Soil microbial diversity across a chronosequence of mixed-oak forest

group selection harvested gaps

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**Introduction:**

Group selection is a silvicultural method aimed at promoting forest stand complexity through mimicking natural disturbance and subsequent regeneration patterns. However, lack of oak regeneration (and other more shade tolerant species) has been observed in gaps created using this method in upland mixed-oak forests in North Carolina (unpublished data). Canopy gap creation alters the microclimate, mainly thermal radiation, impacting soil microbial communities. Forest disturbances reduce soil microbial biomass by a combined average of 29.4% (Holden & Treseder 2013). Additionally, gap creation has been shown to promote soil organic C retention in the form of soil microbial biomass (Liu et.al. 2018). Research has shown soil microbial composition within group-selection harvest sites to recover quickly (between two and four years post-harvest) (Lewandowski et al. 2015). However, gap size has also been shown to be negatively correlated with microbial biomass (Schliemann & Bockheim 2014). Because of the way soil microbial communities directly impact nutrient availability, understanding the soil microbial dynamics for the group selection harvest sites in NC will improve efforts to establish and regenerate oaks in the area.

Microbial communities are highly complex and abundant throughout all ecosystems. Microbes mediate vital ecosystem processes including decomposition, nutrient cycling, carbon storage, climate regulation, and circulation of elements. As such, microbial communities are the backbone to any ecological process or phenomenon observed. There are two main approaches to characterization of biodiversity in ecology: functional and structural. The functional approach is based on the premise that organism functions are of more interest than their identity. The functions that are performed by organisms in an ecosystem, referred to as the functional diversity, provide the missing link between biodiversity patterns and ecosystem functions (Escalas et al. 2019).

Due to the small size, vast abundance, high degree of species diversity, and rapid ability to evolve, characterization of microorganisms has inherent challenges as compared to marcoorganism characterization. Species level measurements are typical for macroorganism trait classification. However, species level measurements for microorganisms is not only challenging, but its use is also highly debated within functional ecology. Excalas asserts that a community-level approach is better suited than a taxa-centered approach to large scale study of natural communities (2019).

Microbial community structure is highly susceptible to changes in microenvironment. However, due to the multifunctional redundancy within a community, microbial community-level function within an ecosystem is not necessarily altered in the same way or degree as community structure. Research indicates weak relationships between microbial diversity and ecosystem functions (Miki et al., 2013). In fact, structurally different microbial communities are often functionally similar. In a study comparing substrate utilization assay and fatty acid analysis of soil microbial communities, the microbial species composition results showed some change over time that was not observed in the microbial community function, indicating community structure is more sensitive to fluctuation than community function. However, Buyer and Drinkwater also point out that measurement of fatty acid composition could be due to factors other than microbial species composition (i.e. fatty acids from plants, changes in soil temperature or changes in substrates) (1997).

In order to broaden the understanding of in situ ecosystem functions, measurement of metabolic activities of the soil microbial community will be assessed. This will be done through measurement of soil microbial functional diversity using Biolog EcoPlates. These multivariate measurements will allow for calculation of diversity indices. Functional diversity and structural diversity are often used in conjunction with one another to provide more in depth community characterization. PLFA is a commonly used structural approach, however, for this study, assessment of functional diversity using EcoPlates will allow for more rapid assessment of if microbial community variation exists across harvest sites.

Biolog EcoPlates were specifically designed for ecological research and provide community-level assessment of microbial dynamics. Each EcoPlate contains 96 wells with 31 carbon assays and a water control in triplicate. This replication provides increased probability that the developed physiological profile accurately represents the soil microbial community as compared to other types of Biolog microplates. The 31 individual carbon substrates fall in to six groups: amines/amides, amino acids, carbohydrates (mainly simple sugars), carboxylic acids, miscellaneous (including phosphorylated and aromatic compounds), and polymers (Xu and Ge, 2015) (Zak et al.). In addition to an individual carbon assay, each well of an EcoPlate contains a colorless tetrazolium redox dye which acts as an indicator of microbial metabolic activity. Following inoculation with soil solution and incubation, microbial respiration will cause reduction of the colorless tetrazolium dye to a violet formazan. Optical density (OD) is then measured spectrophotometrically and analyzed against the Biolog database of distinctive patterns of community-level substrate utilization (CLSU). The result is also commonly referred to as a “metabolic fingerprint” or “community-level physiological profile (CLPP) (Stefanowicz 2006).”

The average well color development (AWCD) indicates the microbial community potential metabolic activity (index of total bioactivity for Biolog plates). Substrate average well color development (SAWCD) can be calculated by subdividing the AWCD values based on substrate groups that are chemically similar (as indicated above) (Feigal). This approach takes into account that the same metabolic pathways are used for chemically similar substrates and therefore chemical structure may act as a proxy for metabolic similarity (Miki et al., 2018). Physiological groups of microorganisms are often used in soil science as indicators of biochemical processes (Galieva et al., 2017). Therefore looking at AWCD classified by substrate guild, indicates different ecosystem functions the microbial communities measured have the potential to carry out.

Additionally, 9 of the 31 substrates in the EcoPlate are reported as constituents of root exudates: 2-Hydroxy Benzoic Acid, 4-Hydroxy Benzoic Acid, D-Malic Acid, D-Xylose, L-Arginine, L-Asparagine, L-Serine, L-Threonine, and L-Phenylalanine. Comparisons of any variation in the utilization of these 9 substrates has potential to provide insight into how the microbial community interacts with the types of vegetation (either woody or herbaceous species) (Campbell et al., 1997).

The Biolog data is used to calculate the Shannon diversity index (H) which is a measure of physiological diversity of microbial communities. Additional measures to consider from these data are substrate richness (SR) and Shannon evenness (E) (Feigl).

Appalachian forests are subject to a high degree of topographical and vegetative variability and have high degree of carbon source variability. Microbial communities in these ecosystems have previously been shown to have correlations with understory vegetation. Strong positive correlations between plant diversity and soil functional diversity have been observed in mixed-oak forests with herbaceous plant and fern understory (Rodriguez-Loinaz et al., 2008). Osburn et al. observed an increase in microbial activity following the removal *Rhododendron* understory through increasing soil C and N availability (2018).

**Significance of research:**

The pre-European settlement forests of the southern Appalachians were highly diverse at stand and landscape scales, but have moved to more homogeneous composition over time. Forest disturbances, including timber harvesting, alter soil microbial dynamics, which directly impact nutrient availability. Therefore, increased understanding of soil microbial dynamics within group selection harvested gaps over time will improve efforts for reestablishment and protection of these landscapes.

**Primary goals and objectives:**

In this study, we aim to provide links between above- and below-ground biodiversity through answering the following questions:

1. Is there a correlation between soil microbial diversity and biomass and oak regeneration patterns?
2. Is there a correlation between soil microbial diversity and harvested mature oaks (stumps) and unharvested oaks (in controls)? If so, are these microbial communities the same?
3. How does above ground species diversity correlated to soil microbial diversity and biomass?

Additionally, correlations between soil physicochemical properties and microbial diversity will be determined in order to account for the vast microclimate diversity that is expected in forest ecosystems.

**Materials and methods:**

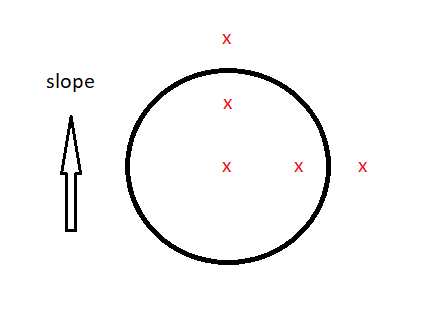
*Study site and study design:*

My research will take place on the Boyd Branch group-selection harvested gaps located on Bent Creek Experimental Forest (BCEF) on Pisgah National Forest located in the Appalachian Mountains of North Carolina. Three group-selection harvests have been completed at Boyd Branch (1986, 2004, 2017) with multiple gaps of varying sizes (averaging 0.08, 0.28, and 0.29 hectares) created each time. For my research I will be focused on the latter two harvests (2004 and 2017) from this site, as these are more comparable in size. 23 and 14 gaps were created in 2004 and 2017 respectively, for a total of 37 gaps of interest.

*Field methods:*

Mineral soil samples 0-15 cm depth will be collected along two transects from the center of each gap to the edge of the gap; one transect going upslope and the other 90 degrees perpendicular to the slope. Sampling points will be at the center of the gap, 2 meters from the edge of the gap, and 2 meters outside of the gap along each transect, for a maximum of 5 sampling points per gap (Fig. 1). Because many of the gaps are directly next to one another, many sampling points will be redundant or within another gap. At each sampling point, four cores will be taken using a 2.5 cm diameter pushtube and combined into one composite sample.

Figure - Sampling Layout



At each sample location, pin flags will be placed and GPS coordinates recorded. A picture will be taken of vegetation at each sample location. If travel is possible, I will return to characterize vegetation in detail. Soil moisture and soil temperature will be measured. Slope and aspect of each gap will be measured.

*Soil Analysis:*

Microbial functional diversity will be determined using Biolog EcoPlates. Microbial biomass C and N will be determined using the chloroform fumigation extraction method. Soil pH will be measured in a 1:2 water solution. Soil C and N will be determined using dry combustion with detection by thermal conductivity. Organic C will be measured by loss on ignition.

*Biolog EcoPlate:*

Soil will be inoculated with a sterilizing phosphate buffer or 0.1145mol L-1 NaCl solution to leach soil microorganism and liquor transferred to EcoPlates. EcoPlates will be incubated at 28-30 degrees and read using the MicroStation (at NCSU soils or forestry department) or OmniLog incubator/reader (if available[[1]](#footnote-1)) every 24 hours for 7 days.

**Time schedule:**

Initial soil measurements will be taken the week of 7/27/2020. Subsequent measurements will be taken seasonally (approx. November, and possibly January and March depending on number of seasons needed). Laboratory procedures and data analysis will be completed by end of spring 2021 semester.

**Plans for dissemination of findings:**

Research findings will be presented as my master’s thesis at North Carolina State University and will also be submitted for publication to journals such as Forest Ecology and Management and the Canadian Journal of Forest Research.

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1. Checking with Dr. Shi [↑](#footnote-ref-1)