METAGENOMIC APPROACHES TO DETERMINE SOIL MICROBIAL COMMUNITIES ASSOCIATED WITH ARMILLARIA ROOT DISEASE

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INTRODUCTION

Armillaria root disease causes extensive damage to tree roots throughout the world, but efficacious management practices are lacking. However, soil interactions among *Armillaria* species, microbial communities, and trees may determine the impact of pathogenic *Armillaria* on the growth and survival of trees. Two species, *A. solidipes* (highly virulent) and *A. altimontana* (less virulent), frequently co-occur in forests of inland northwestern USA. Soil metagenomics and metatransciptomics may provide key insights into how interactions among soil microbial communities and root pathogens influence disease severity. If we can understand how soil microbial communities influence Armillaria root disease, then we can potentially develop novel management techniques that enhance biocontrol microbes and favor microbial communities that suppress disease caused by virulent *Armillaria* species.

The research objective is to provide a baseline for soil fungal and bacterial communities that are associated with two *Armillaria* species with differing ecological roles, *A. solidipes* (high virulence) and *A. altimontana* (low virulence).

METHODS

Data were collected from the Priest River Experimental Forest in northern Idaho within a western white pine (*Pinus monticola*) provenance (seed source) study (Figure 1). Of the original 2,400 planted in 1971, <600 trees remain after ~75% thinning in 1987 and other mortality. Sampling was completed during late June, 2016. From the remaining trees, 63 trees were selected, based by health status and previous *Armillaria* association that was determined in 1987. Rhizomorphs, bulk density soil cores, diameter at breast height (DBH), and tree health status were collected from each sampled tree. Soil DNA and RNA were extracted, and tag-amplicon sequencing of the rDNA ITS2 (fungal) and 16S (bacterial) was completed. Rhizomorph-derived cultures were established. From these, DNA was extracted and the translation elongation factor-1α (*tef*1) was amplified and sequenced for *Armillaria* species identification. Illumina files were cleaned using Trimmomatic and aligned to Silva and UNITE reference

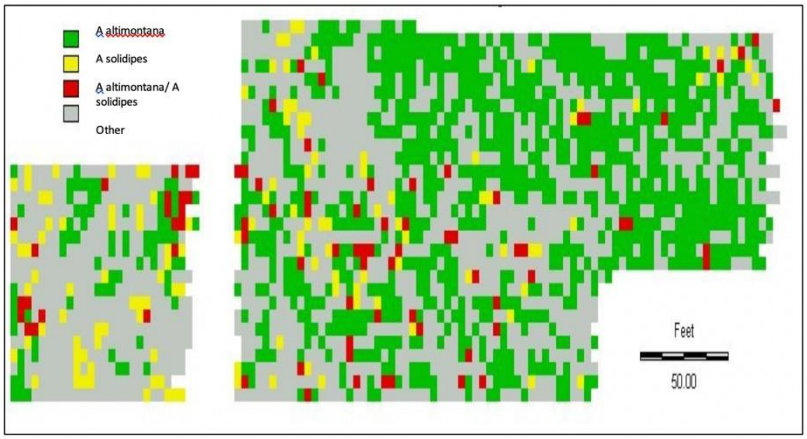

Figure 1. Map of western white pine planting and associated *Armillaria* spp.

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databases for identification. OTU tables were referenced to microbial communities using R (Rstudio Team, 2017). The number of species within fungal and bacterial communities, richness, and the relative abundance, diversity, of samples were analyzed. The analysis was done to determine if soil microbial communities differ in respect to associated *Armillaria* species and tree health status.

RESULTS

Of the total 59 trees sampled, 56 trees were associated with *A. altimontana*, whereas only three trees were associated with *A. solidipes*. *A. altimontana* and healthy trees were associated with more diverse bacterial communities, both in richness and in Shannon's diversity, compared with *A. solidipes* and dead-standing trees. Yet, these differences were only significant for tree health (Figures 2 A & C). Interestingly, *A. solidipes* and dead trees were associated with more diverse fungal communities compared to *A. altimontana* and healthy trees. Yet, there was no significant differences observed for fungal communities (Figures 2 B & D).

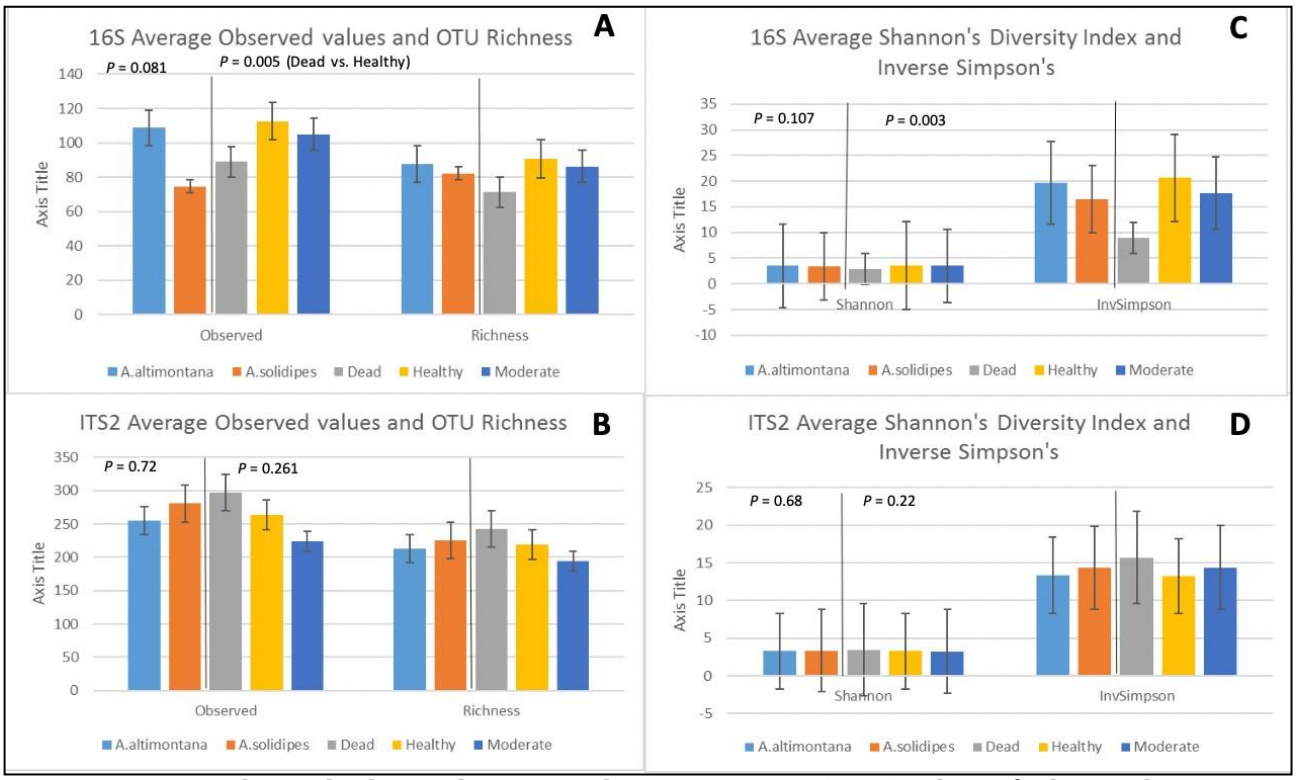

Figure 2. Average observed values and Operational Taxonomic Unit (OTU) richness for bacterial communities (**A**) and fungal communities (**B**) Average Shannon's diversity and inverse Simpson's values for bacterial (**C**) and fungal communities (**D**).

Based on the 712 unique bacterial OTUs identified, more Pseudomonadaceae and Spartobacteria were associated with healthy trees, while more Acidobacteria were associated with dead trees (Figure 3). In respect to *Armillaria* species, more Pseudomonadaceae and Rhizobiales were associated with *A. altimontana*; whereas, more Acidobacteria and Enterobacteriaceae were associated with *A. solidipes* (Figure 3).

Based on the 3,383 unique fungal OTUs identified, more Cortinariceae and Hypocreaceae (e.g., *Trichoderma*) associated with healthy trees, and more Inocybaceae were associated with dead trees (Figure 4). More Trichocomaceae, Cortinariaceae, and Rhizopogonaceae were found in association with *A. altimontana*, while more Mortierellaceae were found in association with *A. solidipes* (Figure 4).

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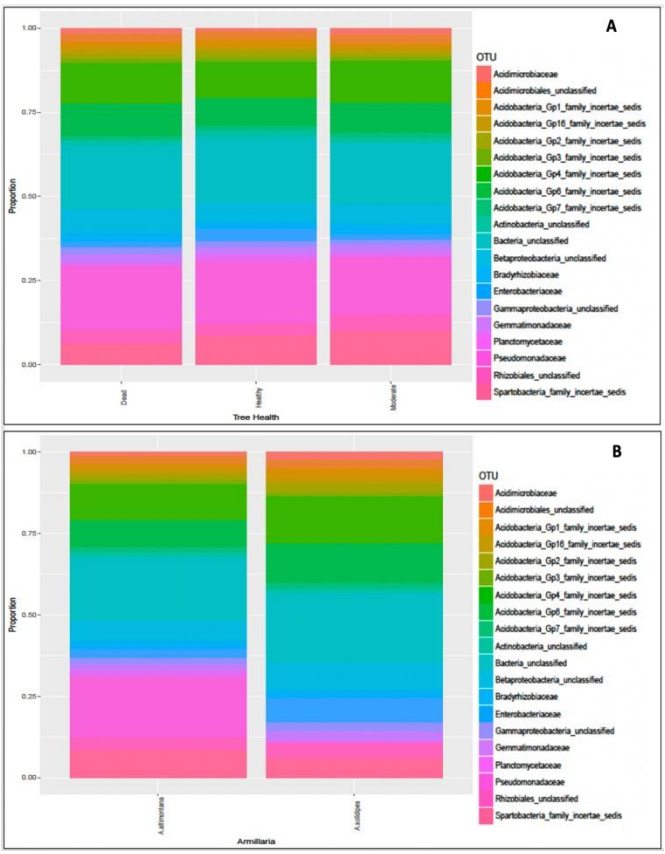

Figure 3. Stacked bar graphs identifying most prevalent bacterial communities; Tree health (A), Armillaria species (B).

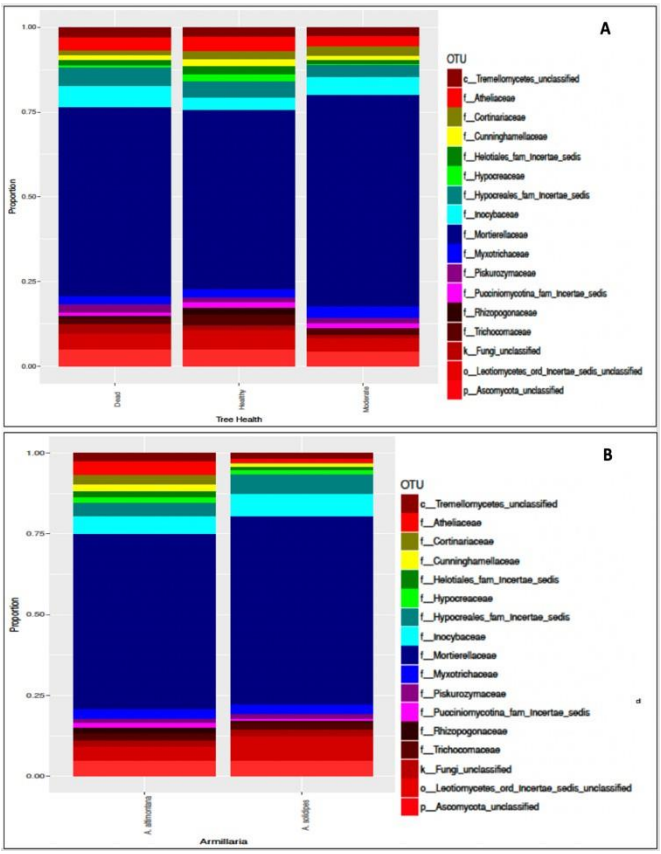

Figure 4. Stacked bar graph identifying most prevalent fungal communities; Tree health (A), Armillaria species (B).

DISCUSSION

Potentially higher bacterial diversity is associated with healthy trees and *A. altimontana*; whereas, higher fungal diversity may be associated with dead-standing trees and *A. solidipes.* When examining OTUs within communities, we found higher levels of Pseudomonadaceae and *Trichoderma* species associated with healthy trees and *A. altimontana.* These organisms are known to be important in biocontrol against pathogens in disease-suppressive soils. Preliminary results suggest novel approaches could be developed for managing Armillaria root disease by fostering soil conditions to favor microbial communities that suppress Armillaria root disease. Results will be correlated to soil physical/chemical properties and efforts are underway to further explore microbial communities associated with *A. solidipes* and *A. altimontana* using artificial inoculations.

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