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FOREST SOIL MICROBIAL COMMUNITIES: USING METAGENOMIC APPROACHES TO SAMPLE PERMANENT PLOTS

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INTRODUCTION

Forest soil ecosystems include some of the most complex microbial communities on Earth (Fierer et al. 2012). These assemblages of archaea, bacteria, fungi, and protists play essential roles in biogeochemical cycles (van der Heijden et al. 2008) and account for considerable terrestrial biomass (Nielsen et al. 2011). Yet, determining the microbial composition of forest soils remains a great challenge due in part to their overwhelming diversity and variability. Until recently, studies of microbial diversity in natural systems have relied on clonal cultures. Early environmental gene sequencing, which cloned specific genes to produce a profile of diversity in a natural sample, revealed that the vast majority of microbial diversity had been overlooked using these direct cultivation methods (e.g., Huber et al. 2007). This is not surprising given the enormous microbial diversity associated with environmental samples (Torsvik et al. 1990) and the fact that only a fraction of microbes can be grown in culture (Amann et al. 1995; Pace 1997; Rappé and Giovanni 2003). The more recent emergence of metagenomics allows us to study the complete microbial community by sequencing DNA extracted directly from an environmental sample (Wooley et al. 2010). The value of metagenomic information relies on associated metadata that provide a context for interpretation and allow for cross-study comparisons. The Forest Inventory and Analysis (FIA) permanent plot network provides an ideal setting for conducting metagenomic studies because these plots sample diverse geographic areas and widely ranging ecosystems in a systematic and unbiased manner. Further, the long-term biological, physical, and historical data associated with each plot are essential for interpreting environmental influences on soil microbial communities and ecological processes. Our objective is to evaluate the efficacy of metagenomics in characterizing soil microbial communities within a small subset of FIA plots established in the Priest River Experimental Forest (PREF), Idaho, USA (Table 1).

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Table 1. Characteristics of selected Forest Inventory and Analysis plots at the Priest River Experimental Forest, Idaho, USA.

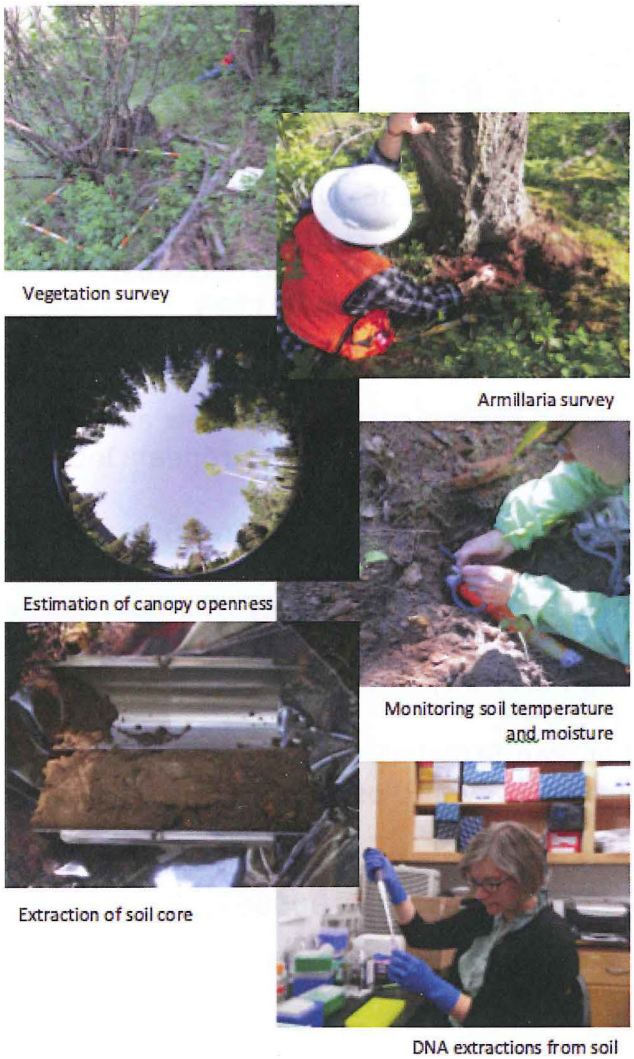
Group	Plot	Habitat Type*	Elevation (m)	Canopy (%)	Slope (°)	Aspect (°)	Understory Diversity ($1m^2$)		Presence of *Armillaria*
							Cover (%)	Richness	
WET	3136	TSHE/CLUN - CLUN PHASE	1052	91.74	32	310	5%	1	No
WET	3161	TSHE/CLUN - CLUN PHASE	917	89.66	32	18	22%	4	Yes
WET	3104	TSHE/CLUN - CLUN PHASE	1218	90.96	20	286	trace	2	Yes
WET	3147	TSHE/CLUN - CLUN PHASE	1190	77.67	24	320	20%	9	Yes
WET	3139	TSHE/CLUN - CLUN PHASE	992	92.26	28	320	2%	4	Yes
WET	3146	THPL/CLUN - CLUN PHASE	1085	91.74	24	183	44%	9	Yes
DRY	3111	ABGR/PHMA - PHMA PHASE	823	55.25	38	322	76%	12	No
DRY	3317	ABGR/PHMA - PHMA PHASE	779	90.70	43	138	47%	10	No
DRY	3106	ABGR/CLUN - PHMA PHASE	863	84.96	32	215	18%	11	Yes
DRY	3381	ABGR/CLUN - PHMA PHASE	810	89.13	12	132	26%	10	Yes
DRY	3276	PSME/PHMA - PHMA PHASE	799	84.96	28	212	100%	11	Yes
DRY	3128	PSME/PHMA - SMST PHASE	891	39.62	8	158	63%	7	Yes

*TSHE = *Tsuga heterophylla*; CLUN = *Clintonia uniflora*; THPL = *Thuja plicata*; ABGR = *Abies grandis*; PHMA = *Physocarpus malvaceus*; PSME = *Pseudotsuga menziesii*; SMST = *Maianthemum stellatum* (Cooper 1991).

APPROACH

DNA and RNA isolated from forest soil cores taken from replicates of contrasting habitat types (relatively wet mesic vs. relatively dry mesic) will be analyzed by amplicon sequencing coupled with metatranscriptomics (Figure 1). Subsequent results will reveal if and how forest soil microbial communities, and their respective levels of gene expression, differ between habitat types. Further, by sampling across time and space, we can address questions related to the scale at which these communities operate and the associated implications for a sampling protocol. Initial sampling has begun within FIA subplots (6 within each contrast) established within PREF. Each subplot was randomly selected after controlling for canopy cover, mean canopy height, and elevation and excluding minority condition subplots. From the center of each selected subplot, soil was sampled at 0 cm, 7.5 cm, and 15 cm below the forest floor as well as from a composite sample. Samples were collected at the midpoint between the bole and drip line of the nearest grand fir (*Abies grandis*) or Douglas-fir (*Pseudotsuga menziesii*). Adjacent trees were surveyed for the presence of *Armillaria* species. RNA and DNA will be isolated from each soil subsample collected in the autumn 2013 and spring 2014 using the RNA PowerSoil Total RNA Isolation and PowerLyzer PowerSoil DNA extraction kits (MoBio, Carlsbad, CA), respectively. Barcoded amplicons (variable regions of bacterial 16S rDNA and the internal transcribed spacer (ITS) and large subunit (LSU) of nuclear-encoded ribosomal RNA genes (rDNA) of fungi) will be sequenced via the

Illumina MiSeq system using 2 x 300-bp, paired-end processing. In addition, RNA will be sequenced on the Illumina HiSeq system at the Institute for Bioinformatics and Evolutionary Studies (IBEST) Core Facility (University of Idaho, Moscow, ID). Composition of soil microbial communities and their respective levels of gene expression will be compared among samples to determine if and how forest soil microbial communities differ between habitat types and to examine how communities compare across space and time.



SELECT PLOTS
- Stratified random sample (12 of 60 FIA plots standardized for canopy characteristics and elevation)
- Confirm habitat type

COLLECT METADATA
- Vegetation survey (1m^2 and 400 m^2)
- *Armillaria* survey (species, genets)
- Canopy cover
- Soil moisture and temperature (5 cm depth, every 60 min for 1 year)

CORE SOIL
- Characterize soil (e.g., water holding capacity, texture, bulk density, pH, salinity, cation exchange capacity, labile C, C:N) Amplicon sequencing - 4 x 0.3 g subsamples in autumn 2013 (0 cm, 7.5 cm, and 15 cm depth as well as a composite sample) and spring 2014 (0 cm, 7.5 cm, and 15 cm depth as well as a composite sample)
- Metatranscriptomics (spring 2014, 7.5 cm depth)

AMPLICON SEQUENCING AND METATRANSCRIPTOMICS
- Illumina MiSeq system (16S rDNA, ITS, LSU)
- Illumina HiSeq system

ANALYSES
- Assign sequences to specific subsamples via barcodes
- Group sequences into Operational Taxonomic Units
- Assess statistical differences in the overall community composition and levels of gene expression

Figure 1. Proposed methodology for using metagenomic approaches to survey forest soil microbial communities on FIA permanent plots at PREF in northern Idaho, USA.

FUTURE APPLICATIONS

As metagenomic technologies continue to develop, costs are expected to decrease as information yields increase. At this time, the utility of metagenomic information seems nearly limitless. Future application of metagenomics to FIA plots will promote an understanding of how biotic communities and their environmental relationships drive forest ecosystem processes. As metagenomic information accumulates across diverse forest habitat

types and across spatial and temporal scales, we will better understand the roles of climate change, fire, management practices, biotic communities, and physical attributes of a site on forest productivity, sustainability, resilience, carbon sequestration, and other ecosystem functions. Further, metatranscriptomic studies could be used to decipher how these communities respond to changes in their environment by examining gene expression under different conditions. Of primary interest is determining whether soil microbial communities can be managed to suppress root diseases in forest ecosystems.

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