Differences in prokaryotic diversity between store-bought soil and natural garden soil

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Research Question

How does prokaryotic diversity change in response to repeated harvesting of *Brassica rapa* plants differ in natural soil and store-bought soil?

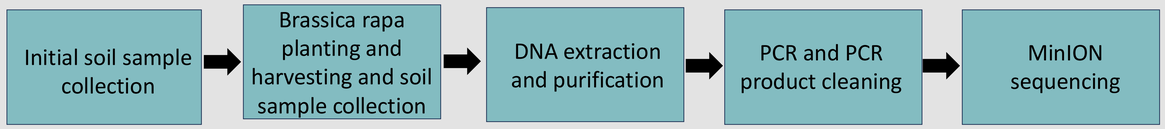
Introduction

Soil microbes offer many benefits to the plants growing within the soil. For example, certain bacteria suppress plant pathogens by producing antimicrobial compounds, while plants provide a habitat for these beneficial bacteria (Das et al., 2022). Additionally, soil microbes enhance nutrient availability for plants, by using their flagella to access insoluble nutrients (Das et al., 2022). Some soil microbes enter plant tissues as endophytes, where they fix atmospheric nitrogen and synthesize amino acids for the plant (White et al., 2022). These endophytic microbes also help plants defend against pathogens that manage to invade the plant (White et al., 2022). Altogether, soil microbes are important to plant survival, occupying various niches that support plant growth and health. Plant's reliance on prokaryotic symbiotes is why the prokaryotic diversity in soil is important for gardeners.

This study aims to investigate the differences in prokaryotic diversity between natural and store-bought soil and how these prokaryotic communities respond to repeated harvesting. By investigating and reporting this, this study hopes to solve the problem of gardeners being uninformed on whether they are depriving their plants of the benefits of prokaryotic diversity because of the soil they are choosing and inspire these gardeners to focus on increasing prokaryotic diversity in their gardens. The hypothesis for this study is: if the soil sample is taken from natural soil, then it will have a higher number of unique 16s rRNA sequences than the samples from the Miracle Gro Garden Soil and the number of unique 16s rRNA sequences in the natural soil will decrease less over *Brassica Rapa* growth cycles.

Methodology

Workflow:



- 500g of soil from and active vegetable garden and 500g of Miracle Gro garden soil was placed in seperate 6-inch terracotta pots
- Soil samples were collected at 5 cm depth from each pot initially and stored at -18°C
- A *Brassica rapa* seed was then planted in each pot
- Soil samples were collected after three-week intervals through 2 *Brassica rapa* growth cycles
- Each pot was under LED Gro Lights for 12 hours a day and given 100mL of water a day
- 0.25g of each soil sample was bead bashed with lysis buffer to extract DNA from cells
- ZymoBIOMICS DNA Kit was used to purify DNA.
- The 16s rRNA gene in the extracted DNA was amplified using endpoint PCR
- Magnetics beads were used to clean PCR product for sequencing
- The PCR Product was sequenced using minION sequencing

Figure 1: Pots with initial samples of soil. Natural soil is on the left and Miracle Gro Garden Soil is on the right.

Figure 2: Pots with soil and *Brassica rapa* plants. Natural soil is on the left and Miracle Gro Garden Soil is on the right.

How does 16s rRNA sequencing measure prokaryotic diversity?

The 16s rRNA gene is a gene that is highly variable in prokaryotes. The minION sequencer will count the number of unique 16s rRNA sequences present in each soil DNA extract. The 16s rRNA gene is not completely unique to each prokaryotic species but can typically differentiate them down to the genus level. Furthermore, some prokaryotes may have multiple unique 16s rRNA sequences. Therefore, the number of unique 16s rRNA sequences is not the number of prokaryotic species present in the soil. However, the number of unique 16s rRNA sequences is still a measure of prokaryotic diversity because a higher number of unique prokaryotes means more unique 16s rRNA sequences will be present in the soil sample.

Results

The amount of unique 16s rRNA sequences in each sample was used to calculate the Simpson's diversity index of prokaryotes for each soil sample. The closer the Simpson's diversity index is to one, the more diverse the sample is.

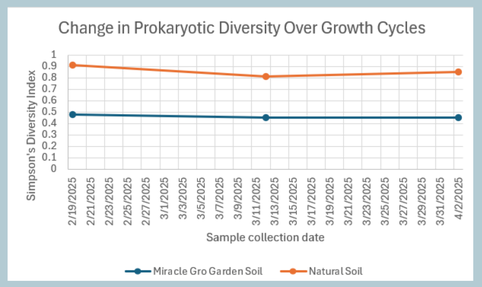


Figure 3: Graph showing how prokaryotic diversity changed over *Brassica Rapa* growth cycles

Conclusion

Overall, the data showed that natural soil has more prokaryotic diversity than Miracle Gro Garden Soil. The *Brassica rapa* growth cycles did not seem to have a big impact on the prokaryotic diversity. More analysis must be done on the specific species of prokaryotes present in each soil to determine which one is better for gardeners in terms of the soil's prokaryotic community. The Miracle Gro Garden soil might have less prokaryotic diversity but more symbiotic prokaryotes.

Sources

Das, P. P., Singh, K. R., Nagpure, G., Mansoori, A., Singh, R. P., Ghazi, I. A., Kumar, A., & Singh, J. (2022). Plant-soil-microbes: A tripartite interaction for nutrient acquisition and better plant growth for sustainable agricultural practices. *Environmental Research*, *214*, 113821. https://doi.org/10.1016/j.envres.2022.113821

White, J. F., Kingsley, K. L., Zhang, Q., Verma, R., Obi, N., Dvinskikh, S., Elmore, M. T., Verma, S. K., Gond, S. K., & Kowalski, K. P. (2019). Review: Endophytic microbes and their potential applications in crop management. *Pest Management Science*, *75*(10), 2558–2565. https://doi.org/10.1002/ps.5527