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ORIGINAL ARTICLE

A meta-analysis of changes in bacterial and archaeal communities with time

Ashley Shade[1], J Gregory Caporaso[2,3], Jo Handelsman[1], Rob Knight[4,5] and Noah Fierer[6,7]
[1]*Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT, USA;* [2]*Department of Computer Science, Northern Arizona University, Flagstaff, AZ, USA;* [3]*Argonne National Laboratory, Argonne, IL, USA;* [4]*Department of Chemistry and Biochemistry and Biofrontiers Institute, University of Colorado, Boulder, CO, USA;* [5]*Howard Hughes Medical Institute, Boulder, CO, USA;* [6]*Cooperative Institute for Research in Environmental Sciences, University of Colorado, Boulder, CO, USA and* [7]*Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO, USA*

Ecologists have long studied the temporal dynamics of plant and animal communities with much less attention paid to the temporal dynamics exhibited by microbial communities. As a result, we do not know if overarching temporal trends exist for microbial communities or if changes in microbial communities are generally predictable with time. Using microbial time series assessed via high-throughput sequencing, we conducted a meta-analysis of temporal dynamics in microbial communities, including 76 sites representing air, aquatic, soil, brewery wastewater treatment, human- and plant-associated microbial biomes. We found that temporal variability in both within- and between-community diversity was consistent among microbial communities from similar environments. Community structure changed systematically with time in less than half of the cases, and the highest rates of change were observed within ranges of 1 day to 1 month for all communities examined. Microbial communities exhibited species–time relationships (STRs), which describe the accumulation of new taxa to a community, similar to those observed previously for plant and animal communities, suggesting that STRs are remarkably consistent across a broad range of taxa. These results highlight that a continued integration of microbial ecology into the broader field of ecology will provide new insight into the temporal patterns of microbial and 'macro'-bial communities alike.
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Introduction

Understanding how communities are structured in time is a fundamental pursuit in ecology. There is a long history of research on temporal dynamics in animal and plant communities (for example, Preston, 1960; Holling, 1973; McNaughton, 1977; Pimm, 1984; Ives *et al.*, 2003; Ives and Carpenter, 2007), and this research has been integral to the development of a wide variety of concepts in ecology. In particular, research on long-term changes in plant and animal diversity have been instrumental in helping ecologists recognize successional dynamics (for example, Lockwood *et al.*, 1997; Chase, 2003), identify relationships between community stability and biodiversity (Cottingham *et al.*, 2001; White *et al.*, 2006) and predict how communities may respond to disturbances, including longer-term global changes (for example, Fraterrigo and Rusak, 2008; Magurran *et al.*, 2010).

Though microorganisms are ubiquitous, abundant and have critical roles in ecosystems, far less is known about the temporal dynamics exhibited by microbial communities relative to those exhibited by communities of larger organisms. A growing collection of site-specific studies in the microbial ecology literature suggests that microbial communities exhibit a wide range of discernable temporal patterns. For example, patterns of primary succession in an infant gut (Koenig *et al.*, 2011) and on leaf surfaces (Redford and Fierer, 2009) as well as patterns of recurring seasonality in aquatic systems (Fuhrman *et al.*, 2006; Shade *et al.*, 2007; Eiler *et al.*, 2012; Gilbert *et al.*, 2012) have demonstrated that some microbial communities change directionally, according to environmental conditions. By contrast, patterns of stability in wastewater treatment systems suggest that some microbial communities are composed of core members that exhibit minimal temporal variability and rarer taxa that exhibit more

Correspondence: N Fierer, University of Colorado, Cooperative Institute for Research in Environmental Sciences, 216 UCB, Boulder, CO 80309-0216, USA.
E-mail: noah.fierer@colorado.edu
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pronounced fluctuations in abundance over time (Werner *et al.*, 2011). Further, in a range of systems, including gut microbiota challenged with an antibiotic (Dethlefsen *et al.*, 2008), lake microbial communities after water column mixing (Jones *et al.*, 2008; Shade *et al.*, 2012b) and soil communities of denitrifiers and nitrite oxidizers after exposure to increased temperature (Wertz *et al.*, 2007), some microbial communities have the capacity to recover quickly after disturbance events, either to the pre-disturbance state or to an alternative stable state (Shade *et al.*, 2012a). Overall, these and other time series highlight that microbial communities, like plant and animal communities, are dynamic and exhibit temporal patterns that can reflect underlying biotic and abiotic processes.

Because changes in microbial community composition are often associated with changes in the functional capabilities of those communities (for example, Bell *et al.*, 2005; Fierer *et al.*, 2007; Strickland *et al.*, 2009), understanding microbial temporal patterns can be critical for understanding ecosystem processes. Despite this importance, our ability to generalize microbial community dynamics has been limited by a focus on site- or habitat-specific research. However, the ever-increasing accumulation of 16S rRNA gene sequence data allows for comparison of microbial communities across habitats. Though microbial communities from different habitats clearly differ in composition (Lozupone and Knight, 2007; Nemergut *et al.*, 2011), it is unknown whether there are common patterns in the dynamics or variability of microbial communities through time and across habitats. To date, there has been no concerted analysis of microbial temporal dynamics across biomes, and thus understanding these dynamics and determining their commonalities are key challenges in microbial ecology.

Aquatic systems are perhaps the most frequently studied microbial communities through time, due, in part, to the relatively large number of long-term ecological studies conducted in aquatic systems. As a result, published time series of aquatic microbial communities have yielded key insights into the drivers of microbial dynamics in marine and freshwater systems. For instance, we know that physicochemical changes in the environment often drive shifts in aquatic microbial communities, as evident from the observation that marine, lake and river communities frequently exhibit pronounced seasonality (Hofle *et al.*, 1999; Fuhrman *et al.*, 2006; Shade *et al.*, 2007; Nelson, 2008; Crump *et al.*, 2009; Eiler *et al.*, 2012). Furthermore, there is evidence that phytoplankton and bacterioplankton communities appear to be synchronous in some systems (Kent *et al.*, 2007), potentially linked by the consumption of phytoplankton-specific exudates by heterotrophic bacterioplankton (Paver and Kent, 2010). These select examples of aquatic microbial time series (culled from hundreds in the literature), demonstrate the importance of time series analysis for identifying the important biotic and abiotic drivers of microbial community structure.

There are clear challenges to studying microbial communities through time. First, it is often difficult to characterize the micron-scale niches for microorganisms, and therefore the immediate environment experienced by many microbial communities remains unknown (Brock, 1987). Additionally, the timescales over which the greatest microbial community changes occur are typically unknown (Shade and Peter *et al.*, 2013). Depending on the habitat, survey efforts may lack the temporal resolution to capture rapid community changes, particularly in systems with actively growing microbial populations, in which generation times may be on the order of minutes. Finally, surveying microbial diversity can be a daunting task. Individual samples often harbor hundreds to thousands of individual microbial taxa (Curtis and Sloan, 2004; Schloss and Handelsman, 2007; Quince *et al.*, 2008), and the majority of microorganisms cannot be identified using traditional culture-based techniques (Pace, 1997; Schloss and Handelsman, 2007). Fortunately, the ongoing development of culture-independent tools and high-throughput sequencing technologies has made it feasible to describe the temporal dynamics of microbial communities at time scales and resolutions that were previously unattainable (Gonzalez *et al.*, 2012).

We conducted a meta-analysis of newly available time series of microbial communities assessed via high-throughput sequencing of the 16S rRNA gene, which permits the detailed analysis of microbial community changes through time. Our objective was to characterize temporal dynamics of microbial communities from a suite of habitats, and when possible, to compare these dynamics with communities of larger organisms. To assess whether temporal patterns were common across both microbial and 'macro'-bial organisms, we applied analyses previously applied to temporal patterns in plant and animal communities. We address the following outstanding questions: How variable are microbial communities over time, and how does this variability compare within habitats? What kinds of temporal patterns are often exhibited by microbial communities, and at what scales are these patterns most apparent? Do microbial communities exhibit species–time relationships (STRs), and if so, are those relationships similar to those for larger organisms?

Materials and methods

Data sets

We compiled bacterial and archaeal time series from 76 sites, spanning a wide range of study durations and habitats, including aquatic, air, brewery wastewater treatment, soil, plant- and human-associated communities. Fungal and other eukaryotic communities

were not included in the meta-analysis. Each site had a minimum of five observations through time, and sites having a destructive sampling regime (for example, soils) were concatenated into a single time series. The compiled time series included eight seasonally sampled temperate bog lakes in Wisconsin, USA, each monitored at two locations: one sample from the upper, mixed layer (epilimnion) and one sample from the lower stratified layer (hypolimnion). These time series included between 1 and 3 years of approximately once or twice weekly observations during the ice-free period (Shade *et al.*, 2008). Additional lake microbial communities were sampled intensively over the month of a whole-ecosystem disturbance experiment at three depths in the lake (Shade *et al.*, 2012b). A second data set included air from near-surface troposphere samples from a mountaintop location in Colorado, USA (Bowers *et al.*, 2012). The air communities were sampled continuously for hours over a few days, with samplings occurring approximately every month for about a year. Nine brewery wastewater treatment communities were sampled once per month for a year, and two of those sites had been previously sampled a few years before (Werner *et al.*, 2011). The six-year English Channel time series, a coastal marine system, provided our longest series of monthly samples (Caporaso *et al.*, 2012; Gilbert *et al.*, 2012). Flower-associated microbial communities were sampled from six apple trees over the lifespan of the flowers (1 week), including five time points from before flowers opened until petal fall (Shade *et al.*, 2013). In a study of human-associated microbiota, the palm, oral and gut microbial communities from two human subjects were collected approximately daily for a year for a male subject, and daily for 6 months for a female subject (Caporaso *et al.*, 2011). Gut microbiota were also sampled from one infant across dietary shifts for the first 2.5 years of life (Koenig *et al.*, 2011). Agricultural soils at the Kellogg Biological Station, Michigan, USA maintained under different management regimes were sampled monthly for 6 months (Lauber *et al.*, 2013) and soils from National Ecological Observatory Network (NEON) sites in Hawaii and Florida, USA (www.neon.org) were sampled once per month for 3 months. Finally, six freshwater streams in Colorado, USA were sampled every 1–2 weeks for approximately 1 year (Portillo *et al.*, 2012). Additional details about each data set are provided in Supplementary Table S1 and in the associated references.

Clearly, these sample sets vary widely with respect to their sampling intensity and study duration. However, this is unavoidable if we want to assess temporal dynamics for microbial communities from diverse environments, and, as has been demonstrated for meta-analyses of plant and animal community dynamics (Nekola and White, 1999; White *et al.*, 2006; Soininen *et al.*, 2007; Korhonen *et al.*, 2010; White *et al.*, 2010), we can still describe patterns that would not be evident if we were to restrict our analyses to a far more limited set of sample types.

Sequence analyses

The microbial communities in each of the 3431 individual samples were characterized by sequencing a portion of the 16S rRNA gene on either the Illumina (San Diego, CA, USA) or 454 (Branford, CT, USA) platforms. The 16S rRNA gene is widely used for determining the phylogenetic and taxonomic composition of bacterial communities and, in all the cases, the data were derived from PCR amplification of environmental DNA using primer pairs designed to amplify the gene region from all, or nearly all, known bacterial taxa. A closed reference operational taxonomic unit (OTU) picking protocol was applied to each data set separately (Caporaso *et al.*, 2012). Briefly, OTUs were assigned based on 97% sequence identity to sequences in the Greengenes reference database (McDonald *et al.*, 2012) preclustered at 97% identity (http://qiime.org/home_static/dataFiles.html). As we used the same reference-based OTU picking strategy for all samples, we could directly compare the relative abundances of taxa across samples. Furthermore, we sub-sampled each individual data set such that all samples from a given data set were compared at an equivalent sequencing depth (Supplementary Table S1). All analyses were performed on the rarefied OTU tables to permit comparisons of patterns in within- and between-community diversity. Our goal was not to quantify the absolute diversity found in any of the samples: this task is difficult, if not impossible, because individual samples may harbor thousands of rare taxa (Sogin *et al.*, 2006). However, as recent work has demonstrated (Shaw *et al.*, 2008; Kuczynski *et al.*, 2010a), it is not necessary to characterize absolute diversity in order to accurately describe changes in within-sample and between-sample diversity within and between habitat types. Pielou's evenness (Pielou, 1969), richness (number of OTUs) and Faith's phylogenetic diversity (Faith, 1992) were used as within-sample (alpha) diversity metrics. Bray–Curtis was used as a taxon-based metric of differences in community composition (beta diversity), and the dissimilarities were calculated from the rarefied OTU tables in R using the vegan package (Oksanen *et al.*, 2011; R Development Core Team, 2011). QIIME (version 1.2.1, (Caporaso *et al.*, 2010) was used for constructing weighted and unweighted UniFrac distances. UniFrac is a commonly used phylogenetic distance metric to assess pairwise dissimilarity in community composition and incorporates information about differences in phylogenetic composition of community members (Lozupone and Knight, 2005; Lozupone *et al.*, 2011), with weighted UniFrac accounting for differences in the relative abundances of community members.

Statistical analyses

All analyses were performed using the R environment for statistical computing (R Development Core Team, 2011), with the aid of the vegan and ggplot2 packages (Wickham, 2009; Oksanen *et al.*, 2011). To compare temporal variability in diversity across habitats having inherently different diversities, we calculated the coefficient of variation (CV) in within-sample diversity for each community (Equation 1)

$$\mathrm{CV} = \frac{\sigma}{\mu} \quad (1)$$

where σ is the s.d. and μ is the mean.

We calculated median absolute deviation (MAD) to compare variability in between-sample diversity (Equation 2).

$$\mathrm{MAD} = \mathrm{median}_i(\left|\mathrm{X}_i - \mathrm{median}_\mathrm{j}(\mathrm{X}_j)\right|) \quad (2)$$

Finally, we calculated *z*-scores of richness to examine the step-wise variability of richness through time, across data sets (Equation 3).

$$z = \frac{\chi - \mu}{\sigma} \quad (3)$$

where χ is the raw value of richness, μ is the mean of the sample, and σ is the s.d. around the mean.

In using the coefficient of variation, median absolute deviation and *z*-scores of richness over time, we compared variability in diversity rather than absolute measures of diversity, which was most appropriate for comparing communities from different habitats that were assessed using different protocols for 16S rRNA short-read sequencing. To assess whether there were patterns in community structure that could be described by time between observations, we related community similarity/distance to time elapsed using Mantel tests with Pearson's correlation on 999 permutations.

We evaluated the decay of community similarity over time (time-decay) using the same methods for calculating decay of community similarity over space (distance-decay; Nekola and White, 1999; Soininen *et al.*, 2007). Though assessment of distance-decay is common in the literature, it is less common to assess time-decay. To assess time-decay in microbial communities, we used a similar approach adopted by Korhonen *et al.* (2010) to the meta-analysis of aquatic community time-decay. A log-linear model was fitted between the change in community structure (assessed by pair-wise similarities or distances, including Bray–Curtis, UniFrac and unweighted UniFrac) and days elapsed. Community dissimilarities were converted to similarities by subtracting from one. Similarities were log-transformed. The slope of the log-linear model is a rate of community change, sometimes referred to as turnover (Nekola and White, 1999). For plotting time-decay examples from each biome, we applied lowess smoothing over windows the length of 5% of the total series. Because time-decay can be sensitive to the duration of the study, we also performed a simple analysis of how quickly microbial communities change at temporal scales from 1 week to 1 month, from 1 to 6 months, from 6 months to 1 year, from 1 to 2 years, from 2 to 3 years, from 3 to 4 years, from 4 to 5 years and from 5 to 6 years. These windows encompass the breadth of time series durations included in the meta-analysis. For all pairs of observations within a community's time series, a rate of change was calculated by dividing Bray–Curtis dissimilarity by the time between observations. Next, all pairs of observations were partitioned into the appropriate temporal window (determined by the time between observations) and an average rate of change for each window was calculated. The global average rate of change was summarized across sites from the same habitat (reported in Supplementary Table S2).

STRs for each site were constructed in R by calculating richness using the moving window approach of White *et al.* (2006). This approach involves partitioning a time series into as many window subsets as possible given the number of observations and fitting the STR model (the power-law relationship between time and richness) at each window. For example, a 250-time point series could divided into one 250-point window, two 249-point windows, and so on. The power function, rather than the lognormal function, was used so that our results would be directly comparable with the results from communities of larger organisms reported in White *et al.* (2006). There is some debate regarding which function is most appropriate for describing the STR (McGill, 2003; White *et al.*, 2006), but White *et al.* (2006) found that the log and power functions produced identical patterns. We compared STR patterns in microbial communities with patterns for communities of larger organisms. However, microbial communities are widely considered to be more diverse than communities of larger organisms, and it remains difficult to make a direct comparison, as discussed at length previously (Fierer and Lennon, 2011).

There are caveats to the data sets used here that are worth highlighting. First, high-throughput sequencing of 16S rRNA genes can introduce biases in the determination of microbial diversity; these limitations have been described elsewhere (for example, Kunin *et al.*, 2010; Haas *et al.*, 2011; Quince *et al.*, 2011; Soergel *et al.*, 2012). Second, because sequencing depth varied across the sample sets (Supplementary Table S1), it is difficult to directly compare the temporal dynamics of individual taxa across the data sets (particularly taxa that are relatively rare). For this reason, we have focused our meta-analyses entirely on the overarching patterns in within- and between-sample diversity, which should be reasonably robust to differences in sequencing depth (Kuczynski *et al.*, 2010b); however, we also tested whether inter-biome differences were a byproduct of differences in

sequencing depth. Third, we picked OTUs using a closed-reference database protocol to compare data sets generated using different primers that target different variable regions of the 16S rRNA gene (as done in Caporaso *et al.*, 2011). Although this method allowed us to directly compare data sets using the same reference phylogeny, any sequences not matching the well-curated database were discarded from the analyses. Therefore, communities that had better representation of taxa in the database (for example, human-associated communities) had a higher proportion of taxon assignments than, for instance, flower communities, where a larger percentage of the sequences lack a close match to those found in the database. However, we found no relationship between the proportion of sequences that matched the reference database and patterns of temporal variability, suggesting that frequency of matches to the reference database did not bias overall patterns.

Results and discussion

Temporal variability in within-sample diversity

We used coefficients of variation calculated over each site's time series to compare the variability in community evenness (equitability of representation of taxa), richness (number of taxa) and phylogenetic diversity (breadth of lineages). Variability in these diversity metrics was generally consistent across sites representing the same habitat (Figure 1a). Evenness exhibited less variability than richness or phylogenetic diversity over time. This is likely due to the exceedingly uneven nature of microbial communities (for example, Dethlefsen *et al.*, 2008; Quince *et al.*, 2008), where there are few very prevalent community members and a relatively large number of rare members (a 'long tail' distribution). Variability in community structure (beta diversity), measured as the median absolute deviation in weighted UniFrac distances, exhibited similar patterns as the variability in other diversity metrics (Figure 1b). Supplementary Figure S1 additionally provides for each time series a visualization of every time point's deviation from the mean richness.

Variability in evenness, richness, phylogenetic diversity and community structure were all highly correlated (Figure 1c), demonstrating that both phylogenetic (phylogenetic diversity and UniFrac) and taxonomic (evenness and richness) approaches to community diversity revealed similar overarching patterns in temporal variability across sites. This was surprising because we did not necessarily

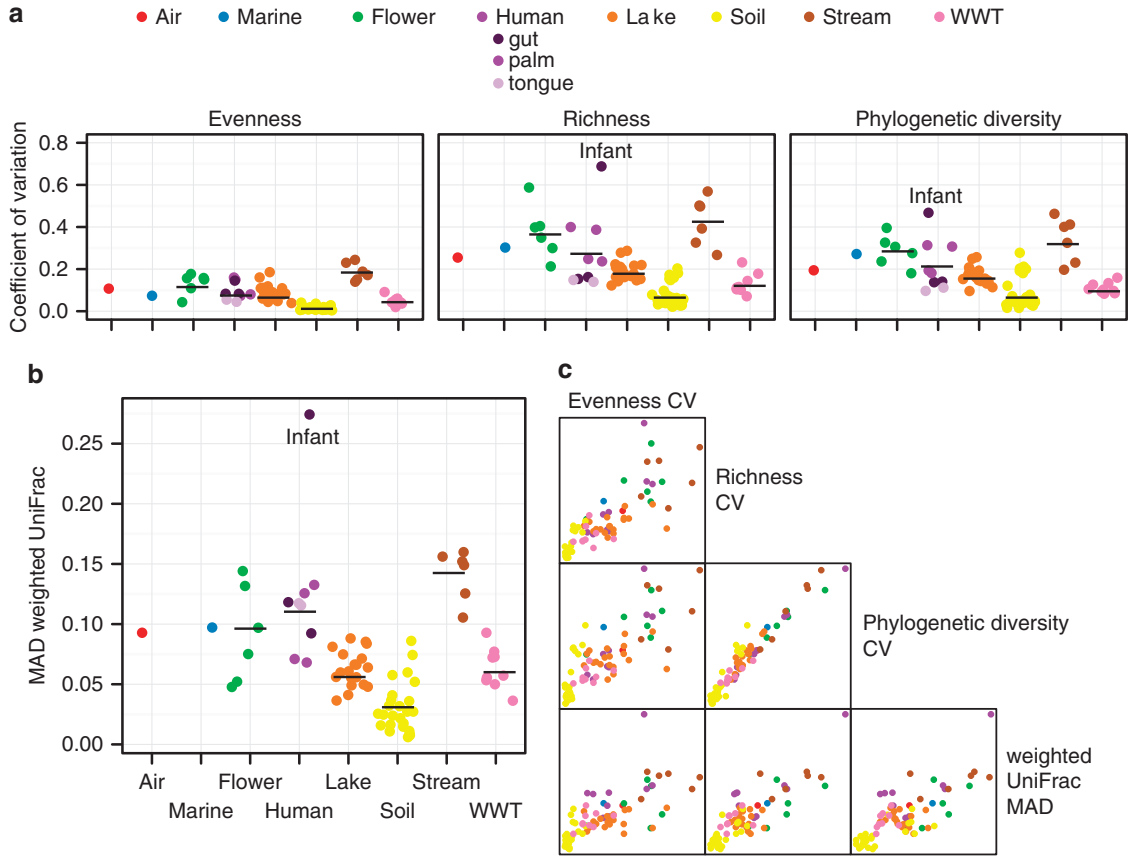

Figure 1 Temporal variability in (**a**) Pielou's evenness (equitability of representation of taxa), richness (number of taxa), Faith's phylogenetic diversity (breadth of lineages) and (**b**) weighted UniFrac distance, a community distance metric (between-sample diversity). Solid lines are the mean within each habitat. (**c**) Correlations in variability for each measure of diversity. All $R > 0.72$ and $P < 0.001$. Coefficient of variation (CV) and median absolute deviation (MAD) are unit-less metrics. WWT, brewery wastewater treatment.

expect phylogenetic metrics, such as phylogenetic diversity, and taxonomic metrics, such as richness, to vary consistently with one another in time. This result suggests that the diversity metric used to investigate temporal variability may not matter, allowing robust comparisons of microbial diversity measured in different ways across different studies. Furthermore, differences in temporal variability across sites and habitats were not likely an artifact of differences in survey effort (the number of sequences per sample) or an artifact of our ability to identify taxa against the reference database (Figure 2, Pearson's correlation, all *P*-values >0.10). These results suggest that there are often clear differences in the temporal variability in both within- and between-sample diversity within bacterial communities from different environment types.

Overall, soil and brewery wastewater treatment communities were consistently less variable than other community types (Figure 1). Though we cannot know *a priori* the drivers governing the observed rates of change, we can generate reasonable hypotheses based on knowledge of the biology and ecology of a microbial habitat. For example, we may expect a relatively low degree of variability in soil communities for two reasons. First, soil environmental conditions may simply be less variable at the timescale of the included studies (6 months) than some of the other habitats considered in this study. Second, soil communities contain a large proportion of dormant organisms (Lennon and Jones, 2011) and because our sequencing approach could not discriminate between active and inactive members, the communities may appear to change relatively little over the given time scale. Soils also have high spatial heterogeneity, which can mask changes in local communities with time because of high community variability across micro-sites. However, brewery wastewater treatment communities may vary minimally for different reasons. Wastewater treatment facilities have been engineered to perform a function (for example, nutrient removal Curtis and Sloan, 2006; Martín *et al.*, 2006; Harris *et al.*, 2012). Thus, a wastewater treatment system may represent an environmental filter for microorganisms that survive in wastewater treatment processes while system performance is maintained. However, operating conditions, such as feeding rate, and other environmental variables (including temperature) change over time in wastewater treatment facilities, as was true for the environmental conditions in the system studied here (Werner *et al.*, 2011). Therefore, these communities may have low variability simply because of an environmental filter, rather than because of dormancy or invariant environmental conditions.

Stream bacterioplankton communities, the infant gut community, flower communities and human palm communities were highly variable in diversity (Figure 1). Again, we can generate hypotheses as to the drivers of this variability based on the knowledge of the microbial habitat. Streams in the subalpine region studied here are flow-through systems with pronounced physicochemical instability. They experience frequent and rapid shifts in environmental conditions as well as shifts in bacterial inputs into the stream channel (from sediment, soil and biofilms) that are likely driven by pulse flashes in hydrological conditions (Portillo *et al.*, 2012). Human palm sites were previously noted to be highly variable (Fierer *et al.*, 2008; Caporaso *et al.*, 2011), and this was attributed to frequent exposure to bacterial inocula from a diverse array of touched surfaces and a high degree of temporal heterogeneity in environmental conditions (likely driven, in part, by the disturbances associated with washing

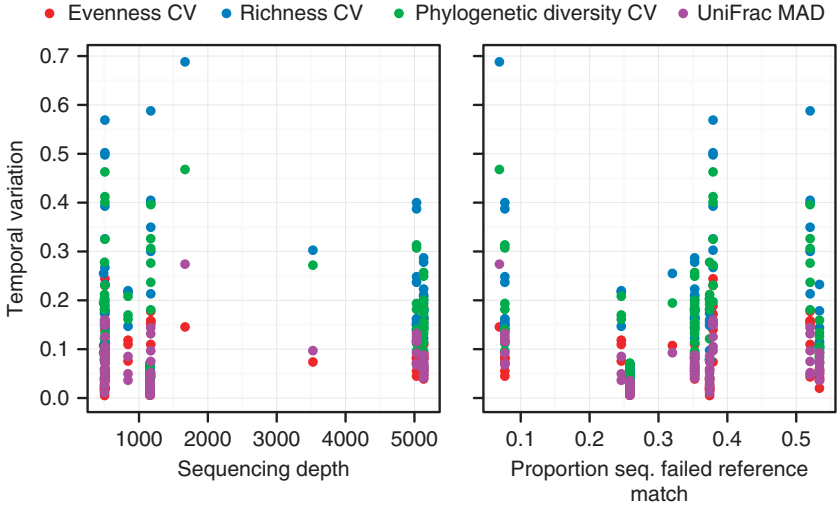

Figure 2 Temporal variability in diversity is not dependent on sequencing depth or the proportion of sequences that could be matched to a reference database for taxonomic assignment (all $P>0.10$). Coefficient of variation (CV) and median absolute deviation (MAD) are unit-less metrics and are used here to describe temporal variability in richness, evenness, phylogenetic diversity, and UniFrac distance.

events). In this way, palm and stream communities both experience environmental conditions that can vary considerably over time.

The time series that represent cases of microbial primary succession, the infant gut and flower surfaces, also exhibited a high degree of temporal variability in diversity. In both cases, there was microbial colonization of a sterile (or nearly sterile) habitat and rapid replacement of members through time as the environment is altered (Fierer *et al.*, 2010). Thus, just as primary succession in plant communities can often lead to rapid changes in diversity and community composition over time, we would also expect a high degree of variability in diversity for microbial communities undergoing succession.

Predictability in microbial community changes over time and across temporal scales

We next asked whether communities sampled close together in time were, on average, more similar in composition than communities sampled further apart. Approximately 26–40% of the 76 sites had community changes that were correlated with time over the entirety of study duration (Figure 3). Specifically, there were 34 significant relationships using Bray–Curtis, 26 relationships for unweighted UniFrac and 21 relationships for weighted UniFrac out of the 76 sites. Thus, in less than half of the sites did we find that samples collected at shorter intervals harbored communities that were consistently more similar than those collected at longer intervals. However, there was at least one site from each biome that exhibited community changes that were significantly correlated with time (Figure 3). Overall, these results suggest that temporal variability is not necessarily predictable solely by considering the time between sampling events.

Those communities that consistently exhibited temporal dependence included air, marine, human and lake communities. The air, marine and lake community time series were sampled over a duration that included seasonal changes, and thus temporal trends are expected for these sites (Shade *et al.*, 2008; Bowers *et al.*, 2012; Gilbert *et al.*, 2012). Further, human-associated microbial communities sampled consecutively over time have been shown to be more similar than those sampled more distantly in time (Costello *et al.*, 2009), which is consistent with our detection of a temporal trend for these communities. These results suggest that overall temporal signals exist for communities from certain habitats and that, for communities from these habitats, the relationship between community structure and time is insensitive to the choice of metric used for evaluating community structure (Figure 3).

Several habitats had no or few communities that exhibited correlations between time and community structure (Figure 3). For example, depending on the metric used, anywhere from five to none of the 25 soil time series exhibited significant temporal dependence (Figure 3). For soil communities, inter-annual changes would not be evident in this data set, as the study durations were $\leqslant$6 months. Thus, it may be that changes in soil communities are not correlated with time at the time scale included but that such relationships could become evident if we had time series extending across full years or multiple years.

It is also not surprising that the structure of brewery wastewater treatment communities was often not correlated with time, as any changes in operation would not necessarily be related to time. For the flower communities, the taxonomic metric, Bray–Curtis, detected a significant temporal signal

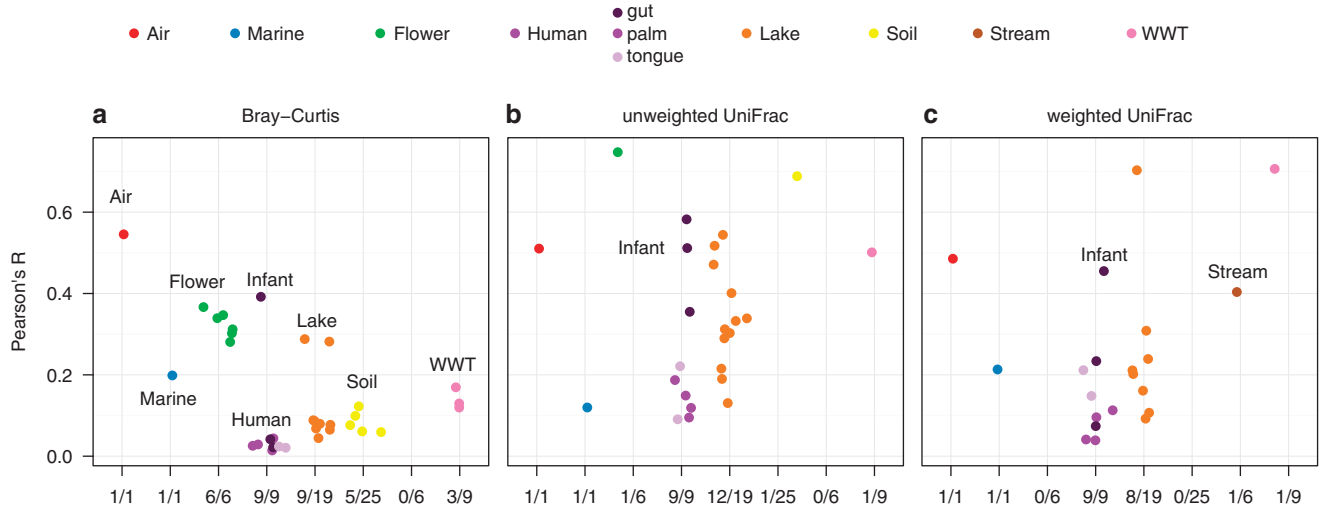

Figure 3 (**a–c**) Changes in community structure were related to time between community observations. The x axes labels give the number of significant relationships over the total sites from the habitat. WWT is brewery wastewater treatment. All points shown had Mantel $P<0.05$ on 999 permutations; tests that were not significant are not shown. For example, all six flower communities (green points) were significant using Bray–Curtis, one was significant using weighted UniFrac and none were significant using unweighted UniFrac metrics.

(Figure 3a) where the phylogenetic metrics did not (Figures 3b and c), likely because closely related taxa were replacing one another (Shade *et al.*, 2013), a change to which a phlyogenetic metric would be less sensitive. Only one of the stream communities was correlated with time, and this correlation was evident only when using the weighted UniFrac distance metric (Figure 3c). Because environmental conditions in the sampled streams are flashy with irregular pulse distances (Portillo *et al.*, 2012), changes in stream communities may not be correlated with time but rather with the occurrence of these infrequent events.

Plant and animal communities often (but not always) become less similar with increasing time or geographical distance, a phenomenon known as similarity-decay (Nekola and White, 1999). To determine whether there was decay of microbial community structure over time (time-decay), we fit log-linear models to community similarity over differences in time between sample collections (Nekola and White, 1999; Korhonen *et al.*, 2010; Figure 4). The rate of temporal change in community structure is the slope of the model, and this slope is a measure of community turnover. A community that is not changing over time would have a slope of zero. More negative, steeper slopes indicate a faster rate of change than less negative slopes. In Figure 4, very gradual time-decay is evident, even for microbial communities that exhibit seasonality, such as the coastal marine and lake sites, as well as for sites that have high temporal variability, such as the stream community. Overall, 31–40% of microbial communities exhibited significant time-decay in community similarity (Supplementary Figure S2, $P<0.05$). Slope was not correlated with richness (all $P>0.05$ for all similarity metrics), suggesting that differences in the number of taxa detected in individual samples did not influence outcomes. Slopes were barely negative in most cases. However, one flower (Gala 2), one soil (HI_R12) and one brewery wastewater treatment (E1) had slopes between -0.02 and -0.03. Aside from these exceptions, the consistency of microbial time-decay suggests that community turnover is generally quite slow.

Time-decay can be sensitive to a study's duration. Therefore, we asked at what temporal scale communities changed the most quickly for the study duration and sampling intensities of observations that were available to us. To do this, we calculated a simple rate of change by dividing pair-wise Bray–Curtis dissimilarity by the time between observations and then partitioned observations into nine broad temporal ranges, from 1 day to 6 years (Supplementary Figure S3), representing the breadth of the available time series. This analysis also showed a consistent trend of slower change with longer durations of time, and additionally reveals gaps in the currently available time series, and points to the temporal resolutions that are yet unknown for certain habitats (Supplementary Table S2).

Notably, just because microbial community turnover is very gradual at long study durations does not mean that there is no change occurring in these communities. Rather, it suggests that there are not drastic or new changes occurring, such as the addition of new or phylogenetically distinct taxa. Also, it is possible that some of the communities may be changing around a 'baseline' of normal variability, following the conceptual model of ball and urn for alternative stable states (Beisner *et al.*, 2003).

STRs

STRs describe the accumulation of richness in a community, over increasingly longer durations (for example, Preston, 1960). The exponent of the STR provides an indication of the rate at which new taxa are observed in a community over time; the higher the exponent the more new taxa are introduced over time. Depending on the scale of the study and the community of interest, STRs can be explained by sampling, ecological or evolutionary effects (Preston, 1960; White *et al.*, 2006, 2010). Over short-time periods, incomplete sampling effort (duration or intensity) often drives STRs, but evolutionary processes such as speciation and extinction become more important for STRs over longer-time periods. Between these extremes, ecological processes such as meta-community dynamics are important for STR, including dispersal of transient tourists into a regional species pool (Magurran and Henderson, 2003). Though the STR spatial equivalent, the species–area relationship, remains actively investigated in microbial ecology (for example, Horner-Devine *et al.*, 2004; Fierer and Jackson, 2006; Green and Bohannan, 2006; Martiny *et al.*, 2006; Woodcock *et al.*, 2006; Bell, 2010), STRs are less often documented for microorganisms. However, STRs have been reported for communities from diverse environments, such as on leaf surfaces (Redford and Fierer, 2009), in streams (Portillo *et al.*, 2012) and in bioreactors (Van Der Gast *et al.*, 2008), hinting that STRs may generally apply to microbial communities.

We found that all communities had significant STRs ($P<0.05$, Figure 5a). Microbial STRs were not related to study duration or sequencing depth (Pearson's correlation $P=0.81$ and 0.36, respectively), suggesting limited, if any, influence of sampling. However, our analysis does not distinguish stochastic processes (for example, random presence and absence of taxa) from deterministic properties in driving the STR, and both of these likely contribute. There was a very consistent taxa–time relationship across microbial communities, ranging from 0.24 to 0.61. Communities had comparable STR exponents within biomes, again highlighting the within-biome consistency. Furthermore, differences in the STR exponents across biomes could be explained by known ecological attributes of

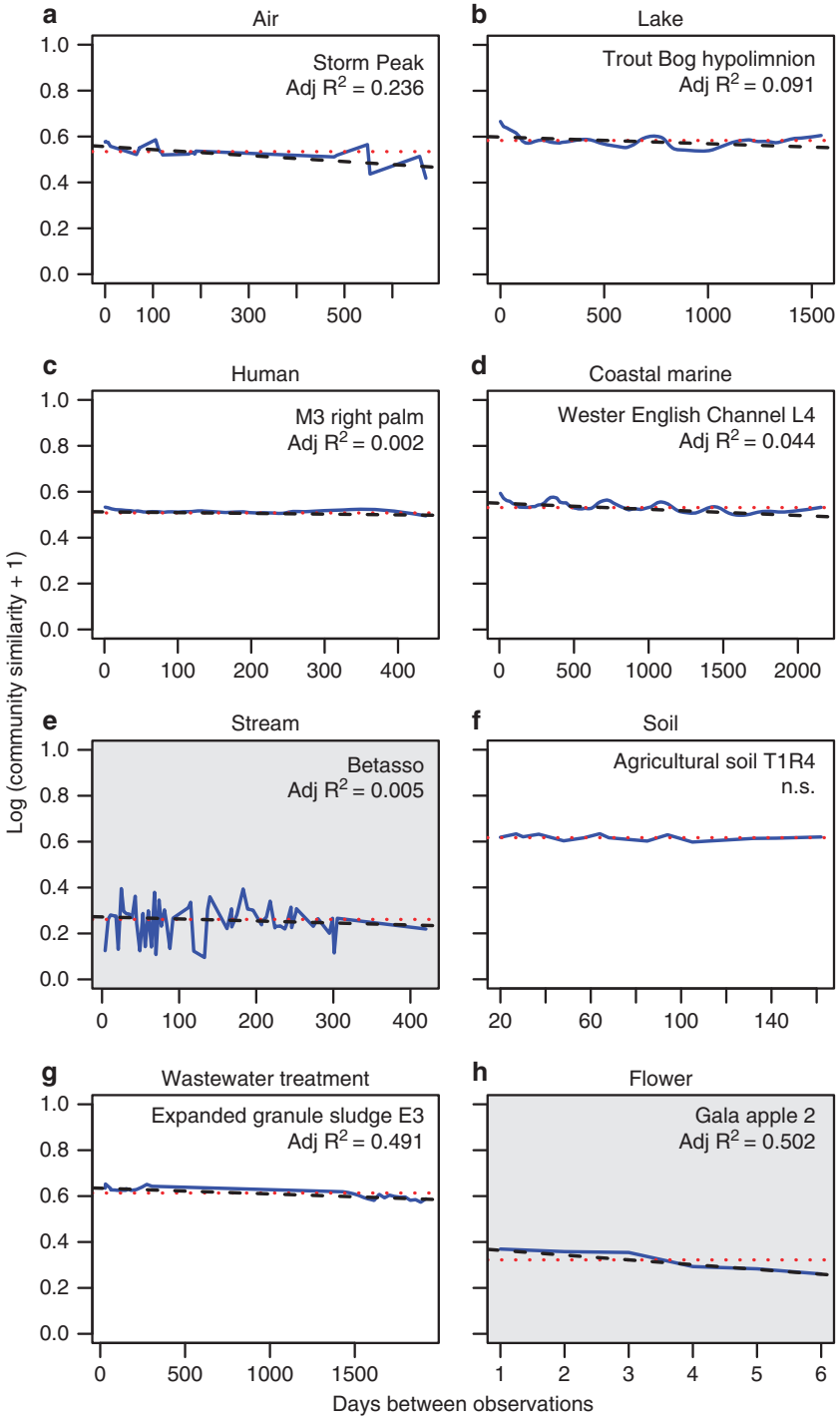

Figure 4 (**a**–**h**) An example of time-decay from each microbial habitat. White chart background shows that the model was fit using weighted UniFrac distances and gray shows that the model was fit using unweighted UniFrac distances. Note differences in x axis ranges. The blue line is the smoothed series of community similarities, the dotted red line is the overall mean similarity and the dashed black line is the log-linear time-decay model. NS, not significant.

the communities. For example, the air and stream communities, with high flow-through and heterogeneity, had high STR. The infant gut and flower primary succession communities also had high STR. The soil communities had the lowest STR exponents, demonstrating that despite their high levels of diversity (for example, Elshahed *et al.*, 2008), the taxa present at a given location do not change appreciably over time (a pattern also noted in Fierer and Jackson, 2006). These results are consistent with the general trends in diversity over time as seen in Figure 1 and suggest that fluctuating abiotic

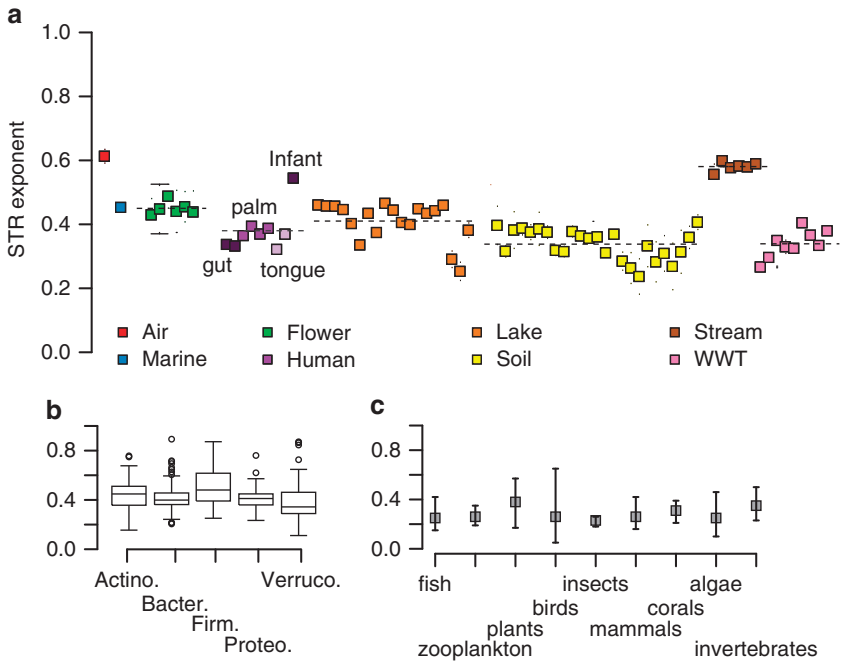

Figure 5 STR exponent, *w*, from power-law fit to the accumulation of taxa detected with time, as per White *et al.* (2006). (**a**) Microbial community STR across habitats. The dashed line indicates the average exponent value for each habitat. Error bars are s.e. but, in most cases, are too small to visualize on the chart. WWT is brewery wastewater treatment. (**b**) Microbial STR exponent for phyla detected across all habitats: Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria and Verrucomicrobia. White circles are samples that had STR values beyond range times the interquartile range (potential statistical outliers). (**c**) Average STR exponent by taxonomic group for larger organisms, reproduced from Figure 3 of White *et al.* (2006). Error bars are exponent ranges for each taxonomic group.

conditions drive higher microbial STRs. These environmental fluctuations potentially allow for time series to effectively encompass a wider range of microbial niches (Shurin, 2007).

We next asked whether STRs could be distinguished at the phylum level. For each community, we calculated STRs for common phyla observed within each habitat (Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria and Verrucomicrobia), at each site. We found a range of STRs at the phylum-level, but the exponents had comparable means across phyla (Figure 5b). This suggests that microbial phyla do not have inherently different rates of replacement but that the local ecology drives the observed STR within a community. One exception to this may be the Firmicutes, which had a slightly higher mean STR and wider upper range than the other phyla. We speculate that this pattern may be a product of many taxa affiliated with Firmicutes being capable of sporulation (Onyenwoke *et al.*, 2004) and may either have higher rates of dispersal into communities or are able to persist in communities in very low abundances when conditions are unfavorable and bloom when conditions become favorable. However, taxa affiliated with other phyla are also capable of persisting in dormant states, and more evidence is needed to address this hypothesis.

It can be difficult to directly compare STR patterns of microbial communities with communities of larger organisms. First, most microbes probably have generation times shorter than those of larger organisms, and the microbial time series investigated here likely included far more microbial generations than comparable study durations of plant and animal taxa. In addition, species definitions as applied to microbes are distinct from how plant and animal species are typically defined (Stackebrandt *et al.*, 2002; Gevers *et al.*, 2005). Finally, microbial communities are often more diverse than communities of larger organisms, with individual samples harboring hundreds to thousands of taxa as compared with the tens of taxa reported in White *et al.*, (2006). However, despite these caveats, the range of STRs for microbial communities was the same as that reported in White *et al.*, (2006) meta-analysis of STR for larger organisms (Figures 5a and c). On average, STR exponents are higher for microorganisms than for larger organisms, and the exponents were less variable within communities. Though White *et al.* (2006) found a relationship between STRs and richness in their meta-analysis, we found no such relationship for microbial communities ($P=0.19$), suggesting that microbial community STRs are better explained by changes in environmental conditions than by local richness. However, the slightly higher average STR for microbial communities over macrobial communities may be due to the generally higher richness of microbial communities. Random changes in the occurrences of these community members may partly be driven by the stochastic process of drift (Vellend, 2010), which could also contribute to the STR. Nonetheless, the overall consistency in STRs

across microbial and macrobial communities is striking, suggesting that this may be an ecological pattern that is ubiquitous across scales, phyla and habitats.

Conclusions and future directions

From this meta-analysis, we gain a preliminary understanding of how temporal patterns in microbial communities compare with each other and with those of communities of larger organisms. There was consistent variability in diversity and STRs across communities from similar habitats. This consistency can be leveraged when making logistical decisions about sampling regimes and for understanding baseline levels of temporal variability. Within a habitat, these temporal patterns may represent the equilibrium from which disturbance events may alter microbial dynamics. This information is important for identifying when a microbial community is experiencing a disturbance, anticipating how quickly a community may recover from such events, and determining if and when a community has recovered post-disturbance (Allison and Martiny, 2008; Shade *et al.*, 2012a; Shade and Peter *et al.*, 2013).

A challenge in conducting this meta-analysis was the availability of directly comparable microbial time series collected across habitats. The same protocols and standards of quality across data sets were necessary for this undertaking, and though there are 76 microbial communities that span 8 distinct microbial biomes, there are obviously many habitats that were not represented in the meta-analysis simply because data were not available. Further, some of the available time series were limited in duration or sampling intensity, and therefore, it cannot be determined whether temporal patterns were not discovered because they truly do not exist or because of a limitation in the sampling effort. For example, we may observe seasonality in soil microbial communities sampled over 6 years instead of over 6 months. With the increased availability of high-throughput sequencing, the number of higher-resolution, longer-term time series will only increase. For example, the Earth Microbiome Project is leading a concerted effort to collect and curate high-quality microbial community sequencing data and corresponding contextual data from diverse environments (Gilbert *et al.*, 2010). Placing microbial community dynamics within the rich context of environmental dynamics will provide insight into the key drivers of those communities, help to explain discrepancies in patterns across communities from similar habitats, and allow ecologists to begin predicting microbial dynamics. Efforts like these will help to build theory for microbial ecology, advancing beyond system-specific observations (Prosser *et al.*, 2007). Additional discussion about challenges with sampling microbial communities in time (including defining a microbial community, sampling the same community longitudinally and accounting for micro-scale spatial heterogeneity) is available as a Supplementary Discussion.

This meta-analysis highlights that microbial communities, like plant and animal communities, are variable with time; that microbial temporal dynamics are dependent on the habitat type in question; and, furthermore, that microbial temporal dynamics are often predictable. Perhaps more importantly, this work demonstrates the utility of time course analyses in microbial ecology. Likewise, this meta-analysis highlights that microbial communities represent a useful system for studying temporal dynamics in communities, dynamics that would be very difficult to explore in plant and animal communities where generation times are often far longer. For example, it is often easier to execute disturbance experiments with microbial communities than with plant and animal communities, and microbial responses can be detected over relatively short-time periods (for example, Dethlefsen *et al.*, 2008; Shade *et al.*, 2012b). These kinds of directed, *in situ* experiments with microbial communities may provide key empirical validation (or invalidation) of theoretical paradigms.

Just as comparing the spatial patterns exhibited by microorganisms versus larger organisms has yielded interesting findings that have allowed us test paradigms in biogeography (for example, Horner-Devine *et al.*, 2004; Fuhrman *et al.*, 2008; King *et al.*, 2010), paradigms almost wholly derived from research on plant and animal communities, we can use microbial communities to build a more comprehensive understanding of time–biodiversity relationships. The continued integration of microorganisms into the broader field of ecology will clearly be advantageous for both 'macro'-bial and microbial ecologists, providing rich insight into the common forces that shape patterns of distribution and diversity for organisms of all sizes.

Conflict of Interest

The authors declare no conflict of interest.

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