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Bacterial Succession on the Leaf Surface: A Novel System for Studying Successional Dynamics

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Abstract Succession is a widely studied process in plant and animal systems, but succession in microbial communities has received relatively little attention despite the ubiquity of microorganisms in natural habitats. One important microbial habitat is the phyllosphere, or leaf surface, which harbors large, diverse populations of bacteria and offers unique opportunities for the study of succession and temporal community assembly patterns. To explore bacterial community successional patterns, we sampled phyllosphere communities on cottonwood (*Populus deltoides*) trees multiple times across the growing season, from leaf emergence to leaf fall. Bacterial community composition was highly variable throughout the growing season; leaves sampled as little as a week apart were found to harbor significantly different communities, and the temporal variability on a given tree exceeded the variability in community composition between individual trees sampled on a given day. The bacterial communities clearly clustered into early-, mid-, and late-season clusters, with early- and late-season communities being more similar to each other than to the mid-season communities, and these patterns appeared consistent from year to year. Although we observed clear and predictable changes in bacterial community composition during the course of the growing season, changes in phyllosphere bacterial diversity were less predictable. We examined the species–time relationship, a measure of species turnover rate, and found that the relationship was fundamentally similar to that observed in plant and invertebrate communities, just on a shorter time scale. The temporal dynamics we observed suggest that although phyllosphere bacterial communities have high levels of phylogenetic diversity and rapid turnover rates, these communities follow predictable successional patterns from season to season.

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Introduction

Succession generally refers to the biological changes that occur in an ecosystem after a clearing or exposure of an area, often resulting in predictable sequences of species composition shifts [37]. Plants and sessile animals have long been the focus of succession studies, primarily due to the perceived physical and ecological dominance of their respective landscapes [8]. Despite their ubiquity, abundance, and diversity, surprisingly few studies have examined succession patterns in microbial communities. This is partly due to methodological reasons, as it is difficult to describe the full extent of microbial diversity in a given sample using traditional, culture-based methods [2, 38]. However, with molecular approaches, we can now rapidly survey and describe microbial community structure and composition at an unprecedented level of detail. Microorganisms therefore provide unique opportunities for studying community-level succession and for testing competing models of successional dynamics.

A handful of studies have examined patterns of microbial succession in environments as diverse as food products [9, 13, 41], artwork [5], compost [35, 40], concrete [43], and the human body [15, 28, 39]. A few

studies have focused specifically on the process and participants of microbial succession and how these patterns are similar to those seen in macrobial succession. For instance, Nemergut et al. [36] examined chronosequences of newly exposed glacial soils and found that successional patterns in these soils were similar to those found in macrobial systems; the soil microbial communities were structured by substrate age and followed predictable composition patterns. Likewise, Jackson et al. [23] examined microbial succession in aquatic biofilms and also found predictable patterns of bacterial succession during biofilm formation. Jackson et al. [23] proposed a conceptual model to describe changes in community richness over time as succession progresses. In their model, species richness initially increases as microorganisms colonize the new surface. As competition increases, species richness decreases, and during later stages of succession, there is another increase in bacterial species richness as resource diversity increases due to the creation of new spatial and ecological niches within the biofilm. Subsequent studies have found patterns of biofilm succession that fit the model proposed by Jackson et al. [23] and have found that bacterial communities cluster according to age of the community [31, 34]. In each of these studies, microbial communities follow predictable, repeatable successional patterns.

The phyllosphere, or leaf surface, offers a unique habitat in which to explore temporal variability in bacterial communities and the process of bacterial community succession. Phyllosphere bacteria are abundant (bacterial densities average 10^6 to 10^7 cells/cm^2 of leaf [3]), diverse [52], and are likely to have an important influence on plant health [29]. In addition, the leaf surface of deciduous species of plants is an inherently ephemeral habitat that emerges anew each year as a presumably sterile surface [24], making it an ideal natural habitat in which to conduct detailed studies of successional processes. There is a long history of research on phyllosphere inhabitants, including studies which have found that leaves sampled at the same time in the growing season had similar communities and that this pattern was predictable from year to year [12, 48]. However, most of these previous studies employed culture-based methods, which have important limitations given that such methods effectively ignore a large portion of the microbial diversity and are less likely to detect microbial community shifts that occur over time [2, 38].

To our knowledge, this study represents one of the first detailed examinations of succession in phyllosphere bacterial communities utilizing culture-independent methods. We chose to focus on a single common deciduous tree species from northern Colorado, the cottonwood, *Populus deltoides*. The leaves of this riparian tree species emerge each year in early May and persist through leaf-fall at the end of October. We used sequence-based phylogenetic methods to characterize and compare the phyllosphere bacterial communities of a single tree across the growing season. We also compared the variation in phyllosphere community composition between several individual cottonwoods on a single day in order to determine how tree-to-tree variation in phyllosphere communities compares to the variation observed over time. Our goal was to determine if the patterns of succession in phyllosphere communities are consistent with the patterns observed in other studies of succession in microbial communities, both in terms of community-level diversity and the turnover of taxa over time. In addition, we wanted to determine how these patterns compare, on a general level, to some of the successional patterns commonly observed in plant and animal communities.

Methods

Sample Collection

Leaves were collected from an individual cottonwood tree (*P. deltoides*) located in an 86-ha natural area in northern Colorado, USA (40°35′ N, 105°5′ W, elevation 1,519 m). We chose to focus on a single tree in order to minimize potential inter-individual sources of variability in leaf conditions that could obscure any temporal patterns in the bacterial leaf surface communities. Collections began on August 31, 2006 and continued once per month through October 26, 2006, at which point leaf senescence had completed for the season. Collections continued the following spring, starting on May 17, 2007, shortly after leaf-out, and continued once per month through August 21, 2007. Additional collections were made from three nearby cottonwood trees on September 7, 2006 (pairwise distances between individual trees ranged from 20 to 170 m). At each sampling, a sterile 500 mL container was filled with 30–35 leaves from around the canopy at a 1–2 m height, as we wanted to factor out any variability in bacterial communities associated specifically with the position of leaves within the canopy. We also did not directly take leaf age into account due to the difficulties associated with directly determining the ages of individual leaves and because previous studies have found that leaves of different ages had more similar communities when sampled at the same time than leaves of the same age sampled at different times [12].

Leaves were weighed inside the containers in order to ensure relatively consistent leaf masses for each sampling. A 1:50 diluted sterile wash solution (1 M Tris–HCL, 500 mM ethylenediamine tetraacetic acid, and 1.2% Triton diluted in sterile water) [24] was then added to the container with the leaves, and the container was shaken at 350 rpm for

5 min. The wash solution was then filtered into two 50 mL centrifuge tubes through sterile glass wool. The tubes were centrifuged at 2,200×*g* for 15 min at 4°C. The supernatant was discarded, and DNA was extracted from the resulting pellet using the MoBio PowerSoil DNA kit according to the manufacturer's instructions (MoBio Laboratories, Carlsbad, CA, USA).

Clone Library Construction

Bacterial 16S rRNA genes were amplified using primers 799f and 1492r, a primer set designed to screen out chloroplast DNA [4]. Each 50 μL polymerase chain reaction (PCR) contained 1.5 mM $MgCl_2$, 200 μM each dNTP, 1× PCR buffer (Invitrogen, Carlsbad, CA, USA), 500 pmol each primer, 0.25 units of *Taq* DNA polymerase, and 2 μl of DNA sample. Amplification conditions were identical for all samples, with an initial denaturation at 95° C for 2 min, followed by 36 cycles of 94°C for 45 s, 52°C for 30 s, and 72°C for 60 s, followed by a final elongation at 72°C for 10 min. Amplicon size was confirmed by agarose electrophoresis with DNA concentrations determined on a NanoDrop-1000 Spectrophotometer (Wilmington, DE, USA). Six replicate amplification reactions from each sample were pooled and then purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA). The purified PCR product was cloned into TOP-10 cells using the TOPO TA kit (Invitrogen, CA), following the manufacturer's instructions. The transformed cells were incubated overnight on Luria agar with 50 μg/mL ampicillin. Isolated colonies were picked, and the cloned insert was amplified under the same PCR conditions described above. The resulting PCR plates were sent to Agencourt Bioscience (Beverly, MA, USA) for sequencing, with 96 single-pass reads per sample.

Sequence Analyses

Sequences were aligned using the NAST aligner [10] and classified (minimum length, 400; minimum identity, 75%) using the Hugenholtz and Pace taxonomies in the Greengenes database [11]. Maximum likelihood trees were built, with 100 bootstrap replicates to incorporate phylogenetic uncertainty, using the GTR+gamma model of evolution in the MPI version RAxML-7.0.0 [47]. In order to test the hypothesis that each community was phylogenetically distinct, we used Treeclimber [46] to run the Phylogenetic (P) test [32, 33]. The majority consensus tree was run through UniFrac in order to obtain a distance matrix describing the pairwise phylogenetic distance between each community [30]. Faith's Phylodiversity index [14] was calculated for each replicate tree using Phylocom [49]. The number of unique operational taxonomic units (OTUs) at the 97% level was determined using the FastgroupII algorithm [53]. The 97% sequence similarity level was chosen because it is commonly used as the level of similarity in the 16S rRNA gene that defines a bacterial "species" [45].

Bacterial Abundance

We used qPCR to estimate the relative abundance of bacterial 16S rRNA genes in each sample as previously described [16]. The qPCRs were conducted in 96-well plates, with three replicates per sample and per dilution standard. Each 25 μL reaction contained 12.5 μL ABsolute qPCR SYBR Green Mix (ABgene, UK), 1.75 μL each primer (Eubac388f and Eubac518r), 8 μL water, and 1 μL template. Reaction conditions were 15 min at 94°C, followed by 40 cycles of 94°C for 30 s, 30 s at 50°C, and 72°C for 45 s, followed by melting curve analysis to confirm the fluorescence signal resulted from specific PCR products and not primer-dimers or other artifacts. Reactions were performed on a Mastercycler ep realplex machine (Eppendorf, Germany).

Estimation of the Species–Time Relationship

Given that we were characterizing the temporal variability in bacterial communities, we wanted to quantitatively compare the species–time relationship for the cottonwood phyllosphere communities to those of other microbial and macrobial communities. To do so, we used a power law function plotted in log–log space to describe the relationship between cumulative "species" richness (S) and time (T) as $S=cT^w$, where c is an empirically derived taxon- and location-specific constant, and w is the slope of the line in log–log space (scaling exponent in linear space) [42]. Although Rosenzweig [44] has suggested that the logarithmic function may provide a better fit to the species–time relationship, in practice, there appears to be little significant difference between the fit of the logarithmic and the power function for the species–time relationship [51]. Moreover, since the power function exponent provides a simple measure of relative species turnover, it is used more often than the logarithmic function to describe the species–time relationship of both microbial and macrobial communities [51, 17]. Cumulative species composition was calculated with a sliding window approach [1] using the FastgroupII results to calculate number of "species" per window, using the species definition described above.

Nucleotide Sequence Accession Numbers

All sequences obtained from this study have been deposited in the GenBank nucleotide database with accession numbers EU448808–EU448879 and EU694441–EU695207.

Results

Changes in Diversity and Abundance Over Time

The phyllosphere communities were phylogenetically diverse. After eliminating possible chimeras and otherwise poor quality sequences, 839 valid sequences remained and were used in subsequent analyses. Out of these 839 valid sequences from all 11 samples, 355 sequences were unique at the 97% sequence similarity level. The rarefaction results from the most and least diverse communities, as well as a community of near-average diversity, indicated that we did not capture the full extent of "species" diversity in most of these communities, as the rarefaction curves failed to reach an asymptote (Fig. 1). Community diversity did change over time, but no clear patterns emerged, whether we estimated phylogenetic diversity using Faith's phylodiversity index or by estimating the percentage of unique sequences at the 97% level per time point (Fig. 2). The most diverse communities were those sampled on August 21, 2007 and September 28, 2006 (Faith's PD=0.28 and 0.27, respectively; 78% and 75% uniques, respectively). The least diverse community was the community sampled on July 19, 2007 (Faith's PD=0.09 and 9% uniques; Fig. 2).

The relative abundance of bacterial 16S rRNA gene sequences in each community ranged from 5.56×10^4 ($\pm7.0\times10^3$) to 8.7×10^5 ($\pm8.4\times10^4$) gene copies/μL DNA extract. There was no discernible pattern in bacterial abundance over time, and there were no significant correlations between bacterial abundance and either the time of sampling, meteorological conditions, or estimated diversity levels ($r<0.3$, $P>0.5$ in all cases).

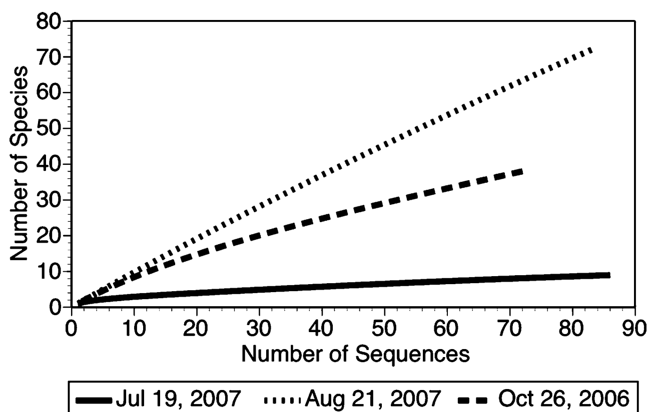

Figure 1 Rarefaction curves for communities of highest, lowest, and midrange diversity

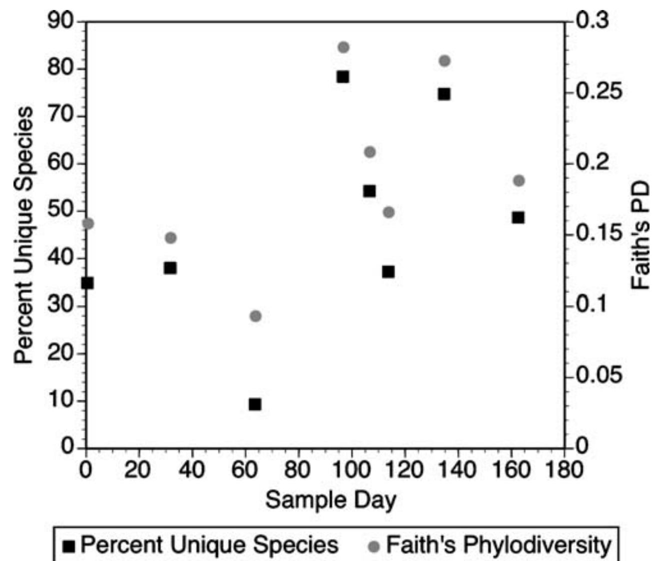

Figure 2 Illustration of two descriptions of diversity for each of the time point communities. Sample days are counted from the earliest sampling date (May 17, 2007) through the latest (October 26, 2006). Dates are in order of time since the start of the growing season rather than chronological order

Changes in Community Composition Over Time

If we considered all samples together, sequences belonging to the phylum Proteobacteria were the most common on the leaves, representing 62% of all sequences. The Gammaproteobacteria was the most common subphylum within Proteobacteria (accounting for approximately 76% of proteobacterial sequences), though members of the Alpha-, Beta-, and Delta-proteobacteria were also found (10%, 11%, and 3% of proteobacterial sequences, respectively). Acidobacteria were also fairly common, comprising about 20% of all sequences. Firmicutes and Bacteroidetes each represented about 7% of total sequences, and members of Actinobacteria, Deinococcus-Thermus, Nitrospira, SC4, and Verrucomicrobia were also detected, though at relatively low levels (less than 1% of the total number of sequences in all cases).

The clustering diagram shown in Fig. 3 illustrates the relative phylogenetic distances between each pair of communities sampled. Based on the TreeClimber results, each of the samples collected from the same tree on different dates harbored a distinct bacterial community (all pairwise P values <0.005). The variability in bacterial communities between different trees sampled on a given date was less than the variability in a single tree over time. The bacterial communities on the four trees sampled on the same date (September 7, 2006) were relatively similar to one another as three out of the four different cottonwood trees sampled on that date were more similar to each other than to any other community (P=0.3, P=0.2, and P=0.03

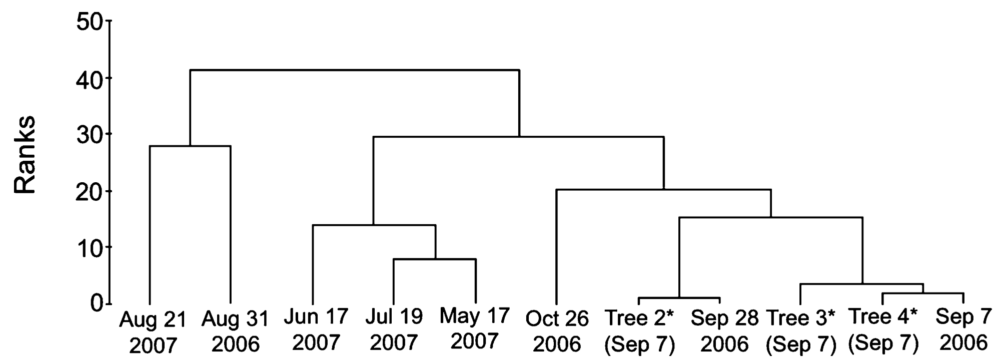

Figure 3 Clustering diagram of the UniFrac distance matrix, illustrating the relative similarity of each of the phyllosphere time point communities. *Asterisks* Other sample trees, collected on September 7, 2006. All other samples are from the same tree (i.e., Tree 1) on date indicated

for those comparisons), and the community on one tree was more similar to the September 28, 2006 community (Fig. 3). The October 2006 community was more similar to the September 2006 communities than to any of the other communities (Fig. 3). The early season communities (May, June, and July 2007) clustered together, and clustered more closely with the late season communities (September and October 2006) than with the peak season communities (August 2006 and 2007). The August communities clustered together to the exclusion of all other time points (Fig. 3).

The meteorological conditions during the sampling period are represented in Fig. 4. No significant correlations were found between average weekly temperature, average weekly precipitation, or daily high and low temperature and the phylogenetic distances between the communities ($r<0.3$, $P>0.1$ in all cases). Even though there were distinct and predictable changes in community composition over time (Fig. 5), the measured meteorological conditions did not appear to predict the observed changes in community composition.

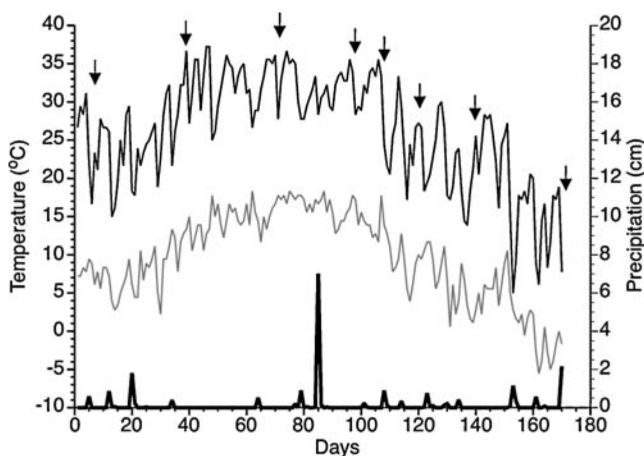

Figure 4 Illustration of climate conditions for the sampling period. Day 1 is May 10, 2007, 1 week before the first sampling. Transition from 2007 to 2006 occurs at day 107, 1 week before the August 31, 2006 sampling date, and day count continues through October 26, 2006. *Arrows* indicate the dates phyllosphere communities were sampled. The *top line* is daily high temperature, the *middle line* is daily low temperature, and the *bottom line* is daily precipitation. Precipitation in each case is rain, with the exception of the last two precipitation events, which were snow events

Figure 5a illustrates the shifts in the relative abundances of bacterial phyla over time. The May, June, and July 2007 samples were dominated by Proteobacteria; over 80% of sequences in each of these months were proteobacterial. The August 2006 and 2007 samples showed a decrease in Proteobacteria and an increase in Acidobacteria. The relative abundances of Firmicutes and Bacteroidetes in August were similar to those observed in the early season samples. Acidobacteria became dominant in the late August 2006 sampling, but their abundances declined within 1 week, and by the September 7, 2006 sampling date, Proteobacteria became dominant again. Acidobacteria and Proteobacteria maintained relatively high abundances, and Bacteriodetes became increasingly numerous in the late-season samples (September 28 and October 26, 2006).

The relative abundances of proteobacterial divisions varied among the samples as well (Fig. 5 b). Across all time points, members of the Gamma-proteobacteria were the most dominant type of bacteria, being the most common in the spring and early summer, and far less common at midseason. Members of the Alpha- and Beta-proteobacteria could be found at nearly every time point, with the only exception being the community sampled in July, which appeared to be dominated by a single group of Gamma-proteobacteria.

We conducted more detailed phylogenetic analyses of the Gamma-proteobacterial sequences related to the Enterobacteriaceae, as this taxon showed the most dramatic shifts in relative abundance across the sampling dates (Fig. 5c). The early-season Enterobacteriaceae were largely composed of sequences closely related to cultured isolates of *Pantoea* sp., though the May sample had a more even distribution of Enterobacteriaceae genera, and the July sample was almost entirely composed of sequences very similar to *Pantoea*. The later season samples had a number of Enterobacteriaceae sequences similar to *Escherichia* sp., as well as other genera within the Enterobacteriaceae, and the sequences most similar to *Pantoea* sp. became relatively rare.

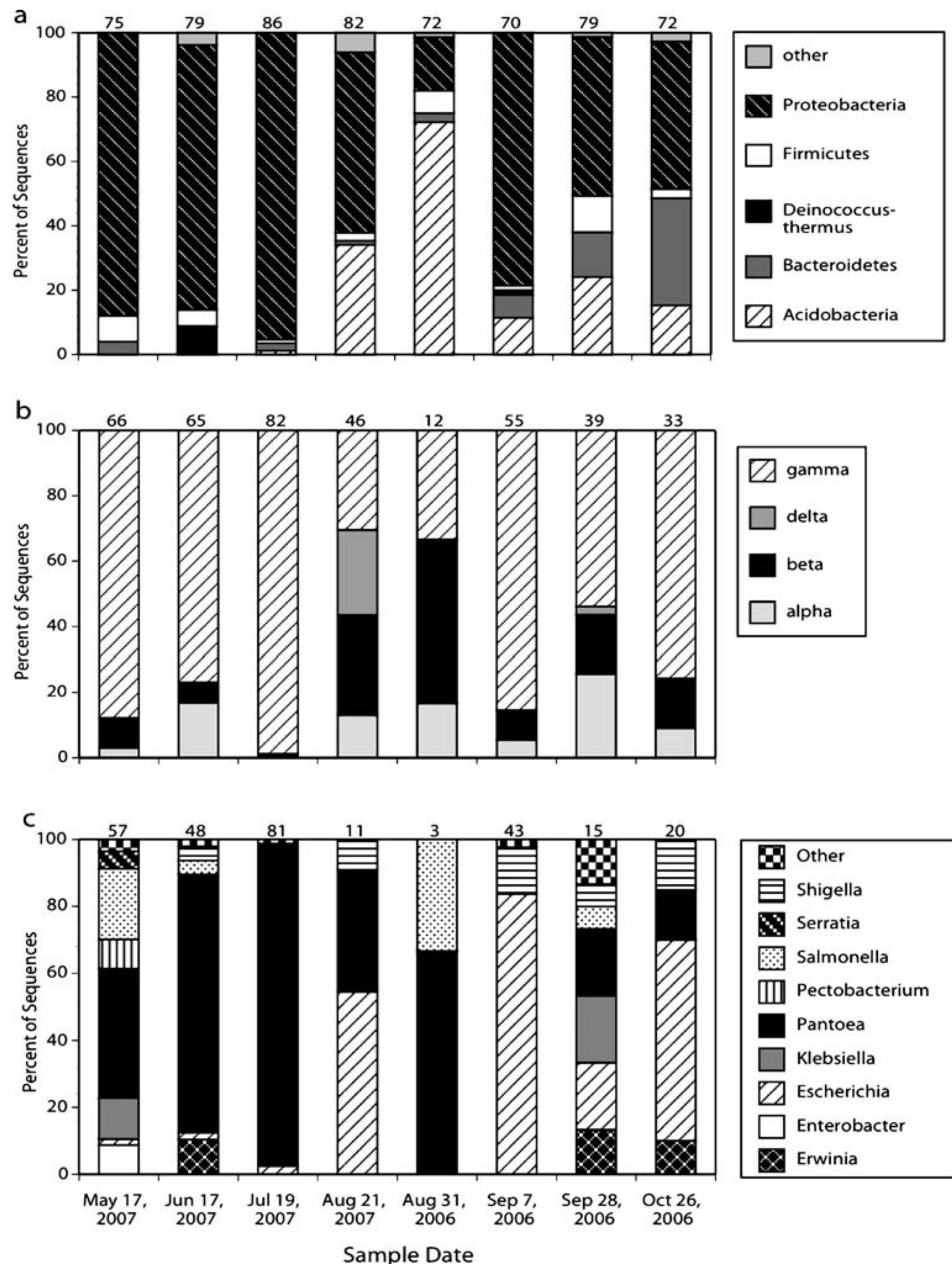

Figure 5 Relative abundances of bacterial taxa in each of the phyllosphere time point community libraries (*numbers at the top of each column* indicate the number of sequences): **a** bacterial phyla, **b** proteobacterial subphyla, **c** Enterobacteriaceae genera (sequences were classified according to the National Center for Biotechnology Information taxonomy in the Greengenes database for this level of resolution). Sample dates are shown in order of time since the start of the growing season rather than chronological order

The species–time relationship for cottonwood phyllosphere communities was described by the function $S=33.3T^{0.318}$ ($r^2=0.92$, $P=0.005$), where S="species" (OTU) richness and T=time (Fig. 6).

Discussion

Changes in Bacterial Diversity Over Time

We did not find predictable changes in bacterial diversity over time (Fig. 2). This result contrasts with the findings of Jackson et al. [23], Martiny et al. [34], and Lyautey et al. [31], who observed predictable changes in bacterial diversity during biofilm succession with the highest levels of bacterial diversity in the earliest stages and in the latest stages of biofilm development, a pattern described as consistent with the intermediate disturbance hypothesis [18, 7]. Our results are more consistent with a study of the bacterial communities within a bioreactor where there was no discernible pattern in the changes in bacterial community richness over time [17]. One possible explanation for the lack of predictable changes in bacterial diversity on the leaf surface is that we are not seeing a continuous successional

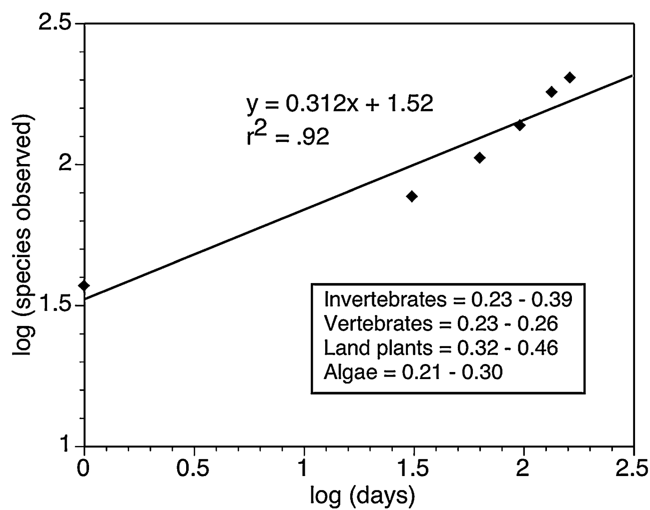

Figure 6 Species–time relationship power function transformed and plotted in log–log space. Slope of the line of best fit shown here is equivalent to power-function exponent from nontransformed data. *Values in the box* are the average slope ranges for taxa examined by White et al. [51]

sequence over the lifetime of the leaf but rather a discontinuous successional sequence that is frequently interrupted by disturbance events. The initial emergence of the leaves likely provides a virtually sterile environment [24], allowing colonization of a number of species following a primary successional pattern. However, disturbances over the growing season due to changes in UV exposure [24], moisture conditions, resource availability, or leaf cuticle properties could essentially prevent the formation of a "climax" community, and neither bacterial diversity nor bacterial community composition would appear to stabilize over time. In this respect, the bacterial communities on the leaf surface may be similar to plant communities living in habitats where disturbances are frequent enough that a climax community per se may never truly exist [8, 21, 22]. If the communities did pass through initial stages of succession upon colonizing the leaf surface, it is possible that these initial stages occurred far too rapidly to be detected using our sampling scheme, even though the leaves were initially sampled less than 2 weeks after leaf-out. Alternatively, since we analyzed bulk leaf samples and our sampling was necessarily destructive, we were not able to track succession patterns on individual leaves where the changes in community diversity over time may be more predictable. It is also important to note the difficulties in accurately sampling the full extent of species diversity present in microbial communities given their immense phylogenetic diversity [25]. However, diversity patterns over the course of succession are known to vary widely and are strongly influenced by environmental conditions and the life-history characteristics of the specific organisms in question [22].

Changes in Community Composition Over Time

The composition of the phyllosphere bacterial communities changed in a consistent manner over time with communities at similar points during the growing season being phylogenetically similar. Phyllosphere bacterial communities sampled throughout the growing season were significantly different from one another in all cases, and the phyllosphere communities clearly cluster into early-, mid-, and late-season categories (Fig. 3). The clustering patterns appear to be consistent from year to year given that the August samples collected in two successive years have communities that are quite similar (Fig. 3). Additionally, the different cottonwood trees sampled on the same date in September all appear to harbor similar bacterial communities suggesting that the variability in community composition over time exceeds that found between individuals of the same tree species located in close proximity. Our results are consistent with two culture-based studies of phyllosphere communities which found that leaf samples taken at the same time of year harbored bacterial communities more similar to one another than to samples taken at different times of year [12, 48]. Though neither of these studies framed these seasonal patterns as succession per se, they support our results and suggest that the temporal variability in phyllosphere bacterial community composition is not random and that the communities follow predictable successional patterns that are consistent from season to season. This, along with the fact that bacterial communities appear to be plant species-specific [27], hints at a more structured relationship between the bacterial communities and their plant hosts.

From this work, we cannot determine the specific factors that influence the observed temporal variability in phyllosphere bacterial communities. The phylogenetic distances among the communities sampled at different times did not correlate with weather changes that occurred across the growing season, which is consistent with a culture-based study of phyllosphere microbes [48]. It is possible that unmeasured changes in environmental conditions (e.g., UV exposure or relative humidity) or changes in leaf surface characteristics (e.g., leaf wax chemistry, nutrient content, cuticle thickness, or the supply of carbon substrates to phyllosphere bacteria) were at least partially responsible for the shifts in community composition. Also, the apparent variability across time may be enhanced by the unavoidable destructiveness of our sampling, given that we were not able to resample the exact same leaves over time. Additional work is necessary to determine the causal factors driving the observed shifts in bacterial community composition over time. In particular, it would be valuable to compare bacterial community succession on plants growing under controlled environmental conditions versus succession on plants growing under field conditions.

Although we observed dramatic temporal variation in phyllosphere bacterial community composition, the functional consequences of these community shifts are unclear given that we know very little about the natural histories of most of the bacterial taxa observed in this study. Even Proteobacteria, one of the best-studied bacterial divisions represented by a relatively large number of cultured representatives, are so diverse that it is difficult to infer function from small-subunit rRNA gene sequences alone. More specific assays of functional genes, enzyme activities, or focused surveys on the dynamics of particular ecotypes of bacteria, such as nitrogen fixers or methylotrophs, would provide more insight into the function of these communities and how they might interact with the plants on which they dwell.

The leaf surface is a unique and sizable environment where the bacterial inhabitants are sufficiently numerous to potentially have a significant role in global ecological processes [29]. Previous work has established that immigration, emigration, growth, and death are all important components of bacterial community dynamics in the phyllosphere habitat [26]. Furthermore, the variability in community structure and size between and even within leaves in a canopy can be quite large, as each individual leaf can experience a unique set of environmental characteristics [19]. Most of this knowledge is based on investigations of single species or functional groups of bacteria, primarily of potentially pathogenic culturable organisms, such as *Pseudomonas syringae* [29]. Though our study was conducted at a relatively coarse level of resolution, it represents one of the first attempts to more comprehensively assay the composition and temporal dynamics of phyllosphere bacterial communities.

The Species–Time Relationship

The slope of the species–time relationship (or more appropriately, the "species"–time relationship given that the definition of a bacterial species is somewhat arbitrary [45]) for the cottonwood phyllosphere bacterial communities is similar to slopes of species–time relationships observed in plant and invertebrate communities [51] (Fig. 6). It is possible that our slope was, to some degree, overestimated due to a sampling effect [6] associated with our inability to survey the full extent of species-level bacterial diversity at each time point. Given the high levels of observed diversity (Figs. 1, 2), we do not have the species abundance data required to fully explore where the sampling effect ends and the ecological effect begins [50]. However, studies that test two-phase models that incorporate a sampling phase show that the transition point between the sampling phase and the ecological phase usually occurs fairly early on in the time sequence [51], and therefore, the sampling effect is likely to have relatively little effect on the estimation of the overall slope. A study of the species–time relationship in bacterial communities within a bioreactor found that lower slopes were associated with bacterial communities that had higher species richness levels [17]; White et al. noted a similar pattern for macrobial communities [51]. Furthermore, van der Gast et al. were able to associate a drop in the species–time slope with an increase in niche-related restrictions within bacterial communities [17]. Our slope was in the mid-range of the slopes found in the gradient bioreactor bacterial communities, which the authors described as suggesting some balance between stochastic and niche-based, deterministic community formation patterns [17]. Overall, our data support the notion that the species–time relationship, as previously demonstrated for the sister index, the species–area relationship [20], can be applied to both microbial and macrobial communities, suggesting similar overarching patterns in species turnover in microbial and macrobial communities.

Conclusions

The data from our study of bacterial phyllosphere communities over the growing season are consistent with our initial hypothesis that patterns of community succession should be similar to those observed in other microbial systems. As in previous studies of microbial community succession, we observed predictable changes in community composition over time given that the phyllosphere bacterial communities appeared to cluster by season. Although the specific changes in community-level diversity do not necessarily correspond with other succession studies, it is entirely possible that there are multiple successional sequences occurring throughout the growing season that are responsible for these differences. Although microbial communities are phylogenetically and functionally very diverse, they appear to exhibit predictable successional patterns, just on much shorter time scales.

On a more practical note, our data demonstrate that studies of microbial communities may need to carefully consider the high degree of temporal variability in their sampling design, given that, in this system, one could go back to the same tree several weeks in a row and find very different phyllosphere communities. This poses a challenge that is unique in magnitude to microbial ecology, but it also represents an opportunity, as temporal change in microbial communities can be observed over time scales that are amenable to more detailed and manipulative studies and experiments. Like the model system of aquatic biofilms, the

leaf surface offers the ability to fairly easily replicate the successional process as leaves re-emerge each year. Plants can be grown under controlled conditions, whether in the field or in the lab, to isolate some of the factors that may influence successional dynamics. In short, the microbes on leaf surfaces provide ecologists with tractable model systems that are fairly easy to manipulate and provide opportunities for more in-depth investigations of temporal dynamics in community assembly patterns.

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