Development and Validation of Incubation Chambers to Manipulate Microbial Dispersal

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Objectives

- Determine if bacterial dispersal can be limited by using stainless steel meshes.
- Employ the tested meshes on field samples to determine their effectiveness through microbial community 16S rRNA analysis.

Background

- Typical bacterial cells are about 1 μm in diameter[1].
- Microbial dispersal is the way that microbial communities move through space.
- Increased dispersal has been shown to lead to increased species richness and diversity[2].
- The 16S rRNA gene is the leading sequence-based bacterial analysis method by clustering sequences and comparing to databases to identify species[3].

Validation of Chambers

- DH5-α *Escherichia coli* expressing ampicillin resistance was used in this study.
- An OD_{600} calibration curve to estimate concentration of E. coli in nutrient broth was created (Figure 1).
- Three sizes of meshes were used in the preliminary and field experiments, 1μm, 25μm, 75μm.
- Meshes were made into open-top cylindrical shape sealed with cold-weld steel reinforced epoxy.
- Placed in 1L-beakers with 0.5L of 100 μg/mL ampicillin nutrient broth. The system was sterilized.
- The system was stirred at 80 rpm and an inoculant was administered into the chamber as well as plated.
- Serial dilutions of the external broth were conducted at various timepoints and plated on ampicillin nutrient agar plated and incubated at 37°C for 24h.
- The plates were counted, and the percentage of *E. coli* dispersed through the chambers at each timepoint was plotted (Figure 2 and 3).

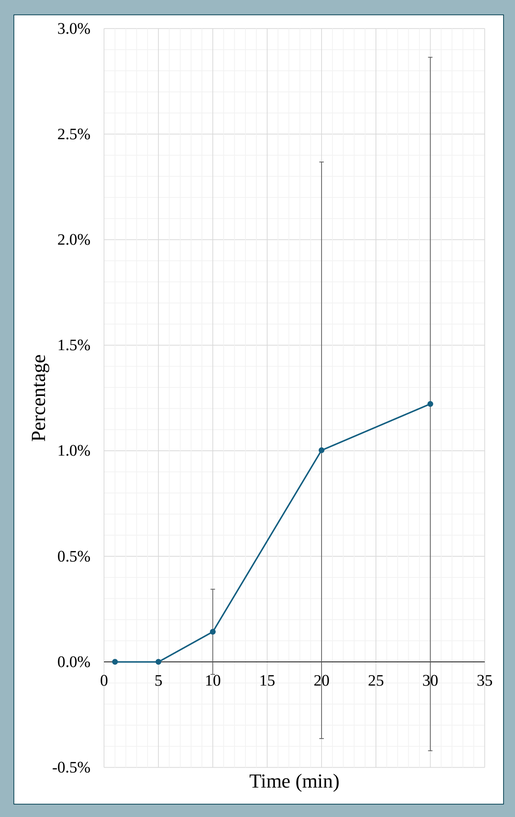


Figure 2. Percentage of inoculated bacteria trespassing the 1μm mesh plotted over time (n=3).

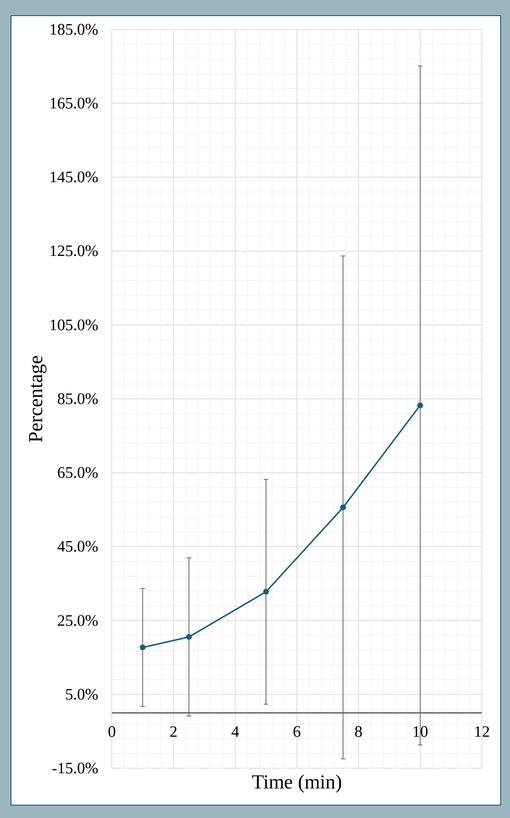


Figure 3. Percentage of inoculated bacteria trespassing the 75μm mesh plotted over time (n=3).

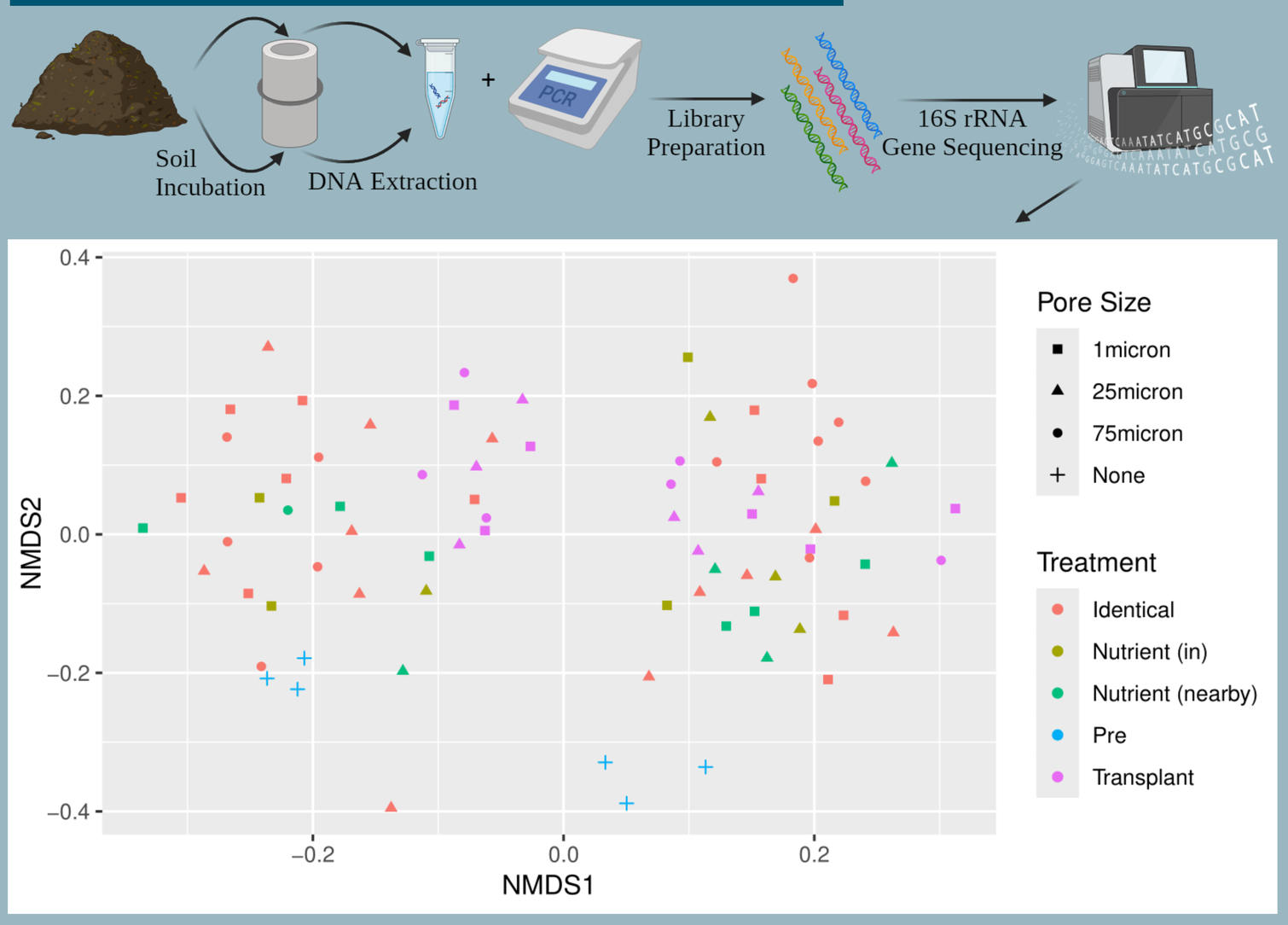
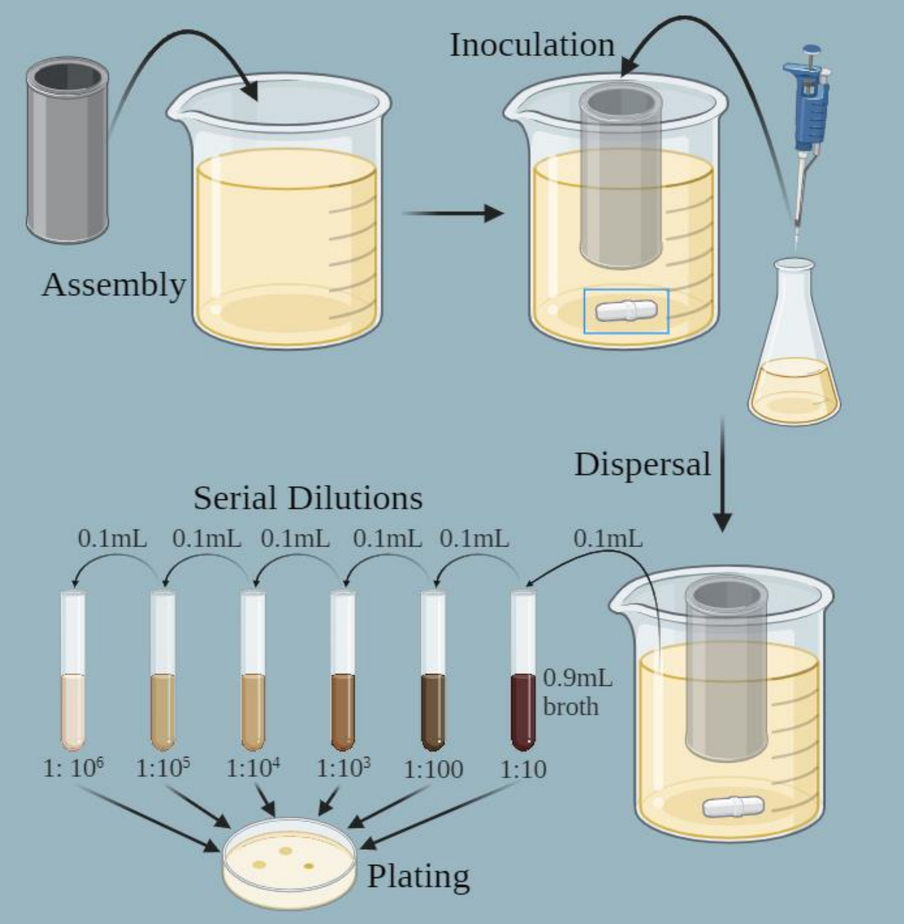


Figure 4. MDS plot demonstrating the Bray-Curtis dissimilarity of samples before and after each treatment.



Manipulating Soil Dispersal

- A grasslands and a forest surface soil sample was collected, homogenized and sieved.
- Soil moisture and density analyses were conducted.
- Nutrients from each soil were extracted into water.
- The soil were moistened with their respective treatments to 95% saturation of water.
- An incubation chamber 3D-model was designed, printed, and assembled with three pore sizes of mesh.
- The wet soils were loaded into the incubation chambers and left to interact for 20 days.
- DNA extractions of the soil from each chamber and before treatment were conducted.
- The 16S rRNA gene was amplified using 341f and 806r primers and sequenced using NGS.
- Data was analysed using phyloseq and vegan in R.

Conclusions

- The different sizes of meshes successfully limit dispersal in *E. coli* cells.
- There was a high anaerobic treatment effect in the incubation chambers creating selection.

Future Work

- Make environment aerobic to limit anaerobic effect without introducing contaminants.
- More time to interact and create a significant difference in community composition.

Acknowledgments

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References