (as opposed to the bark results shown in Fig. S6). More dissimilar colours represent more dissimilar communities using principal coordinate analysis scores from the first coordinate.

**Fig. S8.** Schematic of leaf sampling sites coloured by the relative abundance of the six dominant phyla and classes associated with leaves. Green sites indicate higher relative abundances, and gray sites indicate lower relative abundances. The left side of the subset panels represents south facing branches and the right side represents north-facing branches.

**Table S1.** Phylotypes that had a median relative abundance (%) of at least 1% in each of the sample types.

**Table S2.** Summary statistics for the proportion of sequences that were identified as chloroplast, and the number of quality-filtered sequences obtained per sample after removal of the chloroplast sequences. Only samples with  $\geq 50$  sequences were included.