Using A Metagenomic Approach To Improve Our Understanding Of Armillaria Root Disease

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INTRODUCTION

Metagenomics has illuminated our understanding of how microbial communities influence health and disease. Researchers are beginning to characterize what constitutes healthy microbiota in terms of structure, function, and diversity in a variety of environments. Although investigation lags behind the more well-studied human microbiome, a growing body of research is using next-generation sequencing tools and advances in bioinformatics to explore how microbiota and constitutive microbiomes in soils and plant tissues can affect crop and forest diseases (Damon et al. 2012, Bonito et al. 2014, Penton et al. 2014, Qiu et al. 2014, Štursová et al. 2014). Disease suppression in agricultural systems has been fairly well-studied, with suppression attributed to diverse microbiota that affect pathogen survival, growth, and infection. In these systems, management practices such as no-till and stubble retention, which supply higher levels of available carbon, have been shown to favor diverse microbial communities. Our understanding of disease suppression in forest soils is minimal, even though these ecosystems are home to some of the most complex microbial communities (Fierer et al. 2012) that play essential roles in biogeochemical cycles (Van Der Heijden et al. 2008) and account for considerable terrestrial biomass (Nielsen et al. 2011). Armillaria Root Disease is one of the most important diseases of trees in temperate regions, yet it remains difficult to manage. Results of biological control research suggest that components of the forest soil microbiota may affect Armillaria Root Disease (e.g., Reaves et al. 1990, Reaves and Crawford 1994, Filip and Yang-Erve 1997, Becker et al. 1999, Chapman et al. 2004, Shapiro-Ilan et al. 2014).

Our objective with this work is to evaluate the efficacy of using a metagenomic approach to characterize forest soil microbial communities within a small subset of Forest Inventory and Analysis (FIA) plots established in the Priest River Experimental Forest (PREF), Idaho, USA. This information can be used to design and implement studies to examine how forest soil microbiota relate to Armillaria Root Disease, with the ultimate goal of informing management decisions that manipulate the environment in ways that favor disease suppression.

PILOT STUDY

DNA and RNA isolated from forest soil cores taken from replicates of contrasting habitat types (relatively wet-mesic vs. relatively dry-mesic) within PREF are being analyzed via amplicon sequencing coupled with metatranscriptomics. FIA plots (6 within each contrast) established within PREF have been sampled (Figure 1). Each plot was randomly selected, controlling for canopy cover, mean canopy height, and elevation. Soil was sampled at two time points (autumn 2013 and spring 2014) and three depths (0, 7.5, and 15 cm below the forest floor, as well as from a

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composite sample) at the midpoint between the bole and drip line of the nearest mature Douglas-fir (*Pseudotsuga menziesii*) or grand fir (*Abies grandis*) at a measured distance and azimuth from the geolocated plot center. These trees were surveyed and sampled for the presence of *Armillaria* species, which were identified using a combination of somatic pairing and tef1α sequence variation (Ross-Davis et al. 2012). RNA and DNA have been isolated from each soil subsample using the RNA PowerSoil Total RNA Isolation and PowerLyzer PowerSoil DNA extraction kits, respectively (MoBio, Carlsbad, CA). Barcoded amplicons are being sequenced via the Illumina MiSeq system, and RNA will be sequenced on the Illumina HiSeq system through the IBEST Core Facility (University of Idaho, Moscow, Idaho). 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, across fine spatial and temporal scales, and to examine how these communities relate to their environment.

PRELIMINARY RESULTS AND FUTURE DIRECTIONS

Compared to wet-mesic plots, dry-mesic plots had lower canopy cover, more diverse ground vegetation, more variable soil moisture and temperature profiles (with higher temperature means and maxima, and lower temperature and moisture minima), much earlier Spring warming (i.e., soil temperatures in dry-mesic plots reached > 0 °C in mid-March compared to mid- to late-April in the wet-mesic plots) and were less prone to harbor pathogenic species of Armillaria (Tables 1, 2, and 3). Three species of Armillaria were detected, mostly collected as rhizomorphs. Mycelial fans of *A. solidipes* were present in 2 of the 12 sampled plots (both wet-mesic). Analysis of the forest soil microbiota is on-going.

Table 1. Characteristics of Surveyed FIA Plots at Priest River Experimental Forest, Idaho, USA.

	Plot	Habitat Type*	Canopy (%)	Slope (°)	Aspect (°)	Ground Vegetation (% Cover, Richness)
WET-MESIC	3136	TSHE/CLUN - CLUN PHASE	91.74	32	310	5, 1
	3161	TSHE/CLUN - CLUN PHASE	89.66	32	18	22, 4
	3104	TSHE/CLUN - CLUN PHASE	90.96	20	286	trace, 2
	3147	TSHE/CLUN - CLUN PHASE	77.67	24	320	20, 9
	3139	TSHE/CLUN - CLUN PHASE	92.26	28	320	2, 4
	3146	THPL/CLUN - CLUN PHASE	91.74	24	183	44, 9
DRY-MESIC	3111	ABGR/PHMA - PHMA PHASE	55.25	38	322	76, 12
	3317	ABGR/PHMA - PHMA PHASE	90.70	43	138	47, 10
	3106	ABGR/CLUN - PHMA PHASE	84.96	32	215	18, 11
	3381	ABGR/CLUN - PHMA PHASE	89.13	12	132	26, 10
	3276	PSME/PHMA - PHMA PHASE	84.96	28	212	100, 11
	3128	PSME/PHMA - SMST PHASE	39.62	8	158	63, 7

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

Table 2. Soil Moisture and Temperature Profiles of Surveyed FIA Plots.

	Plot	Soil Moisture m^3/m^3 Mean (Min - Max)	Soil Temperature °C Mean (Min - Max)
WET-MESIC	3136	0.116 (0.0322 - 0.3518)	6.21 (-1.727 - 16.272)
	3161	0.151 (0.0519 - 0.2414)	6.74 (-2.044 - 17.201)
	3104	0.166 (0.0366 - 0.3148)	6.57 (-0.563 - 17.605)
	3147	0.189 (0.0330 - 0.3279)	6.61 (-0.004 - 17.153)
	3139	0.151 (0.0402 - 0.3373)	6.43 (-1.986 - 16.320)
	3146	0.094 (0.0010 - 0.2501)	6.69 (-2.918 - 18.319)
DRY-MESIC	3111	0.114 (0.0010 - 0.2356)	7.89 (-2.363 - 19.080)
	3317	0.116 (-0.0142 - 0.2443)	9.11 (-6.105 - 24.026)
	3106	0.121 (0.0003 - 0.2632)	7.80 (-3.866 - 32.846)
	3381	no data	no data
	3276	0.118 (-0.0150 - 0.2625)	9.54 (-2.654 - 23.833)
	3128	0.158 (-0.0273 - 0.3431)	10.21 (-2.131 - 26.769)

Table 3. Disease Data and Associated Fungi of Surveyed FIA Plots.

	Plot	Armillaria*	Host*	Other fungi	Host*
WET-MESIC	3136	No	.	No	.
	3161	*A. altimontana* – R	ABGR	*Heterobasidion irregulare*	TSHE
		A. solidipes – F	ABGR		
	3104	*A. solidipes* – F	PSME	*Perenniporia subacida*	PSME
	3147	*A. altimontana* – R	**ABGR**	*Leptodontidium* sp.	**ABGR**
				Leohumicola sp.	**ABGR**
	3139	*A. gallica* – R	ABGR	No	.
	3146	*A. gallica* – R	PSME	No	.
DRY-MESIC	3111	No	.	No	.
	3317	No	.	No	.
	3106	*A. altimontana* – R	PSME	*Phialocephala fortinii*	PSME
				Phellinidium sulphurascens	PSME
	3381	*A. gallica* – R	**ABGR**	in progress	**ABGR**
	3276	*A. gallica* – R	PSME	*Penicillium* sp.	PSME
	3128	*A. gallica* – R	PSME	No	.

* TSHE = *Tsuga heterophylla*; ABGR = *Abies grandis*; PSME = *Pseudotsuga menziesii*; R = rhizomorph; F = mycelial fan; hosts in bold were dead.

Figure 1. **A**: Sampling vegetation within a 1m2 plot; **B**: Surveying for Armillaria; **C**: Digital canopy analysis; **D**: Installing data loggers to measure soil moisture and temperature; **E**: Coring soil to sample at set depths below the forest floor; and **F**: Isolating DNA from soil samples.

Future applications of this approach to FIA plots will promote an understanding of how biotic communities and their relationships to the environment drive forest ecosystem processes. As information accumulates across diverse 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.

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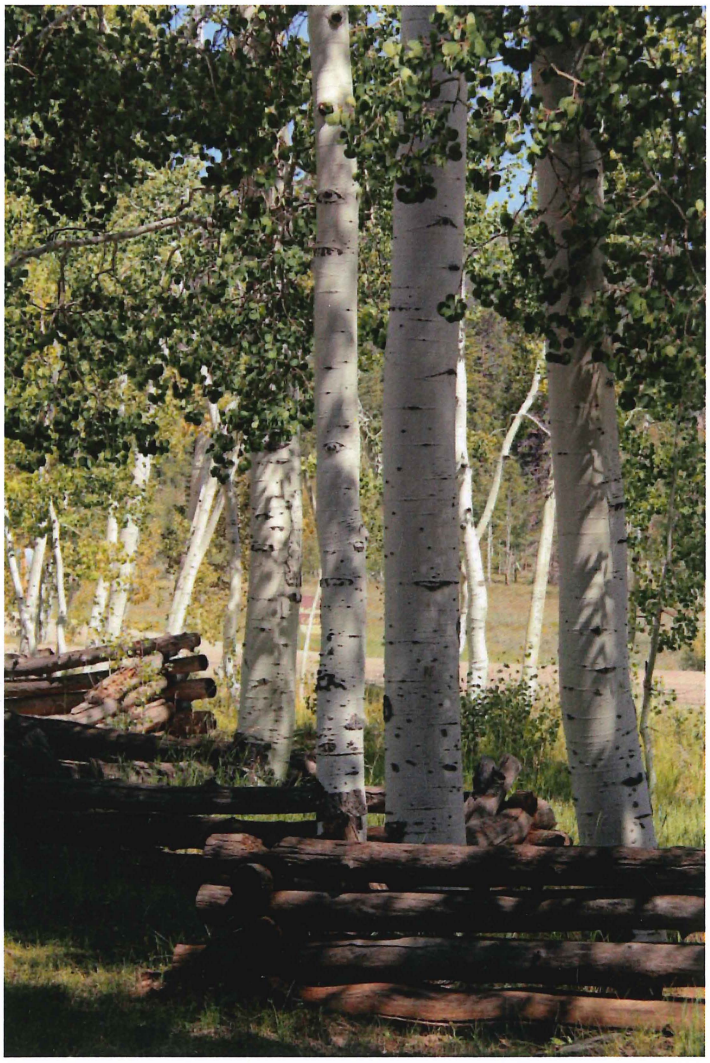

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