ASCC paper title TBD

Kya Sparks, Tim Fegel, Kaela K. Amundson, Emily Bechtold, Charles C. Rhoades, Michael J. Wilkins

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**Abstract**

**Intro**

**Methods**

Study Site and Experimental Design

This study was conducted across three experimental silviculture trials in Colorado, USA: State Forest State Park (SFSP), Taylor Park, and San Juan National Forest (SJNF). These sites represent distinct environmental conditions, differing significantly in geographic location, climate, vegetation, and soil characteristics. Notably, these forests have not experienced recent fire events or significant impacts from mountain pine beetle infestations. SFSP is situated at elevations ranging from 9,143 to 10,626 ft and is predominantly composed of Engelmann spruce, subalpine fir, and lodgepole pine. Taylor Park, with elevations between 9,662 and 10,538 ft, is dominated by lodgepole pine, with smaller components of Engelmann spruce and subalpine fir. SJNF lies at a lower elevation of 7,400 to 8,600 ft and features a mixed conifer forest, including ponderosa pine, fir, aspen, and Gambel oak. Soil characteristics also varied among the sites. The mineral soils at SFSP and Taylor Park were drier and sandier compared to SJNF, while the organic matter layers differed in composition, particularly in needle and duff content. (maybe add: These environmental and biological differences provided a robust framework for exploring microbial community composition and function.)

This study was designed to investigate differences in microbial communities across three coniferous forest sites in Colorado, with the goal of informing strategies for tree reestablishment and assisted migration success. Within each experimental site, microbial composition was analyzed across distinct soil depths to better understand the factors driving community variation and their potential implications for forest management, INSERT SAMPLING DESIGN,

Sampling and Analysis

Between July and September 2023, soils were sampled for microbial, nutrient, and chemical analysis.

Soil Water, Nutrients and Chemistry

Soils were collected with a 6.4 cm diameter bulb corer that was sterilized with ethanol between samples.

Gravimetric soil water content was calculated by oven-drying samples at 105°C for 24 hours.

Water content is typically measured using the gravimetric method, where a soil sample is weighed, dried to remove moisture, and weighed again; the difference in weight indicates the water content as a percentage of the soil’s weight.

Microbial analyses

Subsamples were collected in sterile Whirlpak bags and stored at 4°C during transport, then transferred to a -80°C freezer until further processing. DNA extraction was performed using the Zymo Quick-DNA Fecal/Soil Microbe Miniprep Kit. For soil bacterial communities, the V4 region of the 16S rRNA gene was amplified using the primers 515F (5’-GTGYCAGCMGCCGCGGTAA-3’) (Parada et al., 2016) and 806R (5’-GGACTACNVGGGTWTCTAAT-3’) (Apprill et al., 2015). Soil fungal communities were amplified using primers targeting the first internal transcribed spacer (ITS1) region of the ribosomal DNA, ITS1f (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) (White et al., 1990). Sequencing was performed on the Illumina MiSeq Platform using 251 bp paired-end sequencing at the Microbial Community Sequencing Lab at the University of Colorado Boulder. Raw sequencing reads were processed using QIIME2 (release 2021.2) (cite). Poor-quality ITS reverse reads were discarded. Demultiplexed 16S and ITS samples were merged, filtered, denoised, and binned to infer amplicon sequence variants (ASVs) using DADA2 (cite). Gene read counts for 16S and ITS were INSERT, and bacterial ASVs were assigned taxonomy using the pre-trained SILVA classifier (version 138; Quast et al., 2013; Bokulich et al., 2018; Robeson et al., 2021), while fungal ASVs were assigned taxonomy using a self-trained UNITE database classifier (version 9.0; Nilsson et al., 2019; Kõljalg et al., 2020). Fungal sequences not assigned to the Kingdom Fungi and bacterial sequences assigned to mitochondria or chloroplasts were removed from ASV tables prior to downstream analysis.

Resulting reads were deposited and are available at NCBI under BioProject:

Statistical Analysis

**RESULTS**

***Subheader about soil chemistry***

A suite of soil physicochemical measurements were made across the three sites and depth-resolved samples. Soil %C and %N was consistenly higher in OM layers relative to deeper mineral soils at all three locations. Water content was also significantly higher in the OM layer than mineral soils at the State Forest and San Juan locations, but not Taylor Park. Water extractable fractions including Dissolved Organic Carbon (DOC), Total Dissolved Nitrogen (TDN), sodium (Na+), ammonium (NH4+), potassium (K+), magnesium (Mg2+), calcium (Ca2+), chloride (Cl-), nitrate (NO3-), phosphate (PO₄³⁻), and sulfate (SO42-) were also measured in mineral soil samples. Describe key trends within a given location and between locations at different depths

***Forest surface organic matter hosts greater microbial diversity than mineral soils.***

Consistent alpha diversity trends were observed for both bacterial and archael, and fungal communities across the three sites. Species richness, an alpha diversity metric defined as the number of unique features in a community (cite), was significantly higher in the organic matter (OM) layer compared to the other two depths at both the State Forest and San Juan sites for the bacterial and archaeal portion of the community (fig x). For fungal communities, species richness was significantly higher in the OM layer compared to the other two depths across all three sites (fig x). Shannon’s diversity, another alpha diversity metric that accounts for both richness and evenness, was significantly higher in the OM layer compared to the other two depths across all three sites (fig x) for both bacterial and archaeal, and fungal communities.

Beta diversity analyses (i.e., assessing dissimilarity between communities) revealed significant differences in community composition across all sites and depths for both bacterial and archaeal, and fungal communities (stats) (Figure X, Table S1). The incorporation of soil chemistry data into multivariate analyses indicated that water content, %N, and %C was driving the separation of microbial communities in the OM layers from the mineral soils. When considering just the mineral soils, DOC, PO4 and SO4 were drivers of bacterial and archael community composition in State Forest samples. Converesely, TDN and NO3 were drivers of bacterial and archaeal community composition in San Juan samples. Complementary analyses of the fungal data revealed similar drivers of community dissimilarity between the three sampling locations (Figure X).

***Abundant Microbial Taxa 16S.***

Abundant Microbial Taxa 16S

1. d\_\_Bacteria;p\_\_Verrucomicrobiota;c\_\_Verrucomicrobiae;o\_\_Chthoniobacterales;f\_\_Chthoniobacteraceae;g\_\_Candidatus\_Udaeobacter;\_\_
2. d\_\_Bacteria;p\_\_Acidobacteriota;c\_\_Blastocatellia;o\_\_Pyrinomonadales;f\_\_Pyrinomonadaceae;g\_\_RB41;\_\_
3. d\_\_Bacteria;p\_\_Verrucomicrobiota;c\_\_Verrucomicrobiae;o\_\_Chthoniobacterales;f\_\_Xiphinematobacteraceae;g\_\_Candidatus\_Xiphinematobacter;s\_\_uncultured\_bacterium
4. d\_\_Bacteria;p\_\_Planctomycetota;c\_\_Phycisphaerae;o\_\_Tepidisphaerales;f\_\_WD2101\_soil\_group;g\_\_WD2101\_soil\_group;s\_\_uncultured\_bacterium
5. d\_\_Bacteria;p\_\_Proteobacteria;c\_\_Alphaproteobacteria;o\_\_Rhizobiales;f\_\_Xanthobacteraceae;\_\_;\_\_
6. d\_\_Bacteria;p\_\_Acidobacteriota;c\_\_Vicinamibacteria;o\_\_Vicinamibacterales;f\_\_uncultured;g\_\_uncultured;\_\_
7. d\_\_Bacteria;p\_\_Actinobacteriota;c\_\_Actinobacteria;o\_\_Corynebacteriales;f\_\_Mycobacteriaceae;g\_\_Mycobacterium;\_\_

Bacterial and archaeal ASVs affiliated with the Phyla Verrucomicrobiota and Acidobacteriota were frequently the most abundant across the majority of samples studied here. Specifically, ASVs affiliated with the genus Candidatus Udaeobacter ranged from ~2–38% relative abundance across samples, with ASVs affiliated with the family Pyrinomonadaceae accounting for slightly lower totals. Discuss Xantho here and lead into core analysis.

The most abundant bacterial and archael taxa in our 16S samples are dominated by diverse groups across several phyla. *Verrucomicrobiota*, represented by the genera *Candidatus Udaeobacter* and *Candidatus Xiphinematobacter* are the two most abundant groups across all sites and depths, highlighting their ubiquity across forest soil microbiomes. *Verrucomicrobiota* are known to thrive in nutrient poor soils (cite), which may explain their prevalence within nutrient-poor or OM-rich soil layers. *Acidobacteriota* is another abundant taxon, associated with low pH soils and environments with rich OM content which are conditions within this study. Similarly, members of the *Planctomycetota* are highly abundant and are often linked to anaerobic conditions, highlighting this microbial community’s ability to thrive in low-oxygen environments. The presense of *Mycobacterium* (*Actinobacteriota*) and *Xanthobacteraeceae* (*Protobacteria*) show the importance of nitrogen cycling within our study sites, as they are known to cycle nutrients and breakdown organic matter.

***Abundant Microbial Taxa ITS.***

Abundant Microbial Taxa ITS

1. k\_\_Fungi;p\_\_Basidiomycota;c\_\_Geminibasidiomycetes;o\_\_Geminibasidiales;f\_\_Geminibasidiaceae;g\_\_Geminibasidium;s\_\_Geminibasidium\_sp
2. k\_\_Fungi;p\_\_Ascomycota;c\_\_Leotiomycetes
3. k\_\_Fungi;p\_\_Ascomycota;c\_\_Leotiomycetes;o\_\_Helotiales
4. k\_\_Fungi;p\_\_Mortierellomycota;c\_\_Mortierellomycetes;o\_\_Mortierellales;f\_\_Mortierellaceae;\_\_;\_\_
5. k\_\_Fungi;p\_\_Mortierellomycota;c\_\_Mortierellomycetes;o\_\_Mortierellales;f\_\_Mortierellaceae;g\_\_Podila;s\_\_Podila\_humilis
6. k\_\_Fungi;p\_\_Basidiomycota;c\_\_Agaricomycetes;o\_\_Atheliales;f\_\_Pilodermataceae;g\_\_Piloderma;s\_\_Piloderma\_sp
7. k\_\_Fungi;p\_\_Basidiomycota;c\_\_Agaricomycetes;o\_\_Cantharellales;f\_\_Hydnaceae;g\_\_Sistotrema;s\_\_Sistotrema\_sp
8. k\_\_Fungi;p\_\_Basidiomycota;c\_\_Microbotryomycetes;o\_\_Microbotryales;f\_\_unidentified;g\_\_unidentified;s\_\_Microbotryales\_sp

***Specific microbial groups drive dissimilarity between locations***

Figures – summary plot, also volcano plots

* Clean up volcano plots !

Can talk about broad phyla-level trends between the ecosites

Also look for specific ASVs (from volcano plots) that are important. Discuss these more

Don’t forget FUNGI!!! - are any EMF discriminant between sites?

We did not find any discriminant taxa among fungi when comparing across all sites and depths.

***Diverse forest soils also host conserved microbial taxa***

Figure – play around with different heatmaps (venn diagram??) showing high resolution rather than phyla level

Core analyses

Are any EMF core between sites?

Obviously mention that Xantho is present across all sites

Microbial communities in forest soils are shaped by distinct environmental factors such as geographic location, climate, and vegetation, leading to substantial variability across sites and depths. Despite these differences, identifying core taxa, microbial groups that are consistently present across a majority of samples, can help uncover key players involved in critical ecosystem functions.

To assess microbial taxa that persist across different Colorado forest types, we performed a core analysis. For this analysis, we required a given ASV to be present in XX... In our analysis of bacterial communities, we identified 11 core taxa present in at least 70% of samples across all sites and depths. Notable taxa include Cthoniobacterales, Xanthobacteraceae, and Rhizobium, which are likely important contributors to nutrient cycling processes. Among these, an ASV affiliated with the Family Xanthobacteraceae was particularly prominent, as it was detected in 100% of samples, highlighting its potential ecological significance as a conserved and ubiquitous taxon across forest soils.

Interestingly, when examining interactions between these core taxa and other community members, we observed very few consistent connections across all sites and depths. However, the strongest interactions were observed with members of the Steroidobacterales order, which are known to play important roles in plant-microbe interactions (cite). This suggests that while core taxa are conserved across forest soils, their ecological roles may be influenced by interactions with functionally significant taxa such as Steroidobacterales.

For fungal communities, no taxa were found to meet the 70% threshold for core membership. However, by lowering the threshold to 60%, we identified two core fungal taxa, both belonging to the Microbotryales order within the Basidiomycota phylum. (maybe add -- The consistent presence of these taxa suggests they may play a functional role in forest soil ecosystems, though their specific contributions warrant further investigation.)

***Xantho-specific section***

Talk about functional potential in MAGs – CAZYmes, metabolism

**Role in networks** – possible links with Steroidales (sp?). Figure showing conserved connections between Xantho and other microbes.

* Steroido may be responsible for immobilization of carbon
  + <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0236305>
  + <https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2021.745915/full>
* <https://www.researchsquare.com/article/rs-5675838/v1>

Given that Xanthobacteraceae was detected in all samples (insert exact number?) across all sites and depths, we conducted a deeper investigation into its functional potential and ecological significance. By linking taxa of interest (Xanthobacteraceae) to a database of metagenome-assembled genomes (MAGs) derived from coniferous forest ecosystems, we aimed to predict its functional potential. Three MAGs corresponding to Xanthobacteraceae were identified in the database.

Among the predicted functions, we identified a carbohydrate-active enzyme (CAZyme) belonging to the glycoside hydrolase family 5 (GH5), which is known for its role in the degradation of amorphous cellulose (cite). This enzyme facilitates both backbone cleavage and oligosaccharide cleavage of amorphous cellulose, breaking it down into smaller sugars that can be utilized by microbial communities (cite). The ability to degrade amorphous cellulose suggests that Xanthobacteraceae may play a key role in the decomposition of plant-derived organic matter, contributing to the carbon cycle in forest ecosystems.

While the presence of the GH5 enzyme hints at the potential for autotrophic processes via cellulose degradation, the abundance of organic matter in these forest soils provides a rich carbon source that may also support heterotrophic activity. This functional versatility could explain why Xanthobacteraceae is consistently found across varying environmental conditions, making it a potentially important contributor to soil nutrient cycling and microbial interactions.

TBD – can we use ML to identify any non-linear relationships (work with Dave on this)

  

 