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Spotlight
Does Microbial Diversity Confound General Predictions?

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Microbes show more geographic diversity than previously expected, a serious challenge for ecological prediction. However, a recent study shows that microbial communities from a global grassland plot network responded consistently to nutrient addition. These results highlight the risks of nutrient deposition, but also hope for generalized understanding of microbial communities.

The explosion of DNA sequencing power has finally enabled microbiologists to begin the large task of mapping the diversity of microbial life across the planet. While the high local diversity of microbes has long been appreciated, recent large-scale sequencing studies have revealed much greater degrees of regional variation than previously expected, even in similar ecosystems [1–4]. For example, Talbot *et al*. [1] found that there were almost no species of fungi shared between similar forest types in eastern and western North America. The explanations for this variation are of course complex, and likely involve barriers to dispersal, spatial variation in climate, environment, and resources, as well as competition with other microorganisms. While understanding the ecological and evolutionary factors that generate this diversity is fascinating, high complexity systems have always been challenging for ecological prediction and raise the specter that each regional community may have its own unique rules. As a result, the incredible regional diversity of microbes raises concerns that it will be impossible to develop general theories and predictions about microbial communities.

Making such predictions is critical in the global change era. Microbial metabolic activity and biomass turnover drive the biogeochemical cycles that support human agriculture and natural ecosystems [5]. In the last century, human activities have increased global phosphorus mobilization by fourfold [6] and have doubled the amount of the annual nitrogen fixation in terrestrial ecosystems [7]. As soil microorganisms are involved in critical steps of N and P cycles, the anthropogenic addition of these nutrients in the last decades is likely to impact the soil microbiome. However, individual studies in different parts of the world have shown varying degrees of responsiveness of microbial communities to anthropogenic stressors [8,9]. In addition, most studies tend to look at a single taxonomic component of the microbiome, either Bacteria, Fungi, or Archaea. These groups have both overlapping and unique functions in the soil. For example, both bacteria and fungi are involved in plant nutritional symbioses, but bacterial symbionts obtain N through fixation of atmospheric nitrogen, while fungi scavenge nitrogen and phosphorous from organic and inorganic sources in the soil. All groups have free-living taxa involved with organic matter decomposition, but often with different specializations. For example, anaerobic cellulose decomposition is primarily carried out by bacteria, while fungi are the primary agents of lignin decomposition. Similar patterns are found in key nutrient transformations, such as the mineralization of nitrogen or phosphorus. Archaea are often the most abundant ammonia oxidizers in terrestrial soils, and bacteria are the most common denitrifiers. As a result of these differences, the response of one microbial group to anthropogenic perturbations may not be predictive of others.

For these reasons, it has been difficult to predict whether microbial responses to anthropogenic perturbations will be similar across different geographic regions (Figure 1A,B) or contingent upon local community composition (Figure 1A,C). Against this backdrop, a recent paper from Leff *et al.* in PNAS [10] is particularly noteworthy, because it addresses twin challenges of generalizing microbial responses to nutrient deposition both across the globe, and across major microbial taxonomic groups. Using standardized sampling and DNA sequencing methods, the authors characterized the soil microbial community across a global network of grassland research sites spanning four continents. For the last four years, experimental plots within each site have been subjected to N, P, or N+P fertilization to mimic anthropogenic nutrient deposition [3]. Despite distinct differences in soil physico-chemical characteristics and microbial composition across these 25 grasslands, the authors found a number of consistent responses in the composition of microbial communities. While the paper contains a wealth of details and data that microbial ecologists will mine for years, perhaps the primary finding was that nutrient deposition tended to shift the microbial community toward fast-growing (or copiotrophic) organisms. For bacteria this was indicated by increased abundance of the phyla *Actinobacteria*, *Alphaproteobacteria*, and *Gammaproteobacteria*, relative to *Acidobacteria*, *Planctomycetes*, and *Deltaproteobacteria*. This was accompanied by reductions in expected genome size, and a reduction of particular gene ontology categories, such as metabolism of
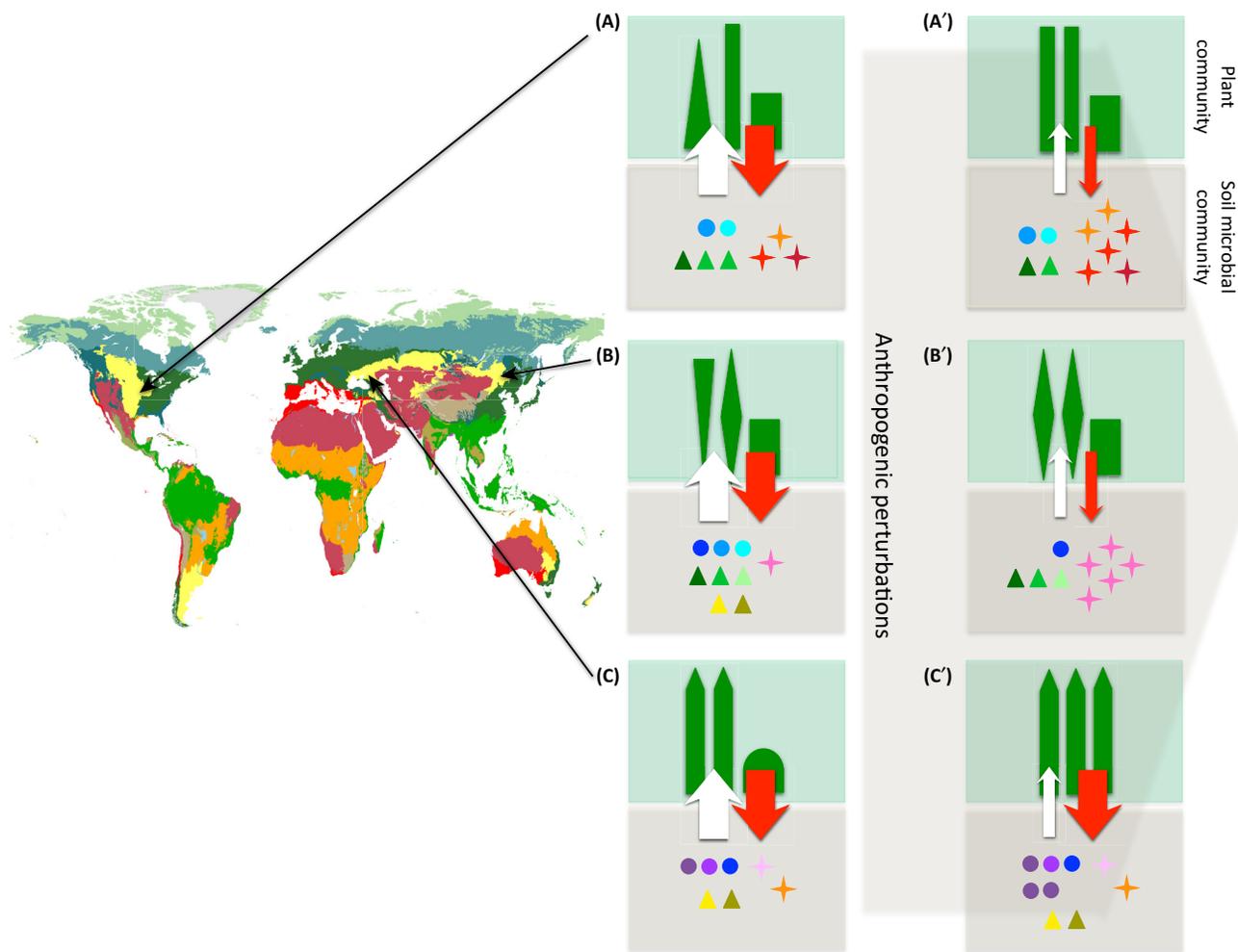
Figure 1. Potential Outcomes of Anthropogenic Perturbations on Soil Microbial Communities. (A–C) represent three different hypothetical ecological systems within the same biome (e.g., temperate grasslands). Composition of microbial communities [(symbols correspond to major phylogenetic groups and colors different operational taxonomic units (OTUs)] differs between the sites, as demonstrated by recent work on microbial biogeography. The figure shows two different scenarios by which these regional differences might alter response to anthropogenic nutrient deposition. (A) and (B) are examples of a deterministic response, where despite differences in OTUs, similar taxonomic groups dominate after nutrient addition. The hypothetical community at (C) responds differently from (A) and (B), indicating that community response is contingent on the unique biogeographic history of this site. The study of Leff *et al*. provides evidence for deterministic response of microbial communities shown in (A) and (B). Whether microbial communities respond to global changes deterministically or contingently may have strong consequences for ecosystem functions, such as symbiotic nutrient uptake by mycorrhizal fungi (Red arrows: carbon transfer; white arrows: nitrogen and phosphorus transfers. Larger arrows show larger transfers). Map modified from [13].

lipid and other amino acids, genetic information processing, enzyme families, folding, sorting, and degradation, signal transduction and transcription, within the soil metagenome. In fungal communities the abundance of fungi involved in symbiotic nutrient uptake decreased while the abundance of Ascomycota - often fast growing saprotrophs - increased. The Archaeal community displayed a decrease in diversity but a higher abundance in

N treatments, likely due to increased abundance of ammonia oxidizing Archaea and a reduction of methanogens. One particularly interesting finding is that initial nutrient content and soil chemistry were not the best predictors of the magnitude of microbial response across sites. Rather, the size of the microbial community response was most strongly correlated with the size of the plant community response to nutrient additions. This finding

may help with prediction (i.e., sensitive grasslands likely have sensitive microbes), but it also means that it is not clear from this study alone if nutrient additions directly affected the microbial community, or indirectly affected it through changes in the plant community.

To some the results found by Leff *et al.* [10] may not come as a huge surprise - many of these results have been shown

individually in other studies – for example, mycorrhizal decline with nutrient addition has been documented many times [11]. However, it is the first time that such a consistent response of soil microorganisms has been shown at the global scale, and it is an important step forward in the global understanding of ecosystems and their response to anthropogenic perturbations. In addition, it is further proof of principle that functional categorizations (phylogenetic, guild, or genomic) can provide a unifying window into the response of microbial communities even when they share few species in common.

In addition to providing a model for future considerations of microbial response to anthropogenic change, the study by Leff *et al.* [10] also raises several challenges for forthcoming studies. First, while Leff *et al.* characterized taxonomic composition of Bacteria, Fungi, and Archaea, current metagenomic methods do not adequately capture genomic diversity of Fungi. Fully characterizing functional response of microbial communities will require better methods for eukaryotic metagenomics. Similarly, it is still necessary to identify the mechanisms (direct vs. indirect) that caused these communities to change, and predict how these changes in the microbial community will influence N, P, and C cycles. In addition, while this study focused on

commonalities across systems (Figure 1 A,B), it is equally important to know to what extent the unique biogeographic history of each site influenced microbial community responses, or how different biomes (i.e., forests, alpine, or agricultural systems) might respond to similar perturbations (Figure 1 A and B versus C). While it is tempting to think of anthropogenic perturbations on microbial communities as having either contingent (Figure 1 A,C) or deterministic responses (Figure 1 A,B), these two paradigms are both relevant as microbial community assembly results most likely from a combination of both [12]. Considering these two concepts and their relative importance in community assembly and dynamics is essential in the attempt to make predictions about global changes.

Answering these questions is critical in the context of global change impacts on ecosystem degradation, biodiversity loss, and eutrophication. Conducting future studies at a global scale, and disentangling the causes and the consequences of the different factors involved, will increase our knowledge about ecosystem functioning and will help us design sustainable management strategies for different biomes. While diversity of microbial communities can often be dazzling, with the right perspective it is possible to see the forest for the trees.

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