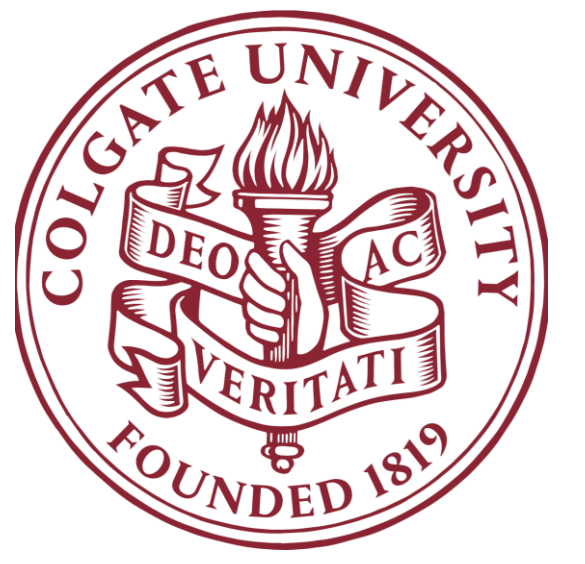


# Household and Environmental Drivers of Gut Microbiome Variation in Ethiopian School Children



Max Warriner. Professor Bineyam Taye Lab  
Colgate University  
Dept. of Biology

## INTRODUCTION

- The human gut microbiome consists of diverse groups of anaerobic microorganisms that have symbiotically co-evolved w/each other and their human hosts
- Microbiome organisms evolved DNA for functions humans cannot do
- Higher microbiome diversity is associated with positive health outcomes
- Childhood gut microbiome profiles form basis for lifelong microbiome interactions
- Studies have shown microbiome profile associations with household and environmental factors
- Vast majority of studies conducted on urbanized, western populations
- Analysis of populations with different living circumstances, nutritional availability, and environmental conditions provides a novel opportunity for the field of gut microbiome research

## OBJECTIVE

To examine how socioeconomic disparities and environmental exposures affect the gut microbiome composition of Ethiopian schoolchildren

## METHODS

- **Study setting and design**
  - School-based cross-sectional study (N=138)
  - Subjects taken from 14 schools in Jimma Town, Southwest Ethiopia

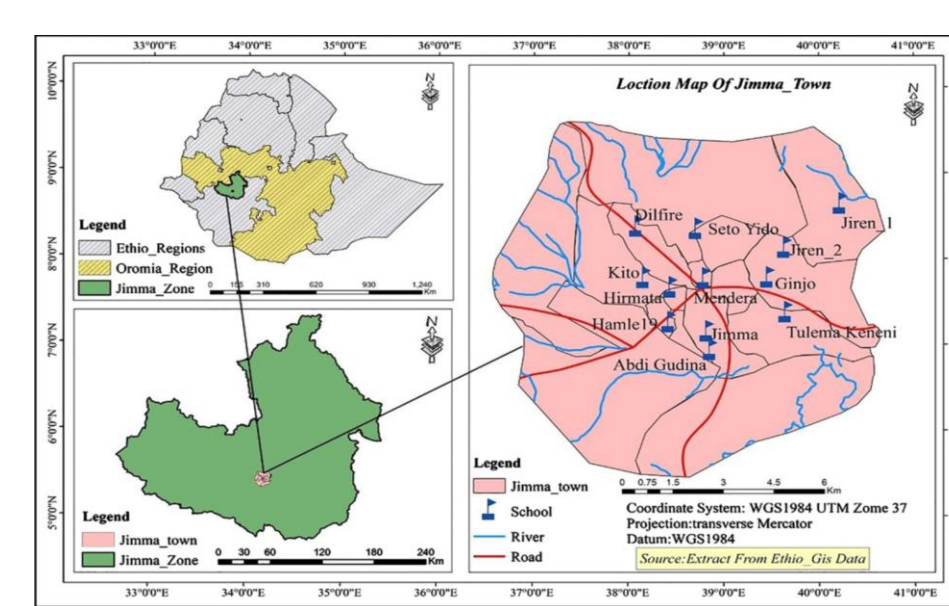
- **Measurements and data collections**
  - Interview-based questionnaire
  - Lifestyle, Clinical, and Socioeconomic Factors

- **Demographic Measurements**
  - Weight, height, ...

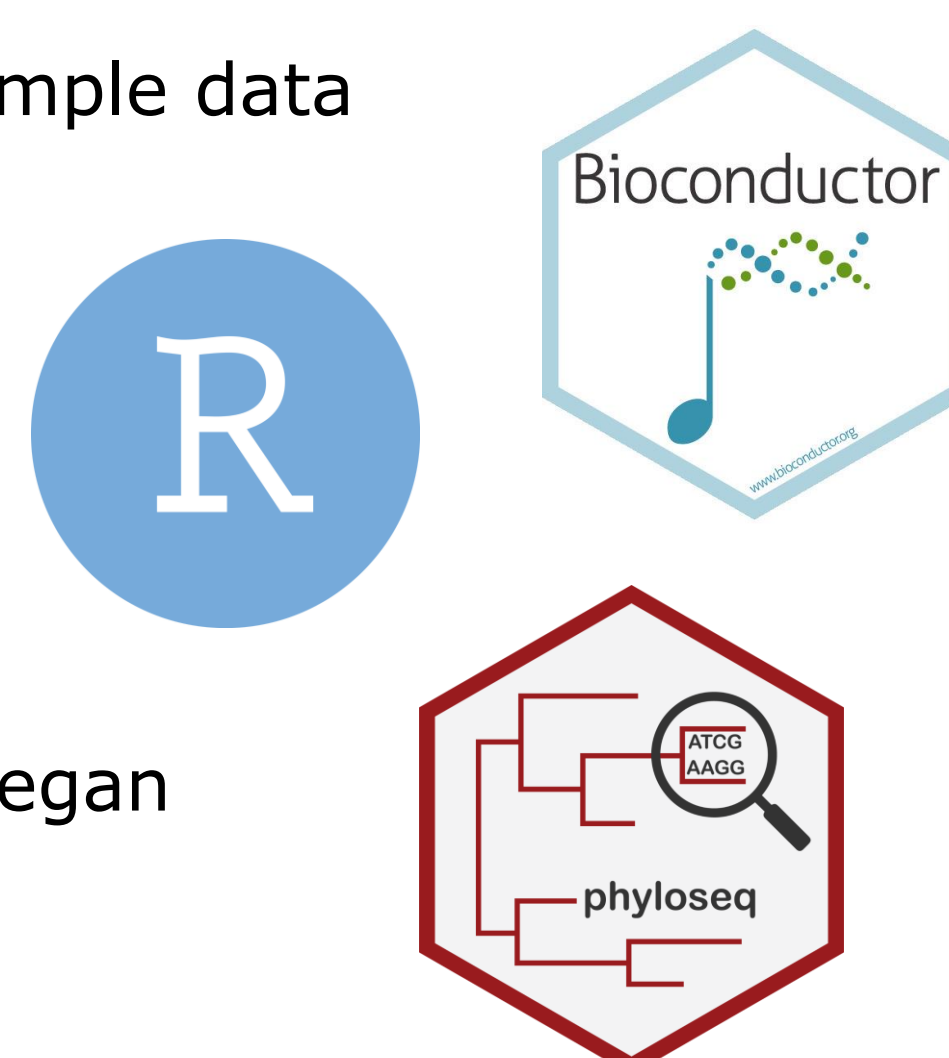
- **Fecal samples for analysis**
  - DNA extraction
  - Amplicon sequencing

- **Data Processing and Analysis**

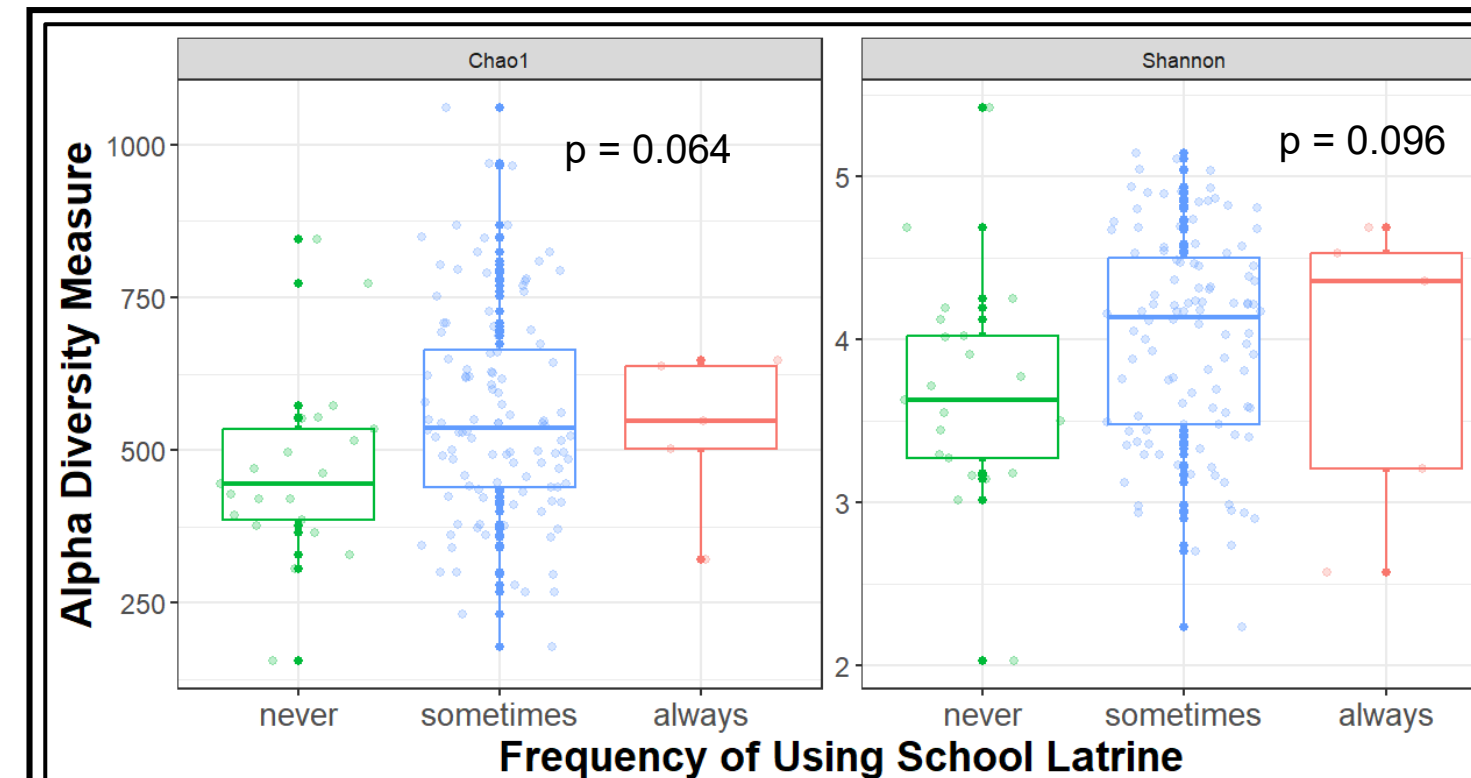
- All analysis done in RStudio
- Data cleaning with dplyr
- Plotting with ggplot2 and ggpvr
- Phyloseq for handling sequencing & sample data
- Alpha Diversity
  - Chao1 and Shannon indices
  - Kruskal-Wallis hypothesis testing
- Beta Diversity
  - PCOA plot w/Microbiota Process
  - Jaccard & Bray distance ordination
  - PERMANOVA hypothesis testing w/vegan
- Differential Abundance
  - Barplots w/Microbiota Process
  - Volcano plots w/ALDEx2
  - LEfSE & edgeR plots w/microbiome Marker



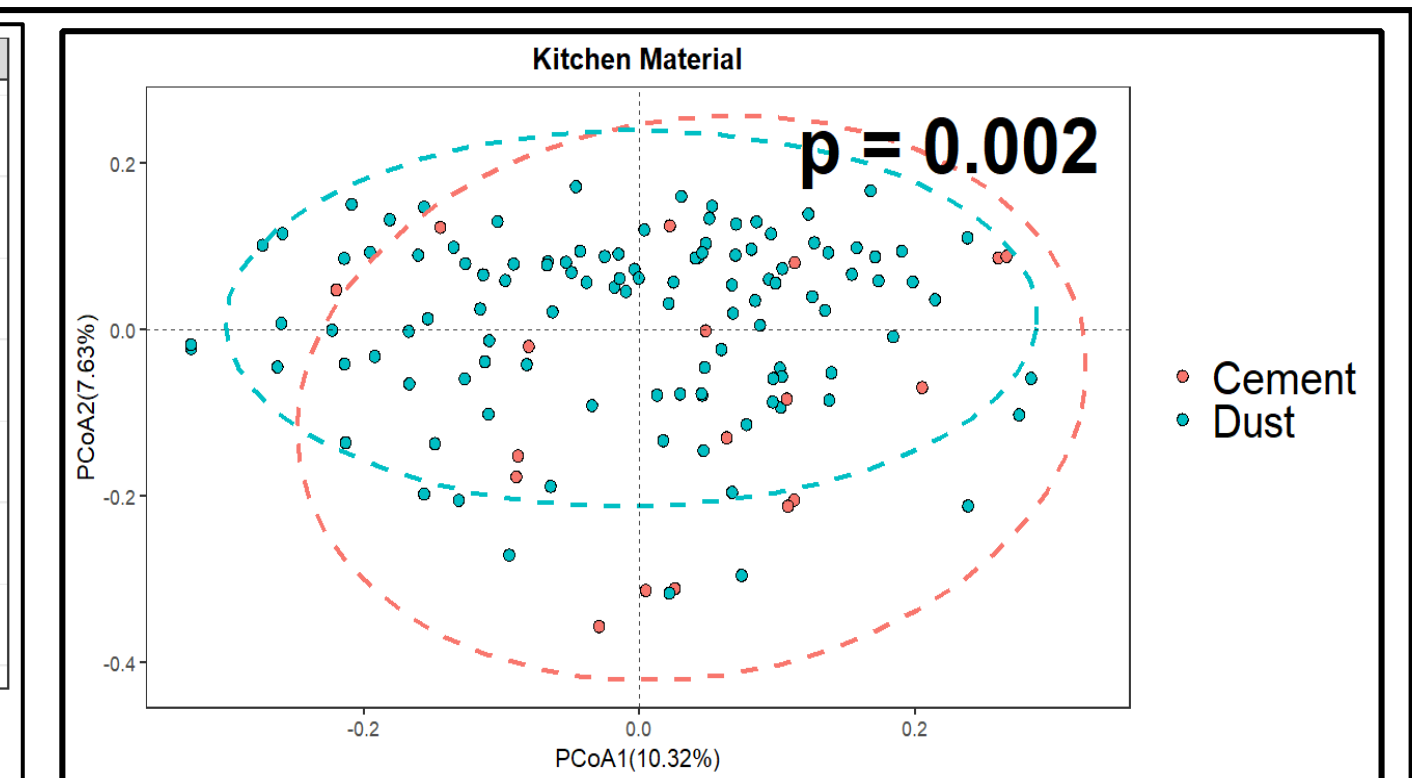
Map of cohort's geographic distribution



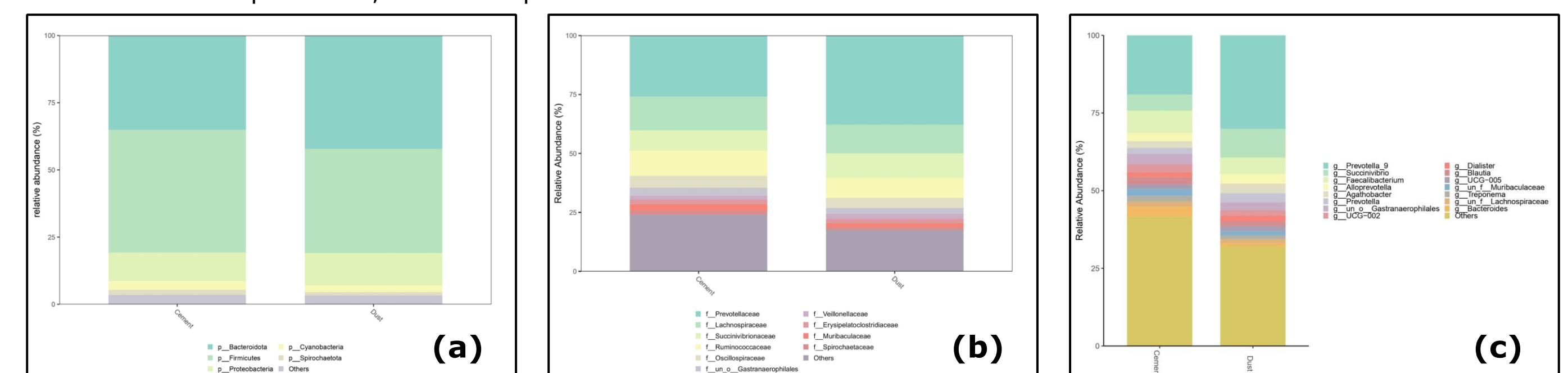
## RESULTS



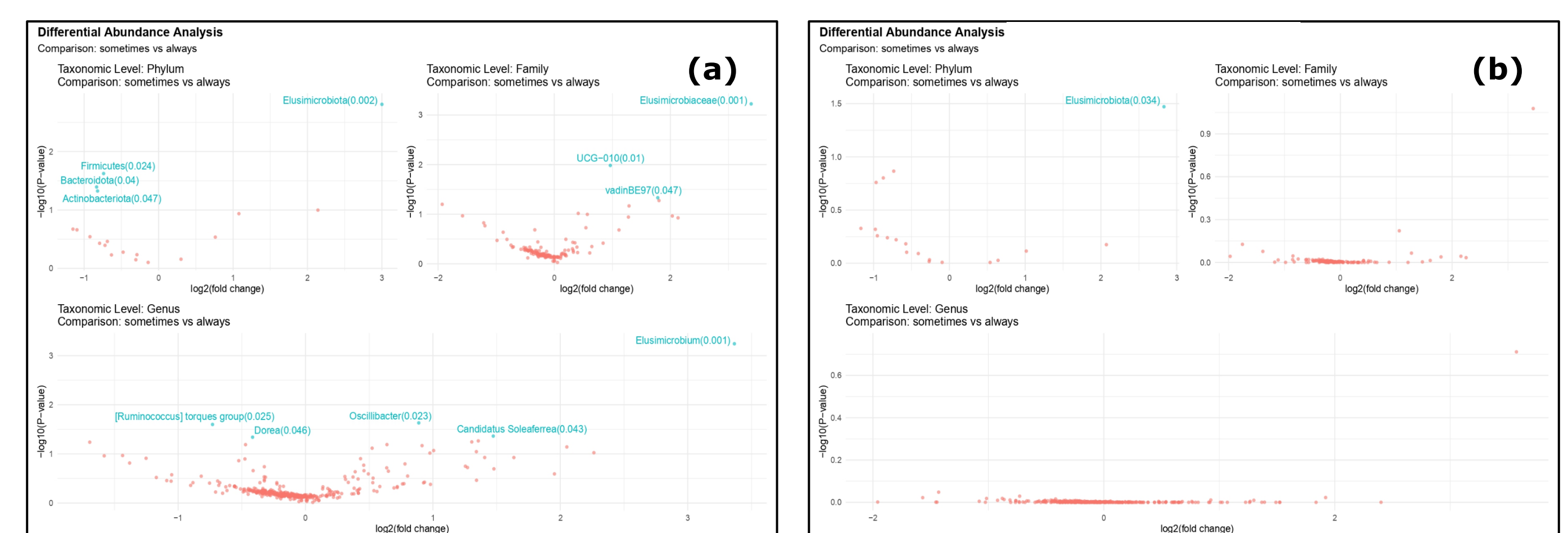
**Fig. 1: Alpha Diversity: School Latrine Use**  
Alpha diversity is a measure of the species richness (and evenness) for one ecosystem/individual microbiome. N = 136 children differed in the frequency of using their school latrine: never - 23(16.7%), sometimes - 108(78.3%), and always - 5(3.6%). A box plot was made for each diversity measure, with group on the x-axis and diversity on the y-axis. A Kruskal-Wallis test of medians was used to assess the differences between the groups' medians across two alpha diversity measures: Chao1:  $p = 0.064$ , Shannon:  $p = 0.096$ .



**Fig. 2: Beta Diversity: Kitchen Material**  
Beta diversity measures the difference in species composition between different groups. N = 131 children differed in kitchen floor material: Dust - 113(81.9%) and Cement - 18(13%). A PCoA plot uses ordination to place an individual microbiome along two axes, with each axis representing a percentage of variation in diversity accounted for. The groups are then outlined with a 95% confidence interval ellipse. A PERMANOVA test of centroids and dispersion of the ellipses was used to assess differences in beta diversity between the two groups:  $p = 0.002$ .



**Fig. 3: Phylum (a), Family (b), and Genus (c) Abundance Across Kitchen Material**  
By using 16s RNA sequencing of fecal samples, Operational Taxonomic Units (OTUs) can be identified and compiled to give a representation of the abundance of different taxonomic levels in an individual's microbiome. The bar plots shown represent the average distribution of taxonomic phyla (a), families (b) and genera (c) across the two groups of children with differing kitchen floor materials (statistics in Fig 2. caption). The two groups differ in the OTUs identified in the phylums (a): Bacteroidota and Firmicutes, the families (b): Prevotellaceae and Lachnospiraceae, and in the genera (c): Prevotella\_9, Succinivibrio, and Faecalibacterium.



**Fig. 4: ALDEx2 Volcano Plot Analysis of Hand Washing After Latrine Use (Before (a) & After (b) Multiple Testing Correction)**

We used the ALDEx2 R package to analyze the abundance of multiple taxonomic groups (phylum, family, and genus) and their differences between two groups separated by hand washing behavior after latrine use. N = 137 children differed in their hand washing frequency after latrine use: never - 1(0.7%), sometimes - 104(75.4%), and always - 23(16.7%). In these plots we're comparing sometimes vs. always. The volcano plots measure the log difference between the abundance of the two groups on the x-axis, and the  $-\log(p\text{-value})$  on the y-axis, with a higher value equating to a lower p-value. The p-values are obtained by ALDEx2 performing a Wilcoxon rank-sum test. Fig. 4a represents the differential abundance before correction. Fig. 4b represents the differential abundance after multiple testing correction with the Benjamini-Hochberg method. There are several significant differences in abundance in phylums, families, and genera before correction. A significant difference still remains after correction in the phylum Elusimicrobiota ( $p = 0.034$ ).

## CONCLUSION

- **Household factors, such as differences in flooring materials, are associated with gut microbiome composition**
  - Groups of children with dust floors compared to cement floors had significantly different beta diversity ( $p = 0.002$ )
- **Lifestyle choices, such as frequency of school latrine usage, are associated with microbiome diversity.**
  - Children who used school school latrines more frequently had marginally significant differences in alpha diversity ( $p = 0.064$  &  $0.096$ )
  - More frequent handwashing is significantly associated with an increase in the Elusimicrobiota phylum of bacteria ( $p = 0.034$ )
- **The most abundant bacteria found by taxonomy were: phylum - Bacteroidota & Firmicutes, family - Prevotellaceae, and genus - Prevotella\_9**
- **Further studies investigating mechanisms of environmental and household factors on microbiome composition are needed**

## REFERENCES

Bäckhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A., & Gordon, J. I. (2005). Host-bacterial mutualism in the human intestine. Science (New York, N.Y.), 307(5717), 1915–1920.  
Zhong, X., Harrington, J. M., Millar, S. R., Perry, I. J., O'Toole, P. W., & Phillips, C. M. (2020). Gut Microbiota Associations with Metabolic Health and Obesity Status in Older Adults. Nutrients, 12(8), 2364  
Nicola Segata, Gut Microbiome: Westernization and the Disappearance of Intestinal Diversity, Current Biology, Volume 25, Issue 14, 2015, Pages R611-R613