Prairie Biotic Research, Inc.
Grant Proposal Form

I. Applicant Information

Date of Proposal December 2013

Researcher Name Christopher K. Black

Affiliation (if applicable) University of Illinois

Address 1500 IGB, 1206 W Gregory Dr **City** Urbana **State** IL **Zip** 61802

Phone 503 929 9421 **Fax** 217 244 6267 **Email address** black11@igb.illinois.edu

How did you learn of PBR's Small Grants Program?

Announcement on ECOLOG listserv.

II. Project Information

Project Title Mapping root communities by MiSeq

Amount Requested (not to exceed $1,000) $1,000

Start Date (month, year) April, 2014 **Completion Date** (month, year) October, 2014

Project Summary (50 words or less)

I propose to map the spatial arrangement of root communities in prairie plots too small for trench excavation. I will collect soil cores and use DNA barcoding to identify the species present in each sample, thus providing a three-dimensional picture of belowground interactions with only minimal disturbance.

III. The Proposal

A. Researcher Qualifications

Briefly describe your qualifications for conducting this project, including relevant current and past activities.

I am a PhD student studying how plant roots interact with their environment and affect ecosystem carbon cycles. I am monitoring the root structure of a restored prairie in Urbana, IL using minirhizotrons and soil cores to track seasonal changes in standing root biomass as the stand matures after planting. I routinely collect soil samples for analysis of microbial and root biomass and chemical composition and have expertise in rapid, minimally-destructive root sample collection without risk of cross-contamination. In addition to these belowground measurements, I have access to a suite of aboveground physiological and phenological data that can be used to correlate root status with whole-community ecological responses. Although this will be my first project using high-throughput sequencing, I have previous experience collecting and analyzing DNA from environmental samples and I will collaborate closely with Drs. Scott Woolbright and James Doroghazi, both of whom work routinely with DNA barcoding and metagenomic datasets. This collaboration will both complement my technical skills and reduce the overall cost of the project by allowing me to 'piggy-back' my samples into the sequencing run for a soil metagenome survey they are currently performing at the same site.

B. Budget

Provide a project budget summary and indicate how PBR grant funds will be used. Note: PBR does not pay for overhead, grant administration or similar costs.

The expected cost of the entire project (each soil sample divided into soil/rhizosphere/root fractions and analyzed with separate primers for plants, fungi, bacteria, archeae, invertebrates = 1500 DNA samples to sequence) is about $11000:

PCR & quantification reagents/consumables: 1500 @ ~$3.50	5250
Indexed primers: sqrt(1500) @ $50	2000
Tissue grinding & DNA extraction: 300 @ ~5.50	1650
MiSeq sequencing run at Keck biotech center	2000
Total	10900

The costs for sequencing and DNA extraction are fixed, and will be paid by my co-investigators working on prokaryotic and invertebrate communities. However, no funds are currently available to cover the per-sample costs (~$10 per soil sample) of identifying root and fungal species. Therefore I am requesting $1000 from PBR to cover the marginal cost of additional reagents and primers; By comparison, it would cost ~$4500 to sequence these root samples by themselves in a separate run.

C. Project Description

1) What do you wish to study?

Prairie plants grow in a complex community where many neighbors compete for scarce belowground resources. To understand these interactions, I want to understand which species are found where in the soil, and especially whether the depth distributions of particular species are stable throughout a community and whether those correspond reliably to particular ecological functions. In highly diverse root communities, visual identification of samples is not accurate enough to answer these questions, so I propose here a method based on deep but minimally-destructive soil cores and DNA fingerprinting to map the species in prairie root community while leaving aboveground vegetation intact.

2) Why is the topic important?

Most of the biomass in a prairie is underground, and root placement may determine drought resistance, nutrient uptake capacity, competitive success, and mycorrhizal status, yet our understanding of how individual species arrange their roots in the community has barely advanced since John Weaver's famously labor-intensive engravings of trench-excavated root systems. Trenching is highly destructive and therefore unsuitable for small restorations or valuable remnants, so much current root research avoids realistic prairies and uses pot-grown plants or very shallow samples that fail to capture the full depth of the root system. Less destructive sampling will provide new insight into the dynamics of real root communities.

3) Describe your methodology (experimental or observational).

At 4 locations within each of 5 experimental blocks, 3 soil cores will be collected to a depth of 1 m and material from each of 5 depths (0-15, 15-30, 30-50, 50-75, and 75-100 cm) will be pooled, for a total of 100 root & soil samples capturing both vertical and horizontal variation across the site. Each sample will be sieved to 0.5 mm to separate roots from bulk soil. All roots from each sample will be collected as a single aliquot, sonicated to remove rhizosphere material, and freeze-dried before grinding in a bead mill and extracting total community DNA using a Qiagen DNEasy kit. Loci from each sample will then be amplified by PCR using barcode primers specific to plants, fungi, bacteria, archaea, and microinvertebrates. DNA content of each sample will be quantified using Sybrgreen fluorometry, adjusted to equal concentration, uniquely labeled using indexing primers, then all 100 samples will be pooled and submitted for sequencing in a single high-throughput paired-end run at the University of Illinois Biotech Center using an Illumina MiSeq V3. The species present in each sample will then be identified by comparing plant sequences against the sequences of leaf voucher specimens collected at the same site in August 2013, and for fungi by comparison against the GenBank database, and species placement will be mapped in both horizontal and vertical space.

4) Where will the study be done?

We will collect samples from a planted 28-species prairie plot being raised for biomass on the EBI Energy Farm in Urbana, IL. This plot is low-diversity by prairie standards, but is complex enough to contain realistic interactions between species and has the advantage of a well-defined composition, which allows easy assessment of barcoding success. In addition, the experimental plots are designed to provide ready access for routine sample collection, and there is an ongoing record of phenology and biomass changes in both the aboveground and belowground communities. I expect my ecological findings to be broadly applicable to other restored tallgrass prairies that contain similar species mixtures. The barcoding and mapping protocol we develop for this project will be species-independent and should be usable with no changes by anyone who wishes to map the root placement of species in other prairie plots elsewhere.

5) What do you hope to learn from this research?

My first research goal is to better understand the distribution of bleowground biomass: By mapping the roots of each species, I hope to determine how closely, and at what spatial scales, the species visible in the aboveground community match the species detectable in the roots. Further, I will ask whether belowground biomass production tends to be dominated by the same species everywhere, or whether there is spatial heterogeneity in the richness and evenness of roots. These observations of physical structural will provide insight on function: Do all species appear to be mostly foraging for the same limiting reource?

Once my co-investigators have compiled the fungal and bacterial metagenomes, we will also ask how the nonplant community affects root placement and success. If mycorrhizal associations change plant root-growth strategies, then host plant species should be found in the presence of arbuscular mycorrhizal fungal species at locations they would not otherwise occupy, while if mycorrhizae simply alter the success of existing roots then mycorrhizal and non-mycorrhizal roots of the same species will be found in similar spatial contexts. Additionally, it will be interesting to ask how species-specific these interactions are: Does this 5-year-old restored prairie contain mostly generalist microbes, or are there specialists that somehow remained in the spore bank through ~150 years of farming?

6) How will the results of your research be presented to others?

The metagenome project will generate at least three papers focused on the spatial distribution of individual groups of organisms (plant roots and their associated fungi and prokaryotes), and at least one synthesis paper, all to be published in peer-reviewed journals. In addition, I plan to use my data on plant species distributions to inform other papers currently in progress on topics such as water usage, biomass allocation, and carbon storage at this site. I and all my co-investigators will present our findings in talk or poster form at scientific meetings such as the Ecological Society of America, the North American Prairie Conference, and the American Society for Plant Biology. Additionally, I will use a simplified version of the root depth distribution results to develop an educational module on roots and grasslands for the midle-school outreach program Plants iView (http://www.igb.illinois.edu/plantsiview/).