

Metagenomic Taxonomical Analysis of Soil Microbial Colonies Via qPCR



Abstract

A novel metagenomics method was produced for taxonomic analysis of a community of soil bacteria via quantitative polymerase chain reaction (qPCR). Taxonomies were confirmed via phylum-specific primers targeting various regions of the 16S DNA gene. Through this method, the effects of common herbicides on soil bacterial communities were determined. This method was verified by direct comparison to shotgun sequencing of the same DNA library used for qPCR. Taxonomic analysis of a similar accuracy as metagenomic shotgun sequencing was achieved. The novel method improves the ability to taxonomically analyze soil bacterial communities without specialized equipment for DNA sequencing.

Introduction

- Microbes are ubiquitous and their presence in the soil is generally beneficial to the ecosystem. Herbicides used in agriculture, however, can have adverse effects on microbial colonies, and thus on soil health.
- The goal of this study was to develop an assay to quantify microbial communities in the soil via qPCR.
- Using this assay, the effects of common herbicides on soil microbial colonies were quantified by comparing soil microbial colonies before and after herbicide exposure.
- The herbicides used were Round-up™ and Dicamba™.
- DNA was extracted from soil before and after being treated with multiple herbicides to directly compare colonies.
- The assay was validated through comparing the qPCR results to a shotgun sequence of the same DNA library used for qPCR.

Conclusion

- Taxonomic analysis of a similar accuracy as metagenomic shotgun sequencing was achieved.
- The novel method improves the ability to taxonomically analyze metagenomic soil bacterial populations without specialized equipment for DNA sequencing.
- Herbicides most affected the populations of actinobacteria and cyanobacteria phyla.
- To increase the accuracy, a more phylum specific acidobacteria primer would need to be designed.
- Because the flow cell was contaminated with excess E. coli, additional runs are necessary to determine proteobacteria populations.
- This method proves to be a more cost effective and accessible than a sequencing method.

References

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Methods

Soil Sampling

- Soil was sampled at a rate of 1/2m² and at a depth of 10cm. Approximately 15 individual samples formed a composite sample.
- For the Effects of Common Herbicides Application, soil samples were taken prior, 24-48 hours after, and two weeks after herbicide exposure.

DNA Extraction

- Soil and Probiotic DNA were extracted via the Qiagen DNeasy PowerSoil and MiniPrep Kit and protocol respectively.
- DNA was kept at -80C prior to qPCR.

qPCR

- qPCR was used to quantify bacterial populations in each application.
- Approximately 75ng of extracted DNA were used in each qPCR well.

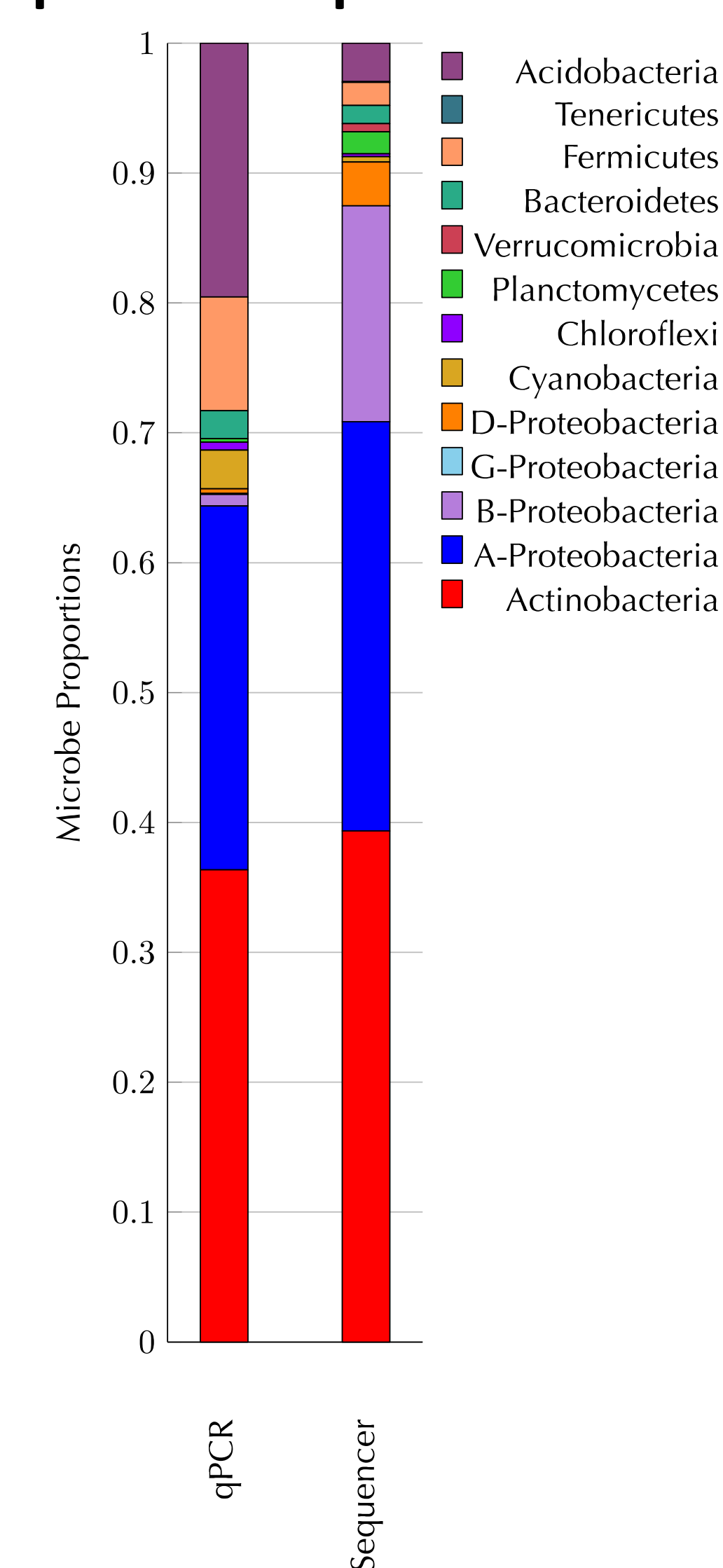
Oxford Nanopore Minion DNA Sequencing

- The Oxford Nanopore 16S and What's In My Pot (WIMP) protocols were followed.
- 10ng of DNA was used in each run.
- Lambda control DNA was used to calibrate the sequencer.
- After each run, the flow cell was washed with wash buffer and stored with storage buffer at 0 degrees Celsius.
- Raw data was interpreted on Epi2me software to provide analyzed quantified bacterial communities.

Mock Community Application

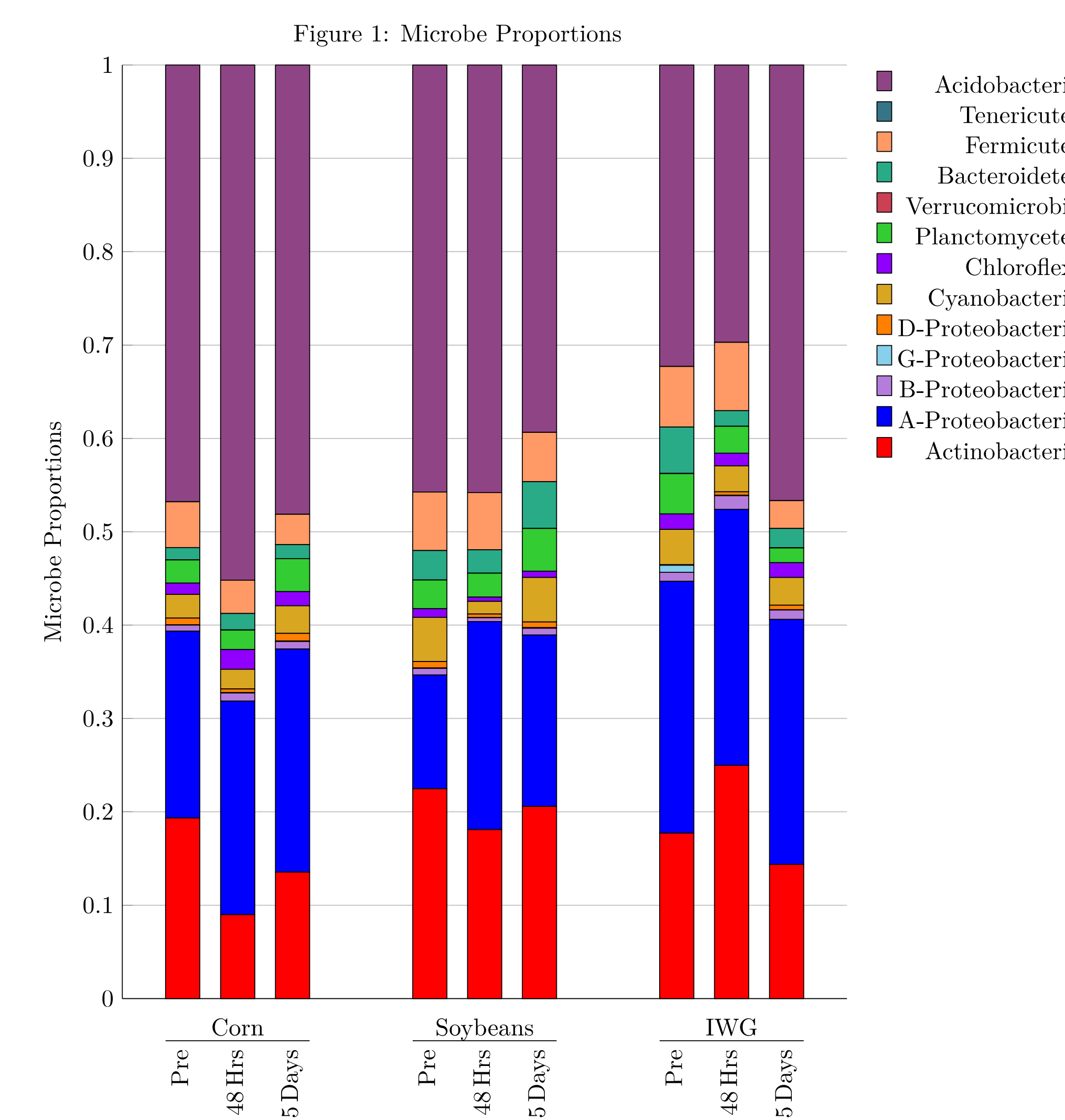
- A probiotic of known composition was used as a mock community that was profiled to verify sequencer accuracy to the genus level.
- Within the community, bacterial communities congruent with the qPCR results were found, including high number of lactobacillus communities.
- Results also included significant values of E. coli communities.
- Because E. coli was not part of the mock community, this proved the proteobacterial bias observed in the soil qPCR vs sequencer results.
- The high levels of E. coli was from previous DNA samples and not inherent to our assay.
- Similar levels of bacteria were found from both methods with the exception of bias from the sequencer concerning high levels of proteobacteria.

Soil qPCR vs Sequencer Results



Discussion

- Gamma-proteobacteria was omitted from the results of the sequencer as it was shown to have been contamination from from a prior run.
- Acidobacteria primer lacked phylum specificity and produced false positives.
- In sequencer, there was residual E. coli from calibration lambda DNA, thus increasing the proteobacteria read in soil samples.
- The soil assay was designed specifically for sandy-loam soils found in Northwest Iowa, thus my not be entirely applicable to other soil types.
- A cost analysis not including the sequencer or qPCR instrument showed that a qPCR run cost approximately \$76 while a sequencer run cost nearly \$100
- Comparing the methods, the qPCR method proves to be cheaper and more accessible. However, each assay need to be designed for a specific area. The sequencer method is less accessible, less user-friendly, and more expensive. However, this method is universal to all areas.
- The method of metagenomic taxonomical analysis should be chosen based off of area and available budget.



Effects of Common Herbicides Application:

- Using the designed assay, the effects of common herbicides on soil microbial populations were determined.
- Samples were taken pre-herbicide testing at a rate of 1/2m² and at a depth of 10cm. This process was repeated 24-48 hours and two weeks after the fields were tested with either Dicamba™ or Round-up™.
- Soil samples were kept at -80C° prior to DNA extraction.
- DNA was analyzed via qPCR using the above primers.
- This method was applied before the ability to compare results with the Minion DNA sequencer, thus the results are not verified.
- This method could again be applied similarly now that the method has been verified.

Results:

- The acidobacteria primer lacked phylum specificity and greater than 100% of the universal primer was read.
- Round-up and Dicamba exposure resulted in a noticeable decrease of actinobacteria and cyanobacteria.
- This assay was appropriate for the loamy-sand soil found in Northwest Iowa. This assay, may not be appropriate for other soil types with different native bacterial phyla.
- Results from control Intermediate Wheatgrass (IWG) soil comparisons fluctuated significantly. This could be due to heavy rains experienced between sampling. Increased moisture can affect bacterial populations. Thus, constant environmental factors are needed to directly compare results.