Next-generation experiments linking community structure and ecosystem functioning

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Limits of ‘omics surveys

A key broad goal in microbial ecology is not just to describe community structure, but to link structure and function. "Structure" refers to the taxonomic composition of the community. "Function" refers to some ecosystem-level impact of the community; ecologically important examples include functions linked to biogeochemical cycles (e.g. the rate and variation of nitrogen fixation, community productivity, decomposition) and to their interactions with the rest of the food web (e.g. plant growth, host mortality). There is also an interest in understanding the link between structure and function in industry, medicine, and agriculture, including, for example, linking microbial community structure to the quality of cheese or wine, the rate and stability of sewage degradation, or the lifespan of a mouse or a person. Understanding the link between structure and function would allow us to move beyond the descriptive natural history of microbial communities, and could result in an ability to manipulate useful or beneficial functions.

There is great hope that ‘omics approaches will solve the problem of linking structure and function by identifying which genes are present and which are switched on while also measuring ecosystem functioning (Gilbert *et al.*, 2016). While ‘omics tools play a key role in structure-function research, there are well-recognised problems in taking a purely observational approach. It is challenging to separate cause and effect when functions, community structure, and environmental conditions covary. Even if a gene is present and transcribed, the degree to which this metabolic effort is realised in the "functions" can be highly context-dependent. For example, the ability of exoenzymes to degrade substrates will depend on temperature, pH, and other factors in the environment, some of which might be modified by the surrounding community. It is therefore clear that hypothesis-generating descriptive studies require external validation through direct manipulations of community structure.

Koch's postulates for ecosystem functioning

Molecular-based surveys of microbial communities have generated more data than they can validate: " ...never before have we witnessed such a rate of development of technology in molecular biology, and the hypothesis-driven science may find it hard to keep up." (Gilbert and Dupont, 2011). Experimental validation has clearly lagged behind the quantity of data produced by molecular surveys, so the time seems appropriate to reverse this trend. I expect the discoveries for the next generation of structure-function studies will result from closing the loop between the hypotheses generated by molecular surveys and experimental validation of those hypotheses.

Unfortunately, experiments that validate molecular surveys are often difficult to conduct. How do we take apart a community to see how it works? One experimental approach has been to assemble communities from their constituent species (Bell *et al.*, 2005). The main criticism of this reductionist approach is that the communities constructed in this way are unlike anything found in nature, and this loss of realism limits their relevance. After all: “If you try and take a cat apart to see how it works, the first thing you have on your hands is a nonworking cat.” (Adams, 2002). If synthetic communities are "nonworking cats", there are a growing number of alternative approaches that make use of natural variation in extant communities (Rivett and Bell, 2018). Discovering which parts of a community can be taken away without altering functioning would tell us about the which components of community structure are important, allowing us to differentiate between a “heart” and a “whisker”.

Consider a case where there is a positive association between a taxon and a measure of functioning. In this case, validation of this link would require two demonstrations: First, experimental additions or increases in the abundance of the taxon results in an increase in functioning. Second, the experimental removal or reductions in the abundance of the taxon results in a decrease in the function. Fortunately, unlike for macro-organisms, microbial community structure can be manipulated in this way at ecologically-relevant spatial and temporal scales while maintaining realistic environmental conditions.

Next generation experiments

In general, experiments would take the extant variation in natural communities as the starting point and either remove or add taxa to the communities to test how the manipulation impacts ecosystem functioning. The key feature of these experiments is that the manipulated and unmanipulated communities have the same environmental conditions- this feature allows analyses that directly explain variance in functioning according to differences in community structure (Rivett and Bell, 2018). There are then 4 plausible manipulations (Figure 1), which have received varied attention in isolation but have not been tied together into a cohesive framework for validating associations.

1. Invasion. The simplest manipulation is to conduct experimental invasions of new or existing populations to alter the presence/absence or relative abundances of the invader taxon. The invasion ecology of free-living microbes is poorly understood, and few experimental releases have been tried in the context of ecosystem functioning (Mallon *et al.*, 2015; Rivett *et al.*, 2018). The main obstacle is in isolating relevant invader taxa in pure culture (i.e. that corresponded to the associations found in observational studies). However, if the taxa of interest were culturable, experimental invasions of by proscribed environmental isolates would provide a clear test that would demonstrate a causal link, at least showing whether the invader was a sufficient explanation for the association.

2. Coalescense. An alternative that does not require isolation of pure cultures is to conduct community coalescense experiments, which compare functioning of communities on their own with functioning of communities that are mixed ("coalesced") (Rillig *et al.*, 2015; Sierocinski *et al.*, 2017). Community coalescence alters community structure, allowing investigations of whether the coalesced community deviates from levels of functioning that are expected from the constituent communities. This is particularly relevant for cases where the combined action of several strains are hypothesised to contribute to a function. The main obstacle to this line of research is in obtaining communities that have relevant structural properties.

3. Fragmentation. The alternative to addition experiments is to selectively remove taxa. The simplest kind of removal experiment is to artificially fragment a community using serial dilution (Franklin *et*

al., 2001; Philippot *et al.*, 2013). Such experiments have been relatively common, but have not been used to test specific hypotheses generated by observational studies. The drawbacks are similar to those of coalescence experiments: the community structures that can be created are constrained by the extant variation in community structure. Serial dilution will progressively retain the most common taxa, so some taxa will always be retained together, which could be problematic for testing some associations.

4. Knockout. The most profitable approach would be the targeted removal of taxa in the community. While removal or inhabitation of broad groups of microbes is possible (e.g. using antibiotics or fumigation) (Degens, 1998; Griffiths *et al.*, 2000), knocking out specific targeted taxa is not possible. Potential future technologies are possible (e.g. using phage with narrow host preferences), but unlikely to be widespread for the foreseeable future.

Living microbiome archives

Combining addition (invasion, coalescence) and removal (fragmentation, knockout) experiments should become the gold standard for validating structure-function associations. Over the next few years, I would expect to see the transition from searching for associations between structure and function, to predicting what will happen when certain taxa are excluded or added in different community contexts. A critical component of this research is to work with communities that are well characterised, that have variable community structure, and critically, that can be used repeatedly. Although repeatability is a crucial component for most research programs that use pure cultures, repeatability has been neglected in research that uses complex communities because of the difficulties of preserving living microbial communities. It is therefore key to develop standard methods for preserving whole microbiomes that preserve the living community while minimising alterations to community structure. Living archives of whole communities could then be used as the raw material for experiments, and would substantially speed up the usage of addition and removal experiments as a standard method for validating associations.

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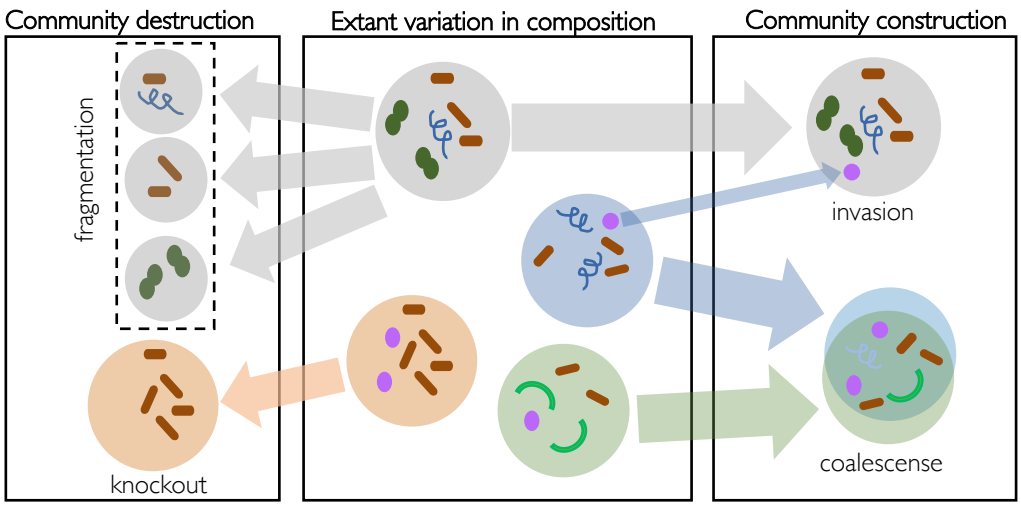
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Figures

Figure 1. Community manipulations to validate structure-function associations. Each circle is a community, and the shapes represent different microbial taxa that inhabit those communities. Extant variation community in community structure is illustrated in the central panel, while left and right panels show the different kinds of addition and removal manipulations.

Figure 1


Community destruction
Extant variation in composition
Community construction
fragmentation
invasion
knockout
coalescense