

# Investigation of the fecal microbiome of raccoons (*Procyon lotor*) in a captive environment

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## Introduction

A microbiome is the genetic collection of all microbes that occupy a specific environment. The genetic profile of microorganisms of the gastrointestinal tract is collectively called as the gut microbiome (represented in fecal microbiome), which plays an important role in the health of its host and ecology (1).

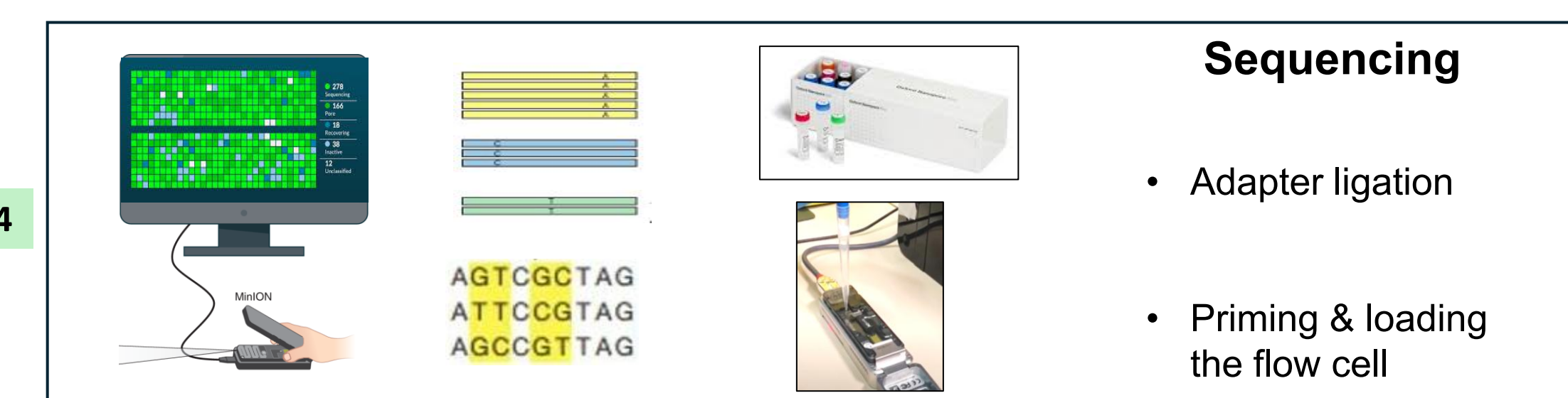
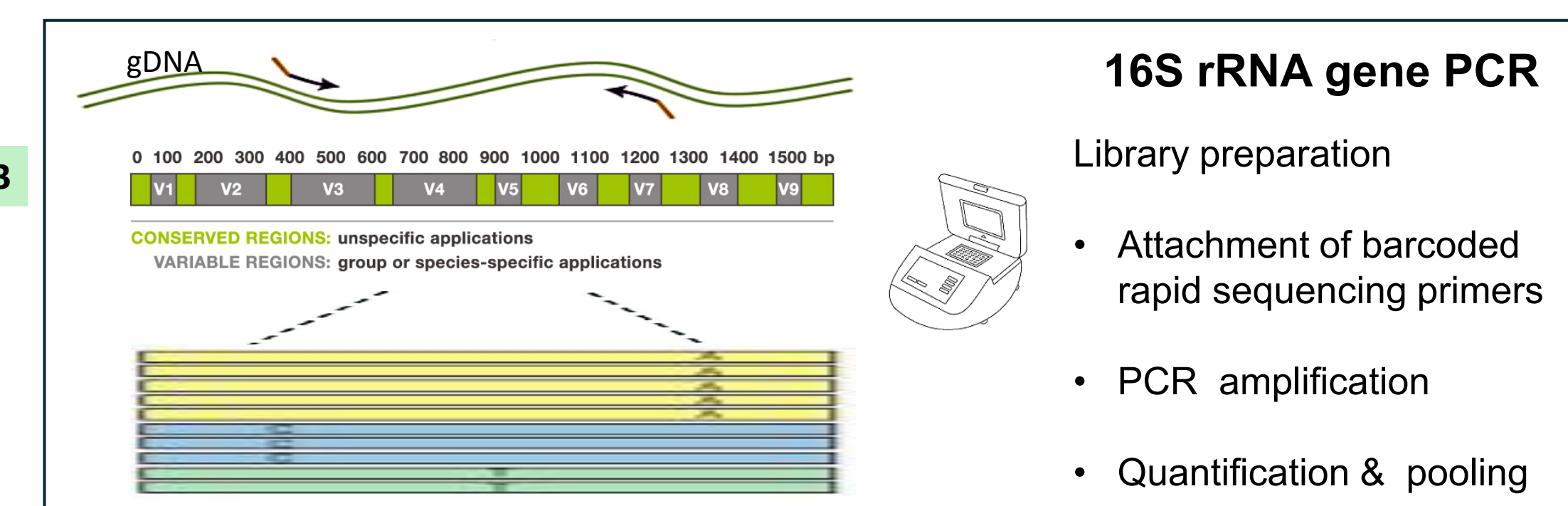
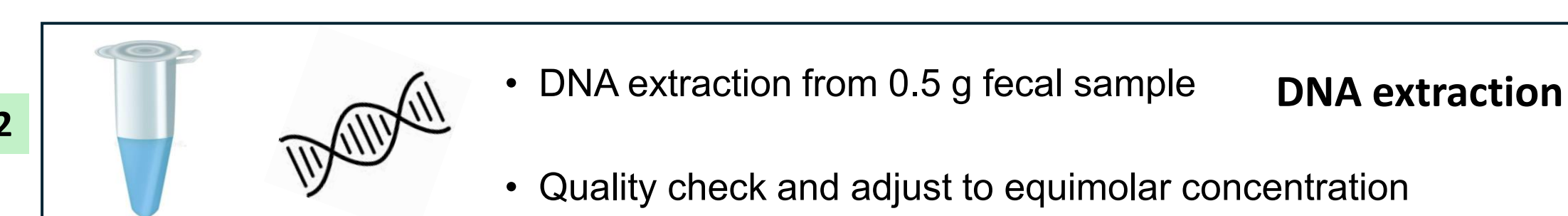
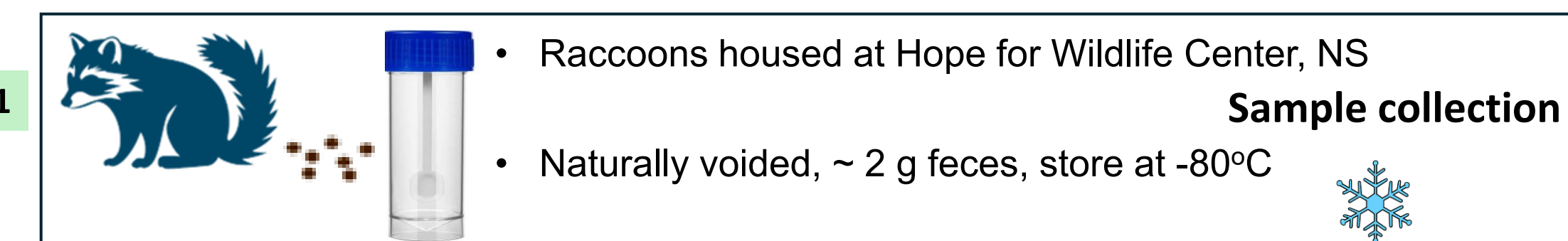
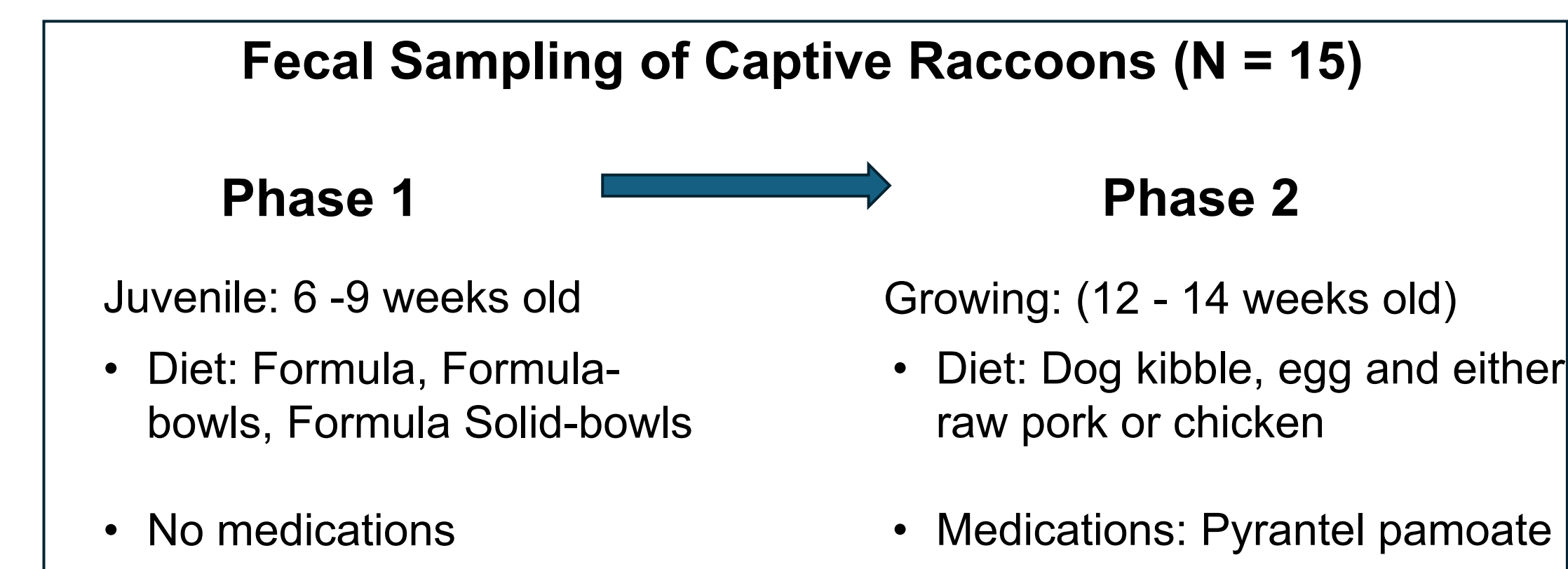
Raccoons are a widely known wildlife species that interface and impact urban ecosystems; however, their gut microbiome is rarely explored (3). The goal of this study was thus to investigate the bacterial community of gut microbiome of raccoons using 16S rRNA gene sequencing method.

## Hypothesis and objective

It is thought that raccoons carry diverse bacteria, which affect the physiology and pathophysiology of the animal through many factors, such as type of diet, gender and age. Besides wild inhabitation, raccoons are also found in urban premises, and it is reasonable to hypothesize that their fecal microbiome impacts urban ecology.

The objective of this study was to investigate fecal microbiome of captive raccoons based on 16S rRNA metagenomic sequencing.

## Method Overview



## Acknowledgements

Funding: AVC Research Fund  
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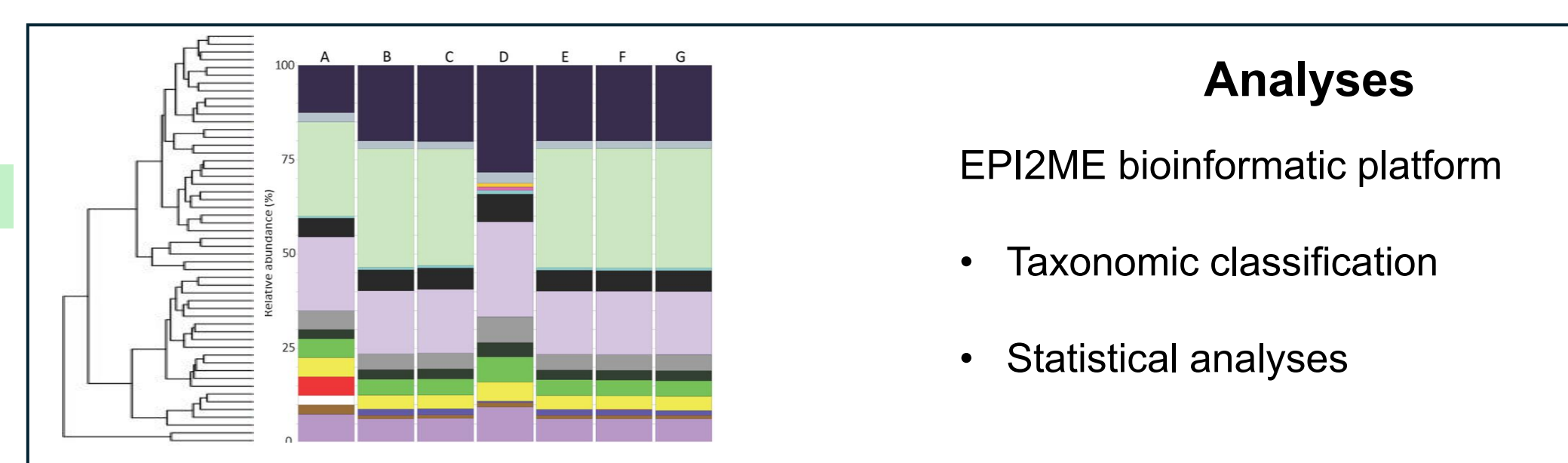


Figure 1. Steps 1 to 5 in 16S rRNA gene analyses of gut microbiome.

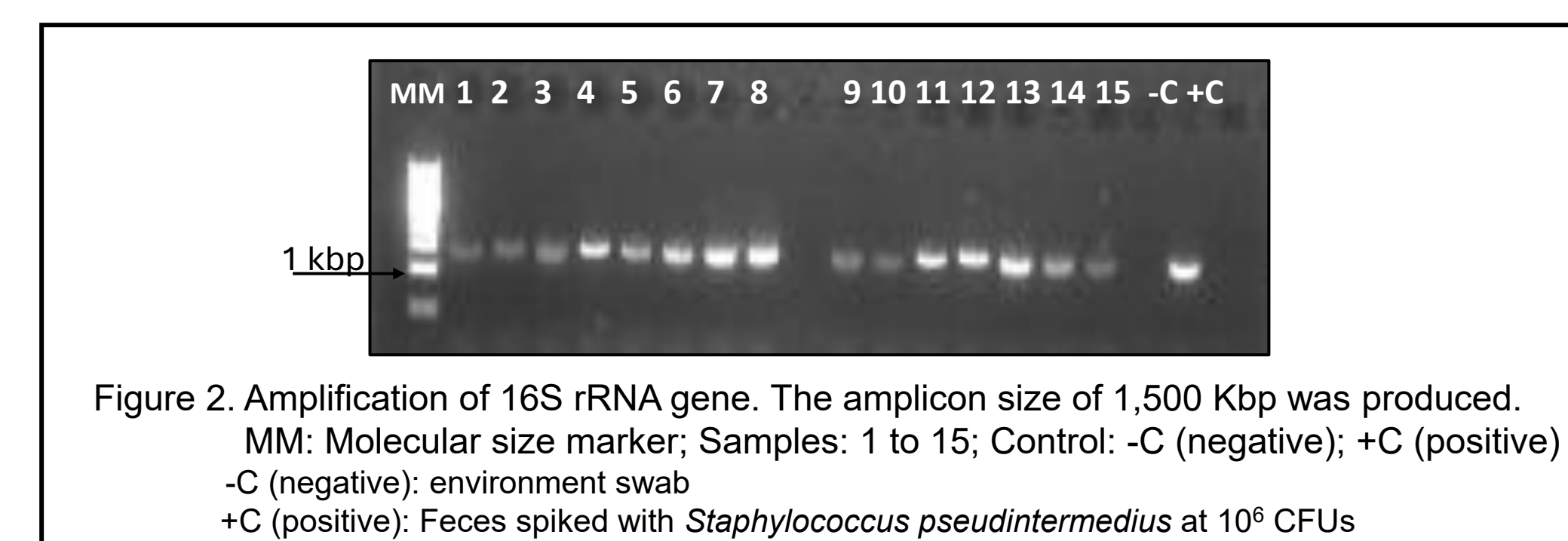


Figure 2. Amplification of 16S rRNA gene. The amplicon size of 1,500 Kbp was produced. MM: Molecular size marker; Samples: 1 to 15; Control: -C (negative); +C (positive): environment swab. +C (positive): Feces spiked with *Staphylococcus pseudintermedius* at 10<sup>6</sup> CFUs

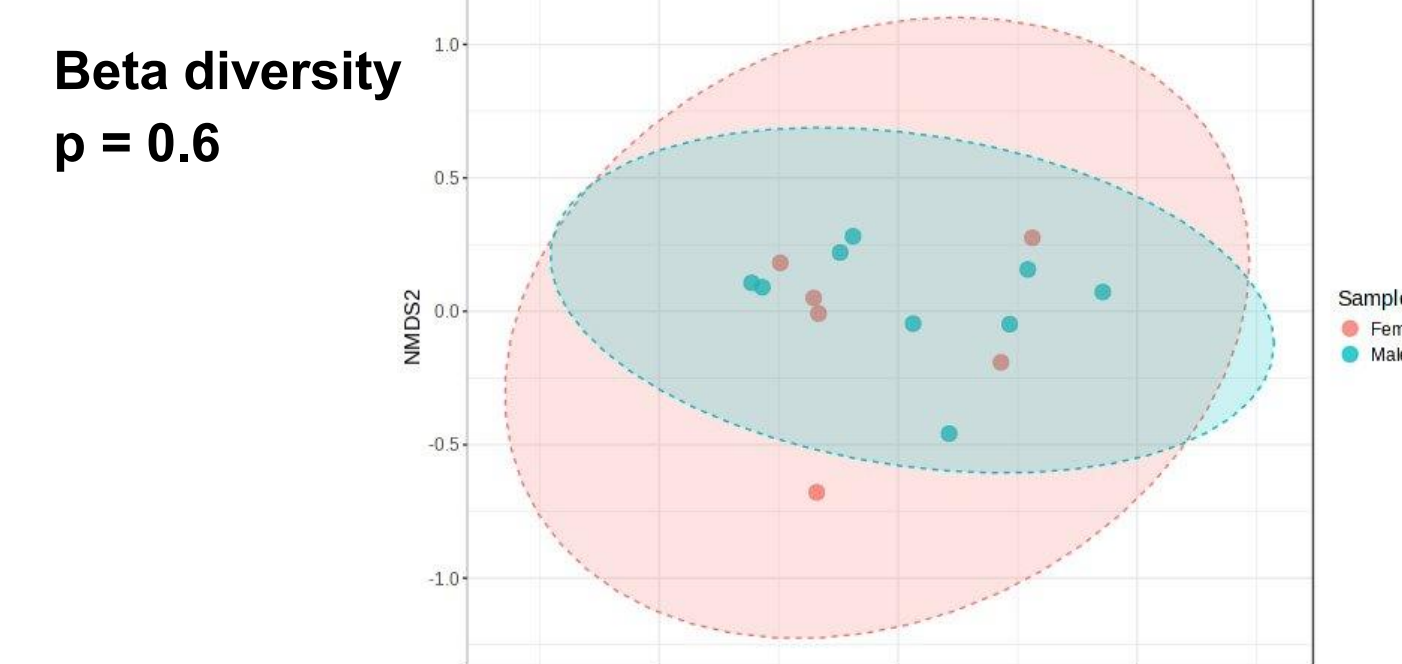
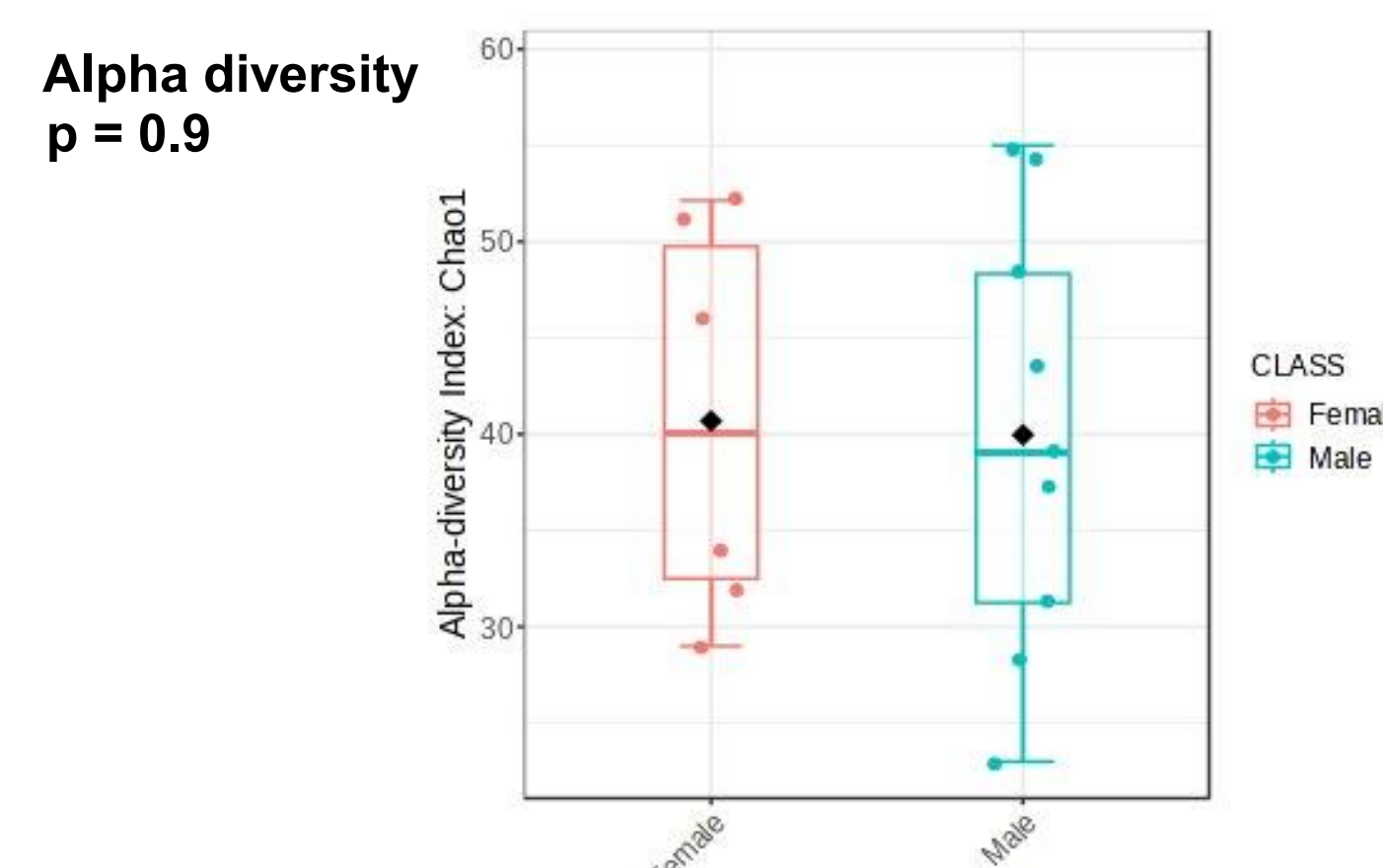


Figure 3. No significant differences in composition of bacterial community of male and female raccoons based on alpha and beta diversity comparisons ( $p > 0.05$ ).

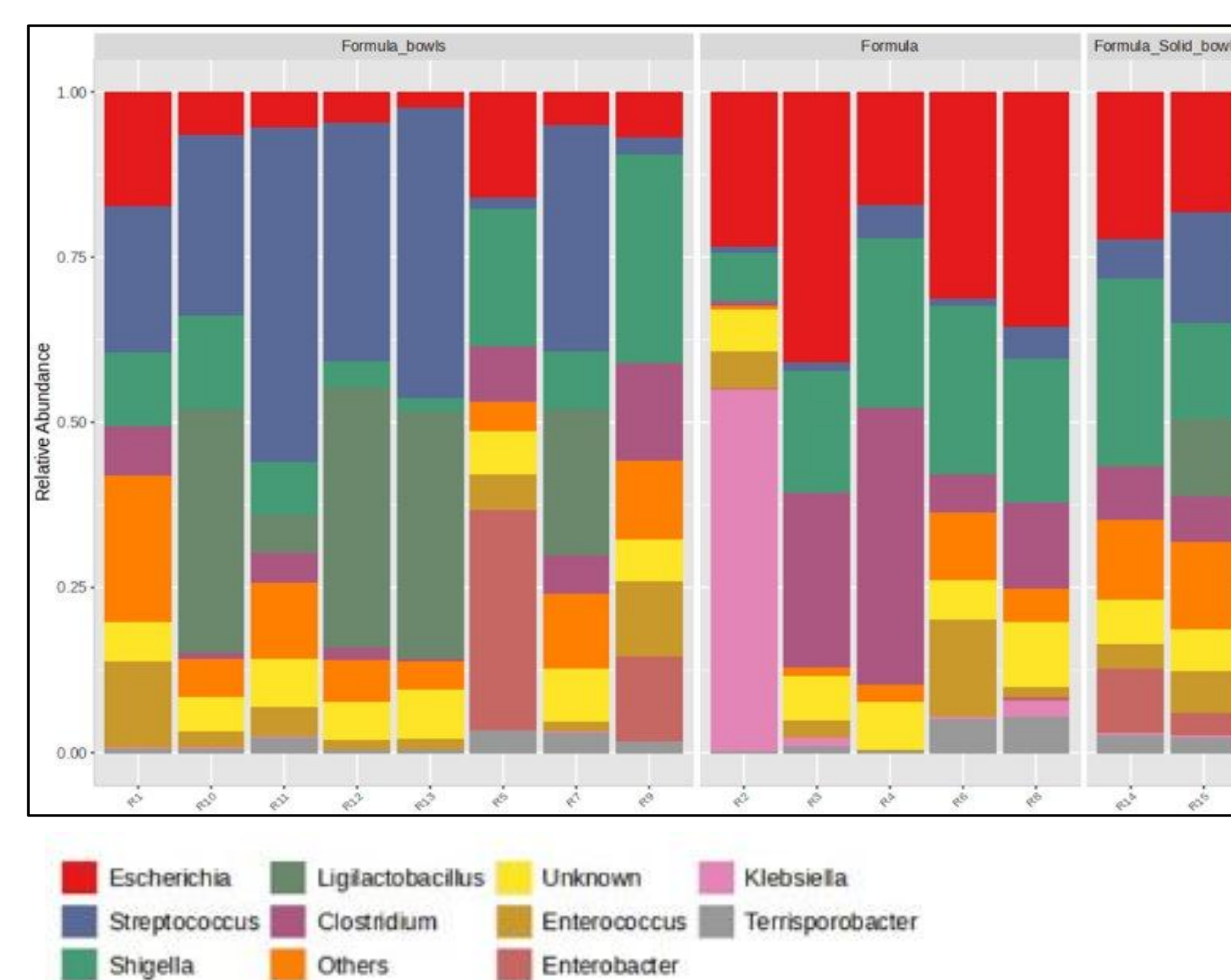


Figure 4. Distribution of top ten genera of bacterial community in feces of raccoons that were fed different types of feed.

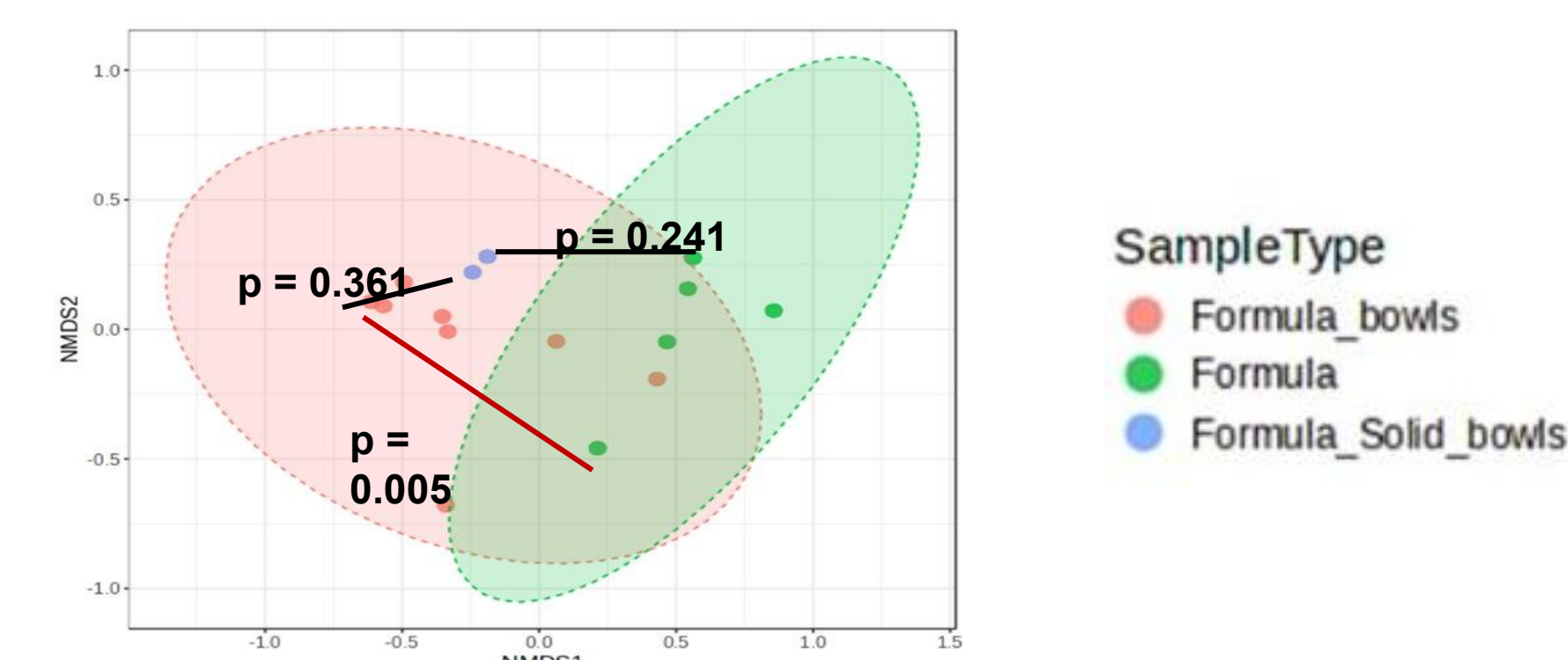


Figure 5. Beta-diversity comparisons showed significant difference in bacterial composition in animals fed formula bowls versus formula diet ( $p < 0.05$ ).

## Results

- The fecal microbiome of 15 juvenile captive raccoons (phase-1) was characterized (Fig 1).
- Among the top ten bacterial genera, *Escherichia* and *Streptococcus* were found in all fecal samples (Fig 4).
- Interestingly, *Shigella* genus was also identified in all the samples.
- The genus, *Ligilactobacillus* was primarily found in formula-bowl fed animals.
- A relative abundance of *Klebsiella* ( $n = 1$ ) and *Clostridium* ( $n = 1$ ) was found in formula-fed raccoons (Fig 4).
- No significant differences in alpha or beta diversity were observed among the genera between samples of male and female raccoons ( $p > 0.05$ ) (Fig 3).
- Beta-diversity comparisons showed significant difference in bacterial composition in animals fed formula-bowls versus formula diet ( $p < 0.05$ ) (Fig 5).

## Discussion

- The juvenile, unmedicated raccoons carried the genus *Escherichia*, which is known to be an early colonizer and normal flora of intestinal tract (2). The organism is an important component in the development of immunophysiology of its host.
- Streptococcus* is a commensal bacteria of skin and oro-pharyngeal mucosa but it is isolated from intestines of humans and animals as well (2).
- While humans and primates are the primary reservoirs of *Shigella*, its identification in raccoon samples is intriguing. Since *Escherichia coli* and *Shigella* share 99% identical 16S rRNA gene, this result will be further confirmed.
- The formula-bowls fed animals showed enhanced colonization of *Ligilactobacillus*, which is a normal inhabitant of intestine and is known for its probiotic properties (2).

## Conclusions

- Raccoons carry diverse group of bacteria in their intestinal tract.
- The diversity and relative abundance of different bacteria is influenced by the type of diet.
- To the best of our knowledge, this is the first study that provides a preliminary information on fecal microbiome of captive raccoons in North America.
- The study warrants further investigation to learn shifts in bacterial profile when the raccoons are released in their natural wild habitat.

## References

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