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# Introduction

Microscopic organisms are among the oldest and most prevalent lifeforms on earth. Accordingly, they play a vital role in the health of ecosystems by affecting processes like carbon dioxide and nitrogen cycling (Martiny *et al.*, 2006). As the worlds climate changes over time understanding how microbes influence the dynamics of ecosystems will be necessary to preserve the environment. Currently, the impacts of climate change on the biogeochemical cycling of microbes are not well understood (Barnard *et al.*, 2005). However, some research suggests microbes may be able to mediate carbon-cycle feedbacks to climate warming (Zhou *et al.*, 2012).

Due to their importance in the function and stability of ecosystems, the biodiversity of microorganisms is vital to the overall diversity of macroscopic organisms. Typically the first objective of many ecological research projects is to determine the which organisms are present and their biogeography (Zimmerman *et al.*, 2014). Previous research suggests the distribution of microbes is heterogenuous and spatially-structured (Maron *et al.*, 2011). Thus, accurate sampling of a wide variety of plots is necessary to understanding how microbes influence a large ecosystem.

For the microbial community, identifying distint species is particularly difficult (Koeppel *et al.*, 2008). Recent advances in molecular biology, including high-throughput sequencing has allowed for genetic identification of microbial species without culturing them. Many researchers use amplicon sequencing of the 16S rRNA gene as a biomarker for the microbial community (Tringe and Hugenholtz, 2008).

The National Science Foundation (NSF) funded National Ecological Observation Network (NEON) was designed to answer how the ecosystem and it’s components respond to changes in climate? (Keller *et al.*, 2008) To this aim NEON collects microbial samples in soil from a number of sites across the United States. I will investigate the UNDE site, near the Great Lakes in Michigan. The UNDE site is managed by the University of Notre Dame Environmental Research Center (UNDERC). The property has a second-growth Northern mesic forest and Evergreen forests. The dominant species are red and sugar maple (*Acer rubrum* and *A. saccharum*), aspen (*Populus tremuloides*), paper birch (*Betula papyrifera*), balsam fir (*Abies balsamea*), cedar (*Thuja occidentalis*), and black spruce (*Picea mariana*). In addition to the deciduous and mixed forests the site has woody wetlands dominated by thickes of alder (*Alnus incana*).

The climate is generally humid, cool, and wet with no true dry season and receives approximately 114 inches of snow annually. The soil families include coarse, loamy, mixed, superactive, frigid, argic fragiaquods.

Before the land was donated to the Notre Dame University in the 1930s, region-wide logging for pine cut most of the forested areas on the property. Timber harvest continued into the 1950s, but was followed by a forest regrowth. Recently, the site is used for recreational, educational, and research goals.

The objective of this study is to determine which microbes are present at the UNDE site, using the 16S barcode sequence, and how this changes over time. This information provides insight into how these ecosystems respond to climate change and their overall health and function.

The data collected by NEON will be processed for quality control, subsampled, trimmed, converted to fasta format and compared to NCBI’s nucleotide database using the BLAST algorithm. The matches to the nucleotide database will be used to make inferences on the type of microbial organisms present.

# Methods

NEON collected and sequenced the samples from the UNDE site, all data can be found in data product DP1.10108.001 (<https://data.neonscience.org/data-products/DP1.10108.001>) (NEON). I analyzed the samples which were formatted and uploaded by NEON to make inferences on the microbial sequences present in each sample over time.

## Sample Collection

Each site contains ten plots that are further sampled one to three times per year. NEON randomly selects three locations for each sample event, with each sampling location is not re-sampled. Soil sampling is collected to a maximum depth of thirty centimeters. After collection, samples are stores in sterile containers, frozen and shipped to a lab for downstream analysis. In the lab, DNA is extracted and the samples are prepared for high-throughput sequence analysis using primers for 16S sequences. The sequences are delivered to NEON for quality control and acceptance. After acceptance, NEON formats and uploads the files in repositories for public use.

## Analysis

I downloaded all unique 16S files for the UNDE site from the NEON database. Then, I checked each files quality using fastqc. I subsampled each file to make the dataset computationally tractable. I trimmed each file in order to capture high quality reads and converted the fastq files to fasta files. Next, I compared each fasta file to a nucleotide database to make inferences on which microorganisms were present in the samples from the UNDE site. Finally, I used those BLAST results to BLANK!!! FILL THIS IN!!!!

### Downloading Data

I downloaded all unique files for the target gene 16S from the NEON database for the UNDE site. The script to download the data used the R library neonUtilities and the NEON API to download the data product. I used the data product ID DP1.10108.001. In addition, I downloaded the metadata for the raw data to include information on when the data was collected and where the data was collected from.

### Fastqc

I extracted the R1 files from their zipped folders. Then, I ran fastqc on each file to assess their quality and identify any problematic files (Andrews, 2010).

### Subsampling

I randomly subsampled 0.5% of the sequences in each file to make the data computationally tractable. For this, I used the srand function and the random seed 1234.

### Trimming

I trimmed the subsampled files using TrimmomaticSE (Bolger *et al.*, 2014). I used four threads, phred33 base quality encoding, a minimum quality threshold of 5 for both leading and trailing bases, and a minimum length of two-hundred base pairs. Addtionally, I implemented a sliding window with window size eight and required quality of twenty-five.

### BLAST

I used bioawk (<https://github.com/lh3/bioawk>) to convert the subsampled, trimmed fastq files to fasta files for downstream BLAST analyses. I compared each fasta file to the GenBank’s nucleotide database (Benson *et al.*, 2000). I used the BLASTN algorithm, which uses a nucleotide query sequence to search the nucleotide database (Camacho *et al.*, 2009). I used four threads, the ‘10 sscinames std’ format, and returned one match for each sequence. I excluded 2018-09-19\_environmental\_sequence.gi to reduce the number of uncultured or environmental matches.

### Analyzing Blast Data

WRITE OUT WHAT I DID AFTER I DO IT

# Results

## Subsections are ok in the results section too

# Discussion

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