

Phylogenetic Estimation of Ecologically Important Traits

Illuminates Microbial Community Responses to Change in Natural and Agricultural Ecosystems



Kelly Gravuer¹ (kgravuer@ucdavis.edu), Anu M. Eskelinen^{2,4}, Priyashiela Singh³, Susan Harrison² and Kate M. Scow³
 Department of ¹Plant Sciences, ²Environmental Science & Policy, and ³Land, Air & Water Resources, University of California, Davis, and ⁴Department of Biology, University of Oulu, Finland

Problem

Increasing studies based on 16S rRNA data have shown that many environmental differences result in “different” soil microbial communities. However, we lack useful ways to compare these “differences” across studies or locations, or to use them for prediction or modeling.



How to compare so many lists of OTUs?

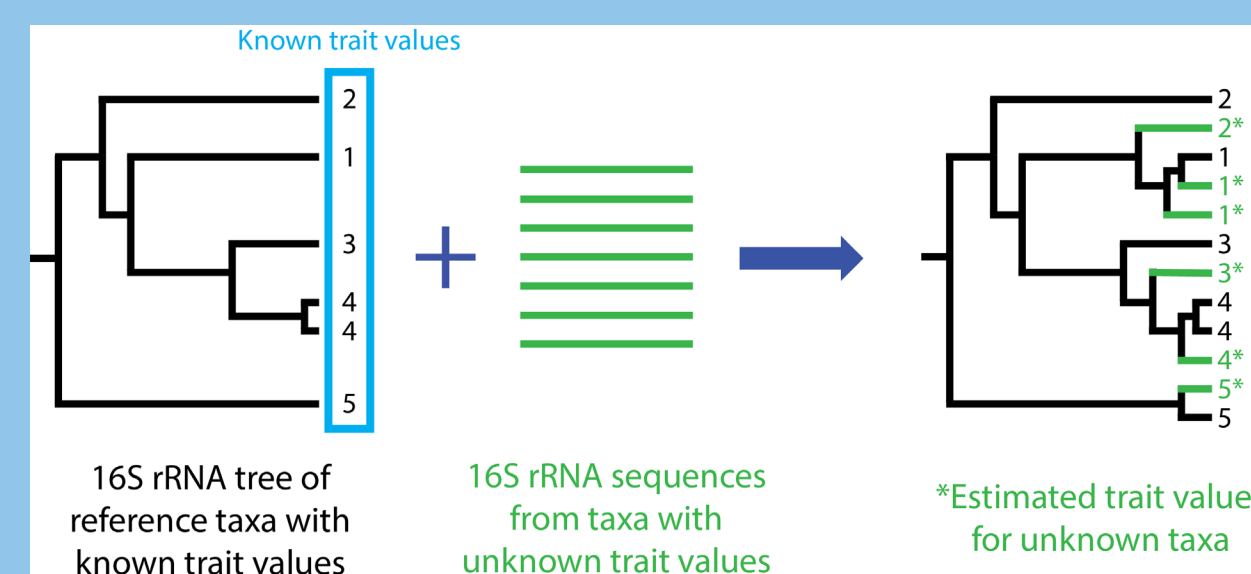
Proposed Solution

Estimating values of ecologically important traits for all microbial taxa in a community can provide clues into the ecological meaning of community shifts, as well as a common currency for comparison across studies. Here, we illustrate three uses of this technique, using data from two experiments.

Methods

In 2012, Kembel *et al.* (PLoS Comput Biol 8(10): e1002743) proposed that ancestral state estimation could be used to estimate unknown microbial trait values based on a reference tree of taxa with known trait values. We constructed such a reference tree using fully-sequenced and annotated genomes downloaded from public databases (e.g., IMG).

Figure 1. Conceptual drawing of strategy developed by Kembel, Wu, Eisen and Green for the estimation of values of phylogenetically-conserved traits across entire microbial communities, based on their 16S rRNA gene sequence data.



For our empirical 16S rRNA soil microbial community data sets, we then estimated ecologically important traits that had (1) data available for many reference taxa, (2) strong phylogenetic signal, and (3) good ability to re-estimate trait values of reference taxa held out from the reference tree, based on initial tests we conducted (Figure 2).

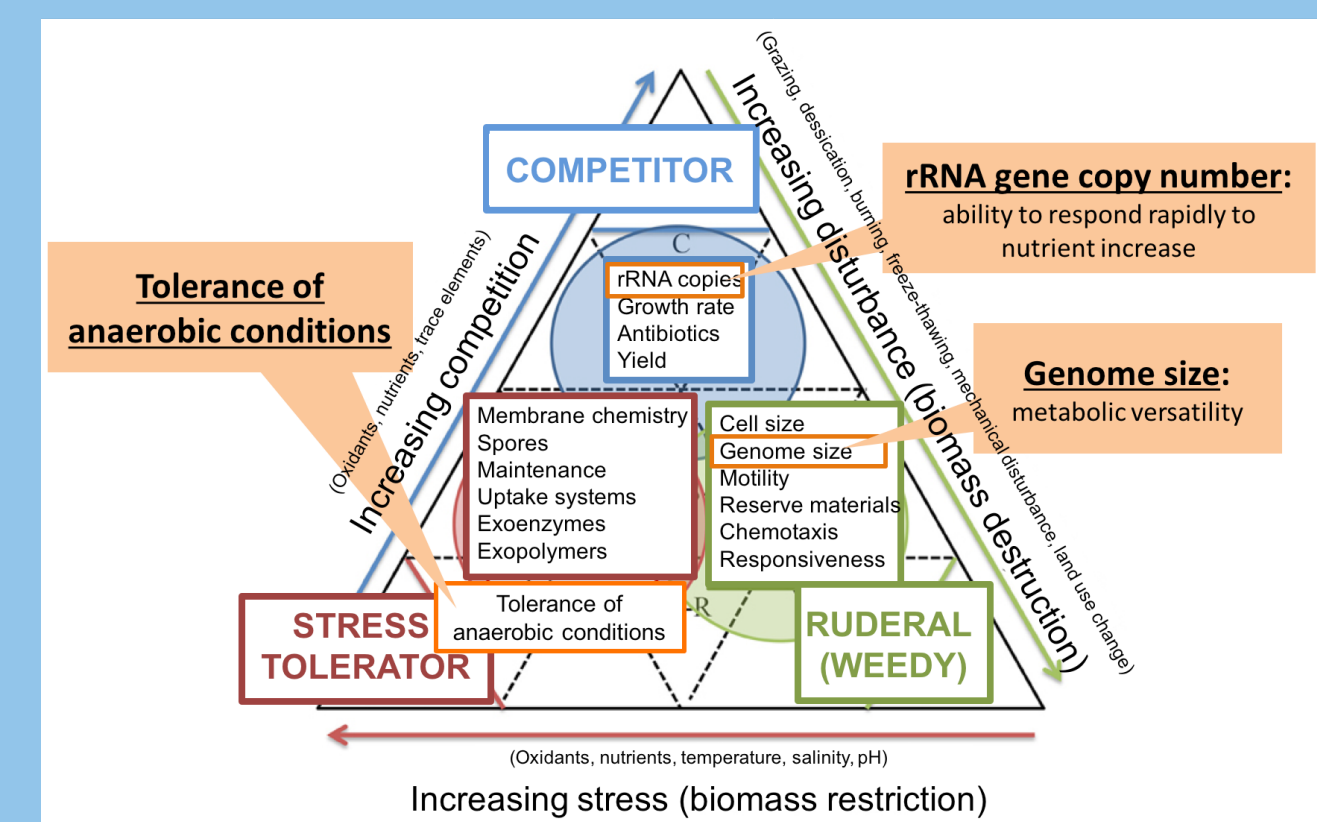


Figure 2. Krause *et al.* (2014, *Frontiers in Microbiology* 5: Article 251) proposed a classification of microbial traits using the three ecological strategies first proposed by Grime (1977, *American Naturalist* 111: 1169-1194). Their figure is modified here to indicate traits estimated in this study, one for each of the three ecological strategies.

Study Systems

A. Grassland environmental change experiment: conducted on two adjacent soil types in the northern Coast Range, CA: (1) “harsh” with coarse texture and low nutrients, and (2) “rich” with finer texture and higher nutrients. Ten plots on each soil received the following treatments: fertilizer (NPK each 10 g/m²/year), supplemental late spring precipitation (1 large event/week for 8 weeks), both treatments or neither (Fig 3).

B. Long-term agricultural management systems experiment: plots on Yolo silt loam soil maintained since 1994 as: organic (tomato-corn rotation, tilled and irrigated with legume cover crop and compost inputs); conventional (tomato-corn rotation, tilled and irrigated with synthetic fertilizer inputs); and no input (wheat-fallow rotation, tilled but not irrigated, no nutrient inputs), in the northern central valley, CA.

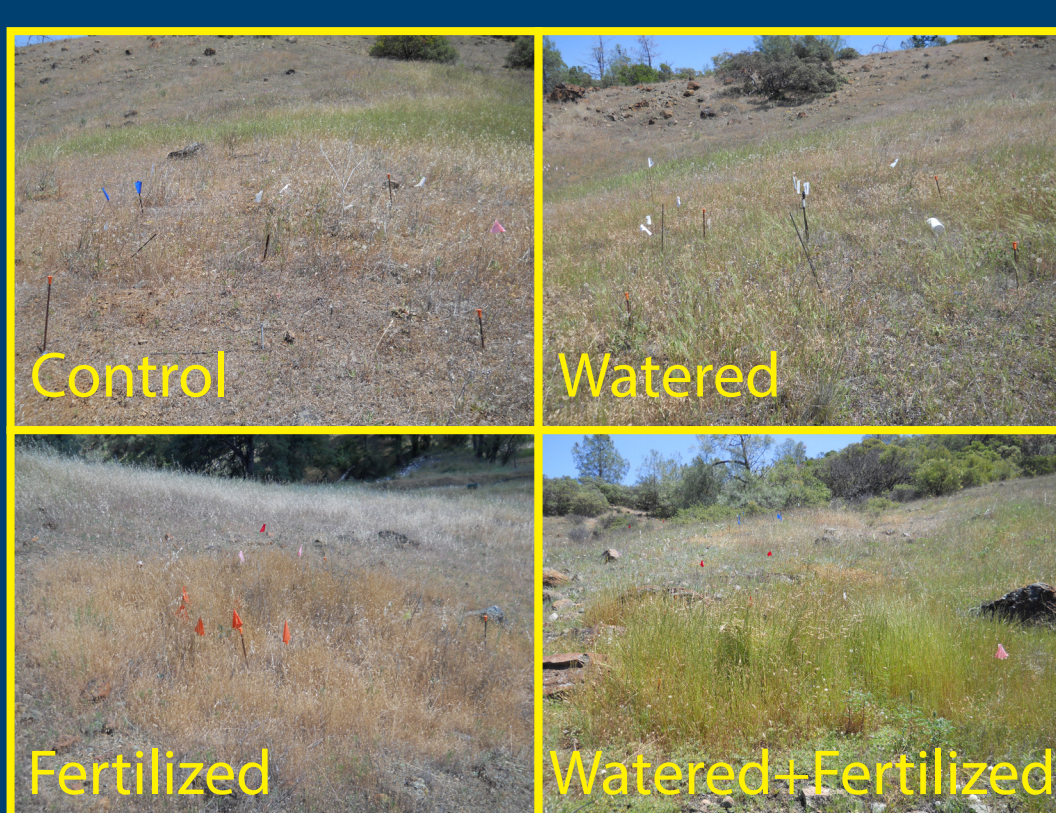


Figure 3a. Harsh soil plots from grassland environmental change experiment (experiment A).



Figure 3b. Rich soil plots from grassland environmental change experiment (experiment A).

1. Using traits to make comparisons across study systems

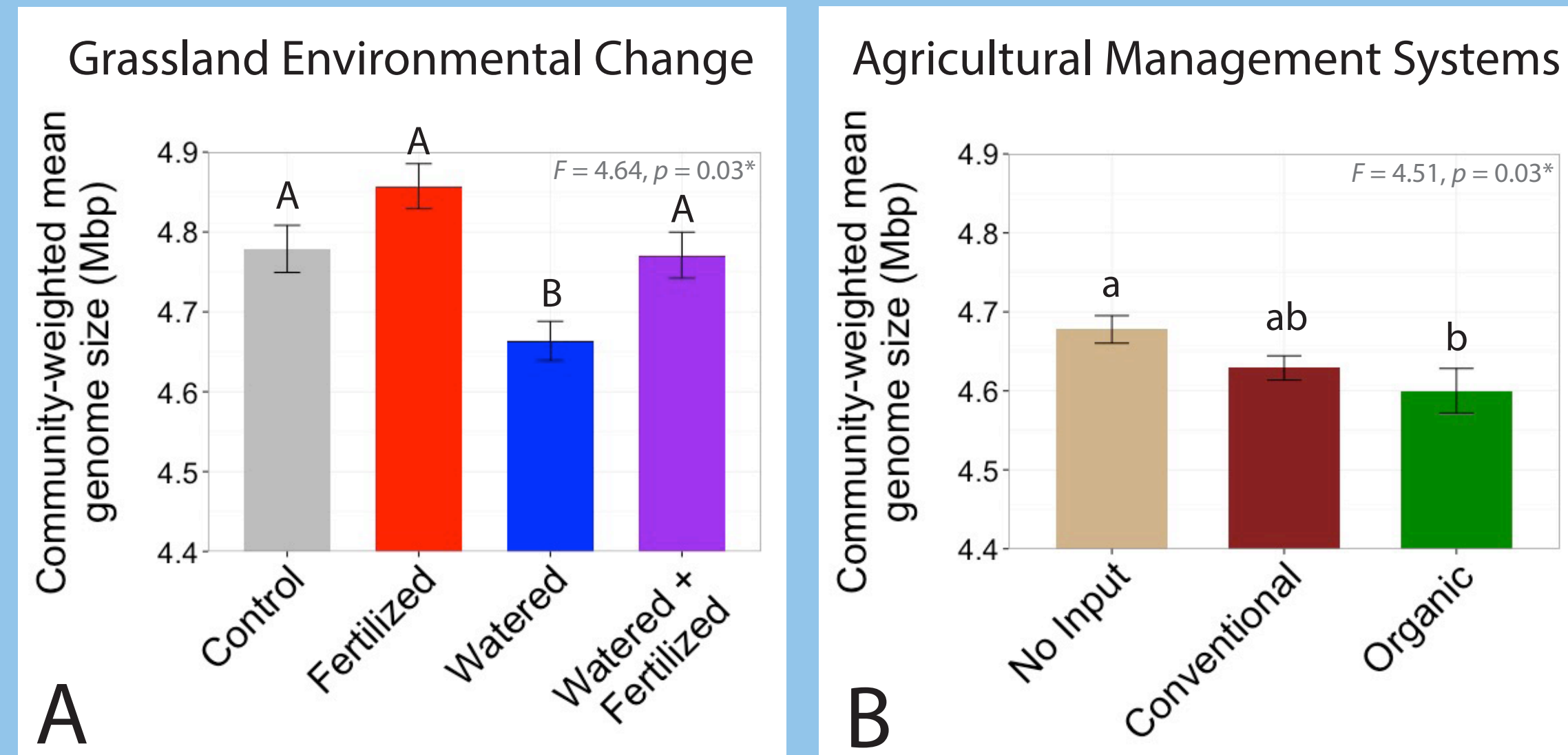
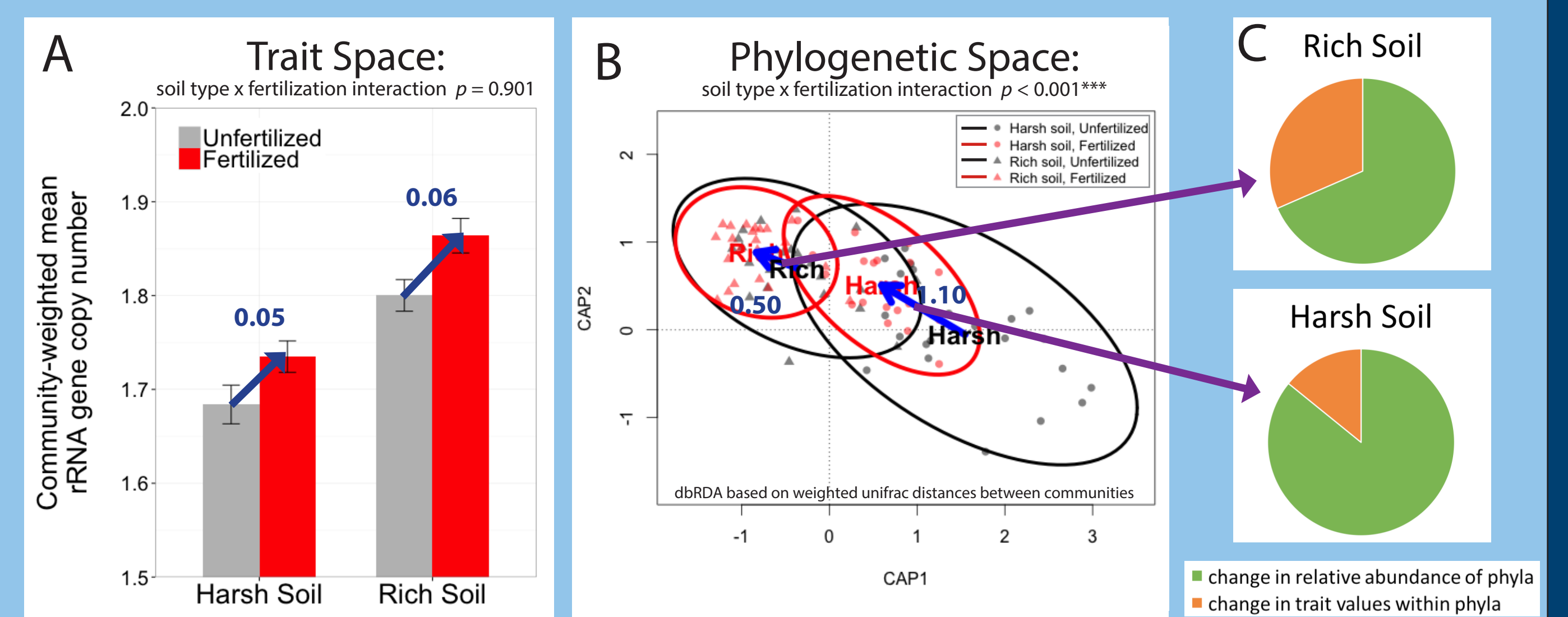


Figure 4A-B. Estimating ecologically-important traits allows direct comparison of two experiments investigating: (A) environmental change manipulations (fertilizer and late-season precipitation addition) in a natural grassland and (B) long-term consequences of different agricultural management systems (organic, conventional, and no input). Comparison reveals that genome sizes were larger (suggesting a broader range of metabolic capabilities) and the effects of treatments were greater in the grassland vs. the agricultural experiment. Traits could also be used to compare, for example, experiments set up across a gradient.

2. Exploring how compositional change relates to community trait change

Figure 5A-C. In response to fertilization, change in rRNA gene copy number was similar in the two soils (A), but change in community phylogenetic composition was greater in the harsh soil than in the rich soil (B). Communities in the two soils thus accomplished similar trait change via different phylogenetic mechanisms, with changes in relative abundance of phyla playing a larger role in the harsh soil and changes in trait values within phyla playing a larger role in the rich soil (C).



3. Testing ecological theories developed for plants and animals

In microbes, high rRNA gene copy number indicates an ability to respond rapidly to a resource increase and is correlated with high maximum growth rate. Fertilization of grassland soil selected for more rapidly-responding and/or faster-growing microbes (Fig 6C), which is similar to patterns observed for both short- and long-term soil nutrient increase in plants (Fig. 6A-B). In contrast, watering selected for more slowly-responding and/or slower-growing microbes, which is opposite to patterns observed for short-term water input increases in plants, but similar to patterns for long-term water input increases (Fig. 6D-E). Microbial responses in watered plots may have resembled long-term rather than short-term plant responses to watering because microbial samples were taken 8.5 weeks after onset of watering in the experiment’s third year - possibly a long term for microbes. This suggests the importance of considering the temporal dimension when predicting microbial community responses.

Figure 6A-B. Short- and long-term effects of nutrient increases on plant community traits. In plant communities, traits associated with fast growth including high Leaf Area Index and Specific Leaf Area (SLA) tend to increase in frequency with both short-term N increases (A; plot experiment) and greater long-term soil N (B; global analysis).

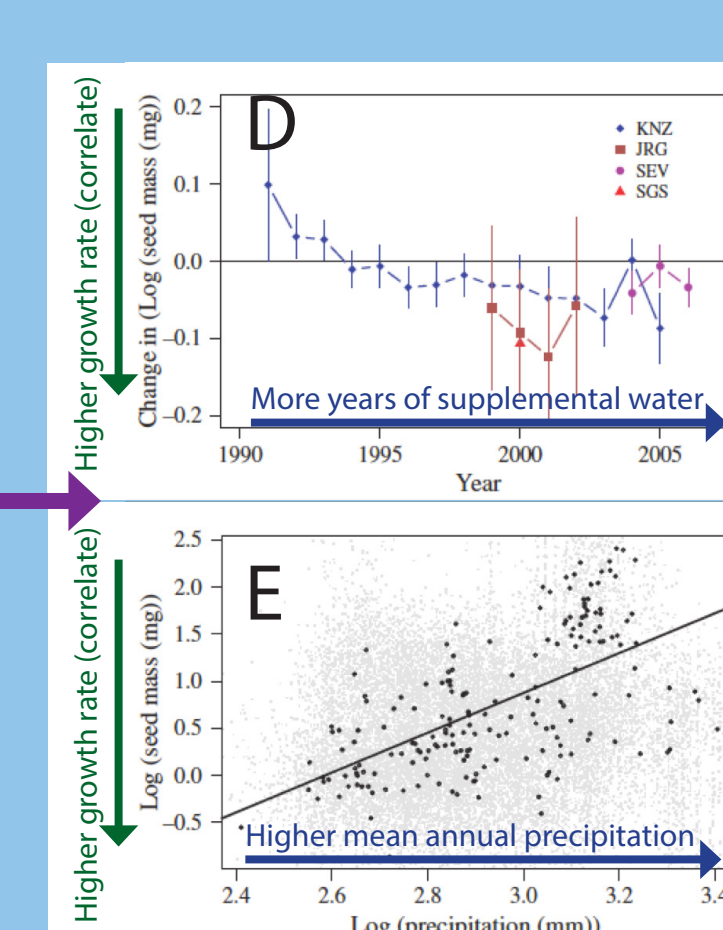
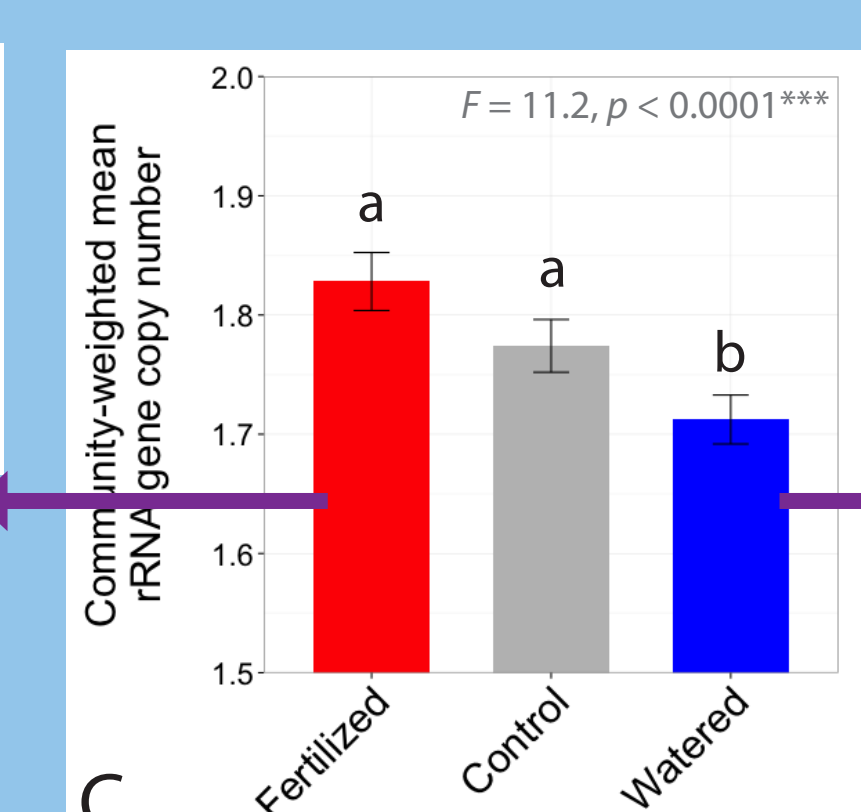
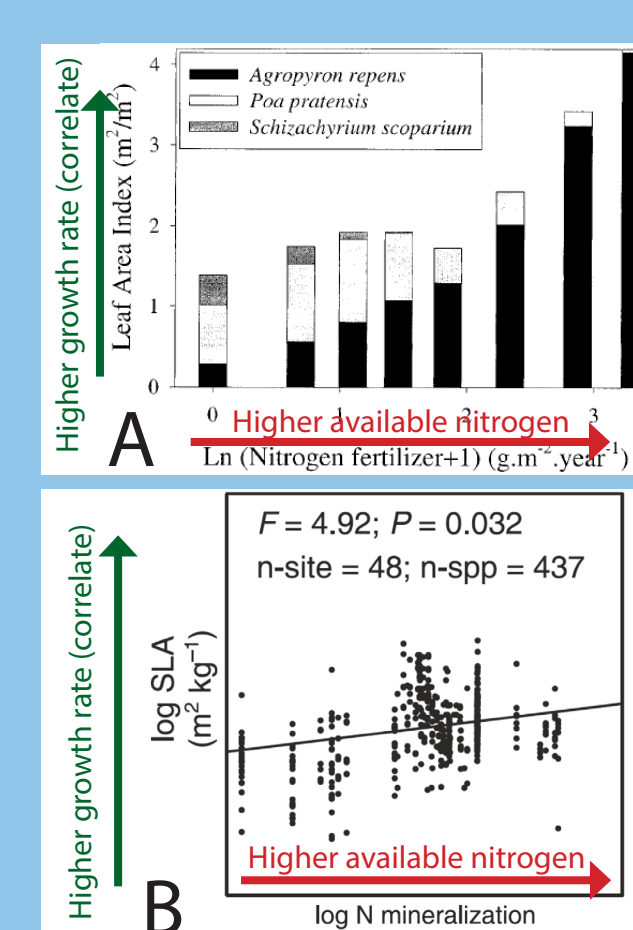


Figure 6D-E. Short- and long-term effects of water increases on plant community traits. In plant communities, traits associated with fast growth including small seed size tend to increase in frequency with short-term water additions (D), but to decrease in frequency with greater long-term precipitation (E). **FIGURES FROM:** Sandel *et al.* (2010) *New Phytol.* 188: 565-575. Fig. 6d: effects of experimental water addition at four sites (represented by colors and letter codes), Fig. 6e: observational plot data from across continental USA.

Figure 6C. Effects of treatments on microbial community trait in grassland expt (this study)

Future Directions

Synthesis and Prediction: This trait estimation method can be applied to publically-available 16S rRNA data sets to address questions such as: Does drought cause similar ecological changes in microbial communities at different locations? How do initial community traits affect microbial response to warming? As we start to see patterns in these relationships, we can use them to make predictions for as yet unsampled communities.

Modeling: Community-wide traits provide a quantitative mechanism by which to explicitly include microbial communities in biogeochemical models (Figure 7), potentially facilitating urgently-needed model improvements.

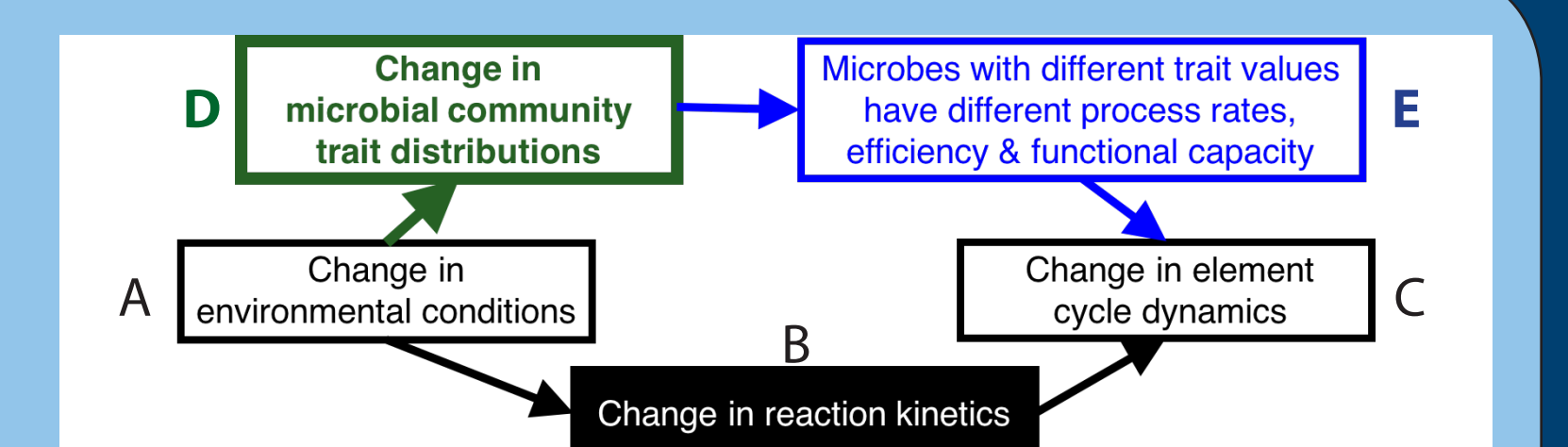


Figure 7. Most biogeochemical models include only boxes A, B and C, treating microbes as black box with behavior completely predicted by environmental conditions. By explicitly including how microbes respond to their environment (box D, this study) and how they affect their environment (box E, future work), we can improve model predictions.

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