

Introduction

Food safety concerns with fresh produce arise from the fact that it is typically consumed raw, and lacks a “kill” step to eliminate pathogens prior to consumption. The critical preventive control step for fresh produce including tree fruit is the sanitizing wash water systems. The Food Safety Modernization Act (FSMA) requires proper validation and verification of any preventive controls. On-site validation of tree fruit wash water systems is nearly impossible, as actual pathogens pose contamination dangers, good indicator organisms are lacking, and natural pathogen contamination is too rare and inconsistent for effective validation, but an effective bacterial index based on the natural bacterial communities could be developed to overcome these problems. The advent of next generation sequencing allows for in-depth characterization of bacterial communities, however to date, there is very limited 16S rRNA gene amplicon sequencing data on the bacterial community composition of various fruits and vegetables. Leff and Fierer provided the most comprehensive characterization of bacterial communities on fresh fruits and vegetables including apples and peaches¹, however these were all commercial produce and not at the harvest stage. In fact, with the exception of a recent small European study on bacterial communities of apples at time of harvest² there is no data on bacterial community composition of carpospheres at pre-harvest or point of harvest from different tree fruit commodities. Furthermore, there is currently no data on the bacterial communities of any citrus fruit including oranges, lemons or limes at pre-harvest, harvest, post-harvest or consumer point of purchase (commercial). Thus, there is currently a very limited amount of information on the bacterial community composition, shifts during the growing season, or changes between seasons of the carpospheres of different commodities of the tree fruit industry.

Objective: The goal of this project is to determine if the natural bacterial communities at the point of harvest for three types of tree fruits, apples (pome), peaches (stone), and navel oranges (citrus), are consistent enough for the development of an effective bacterial index for validating the efficacy of wash water systems.

Materials and Methods

Harvest Fruits

Apples (early, middle, late)
Orange (middle, late)
Peaches (early, late)

DNA extraction

Rinse fruits
Concentrate rinsate
PMA treatment vs total DNA
DNA extraction from sediment

PCR amplify

Chloroplast excluding primers (799F – 1115R)*
V4-V5 primers (515F – 926R)
*Denotes there were technical issues with these primers

Amplicon sequencing

Illumina MiSeq (v2, 300-cycle kit)

Data analysis

Alpha and Beta diversity (QIIME v1.8)
Statistical analysis (R v3.5.0)

Results

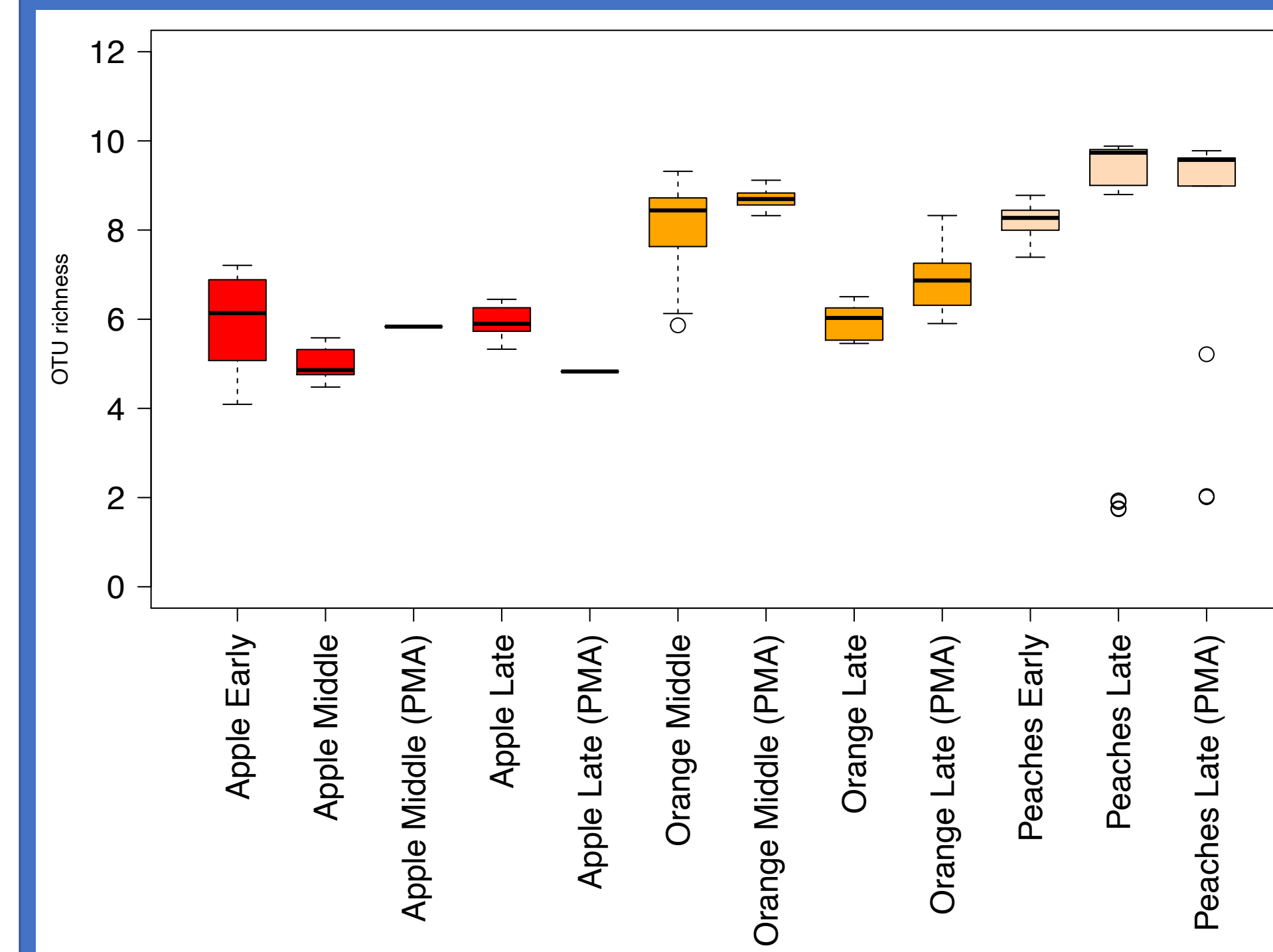


Figure 1. Boxplot of Shannon Diversity Index for type of fruit, harvest time and if treated with PMA. Samples were rarefied at 5,697 sequences per sample. Circles represent outliers.

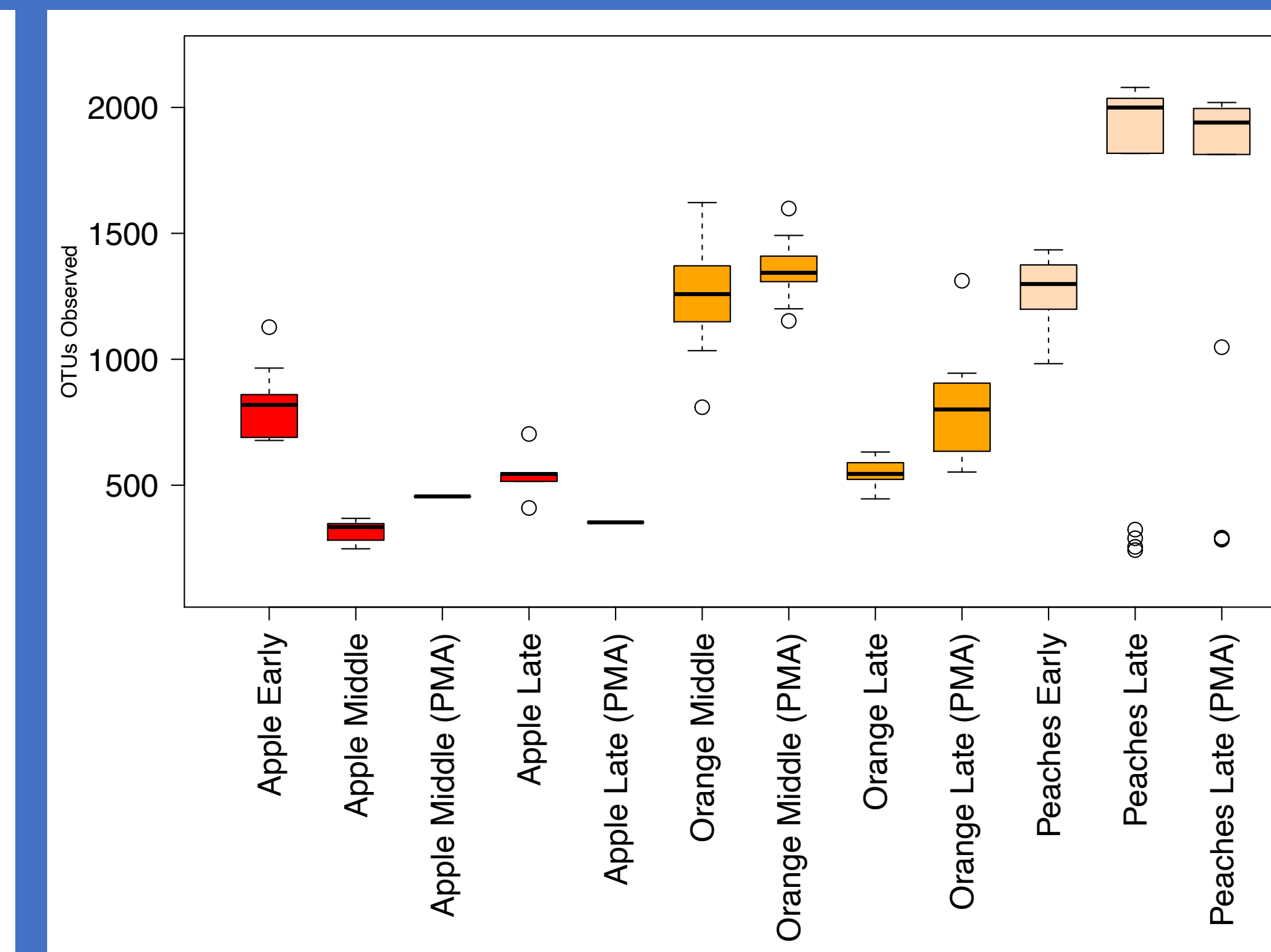


Figure 2. Boxplot of number of observed species for type of fruit, harvest time and if treated with PMA. Samples were rarefied at 5,697 sequences per sample. Circles represent outliers.

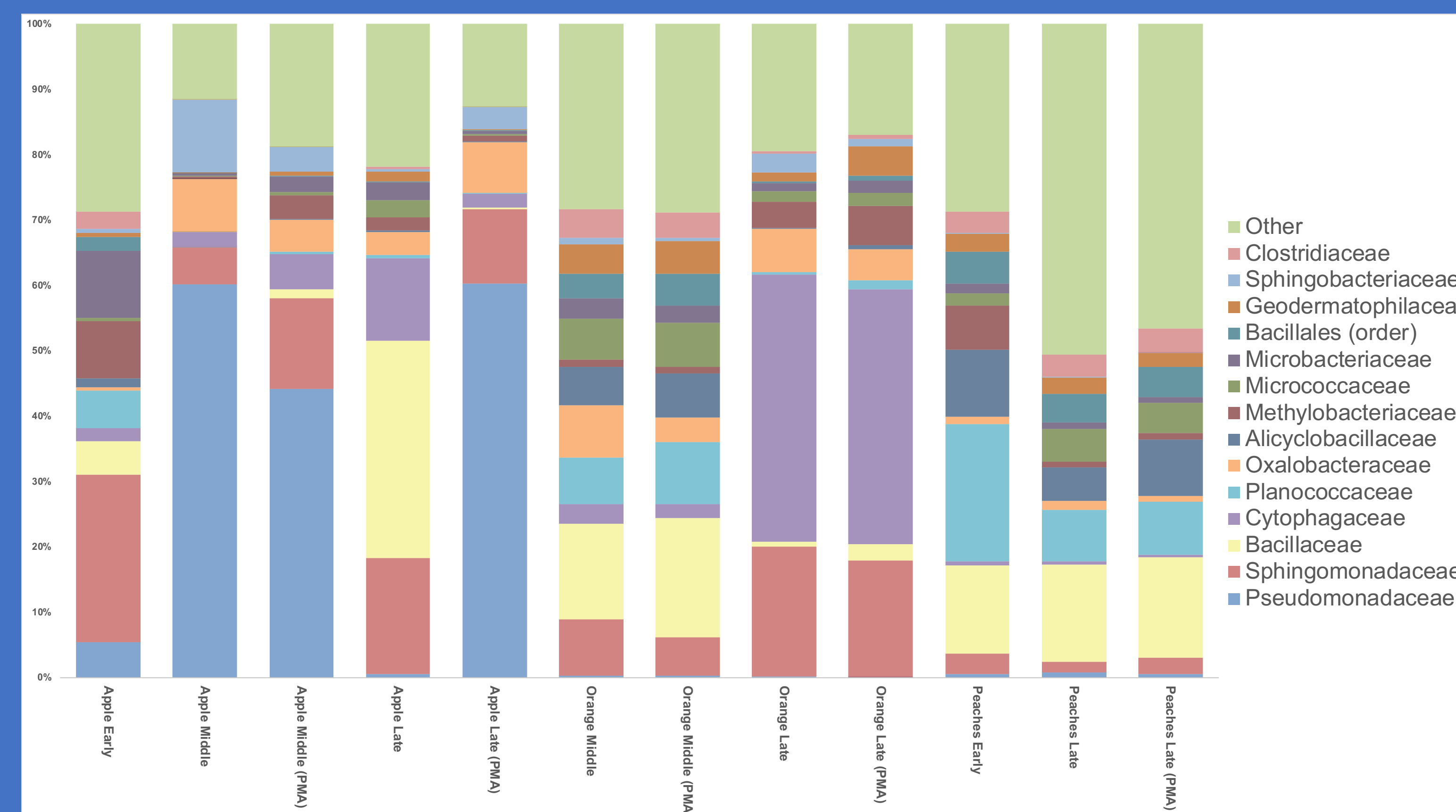


Figure 3. Relative abundance of bacterial families for type of fruit, harvest time, and if treated with PMA. Top 14 families represented, remaining families are summarized in “Other” classification.

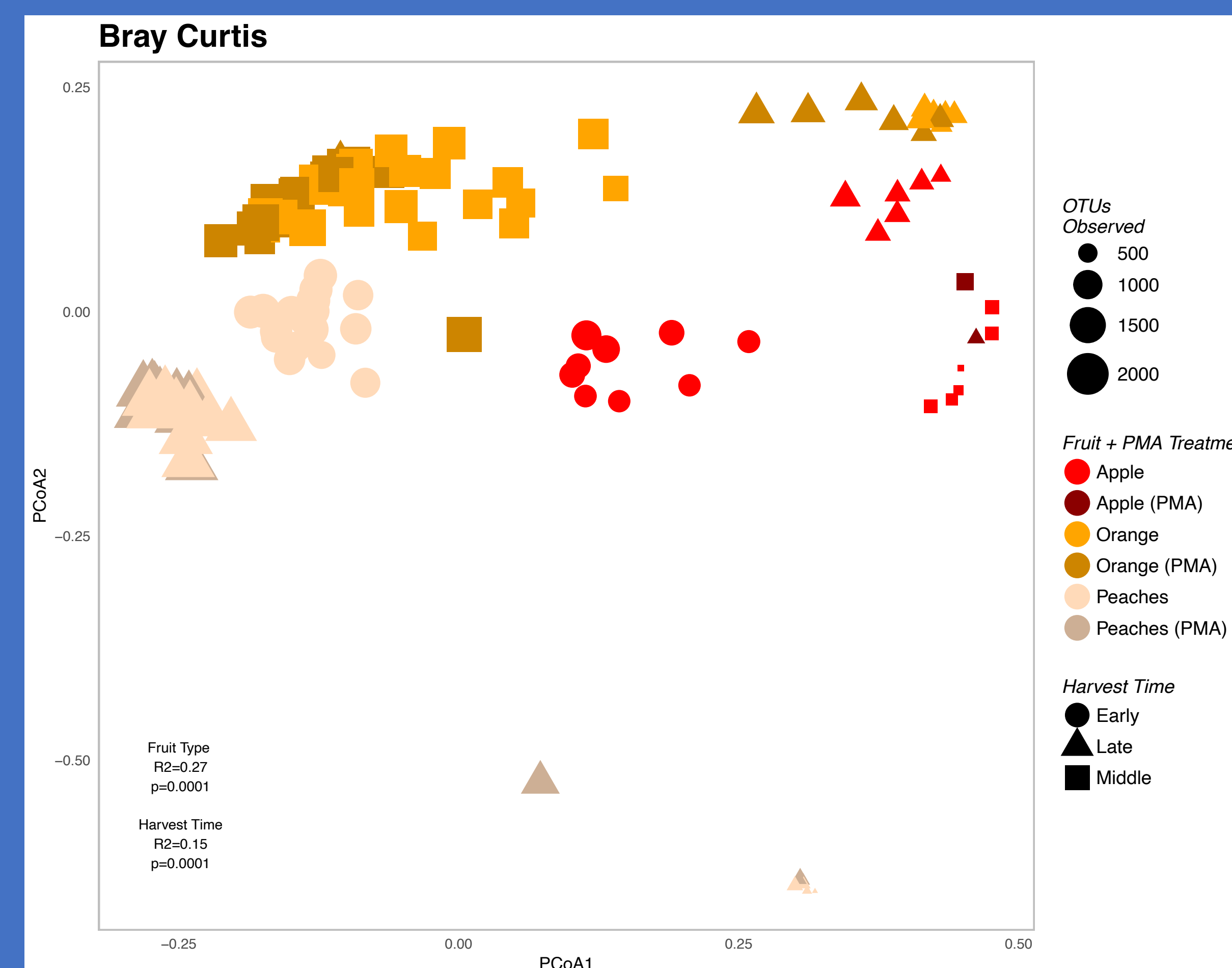


Figure 4. Principal coordinate analysis plots showing differences in bacterial communities. Plot is based on Bray-Curtis dissimilarities between types of fruit, harvest time and if treated with PMA. P-values were calculated using PERMANOVA.

Table 1. Top taxa drivers of differences in bacterial communities

| | Order* | Family* | Genus* |
|-----------------|--|--|---|
| Fruit type | Sphingomondales Sphingobacteriales Burkholderiales | <i>Geodermatophilaceae</i> <i>Sphingobacteriaceae</i> <i>Sphingomonadaceae</i> | <i>Arthrobacter</i> <i>Hymenobacter</i> |
| Time of Harvest | Sphingomondales Sphingobacteriales Burkholderiales | <i>Microbacteriaceae</i> <i>Oxalobacteraceae</i> <i>Sphingomonadaceae</i> | <i>Frigoribacterium</i> <i>Hymenobacter</i> <i>Pedobacter</i> |

*P-value <0.001; P-values were calculated using Kruskal-Wallis test.

Table 2. Potential bacterial index targets

| Index Targets | Relative Abundance of Targets | | |
|------------------------------|-------------------------------|---------|---------|
| | Apples | Oranges | Peaches |
| <i>Sphingomonas</i> sp. | 0.77%* | 1.77% | 1.45% |
| <i>Bacillus flexus</i> | 6.23%* | 3.23% | 0.64% |
| <i>Microbispora rosea</i> | 0.13%* | 1.33% | 1.36% |
| <i>Planococcaceae</i> family | 0.42%* | 1.33% | 2.9% |
| <i>Frigoribacterium</i> sp. | 1.01% | 0.69% | 0.06%* |
| <i>Bacillus muralis</i> | 0.19%* | 1.34% | 1.05% |

*Denotes not present in all samples of that type of fruit

Conclusions

- Peaches appear to have the highest amount of bacterial diversity followed by oranges, then apples.
- Significant differences exist between the bacterial communities of the different types of tree fruit.
- There are significant changes in the communities during the growing season regardless of the type of fruit.
- Potential bacterial index targets were identified at different taxonomic levels such as *Planococcaceae* family, *Sphingomonas* sp., and *Bacillus flexus* that might be used for validation of wash water systems.
- However, initial studies suggest that individual commodity-specific bacterial indexes might have to be developed for validation.

Future Directions

- Finish adapting chloroplast excluding primers (799F – 1115R) that were developed for 454 technology to function with Illumina technology.
- Continue collecting samples from all three types of tree fruit for a second growing season to identify any bacterial community variations between seasons.
- Continue analysis including examining bacterial index for each commodity as an individual, including using a random forest model to predict features in the microbiome that are unique.

Acknowledgements

The authors thank Chris Chabot for technical assistance throughout the project, and also Courtney Sams and Cristina Alcaraz for assistance with different aspects of the project. Funding provided by United States Department of Agriculture (USDA), National Institute of Food and Agriculture (NIFA) Award #2017-67018-26173 awarded to Dr. Kerry Cooper.

References

- 1Leff JW, Fierer N. (2013). Bacterial communities associated with the surfaces of fresh fruits and vegetables. PLoS ONE, 8(3): e59310. doi:10.1371/journal.pone.0059310.
- 2Vepstaite-Monstavice, I., et al. (2018). Distribution of apple and blackcurrant microbiota in Lithuania and the Czech Republic. Microbiol Res, 206:1-8. doi:10.1016/j.micres.2017.09.004.