Supplementary Discussion

for

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

by

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There are three particular challenges to sampling microbial communities in time that require additional discussion. These challenges include: 1) using a metagenomics approach to observe microbial communities inclusive only of ecologically meaningful members, 2) repeatedly observing the same microbial community over time, and 3) sampling distinct microbial communities on a scale appropriate to the micro-spatial niches in an environment.

Here, we adopt a definition of a microbial community discussed by Konopka (2006), which includes two potentially overlapping components: interactions among members of community members, and also interactions of those community members with their local environment. Metagenomics approaches may include many microbial taxa that are arguably irrelevant for the community, including very rare or transient taxa (*e.g.,* Shade and Handelsman 2012). The ecological roles of these rare organisms, sometimes referred to as the "rare biosphere" (Sogin *et al.* 2006), are largely unknown, and so it is difficult to justify removing or including them from analysis, though there are statistically methods available for removing them prior to analysis (Gobet *et al.* 2010). Therefore, we have chosen to maintain the rare members of the community in the meta-analysis. Despite their inclusion, ecologically interesting patterns have emerged from the meta-analyses, suggesting that at least some of the detected rare members may be contributing to a microbial community's temporal dynamics.

Second, sampling issues (*e.g.,* destructive sampling of material or being unable to return to the precise location of the previous sample) often prevent microbial ecologists from observing the same community over time. This is a common concern in microbial ecology. However, it is a concern that we can begin to investigate with the datasets in hand. Time series analysis provides an informative method for understanding potential interactions among microbial taxa and their environment, allowing us to investigate the fundamental parts of microbial communities, through patterns of co-occurrence and persistence among microbial taxa (*e.g.,* Shade and Handelsman 2012) and through analysis of data in a series rather than in un-replicated snapshots (Lennon 2011). For example, if we consistently and repeatedly detect a certain taxon in a locality, there is a high likelihood that this taxon is a member of the community rather than a transient passer-by. A few studies have taken advantage of time series to understand co-occurrences of taxa and narrow the definition of the microbial community (*e.g.,* Beman *et al.* 2011, Eiler *et al.* 2011), and studies like these further support that at least a subset of the sampled community likely have interactions with each other or with the environment. We suggest that considering microbial communities in time provides a baseline for community changes, and for quantifying and distinguishing stochastic noise that can come from sampling issues, and then separating these processes from deterministic responses. These questions will have to be addressed for each habitat before comparing across ecosystems, and there are some habitats for which more or longer examples of time series are needed (*e.g.,* soils).

Finally, microorganisms experience great habitat heterogeneity over relatively small spatial extents, and samples of microbial communities likely include a lot of microhabitats in which possibly distinct microbial communities live. It remains a difficult challenge to compare the spatial heterogeneity across very different habitats, as comparable masses, volumes or surface areas, for instance, of soils and human fecal matter and freshwater, do not necessarily represent the same level of heterogeneity within these systems. This is an important consideration for the meta-analysis of this work and well worth noting. We have not investigated spatial questions specifically because the heterogeneity in space and distribution of microbial taxa in space is a fairly common question that has been investigated in an array of microbial communities. For example, microbial community diversity has been considered in the context of: biogeography (*e.g.,* Martiny *et al.* 2006), latitudinal gradients (*e.g.,* Furhman *et al.* 2008), cross- ecosystem gradients (*e.g.,* Fierer *et al*. 2012), and altitude gradients (*e.g.,* King *et al*. 2010). However, the temporal gradients in microbial communities have been investigated less frequently, and cross-biome temporal analyses remain especially rare. Therefore, we have chosen to focus this work on the relatively unexplored aspect of the temporal analyses, which until recently was impossible because high quality, sequencing-based time series of microbial communities simply did not exist before high throughput sequencing became cost-efficient.

Additional References

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