

Integrating microbial diversity in citizen science BioBlitz projects; combining basic ecology investigation with science communication



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Abstract

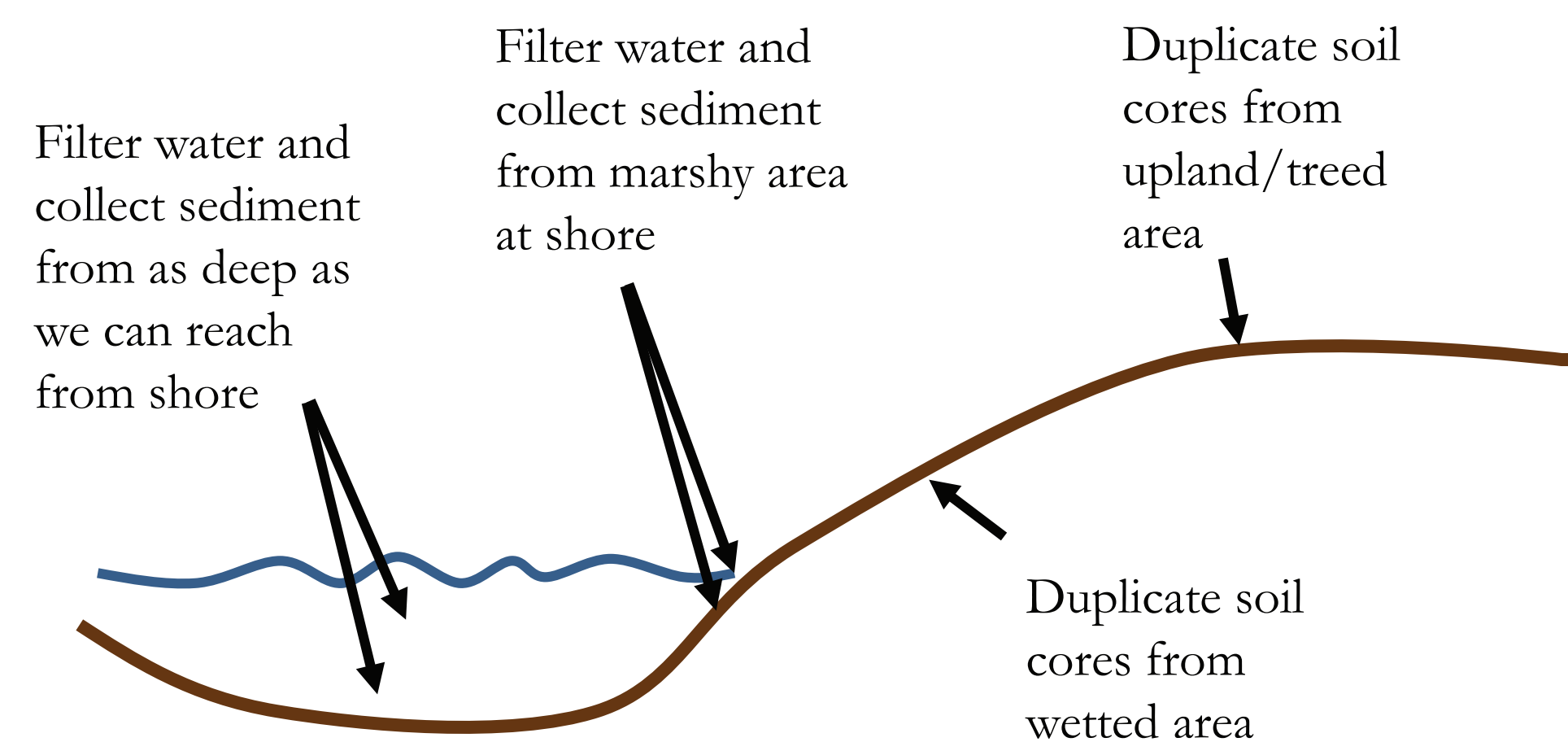
Microbes mediate nearly all biogeochemical cycles, yet our understanding of microbial distribution is still nascent. Advances in molecular techniques, especially metabarcoding, has drastically improved our ability to assess the diversity of microbes in situ. BioBlitz are a blend of data collection and public science communication through interaction with scientists as they collect specimens in an urban or suburban ecosystem. We collected samples for bacterial community analysis in Weir Farm National Historic Site (suburban ecosystem) and in the greater Hartford CT (urban ecosystem) area with primary focus around Three Rivers Middle School which is built on reclaimed industrial land. In both sites we collected duplicate samples from a transect perpendicular to a water body; deep and shallow water and corresponding sediment, wetted soil, and dry soil. Students from Three Rivers Middle School were involved in planning and executing the sampling plan. DNA was extracted then bacterial 16S rRNA v4 was amplified and sequenced at the University of Connecticut MARS facility.

Field Methods

Students from Two Rivers Middle School (a magnet school focusing on science, technology, and the environment) helped design the sampling plan. Samples were collected from transects in duplicate 4-6 weeks before the BioBlitz.



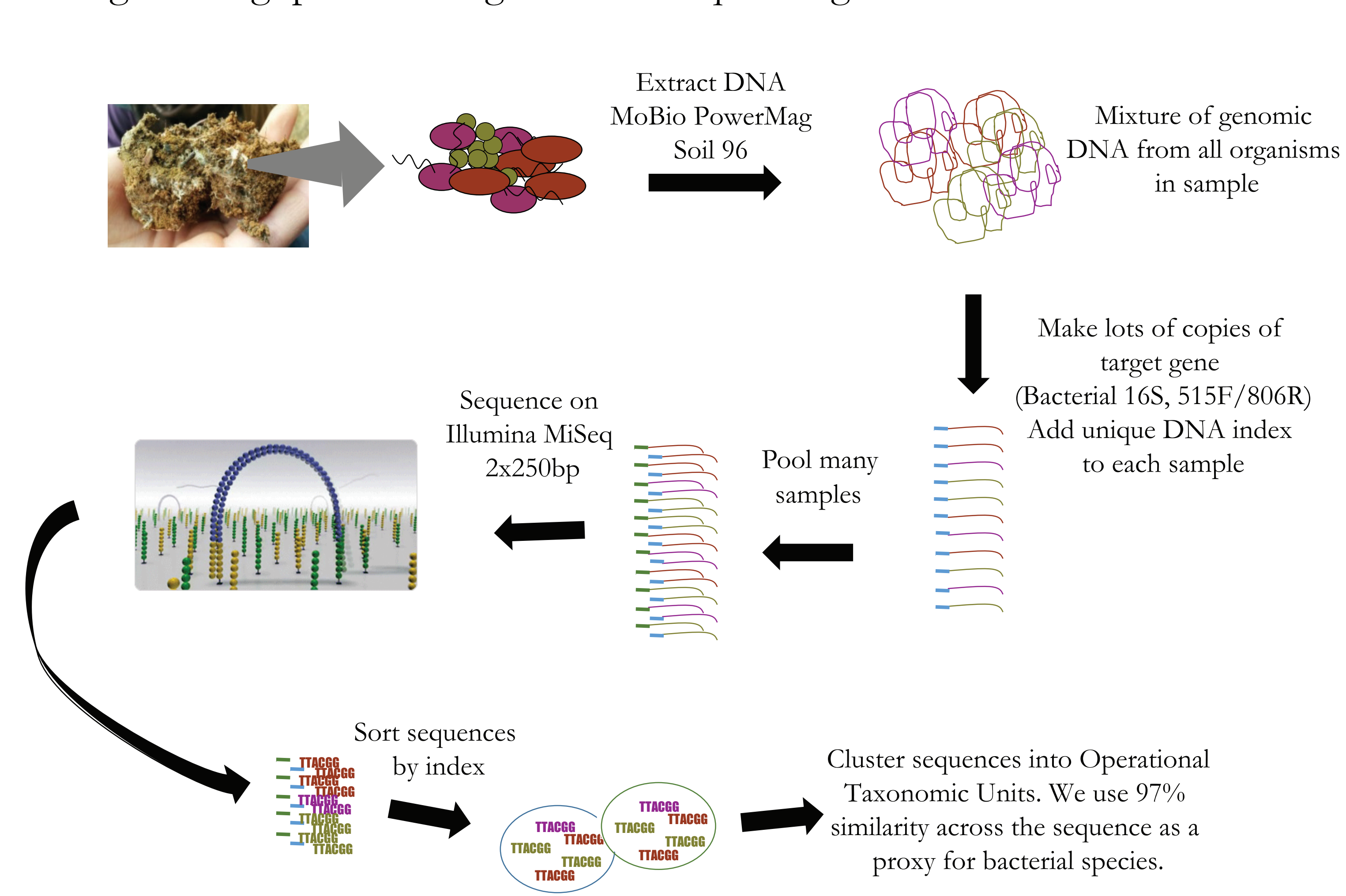
Sampling a pond near Two Rivers Middle School. Top. Site view of transect from pond to dry upland area. Middle. A student collecting "deep water" sample. Student filtering water sample onto 0.2 μ m filter, DNA was extracted from the filter. Bottom. Students collecting soil sample by coring with a cutoff syringe.



Molecular Methods

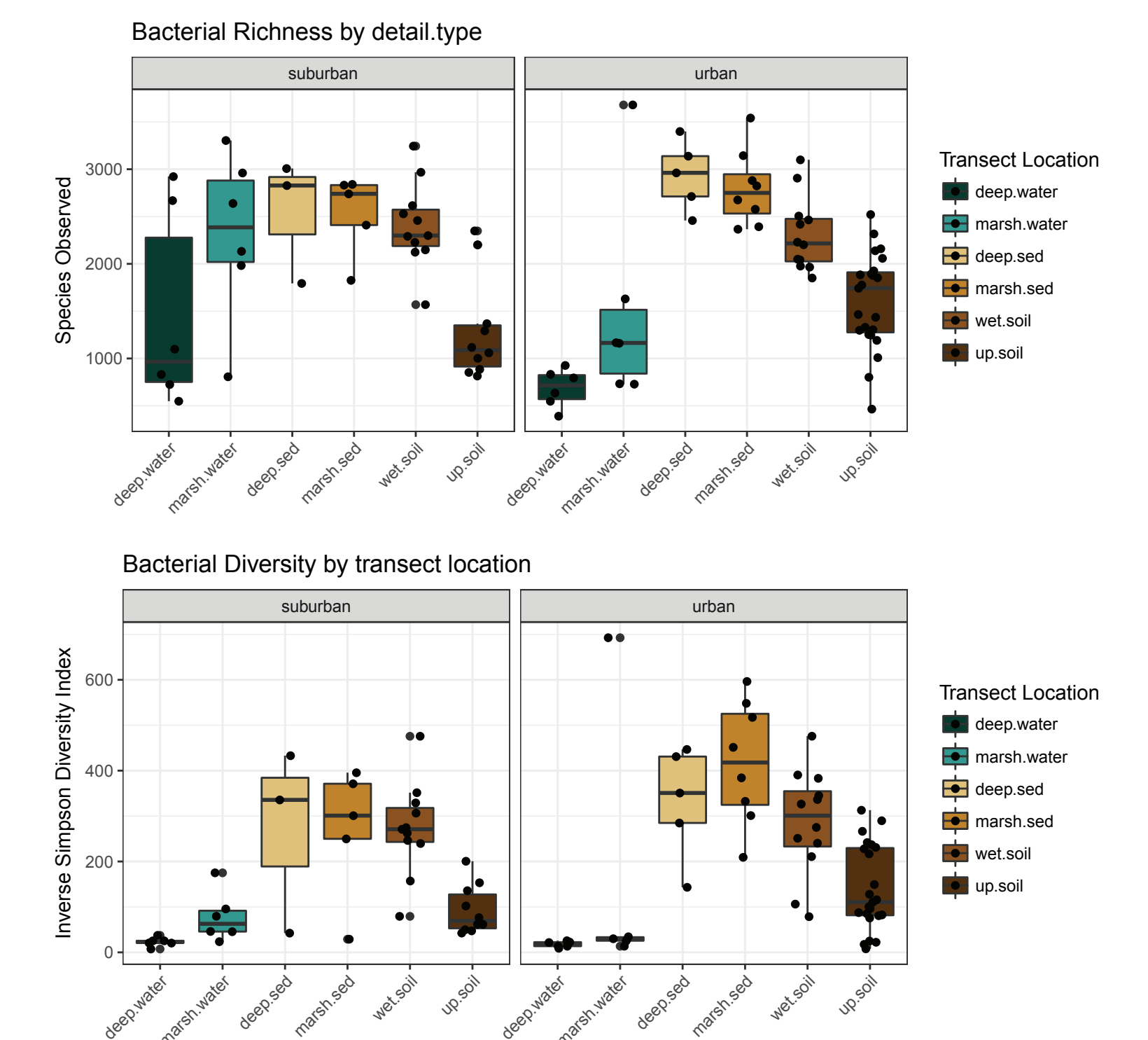
Unfortunately, we were logistically unable to have any students participate in the DNA extraction or sequencing library generation. Instead the MARS facility at UConn processed the samples according to our standard protocols (mars.uconn.edu/SOP). Sequence data processing and statistical analyses Sequences were processed in Mothur v. 1.39.1 following the MiSeq SOP (Kozich et al., 2013). After demultiplexing and quality checking steps the sequences were cluster at 97% similarity. Alpha and beta diversity statistics were calculated by subsampling to 10,000 reads per sample. NMS and Permanova were run using the vegan package (Oksanen 2015) in R 3.2.0.

High throughput or next generation sequencing for mixed communities



Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., and Schloss, P.D. (2013). Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. *Appl. Environ. Microbiol.* 79, 5112–5120.

Jari Oksanen, F. Guillaume Blanchet, Roeland Kindt, Pierre Legendre, Peter R. Minchin, R. B. O'Hara, Gavin L. Simpson, Peter Solymos, M. Henry H. Stevens and Helene Wagner (2015). *vegan: Community Ecology Package*. R package version 2.3-0. <http://CRAN.R-project.org/package=vegan>



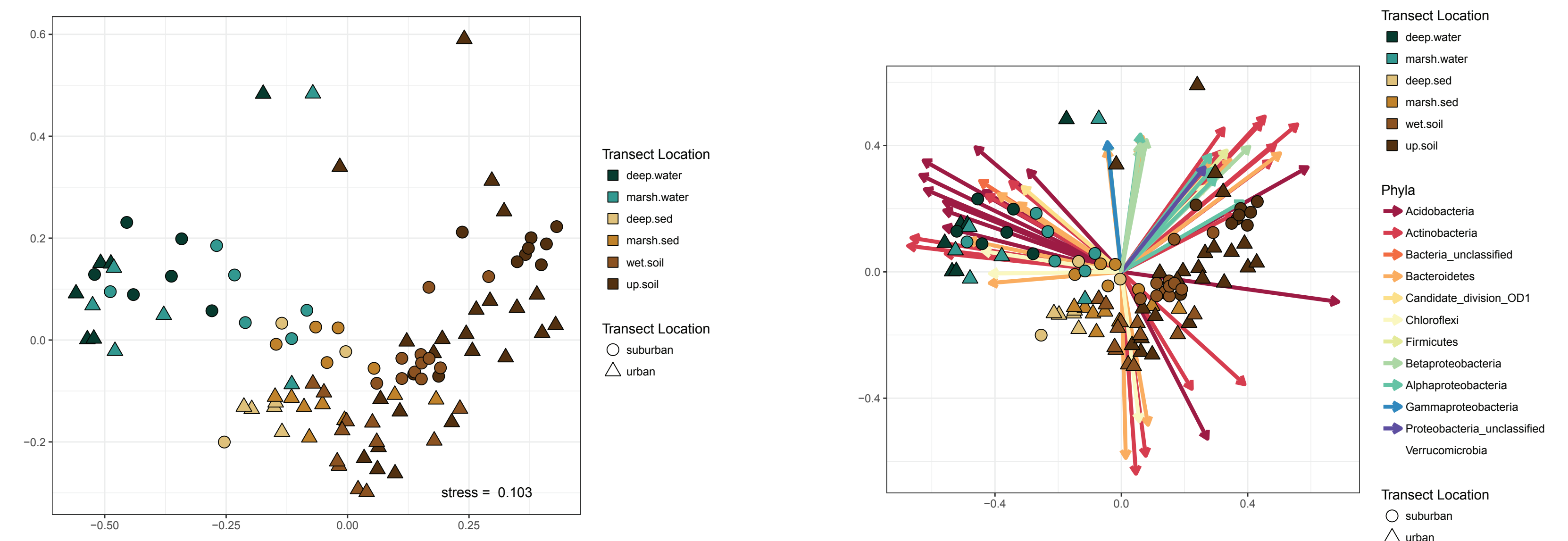
Alpha diversity measures by transect position, separated by land use type. Top species observed, bottom inverse Simpson diversity index.

Ecology Results

We recovered bacterial community data for 122 samples. Species richness (97% OTUs) ranged from 300 in some of the deep water samples to over 3000 in the sediment and soils. There were no overarching trends in alpha diversity between the urban and suburban sample transects, but some sample types did show differences in alpha diversity (i.e. dry suburban soils had lower diversity than dry urban soils, but wetted soils showed no difference in alpha diversity between urban and suburban). Beta diversity ordination testing shows that the samples follow a gradient that mimics the transect layout, evidence of environmental filtering. It also shows the great diversity in microbial communities even at small scales, the biological replicates are slightly different from each other.

Outreach Results

Beyond the species observations, we also interacted with several distinct groups of nonscientists. The middle school students had the most exposure to the microbial communities through exercises involving them in planning the experiments. They saw the variety of microbial communities surrounding them and the variability between microbial communities along short transects. At Weir Farm NHS, we were able to educate park resource personnel on a different type of biodiversity than they typically consider. Also on the day of the BioBlitz at Weir Farm, we guided the general public on a nature walk discussing the bacterial diversity all around us. Addition of microbial diversity into a BioBlitz is a potentially potent avenue for increasing scientific literacy and expanding scientific knowledge of local environments.



Nonmetric multidimensional scaling of the Bray-Curtis dissimilarity index. This shows the relationship between individual samples based on the species and their abundances. Both ordinations are of the same data, but the ordination on the right also includes an overlay of the OTUs that are significantly correlated with this ordination colored by Phyla.

Future directions

This dataset has the potential to be very useful in explaining ecological concepts and introducing the incredible diversity of microbial communities. I don't think that I executed that part of the project as well as I could have. Please use the sticky notes and leave suggestions for how to improve public engagement with this data or microbial ecology in general.

