The Comparison and Quantification of Microbial Abundances in Ponderosa Pine Forest and Mixed Conifer Ecosystems in Northern Arizona

NORTHERN ARIZONA UNIVERSITY
College of Engineering, Forestry, and Natural Sciences

Shavindi Ediriarachchi[1] Alicia Purcell[1,2]

Department of Biological Sciences, Northern Arizona University[1]
Center for Ecosystem Science and Society, Northern Arizona University[2]

Abstract

Soil microbes are taxonomically diverse and abundant and play instrumental roles in nutrient cycling. Although there has been a plethora of research analyzing the activity of soil microbiomes, it is challenging to quantify microbes accurately.

The purpose of this research is to compare the microbial abundances using two methods from soil samples collected from the Ponderosa Pine Forest and Mixed Conifer Forest ecosystems in Flagstaff, Arizona. Cells were enumerated using fluorescence microscopy and qPCR was used to determine total 16S rRNA gene abundance.

We predict that microbial abundance will be higher in the Mixed Conifer soil, due to the higher plant density. These findings will pave the way towards future research on microbes and their influence on biogeochemical cycles[1]

Introduction

Fig 1: Mixed Conifer Site, Flagstaff, AZ

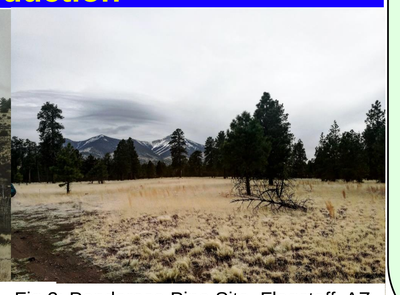
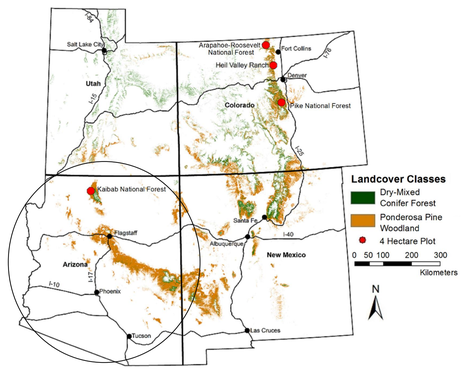


Fig 2: Ponderosa Pine Site, Flagstaff, AZ

- The study sites were the two highest elevation sites of the C. Hart Merriam Elevation gradient.
- Studies comparing these two methods (Cell enumeration and 16S rRNA copies) lack in soil research.
- The objective of this study is to determine the relationship between these two measures.



Methods and Materials

Sample Collection

- **8 samples** were collected from four cardinal directions at each site.

Cell Enumeration and Fluorescent Microscopy

- **Soil Slurry Prep**
  - ❑ 1-1.5g of soil
  - ❑Phosphate-buffer Saline
  - ❑Paraformaldehyde. (4%)
- **Preparation of filter tower setup**:
  - ❑ 0.2um polycarbonate filter
- **Into the filter tower:**
- Cells were stained with SYBR GOLD Nucleic Acid stain.
- **Cell Enumeration**
  - ❑ Cells were counted through the microscope, under the oil immersion at x1000,

Method used was a slight modification of McMurdo LTER and Purcell (unpublished)

qPCR determination of 16S rRNA copy number

- DNA extraction and purification
- Standards were calculated.[2]
- The qPCR proceedings:

Conditions	Primers used
95°C 2 min 95°C 5 sec 59°C 10 sec 72°C 10 sec (40 x) 55-95°C 0.5°C steps- for 30 secs each	Eub338 Forward and EUB518 Reverse[3]

Fig 3 – Shavindi collecting samples from the ponderosa pine site.

Fig 4 : The soil samples collected from the sites.

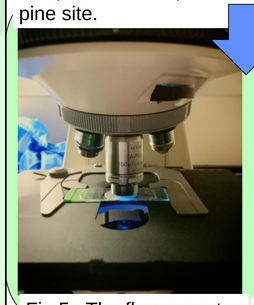


Fig 5 : The fluorescent microscope setup used for enumeration.

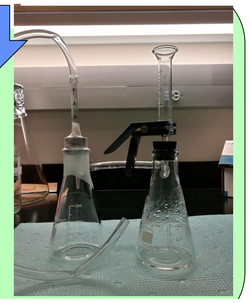


Fig 6 :The filtration setup used for cell enumeration.

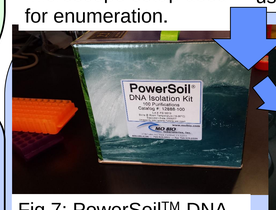


Fig 7: PowerSoil™ DNA extraction kit used in the analysis.

Fig 8 : BIORAD™ MyCycler PCR setup used in the amplification of the DNA samples.

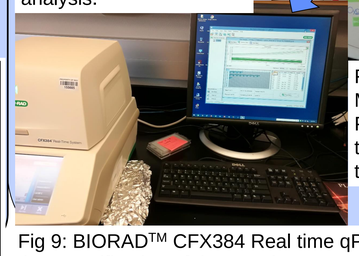


Fig 9: BIORAD™ CFX384 Real time qPCR setup used in the quantification of the templates.

Results/Discussion

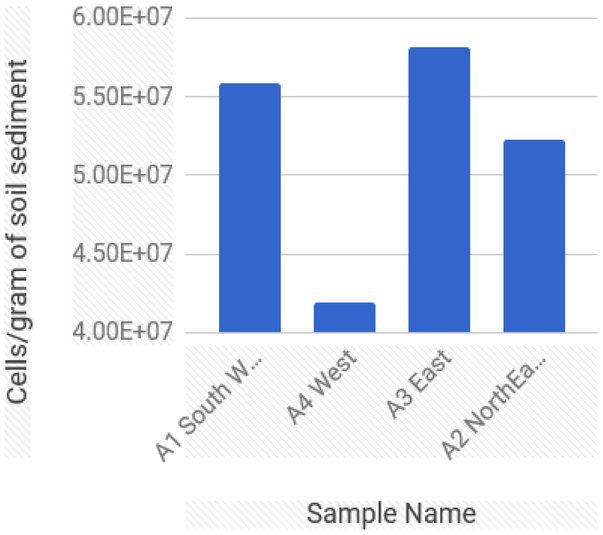


Fig 10 : Cell enumeration results aided by fluorescent microscopy of the soil replicates taken from the C. Hart Prairies site

- The qPCR quantifications for Bacterial 16S rRNA for the two sites are to be determined.

Future Research

These findings in related to cell enumerations and 16S rRNA copy numbers will foster support for other extended research on determining activity of different microbial populations in soil, in a quantitative manner, especially in the microbial populations in higher elevations.

References

1. Schloter, M., Dilly, O. & Munch, J. C. **Indicators for evaluating soil quality**. Agr Ecosyst Environ **98**, 255–262 (2003)
2. Ritalahti, K. M., Amos, B. K., Sung, Y., Wu, Q., Koenigsberg, S. S., and Löffler, F. E. (2006). **Quantitative PCR targeting 16S rRNA and reductive dehalogenase genes simultaneously monitors multiple Dehalococcoides strains.** Applied and Environmental Microbiology, 72(4), 2765-2774.
3. Fierer, Noah & A Jackson, Jason & Vilgalys, Rytas & B Jackson, Robert. (2005), Assessment of Soil Microbial Community Structure by Use of Taxon-Specific Quantitative PCR Assays. Applied and environmental microbiology. 71. 4117-20. 10.1128/AEM.71.7.4117-4120.2005.

Acknowledgements

•I extend my gratitude to the Department of Energy for funding this research.
•I further acknowledge the support provided by the staff, especially my mentor Ms. Alicia Purcell in facilitating myself in the Center for Ecosystem Society and Science of Northern Arizona University (ECOSS)
•Many thanks to Dr. Karen Haubensak and Dr. Jane Marks for mentoring me throughout this research during the Spring 2018 semester.