Using Molecular Tools to Assess Microbial Diversity

Lacy Loos, Nathan Butler, and Julie Anderson ▪ Biology Department ▪ University of Wisconsin-Eau Claire

Objectives

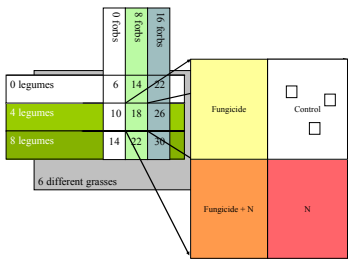
- We propose to measure the diversity of soil microbes across study plots in years one and three of a large prairie restoration project.
- A molecular biological technique, ARISA, will be used to analyze bacterial diversity in natural environments.
- The data collected will contribute to our understanding of the responses of microbial communities to different drivers of plant communities.

Background

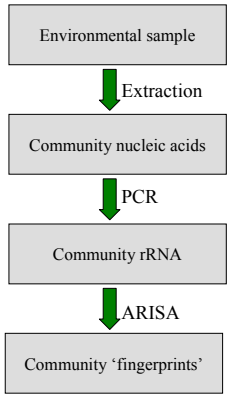
In 2003, an ecological restoration project was started on a 20 acre plot of prairie land in Eau Claire County, Wisconsin (see picture above). The purpose of this project is to better understand what drives the complexities of community assembly and other ecological processes. Through advancements in biochemical research, the central role of soil microbes in such processes has been revealed (1). However, the makeup and dynamics of microbial ecosystems is not completely understood. Molecular studies on microbial diversity in natural environments have since been carried out to help explain the forces driving the development of community structure (1). Such research is imperative to the understanding of how plant/soil microbial communities are assembled and function.

The 20 acre restoration area was divided into 45 plots. Each plot was planted with six different grasses, and one of nine different combinations of legumes and forbes (see diagram below). Each plot was subdivided into four subplots, that underwent four different treatments and are indicated by four colors: control-white, fungicide-tan, fungicide/nitrogen-orange, and nitrogen-red (see diagrams above and below). Soil samples taken from three different locations in each subplot were brought into the lab for analysis of microbial diversity.

9 functional combinations x 5 = 45 unique mixtures



Experimental Design



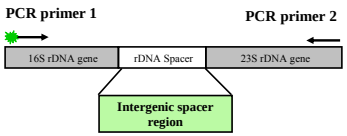
Nucleic acids were extracted from environmental soil samples taken from the prairie restoration site.

Bacterial ribosomal RNA gene sequences were amplified from the community DNA using the polymerase chain reaction or PCR (2).

The various sequences of the community PCR products were separated using an electrophoresis technique named ARISA to generate a community 'fingerprint' for each plot (2).

ARISA

(Automated ribosomal intergenic spacer analysis)

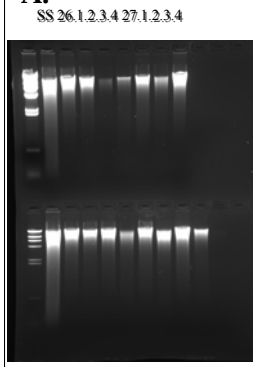


Automated ribosomal intergenic spacer analysis

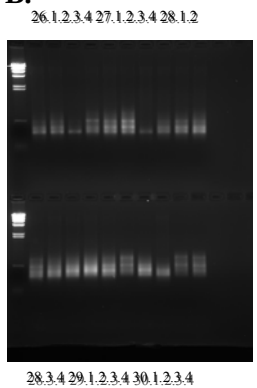
Community ribosomal intergenic spacer fragments are fluorescently labeled, and separated by size using an automated capillary electrophoresis system like the one shown here.

Results

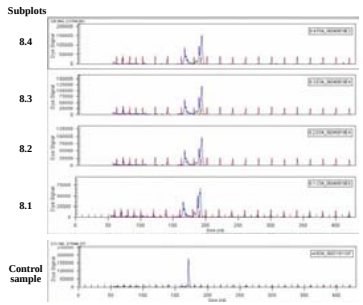
A.



B.



C.



A. An agarose gel electrophoresis digital image of a DNA isolation from subplots 26.1-4, 27.1-4, 28.1-4 and 29.1-4. The far left lanes are standardized size markers. The SS lanes are of salmon sperm for comparison to the isolated microbe DNA.

B. An agarose gel electrophoresis image of PCR results from subplots 26.1-4, 27.1-4, 28.1-4, 29.1-4 and 30.1-4. The far left lanes are standardized size markers. The presence of DNA indicates that the primers amplified the ribosomal intergenic spacer region from the microbial DNA.

C. ARISA profiles from pilot experiments using DNA isolated from subplots 8.1-8.4. The blue peaks indicate fragments of different lengths and the red peaks correspond to molecular weight markers. The control sample is amplified DNA from a single species of bacteria

References

1) Ranjard, L., & Nazaret, S. (2001). Characterization of Bacterial and Fungal Soil Communities by Automated Ribosomal Intergenic Spacer Analysis Fingerprints: Biological and Methodological Variability. *Applied and Environmental Microbiology*. *67*, 4479-4487.

2) Kent, A., & Triplett, E. (2001). Microbial Communities and Their Interactions in the Soil and Rhizosphere Ecosystems. *Annual Review of Microbiology*. *56*, 211-36.

University of Wisconsin Eau Claire

This work is done in collaboration with Evan Weiher at UW–Eau Claire and Tali Lee at University of MN-Duluth with grant support from NSF DEB-0415383 and the Office of Research and Sponsored Programs.