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# Role of Probiotics in Mitigating Bean Beetle Infestation

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Science Honors Research Pinnacle Project

## Abstract

Our overall purpose is observing how bean beetles are affected by human probiotics. This project explores if treatment prevents infestation on black-eyed pea beans by hindering oviposition and its effects on the bean beetles' microbiome.

The main stakeholders in our research are farmers and bean farms in Georgia as we aim to mitigate infestations on stored beans. By focusing on human probiotics, we are hoping to find an accessible, cheaper, and environmentally sustainable solution to this agricultural crisis. Our methodology involves a control group and a probiotic dilution series, and a Nanopore gene sequencing process. Results from our research suggest a notable change in bean beetle microbiomes and initial disruption of oviposition on black-eyed peas. We faced and overcame complications related to bean beetle handling and during probiotic treatment formulation.

## Background

Georgia farmers often deal with pests that damage and destroy crops, one of these many pests being bean beetles. Bean beetles lay eggs on bean crops and stored beans. As larvae grow, they burrow into the bean to feed—rendering the beans unusable. Currently, the way to prevent this is by using pesticides. However, pesticides carry potential health risks such as cancer to exposed individuals. Looking for alternative ways to prevent pests could be valuable to the farming community and the public. Probiotics are a potential alternative for preventing bean beetle infestation. The probiotic powder could provide a barrier between the beans and beetles. We hypothesize probiotic treatment will make black-eyed pea bean surfaces less suitable for oviposition which will prevent infestation. Also, beetles that interact with probiotic treated beans will have more microbiome diversity than those who have not.

## References

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## Methodology

### I. Probiotic Treatment Testing

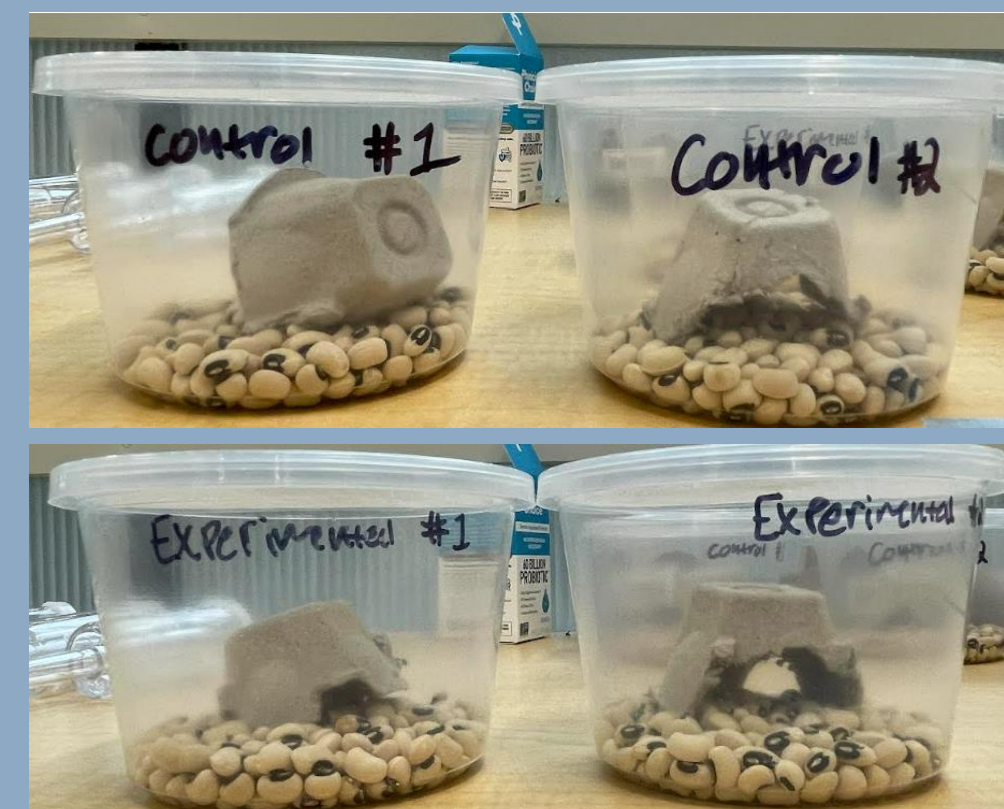


Fig 1: The first round of probiotic treatment testing involved 2 control and 2 experimental containers with 14 beetles each (7 of each gender).

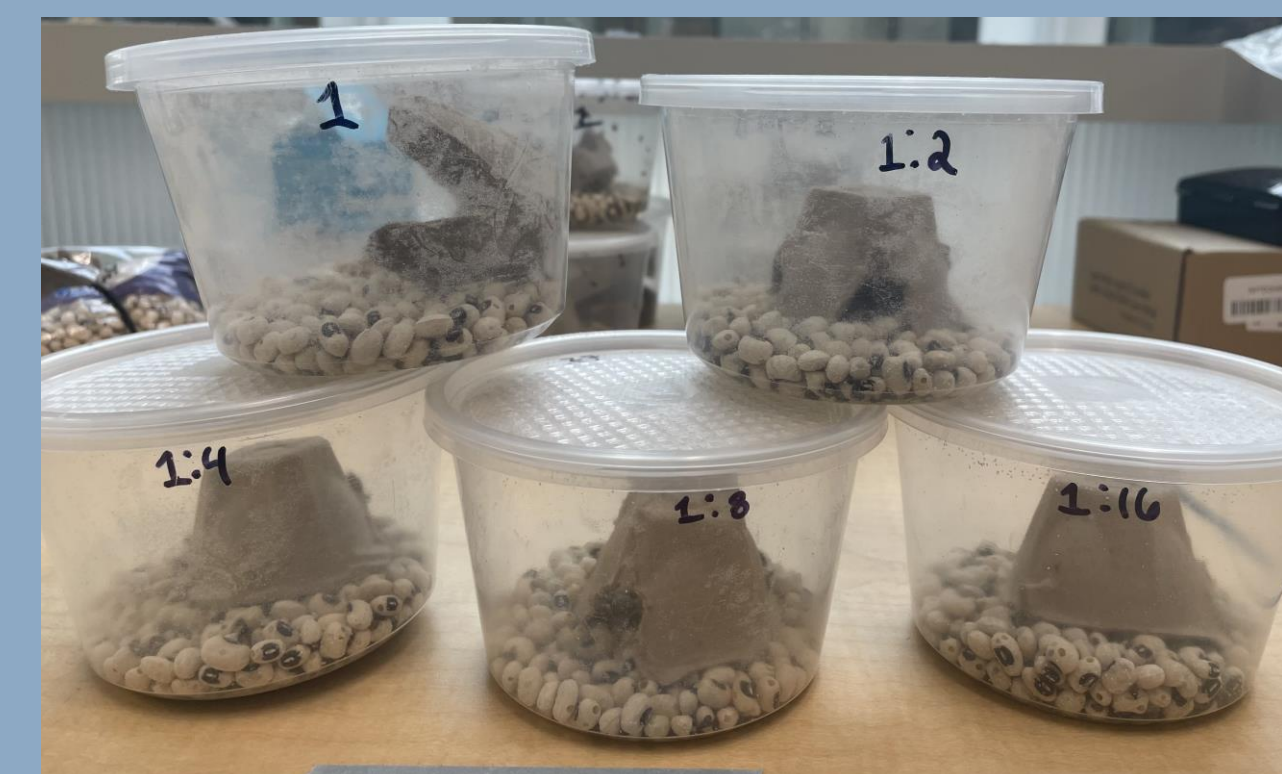


Fig 2: The second round of probiotic treatment testing was done through a dilution series using a dilution factor of 1:2. Dilution 1 has the highest probiotic powder content while Dilution 1:16 has the lowest. Beetle counts were identical to our control and experimental containers.



Fig 3: Bean beetle egg on bean surface as seen through a microscope (left). Emerging beetles leave holes in stored beans (right) after finishing their developmental stages within the beans themselves.

### II. DNA Extraction and Purification



Fig 4: DNA extractions began with the isolation and freezing of individual beetles. Live beetles were transferred to 1.5mL MCTs and then placed in the freezer and allowed to peacefully pass away.



Fig 5: The bead bashing process breaks apart cells and exposes DNA needed for gene sequencing. This image shows 24 crushed beetle samples, along with lysis buffer, added to bead bashing tubes.

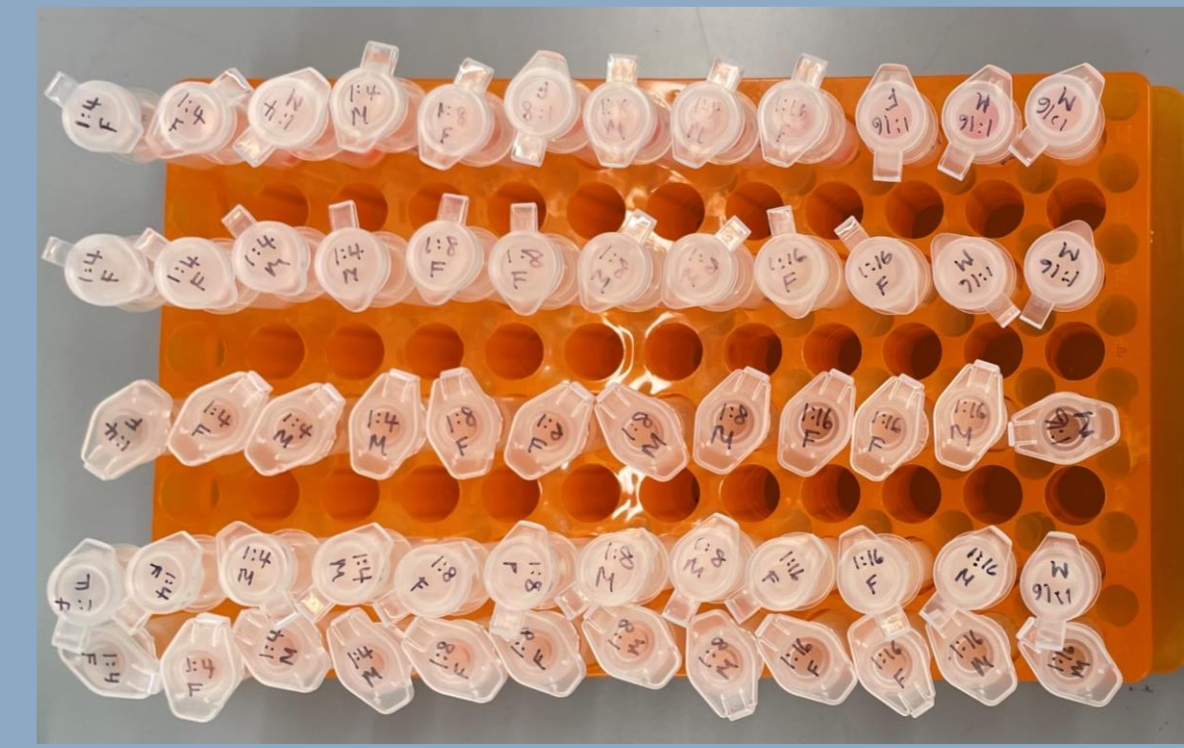
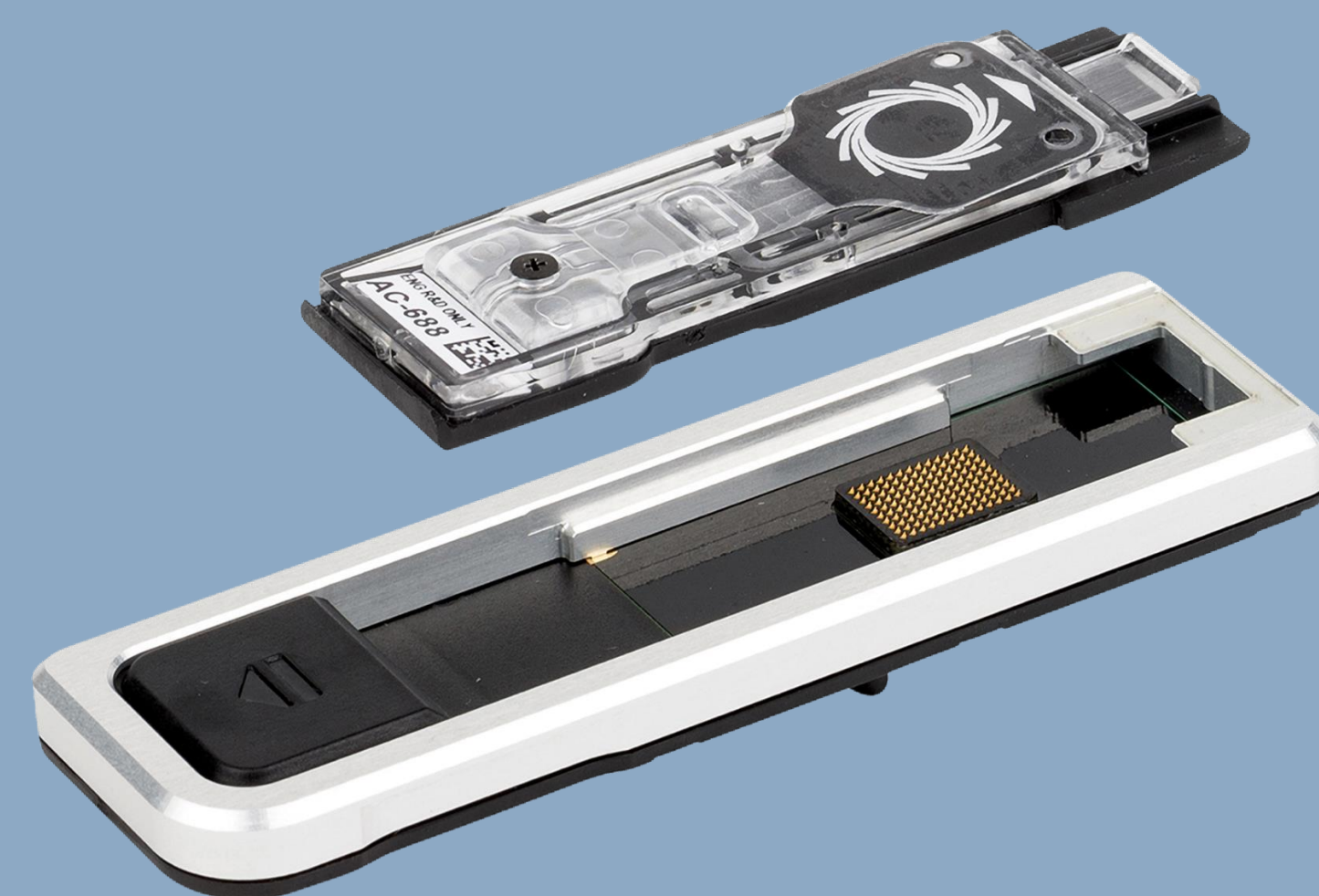


Fig 6: A ZymoBIOMICS DNA Miniprep Kit was utilized to purify the DNA extracted from the bean beetles. The purification process allows us to isolate DNA from cell debris and other substances.

### III. MinION Nanopore Gene Sequencing



- A MinION Flongle machine was used to perform nanopore gene sequencing on our 24 bean beetle samples. This allowed us to identify the various bacteria present in each beetle's microbiome (mainly gut microbiomes).
- Comparisons were drawn between control beetle microbiomes and dilution beetle microbiomes to evaluate our 2<sup>nd</sup> hypothesis.
- Gene Sequencing Process:
  1. DNA Quantification
  2. Dilution of DNA
  3. PCR
  4. Barcoding
  5. DNA pooling
  6. Quantification
  7. Flow Cell Loading
  8. Sequencing Overnight



## Results

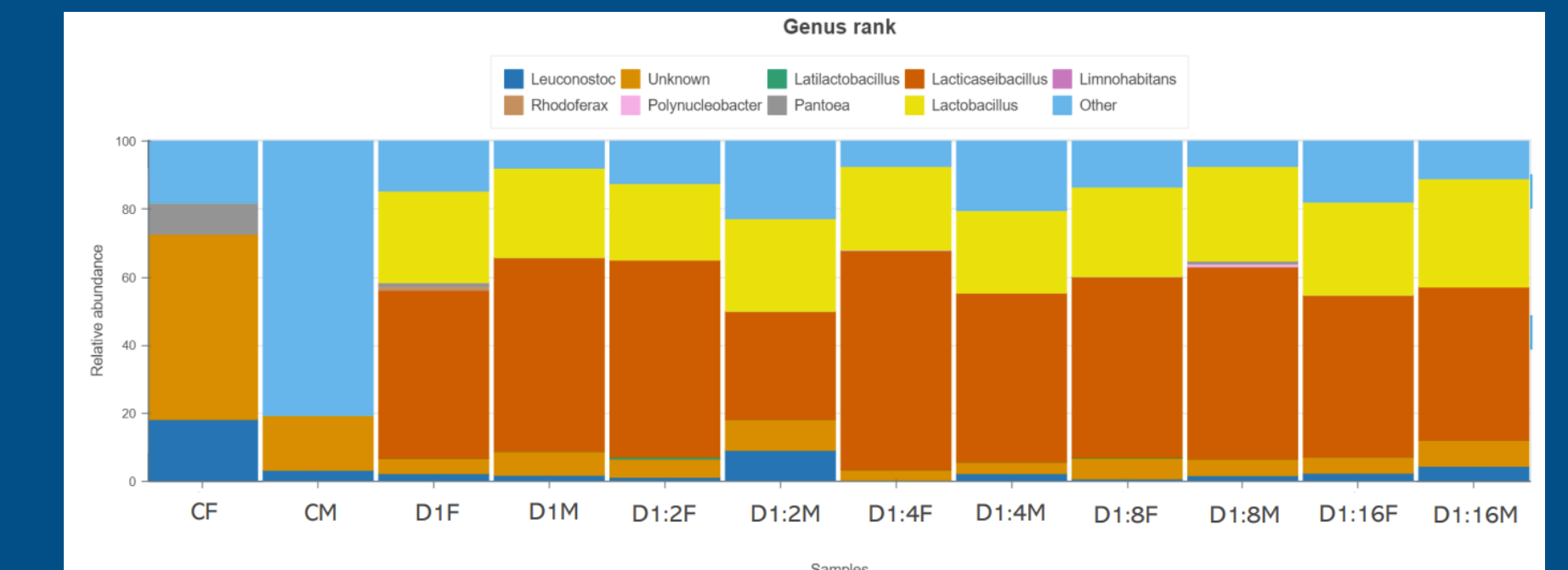


Fig 7: The graph shows the results of the nanopore sequencing. Each column represents a different barcode with the first two being the controls.

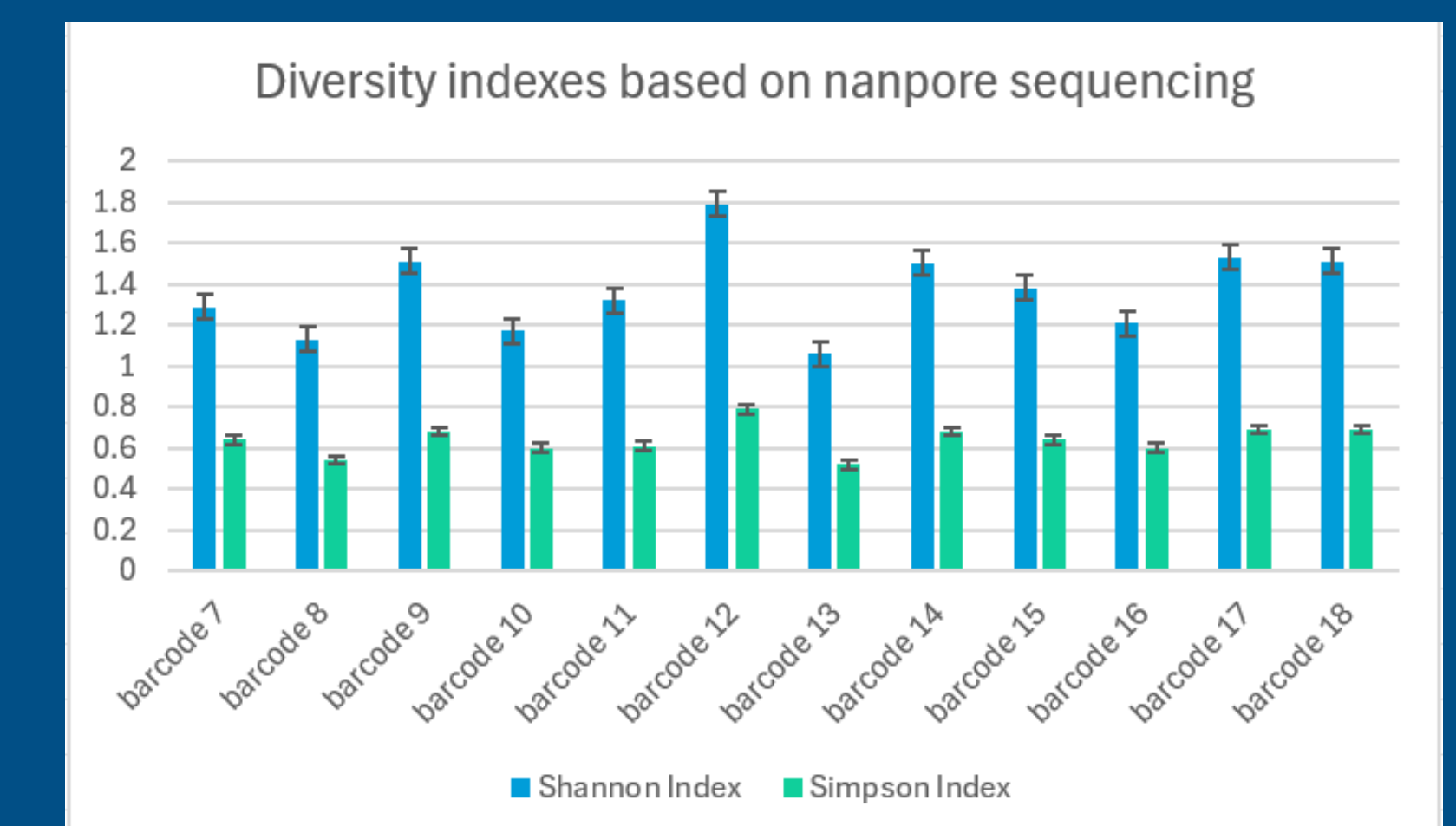


Fig 8: Showcases the Shannon and Simpson indexes of diversity. Indicates the amount of bacterial diversity per barcode.

### Observed Results:

- Longer period for beetles to emerge in serial dilutions and experimental containers compared to controls
- Less eggs present on experimental vs. control
- Beetles still lived and emerged when eggs were laid

## Discussion

- As seen in Fig 7, the differences in bacterial readings between the control (barcodes 7 and 8) and dilutions showcase that interaction with probiotics changed the beetle's microbiome.
- The probiotics did disrupt oviposition initially. However, the disruption was not enough to make probiotics an alternative to pesticides as most eggs fully matured.

## What We Learned

- We learned how to work with insects.
  - We've never worked with insects making this was an educational experience on how to work with them.
- How to use gene sequencing to determine types of bacteria from samples
  - Using nanopore and PCR we did a new procedure to see the bacteria present in the bean beetles.
- How to create our own methodologies to answer a research question
  - We experienced setbacks when creating methodology regarding how to treat the beans