

ASSESSING SOIL MICROBIAL COMMUNITIES ASSOCIATED WITH TREE HEALTH IN A WESTERN WHITE PINE (*PINUS MONTICOLA*) STAND

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At a landscape level, measurements of forest health can be a fluid statistic as it relates to the specific goals of management, which differ from stand to stand (Crann et al. 2015). As a result, analyses of trees at the individual or stand level may discover previously unknown factors that influence specific tree health metrics. Above-ground dynamics, such as productivity and foliage integrity, are common measures of tree health (Trumbore et al. 2015); however, roots and their interactions with soil and microbes may have a greater impact on tree health than the above-ground dynamics.

Soil acts as a substrate for trees to grow, while microbial communities associated in the rhizosphere and adjacent soil facilitate water and nutrient uptake (Baldrian 2017, Crann et al. 2015, Lee Taylor & Sinsabaugh 2014, Robertson & Groffman 2014). Additionally, microbes can act to impede the establishment of harmful pathogens (Baumgartner & Warnock 2006, Mesanza et al. 2016, Xiong et al. 2017, Warwell et al. 2019). Currently, root diseases are hypothesized to cause the largest damage in forests of the United States (Krist et al. 2014). The assessment of soil microbial communities will increase our awareness of the soil ecosystem associated with tree health, while increasing information for root disease management. The goal of our research was to identify how soil microbial communities differ among tree health statuses (healthy, declining, and dead) within a western white pine (*Pinus monticola*) plantation in the Priest River Experimental Forest of northern Idaho. The research question was to evaluate if soil microbial communities shift in association with changes in tree health. This study occurred in a stand historically infected by two *Armillaria* species, *Armillaria solidipes* which is well-known primary pathogen in many conifer species and *A. altimontana*, a species that appears to not harm western white pine while providing protection from pathogenic *A. solidipes* (Warwell et al. 2019).

Sixty-three trees were selected based on the historic tree health status, from a full inventory conducted in 1987 sampled in 2016. In total, 38 trees were healthy, 12 were declining, and 13 were dead. Soil samples were collected in association with each tree and DNA was extracted. Soil DNA was sequenced at the internal transcribed spacer (ITS2) (fungal) and 16S ribosomal (bacterial) DNA regions to determine microbial communities associated with each tree health status using the MiSeq Illumina platform.

Slight fungal community differences were observed between soils associated with declining and dead trees ($p = 0.055$), while no differences were observed between healthy and dead trees ($p = 0.18$) (Figure 1). Soils associated with dead trees had the greatest fungal diversity and richness within samples in comparison to healthy and declining trees (Table 1). Additionally, soils associated with healthy trees had a greater proportion of Hypocreaceae (e.g., *Trichoderma* spp.), which can act as biocontrol agents in disease-suppressive soils.

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Table 1: Richness and diversity indices for soil microbial communities associated with tree health.

Fungi			
	Richness	Shannon	Inverse Simpson
Healthy	216 ± 11.1	3.26 ± 0.074	13.1 ± 1.00
Declining	182 ± 24.5	3.05 ± 0.162	10.0 ± 2.21
Dead	243 ± 18.0*	3.45 ± 0.119*	15.7 ± 1.62*

Bacteria			
	Richness	Shannon	Inverse Simpson
Healthy	224 ± 15.2	4.29 ± 0.205*	27.32 ± 4.06*
Declining	217 ± 35.4	4.16 ± 0.476	22.45 ± 9.45
Dead	177 ± 33.8	3.30 ± 0.321	9.08 ± 6.37

*Identifies a significant difference (p = 0.05)

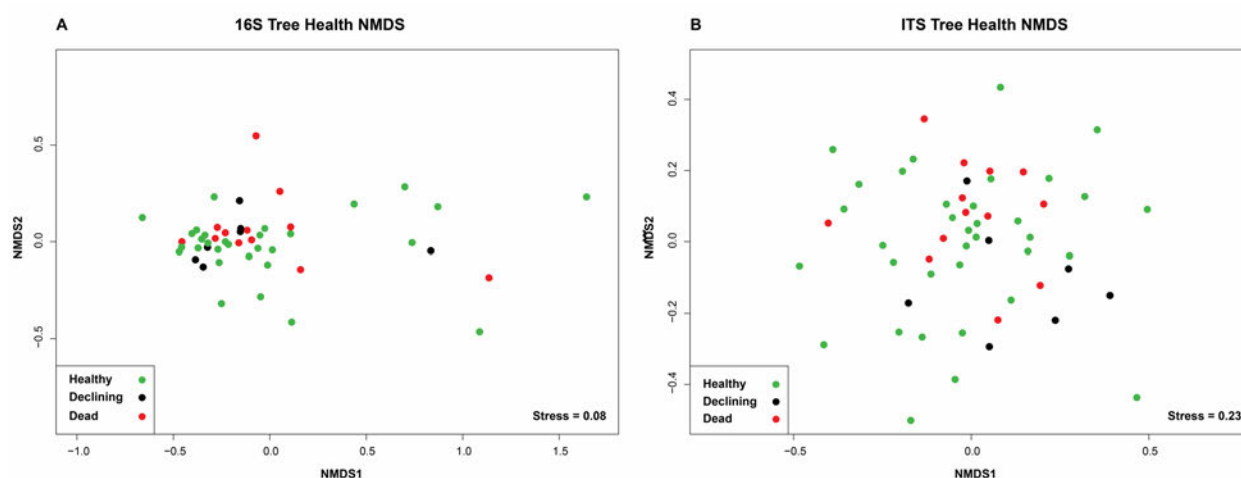


Figure 1: Non-metric multidimensional scaling plots used to determine dissimilarities between soil microbial communities associated with tree health. Tree health status for each tree is indicated by specific colored dots (Healthy = green; Declining = black; Dead = red). A) Determined high overlap between 16S (bacterial) soil communities associated with tree health with some outliers for each tree health. B) Identified similar, high overlap, between ITS (fungal) soil microbial communities associated with tree health.

Minor dissimilarity occurred between soils associated with dead trees and healthy (p = 0.02), indicating slight differences in bacterial communities, while no differences were observed between healthy and declining trees (p = 0.88). Although no differences were observed in the overall community structure, clear patterns were found when examining the most abundant bacterial taxa (Figure 2). Soils associated with healthy trees had the greatest bacterial diversity and richness in comparison to declining and dead trees, with dead trees having the lowest diversity and richness (Table 1). Soils associated with healthy trees had a greater abundance of Chthoniobacteraceae, Nitrosomanadaceae, Acidobacteria subgroups 2 & 6, and Xanthobacteraceae, all of which function to increase soil health. Interestingly, a greater abundance of Pseudomonadaceae and Enterobacteraceae was associated with dead trees, and these organisms are important in biocontrol against pathogens in disease-suppressive soils.

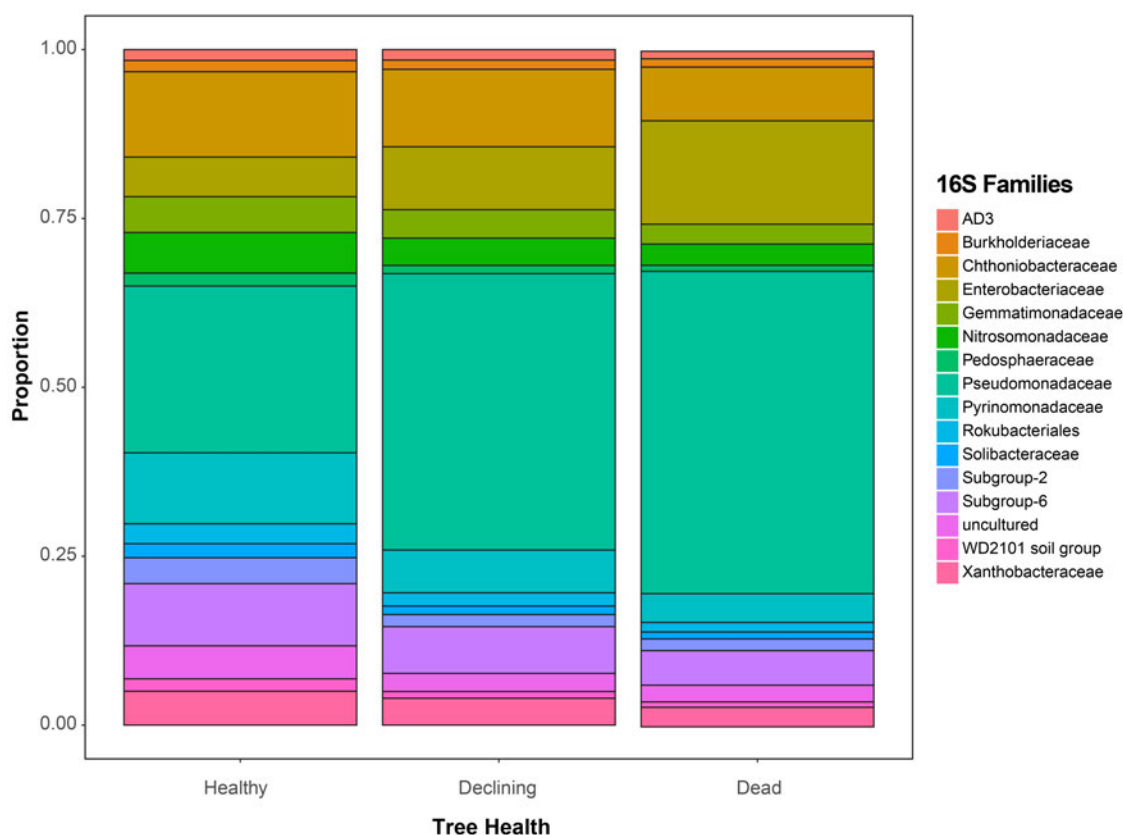


Figure 2: Bar graph determining the most abundant bacterial (16S) families associated with each tree health.

To further determine factors that may influence soil microbial communities, soil properties were assessed at each tree location. The most significant soil property correlated to fungal community composition was pH ($p = 0.001$), while pH ($p = 0.015$) and moisture ($p = 0.010$) were also significantly correlated to bacterial community composition. Additionally, carbon ($p = 0.001$, $p = 0.0232$), nitrogen ($p = 0.017$, $p = 0.01$), and organic matter ($p = 0.0003$, $p = 0.007$) may have an indirect influence on microbial communities as these factors showed a significant relationship to soil pH and/or moisture.

Further analyses are needed to fully determine how soil microbial communities interact with tree health status. Functional characteristics may elucidate this relationship because many microbes are functionally redundant within the soil. Ideally, these studies will increase our knowledge of the dynamics of soil microbial communities with the goal to uncover novel approaches for managing tree health by managing forest soils to favor beneficial soil microbes that suppress root diseases or perform other beneficial functions.

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