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Genomic Characterization of Belowground Ecosystem Responses to Climate Change

S. P. DiFazio,[1] J. Zhou,[1] L. E. Gunter,[1] C. C. Brandt,[1] R. J. Norby,[1] J. C. Schryver,[2] and J. F. Weltzin[3]
[1]*Environmental Sciences Division*
[2]*Computer Science and Mathematics Division*
[3]*Department of Ecology and Evolutionary Biology, University of Tennessee*

Belowground processes are key to predicting the trajectory and effects of global change on terrestrial ecosystem responses to atmospheric and climatic perturbations. However, these processes remain poorly understood because dated and cumbersome methodologies are being applied to highly complex and heterogeneous biological and geochemical systems. We are developing quantitative, species-specific molecular assays to analyze the plant root composition of soil cores, thereby characterizing belowground competitive interactions with unprecedented precision. We are also assessing functionally significant changes in plant-associated microbial communities by assaying the relative abundance and expression of nitrogen-cycle genes using microarray technology. This research will provide a unique view into the inscrutable yet important world of belowground biota, thus contributing to a mechanistic understanding of responses of entire plant and microbial communities to climate change.

With the rapid development of high-throughput techniques for genotyping and expression analysis, exponentially expanding sequence databases, and rapidly developing computational and bioinformatics resources, the field of genomics has the potential to revolutionize ecological research by enabling the study of obscure and recalcitrant systems with unprecedented precision.

The project has three main tasks: (1) development of quantitative real-time PCR (QRT-PCR) assays that can be used to determine the abundance of different plant species in root samples, (2) development of a microarray for assaying abundance and diversity of organisms involved in nitrogen cycling, and (3) application of the developed techniques to determine the relationship between plant species abundance and microbial diversity in an experiment involving seven plant species and altered [CO_2], temperature, and moisture (the OCCAM facility).

Task 1.1. DNA extraction from roots. We have propagated all seven plant species involved in the OCCAM facility and determined that processing with a ball mill provides the most uniform and consistent tissue for DNA extraction. After testing a wide variety of extraction methods, we determined that a silica gel column provides the most consistent yields of high-quality DNA from the target species.

Task 1.2. Species-specific PCR assays. We designed PCR primers for each target species and two reference species using publicly available nuclear DNA sequences to amplify a species-specific, 200-bp PCR product. We optimized PCR conditions for each species and tested primers against each of the target species as well as nine weed species that occur in or near the study plots. Our PCR assays were highly specific for the target species, even differentiating *Trifolium pratense* from the two congeneric weeds *T. repens* and *T. campestre* (Fig. 1).

Task 1.3. QRT-PCR using reference samples. We have completed pure dye calibrations on the BioRad iCycler and are currently analyzing QRT-PCR conditions on a standard thermocycler using Sybr Green I label in the

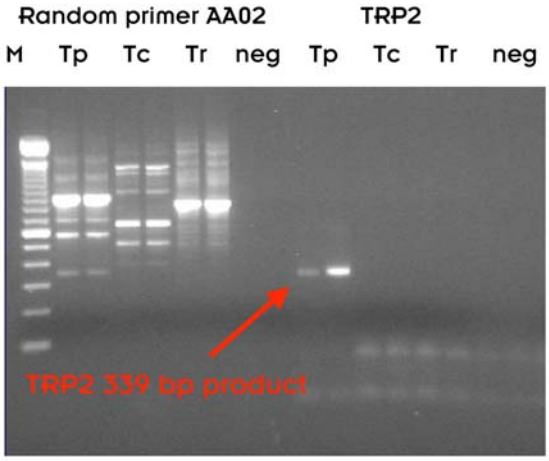

Fig. 1. Specificity of Trifolium pratense *(Tp) primers. DNA from* T. campestre *(Tc) and* T. repens *(Tr) was not amplified with the Tp-specific TRP2 primer (targeting a region of the polyphenol oxidase 2 gene). Amplification with the random decamer primer AA02 indicated that the DNA from all species was of sufficient quality to ensure amplification with matching primers.*

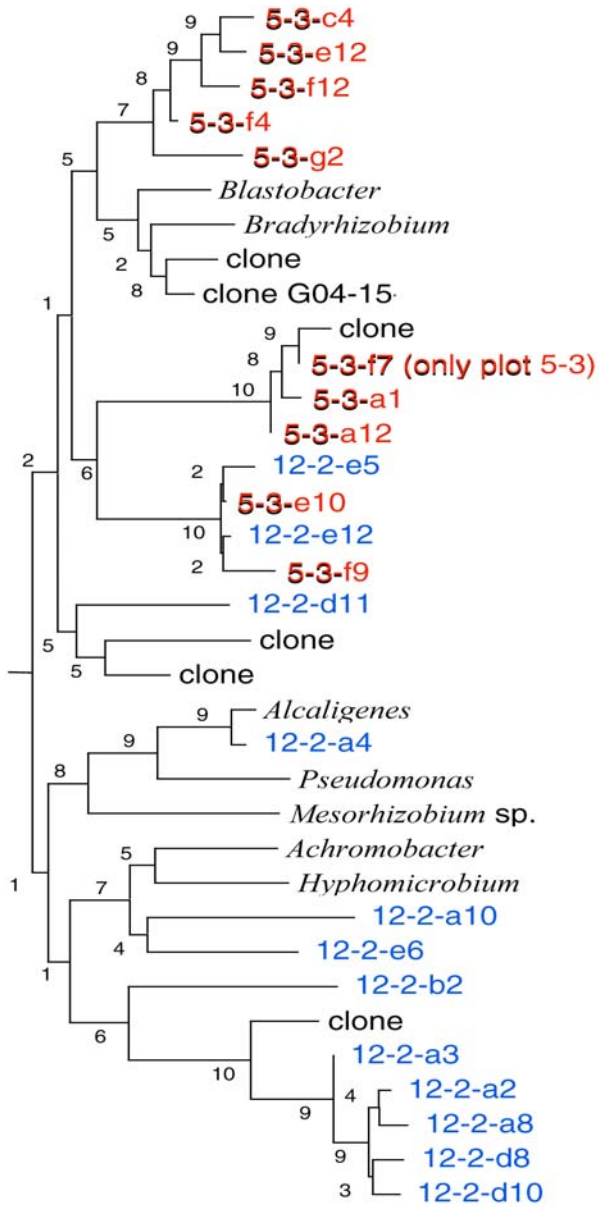

Fig. 2. Neighbor joining tree showing relationships among PCR-amplified fragments of nir*K (Nitrite Reductase K) genes from plots 5 (blue) and 12 (red) from the OCCAM study and previously characterized* nir*K genes. The two plots contained substantially different nitrogen-reducing bacterial communities, and many of these organisms were previously unrepresented in public sequence databases.*

reactions and are in the initial stages of performing QRT-PCR on our salmon reference sample. *Future Plans*: We will derive QRT-PCR calibration curves for each of our target species in isolation and in mixtures with DNA from other species. We will then test the assay on field-collected samples spiked with known quantities of DNA from reference species. Finally, we will test species quantification using mixtures of known quantities of roots.

Task 2.1. DNA and RNA extraction from root-associated soil. Our previous protocol for the extraction of nucleic acids was modified to account for samples derived from soil cores, and in particular to enrich for rhizosphere-associated microbes. With a soil sample of 50 g, a root wash yielded 3 to 5 µg of DNA, and the surrounding soil yielded approximately 100 µg. Because of required replicates and dye switching for microarray experiments, approximately 20 µg is needed.

Task 2.2.1. Database sequences. We have extracted all known *Amo*A, *Nir*S, *Nir*K, and *Nif*H genes from public sequence public databases.

Task 2.2.2. PCR amplification of N-cycling genes from root-associated soil. Clonal libraries have been constructed from PCR amplification of pooled OCCAM rhizosphere DNA using conserved primers for nitrogen cycle genes. We have sequenced portions of these libraries to characterize the microbial communities of two of the OCCAM plots prior to treatment. We found that there were substantial differences between plots for the *nir*K gene and that 84% of the sequences discovered were not represented in current databases (Fig 2).

Task 2.3.1. Microarray construction. More than 5000 unique probes have been designed and spotted onto oligonucleotide arrays. *Future Plans*: Available freeware is being modified for the design of unique probes to account for the alignment of segments common to all sequences, comparison to our growing sequence databases, and comparison back to public databases to test "overall" uniqueness of probes.

Task 2.3.2. Microarray hybridization and scanning. We have tested the arrays using DNA from bulk soil from OCCAM cores. Preliminary results indicate that *nir*K, *nir*S, *amo*A, and *nif*H are present. Two *amo*A sequences (n = 48) and four *nif*H sequences (n = 220) predominated, and these were principally derived from Tennessee groundwater and forested upland soils.

Task 3.2. Data Analysis. We will analyze treatment effects and associations between plant species and microbial diversity using a variety of linear and nonlinear multivariate techniques, including multivariate analysis of variance, principal components analysis, partial least squares, correspondence analysis, canonical correspondence analysis, and artificial neural networks.

Ecosystem genomics is an exciting new field of inquiry where the tools and information from the field of genomics are providing unprecedented insights into the structure and functioning of biological communities. This project is a good example of such work. The methods we are developing will allow ecologists to gain unprecedented insights into the composition of belowground communities, and the responses of those communities to perturbations such as elevated CO_2 and climatic change.