**Introduction**:

Current unknowns and controversies in the literature (Ex: @Luyssaert\_2008 --> @Gundersen\_2021 --> @Luyssaert\_2021) regarding old growth forests demand a more collective understanding of the function and fate of these systems. We believe it is the misrepresentation of the below ground community significantly contributing to not only such contrasting findings, but also to the lack of clarity in soil science and soil ecosystem functionality as a whole. The general aim of this study is to analyze soil systems in terms of carbon functionality as successional stages (SS) progress. We want to see, given different levels of disturbance, if soil carbon functionality changes with time and community composition. To do this we have selected old growth forests as our study system due to the few available areas with remaining longevity, and fungi our model organism as they are the stewards of soil carbon dynamics. To achieve our aims, the study will be broken down into three parts: Part 1 consists of a proof-of-concept experiment that will allow us to determine the impact of analyzing a fungal community through three different genome analysis methods. We will test the capability of amplicon sequencing, whole genome sequencing, and whole transcriptome sequencing in analyzing fungal communities among three different experiments. These experiments will test the influence of carbon-substrate type, soil type, and the level of microbial interference on the ability of these methods to accurately represent the given fungal community. These tests will be conducted on a known community consisting of 9 different saprotrophic fungi, containing (3) early, (3) middle, and (3) late successional stage species. The results from Part 1 will dictate the methods used to analyze fungal communities in soil samples from the field. Part 2 will involve soil sampling and physiological measurements in attempts to capture any mechanisms or parameters associated with changes in fungal community as succession progresses. Soil samples will be extracted and sent for sequencing based on the results from Part 1. Part 3 will involve a greenhouse experiment taking soil samples from different SS's and testing carbon attributes such as enzymatic production, respiration rates (flux), and fractionation of carbon pools. This will allow us to further analyze functional differences among soils from varying SS's. Pending the results from Parts 1-3, we hope to repeat these methods in search for trends among different types old growth forests. The overall gap this research aims to fill is whether there are distinct soil functional differences based on carbon mechanisms provided by fungal community in forest succession. Providing insight to this gap will result in a number of opportunities for future research. This project appears to be the first to attempt to specifically determine if there are functional differences among soils along a forest successional gradient. While we are only analyzing carbon functionality and fungal communities, if trends arise, it opens a massive area of research in learning how soils differ functionally among different microbial communities in different systems (i.e. old growth vs managed). Understanding these concepts will likely drive future ecosystem management and restoration projects. Alternatively, if no trends are observed, and the null hypothesis is supported, it can provide reason for future researchers to save significant amounts of time and money on genomic analysis. These old growth ecosystems are natures natural responders to disturbances such as climate change. If they are truly able to functionally adapt and respond to such changes, they can provide insight for future ecosystem protection and preservation.

**Paragraph 1 topic:** Discussing evidence in the research showing what we know about forests changing over time. (could be broken into a few sub paragraphs)

* Forest parameters changing with succession: soil characteristics, nutrient cycling, etc?
* Microbial communities shifting with succession (i.e. bacterial to a fungal dominated system)
* Disturbance (“reverse succession”) and its impact on microbial community in forest soils?

**Paragraph 2 topic:** Discuss fungi and their role in carbon and soil functionality

**Paragraph 3 topic:** Discuss fungal genomics and (eukaryotic) limitations? Include progress made in prokaryotic.

**Paragraph 3 topic:** Final paragraph discussing the lack of clarity among old growth systems and the lack of consideration in belowground community analysis?

**Lead into methods/experimental design/etc.**