FINE-SCALE VARIABILITY OF FOREST SOIL FUNGAL COMMUNITIES IN TWO CONTRASTING HABITAT TYPE SERIES IN NORTHERN IDAHO, USA IDENTIFIED WITH MICROBIAL METAGENOMICS

Amy Ross-Davis[1], *Jane E. Stewart*[2], *Matt Settles*[3], *John W. Hanna*[1], *John D. Shaw*[4], *Andrew T. Hudak*[1], *Deborah S. Page-Dumroese*[1], and *Ned B. Klopfenstein*[1]

INTRODUCTION

Forests are home to some of the most complex microbial communities (Fierer *et al.* 2012) which drive biogeochemical cycles (Clemmensen *et al.* 2013; van der Heijden *et al.* 2008) and account for substantial terrestrial biomass (Nielsen *et al.* 2011). Fungi, through their ecological roles as decomposers, mutualists, or pathogens, are particularly important in breaking down organic matter and mediating plant nutrition. Despite this, little is known about the variability, composition, and structure of forest soil fungal communities (Tedersoo *et al.* 2014). In fact, much of global fungal diversity remains undocumented (Blackwell 2011; Hawksworth 2012).

Our objective with this work is to apply next-generation sequencing technology to characterize forest soil fungal communities within a small subset of permanent plots established in two contrasting habitat type series within the Priest River Experimental Forest (PREF), Idaho, USA. Specifically, we are interested in exploring how the forest soil fungal community relates to environmental gradients and determine if and how it differs with depth below the forest floor and between habitat type series and seasons.

In: Ramsey, A. & P. Palacios (Comps). Proceedings of the 63rd Annual Western International Forest Disease Work Conference; 2015 Sept. 21-15; Newport, OR. [1]USDA Forest Service, Rocky Mountain Research Station (RMRS), Moscow, Idaho. [2]Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, Colorado. [3]University of California Davis Genome Center, University of California, Davis, CALIFORNIA. [4]USDA Forest Service, RMRS, Forest Inventory and Analysis, Ogden, Utah.

MATERIALS AND METHODS

Soil cores were sampled from the center of each of 12 Forest Inventory and Analysis (FIA) subplots established within PREF at three depths below the forest floor (i.e., 0, 7.5, and 15 cm) at the midpoint between the bole and drip line of the nearest grand fir (*Abies grandis*) or Douglas-fir (*Pseudotsuga menziesii*) at two time points: 16-19 September 2013 and 3-6 June 2014. Six plots were established in the western redcedar (*Thuja plicata)*/western hemlock (*Tsuga heterophylla)* habitat type series (THPL/TSHE; Cooper *et al.* 1991) and six in the Douglas-fir/grand fir habitat type series (PSME/ABGR) providing a comparison of varying soil moisture levels. Trees were surveyed for the presence of *Armillaria* species which were identified using a combination of somatic incompatibility tests and *tef1α* sequence variation (Ross-Davis *et al.* 2012).

DNA was isolated from each soil subsample using the PowerLyzer PowerSoil DNA extraction kits (MoBio, Carlsbad, CALIFORNIA). Double-barcoded amplicons of the internal transcribed spacer 1 (ITS1) ribosomal DNA (located between the 18S and. 5.8S rRNA genes) generated from replicated PCR were sequenced at the I-BEST Core Facility (University of Idaho, Moscow, Idaho) on a paired-end 300bp Illumina MiSeq run in a four file format: Read 1, Index Read 1, Index Read 2, and Read 2.

Raw fastq files were screened for presence of template-specific primer sequences and separated by primer sequence (maximum allowed mismatches 4) and index sequence (maximum allowed mismatches 1). Sequences

were overlapped using Flash (maximum proportion of mismatch 0.25 = number of mismatches/length of overlap) and then classified with the fungal database. Bootstrap cutoff was 0.5. The resulting fixrank file for the amplicon target was parsed to generate abundance of taxa by proportion and number of reads per sample as well as summaries of taxonomic classifications.

A UPGMA cluster dendrogram was created using the plot function in R to visualize patterns of soil fungi composition among plots, depths, and seasons. Environmental data and fungal richness were compared among samples to determine if and how they differ between habitat type series and seasons and across small spatial scales using SAS version 9.2 Software (SAS, Inc., Cary, North Carolina) via PROC GLIMMIX with type III tests of fixed effects used to examine interactions and main effects.

RESULTS AND DISCUSSION

Compared to THPL/TSHE plots, PSME/ABGR plots had thinner forest floors ($p = 0.0030$) with more abundant ($p = 0.0208$) and diverse ground vegetation ($p = 0.0067$). PSME/ABGR plots were also more variable with regard to soil moisture and temperature profiles (Figure 1): soil temperatures in PSME/ABGR plots warmed up to > 0°C in early-March compared to mid- to late-April in the THPL/TSHE plots. PSME/ABGR plots also had greater mean soil temperatures ($p = 0.0004$), with lower minima ($p = 0.0471$) and higher maxima ($p = 0.0033$), compared to THPL/TSHE plots, and volumetric water content reached significantly lower minima among PSME/ABGR plots ($p = 0.0009$). No significant differences were detected between habitat type series for most of the measured physical and chemical variables of either the forest floor or mineral soil.

Table 1. *Richness and diversity (Shannon's index) of soil fungi by season, soil depth and habitat type series based on ITS1.*

Treatment	Level	Richness	Diversity
Season	Spring	126.17 A	2.3387 A
	Fall	109.97 B	2.2768 A
	SE	5.4566	0.06238
Depth	0	125.75 A	2.3861 A
	7.5	112.96 A	2.2204 A
	15	115.50 A	2.3167 A
	SE	6.6830	0.07640
Habitat type series	PSME/ABGR	128.81 A	2.3667 A
	THPL/TSHE	107.33 B	2.2487 A
	SE	5.4566	0.06238
Type III Test of Fixed Effects	df	F (Pr > F)	F (Pr > F)
season	1/60	**4.40 (0.0401)**	0.49 (0.4853)
depth	2/60	1.03 (0.3644)	1.19 (0.3125)
season*depth	2/60	1.75 (0.1822)	**6.11 (0.0038)**
habitat type series	1/60	**7.74 (0.0072)**	1.79 (0.1863)
season*habitat type series	1/60	1.76 (0.1891)	0.55 (0.4594)
depth*habitat type series	2/60	0.13 (0.8809)	1.10 (0.3406)
season*depth*habitat type series	2/60	0.40 (0.6726)	0.50 (0.6067)

A total of 605 fungal taxa was recovered from the forest soil samples, most of which were identified to genus (77%). The most ubiquitous genera were the common woodland agaric *Hygrocybe* and the ectomycorrhizal genera *Russula* and *Wilcoxina*. Fungal richness (number of taxa) differed significantly between habitat type series and seasons but not among soil depths, with no significant interactions among main effects (Table 1). A greater number of fungal taxa were recovered from PSME/ABGR plots compared to THPL/TSHE plots and from spring samples compared to fall. Community diversity was significantly higher just below the forest floor (0 cm) compared to greater sampling depths (7.5 and 15 cm) in the fall only.

The UPGMA cluster dendrogram did not reveal any clear patterns associated with depth, season, or habitat type series (Figure 2). However, when specific taxa were examined individually, patterns emerged. For example, three genera were much more common in the spring relative to the fall (*Hohenbuehelia*, *Diversispora*, and *Conocybe*) while some were more common in the fall (*Cordyceps*, *Hebeloma*, *Brevicellicium*, and *Boletellus*). Several taxa were much more abundant among PSME/ABGR plots (*Cordyceps*, *Amanita*, *Calycina*, *Tuber*, *Gilkeya*, and *Conocybe*), while others were more abundant among the THPL/TSHE plots (*Hebeloma*, *Ramaria*, *Tylospora*, *Sarcosphaera*, *Leucogaster*, and *Boletellus*). For some taxa, abundance increased with soil depth (*Cordyceps*, *Hohenbuehelia*, *Leucogaster*, *Ramaria*, and *Brevicellicium*) while for others, abundance decreased (*Tylospora*, *Sarcosphaera*, *Conocybe*, *Amanita*, and *Boletellus*). Some patterns clearly relate to timing of fruiting (e.g., for *Hebeloma* and *Boletellus*). In-depth analysis is currently underway.

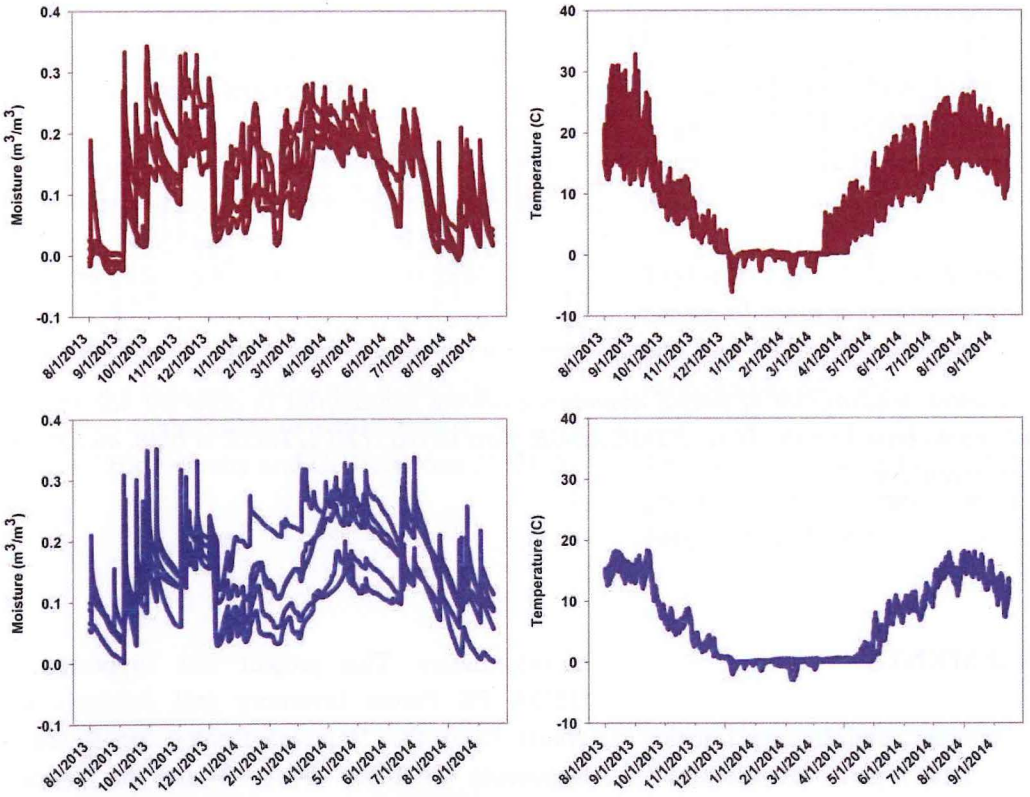

Figure 1. *Profiles of soil moisture (left) and temperature (right) measured at 5 cm below the forest floor in each habitat type series (PSME/ABGR plots in red and THPL/TSHE plots in blue) every hour from 8/1/2013 through 9/15/2014.*

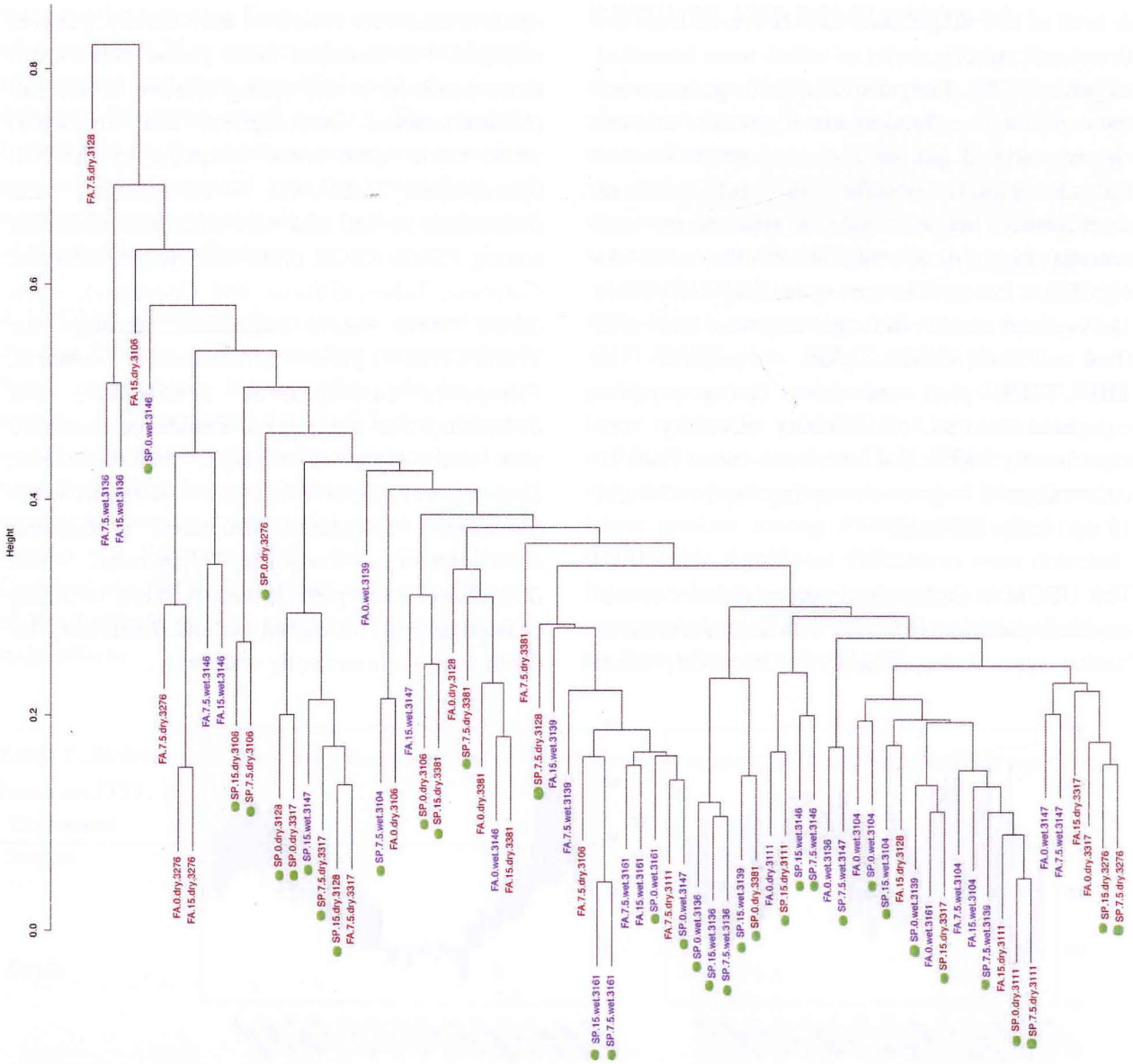

Figure 2. *UPGMA cluster dendrogram of fungal abundance among subsamples in different habitat type series, seasons, and depths based on the ITS1. PSME/ABGR plots in red, THPL/TSHE in blue, and spring samples indicated by green dot.*

ACKNOWLEDGEMENTS

Eric Pitman, Sara Ashiglar, and Joanne Tirocke helped with field work and plant identification. Joanne Tirocke and Eric Doubet assisted with soil analysis and Dan New and Alida Gerritsen assisted with PCR, next-generation sequencing, and bioinformatics. This project was supported by USDA FS Forest Inventory and Analysis and grants from the National Center for Research Resources (5P20RR016448-10) and the National Institute of General Medical Sciences (8 P20 GM103397-10) from the National Institutes of Health.

REFERENCES

Blackwell, M. 2011. The fungi: 1, 2, 3 5.1 million species? American Journal of Botany 98:426-438.

Bonito, G. *et al.* 2014. Plant host and soil origin influence fungal and bacterial assemblages in the roots of woody plants. Molecular Ecology 23:3356-3370.

Buée, M. *et al.* 2011. Influence of tree species on richness and diversity of epigeous fungal communities in a French temperate forest stand. Fungal Ecology 4:22-31.

Clemmensen, K.E. *et al.* 2013. Roots and associated fungi drive long-term carbon sequestration in boreal forest. Science 339:1615-1618.

Cooper, S.V., Neiman, K.E., and Roberts, D.W. 1991. Forest Habitat Types of Northern Idaho: A Second Approximation. USDA Forest Service General Technical Report INT-236

Fierer, N. *et al.* 2012. Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. Proceedings of the National Academy of Sciences 109(52):21390-21395.

Hawksworth, D.L. 2012. Global species numbers of fungi: are tropical studies and molecular approaches contributing to a more robust estimate? Biodiversity and Conservation 21:2425-2433.

van der Heijden, M.G.A., Bardgett, R.D., and van Straalen, N.M. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11:296-310.

Nielsen, U.N., Ayres, E., Wall, D.H., and Bardgett, R.D. 2011. Soil biodiversity and carbon cycling: a review and synthesis of studies examining diversity–function relationships. European Journal of Soil Science 62(1):105-116.

Oehl, F. *et al.* 2010. Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. Soil Biology & Biochemistry 42:724-738.

Ross-Davis, A.L., Hanna, J.W., Klopfenstein, N.B., and Kim, M.-S. 2012. Advances toward DNA-based identification and phylogeny of North American *Armillaria* species using elongation factor-1 alpha gene. Mycoscience 53(2):161-165.

Talbot, J.M. *et al.* 2014. Endemism and functional convergence across the North American soil mycobiome. Proceedings of the National Academy of Sciences 111(17):6341-6346.

Taylor, D.L. *et al.* 2014. A first comprehensive census of fungi in soil reveals both hyperdiversity and fine-scale niche partitioning. Ecological Monographs 84:3-20.

Tedersoo, L. *et al.* 2014. Global diversity and geography of soil fungi. Science 346(6213):1256688.