MODELING POTENTIAL IMPACT OF GLOBAL WARMING ON SOIL BACTERIA COMPOSITION

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INTRODUCTION/OBJECTIVE

Ranking among the most diverse and plentiful organisms on Earth, soil microbes play crucial roles in nutrient cycling, organic matter decomposition, and plant health [1]. Warming soil has various effects on microbial communities; it may alter their composition, abundance, and diversity, which can subsequently impact nutrient availability, carbon storage, and greenhouse gas emissions [2]. By studying the microbiome in heat treated soils, a perspective into how climate change may impact this environmental parameter may be ascertained. This study aims to predict the impact of increasing temperatures on soil bacteria composition. This study is designed to add to the body of evidence needed to inform future projected climate-related changes in the field of agriscience, and help support development of future studies in this area.

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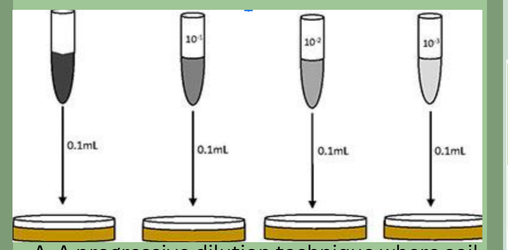
HYPOTHESIS

Soil subjected to temperatures expected in 2100 will exhibit a distinct bacterial composition compared to the microbiota of New Jersey (NJ) soil in 2023.

METHODS

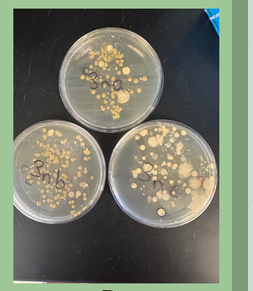
- Soil samples were exposed to extreme conditions mimicking 2100 NJ soil temperatures (30°C) and current NJ soil temperatures (21°C) for 2 weeks.
- Only the 3rd dilution for both samples was used to isolate individual colonies.
- Topsoil was mixed thoroughly and incubated in two equal parts - one at 30°C and the other 21°C - for 2 weeks.
- The following experiment was conducted for both samples: 1g soil was added to 10mL of sterile 0.9% NaCl and vortexed for 30 seconds and left alone for 5 minutes. 3x500µL were transferred to 3 sterile 1.5 mL tubes to be made the undiluted samples - 0na, 0nb, 0nc for the lower temperature sample and 0va,0vb,0vc for the higher temperature sample.
- The tubes were vortexed for 30 seconds and instantly 100µL was transferred to 3x3 sterile microcentrifuge tubes labeled 1na,1nb,1nc or 1va,1vb,1vc containing 900µL of 0.9% sterile saline.
- The steps above were repeated for the 2nd and 3rd dilution triplets
- Tryptic Soy Agar (TSA) plates corresponding to the tubes above were labeled and 100µL of each of the dilutions were plated and kept at their respective temperature with a bowl of water. These were observed daily for 5 days.
- To account for the 20k dilutions to do this experiment, the Colony Forming Unit (CFU) count was calculated back to the original 1 g samples (see results)

FIGURE 1



A. A progressive dilution technique where soil culture was diluted to isolate individual colonies. The final and each of the intermediate dilutions were plated on TSA plates. [3]

An image of bacteria grown from serial dilutions before isolating individual colonies. The colonies to be isolated were labeled with a shape, making organizing easier.



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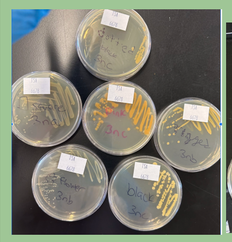

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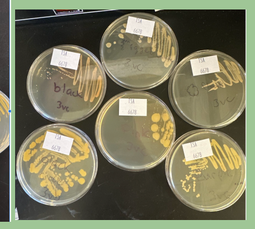
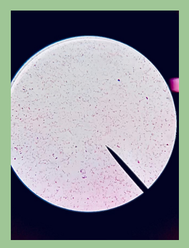
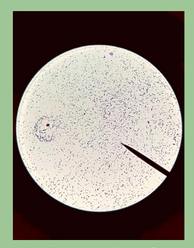


D.

C shows 6 isolates from 2 of the 3 lower temperature triplets (3nb and 3nc). Bacteria from sample 3na was not isolated due to lack of diverse bacterial colonies in that sample. D shows an illustration of the streaking technique for isolation of individual pure colonies from the triplets (3va,3vb,3vc).



E. Gram negative bacteria identified under the lower temperature sample conditions.



F. Example of gram positive bacteria identified under the lower temperature sample conditions.

images B-F were all taken by student.

RESULTS

FIGURE 2

Soil conditions	Bacterial count CFU/g of soil	# gram positive	# gram negative
2023 soil (21°C)	1.6 x 10^6	5	1
2100 soil (30°C)	0.7 x 10^6	6	0

- The 3rd plated serial dilution was found to have the most distinct CFU using the serial dilution method from 1 gram of soil.
- A substantial difference in bacterial CFU was found between samples, with 21°C incubated soil containing 80 CFU per 1/20k of a gram compared to only 35 CFU per 1/20k of a gram in 30°C incubated soil.
- The CFU numbers found in the dilutions (35 per gram in 30°C and 80 per gram in 21°C) were multiplied by 20k to get the number of CFU per gram of soil.
- The CFU count dropped by more than 2 times when comparing the colonies from soil incubated at 21°C vs. at 30°C, respectively.
- Colonies grown at 21°C were tentatively identified as *Azotobacter, E. coli, S. fradiae, S. aureus, B. pumilus,* and *B. cereus* through Gram staining and literature search. Gram staining identified 11 out of 12 total isolates as gram-positive.
- Based on Figure 2, it is probable that, with the sample incubated at 21°C having 5 gram positive and 1 gram negative, vs. the sample incubated at 30°C having 6 gram positive, there is less microbial diversity in the species (also see future research).

LIMITATIONS

The worst case scenario was assumed for global warming impact with only a short term exposure of soil to higher temperature, which may have limited the bacterial species' ability to evolve. The growth of soil bacteria on agar (artificial conditions) could have suppressed growth of some bacteria present in the initial sample. With the resources accessible, only topsoil was used in this experiment.

CONCLUSION

Fewer bacterial colonies with less diverse morphologies were discovered in the soil incubated at 30°C modeling the year 2100 than 21°C of 2023. These findings can provide crucial insights for developing strategies to mitigate the adverse effects of climate change and preserve soil biodiversity and ecosystem resilience. Soil is a non-renewable resource, so microbes can be the solution to preventing a food crisis.

FUTURE RESEARCH

Further morphological, biochemical and genetic tests can establish the precise species composition of the soil bacteria. (e.g., 16S rRNA gene sequencing, antibiotic resistance, Gram staining with controls, mass spectrometry). In future research, a larger scale experiment would be needed to make a more definitive conclusion about bacterial diversity.

BIBLIOGRAPHY

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[3] Soil macromorphology. Soil Macromorphology - Microbiology Resource Center - Truckee Meadows Community College. (n.d.). https://www.tmcc.edu/microbiology-resource-center/lab-protocols/soil-macromorphology