A Molecular Exploration of the XROOTS Hydroponic/Aeroponic System on ISS

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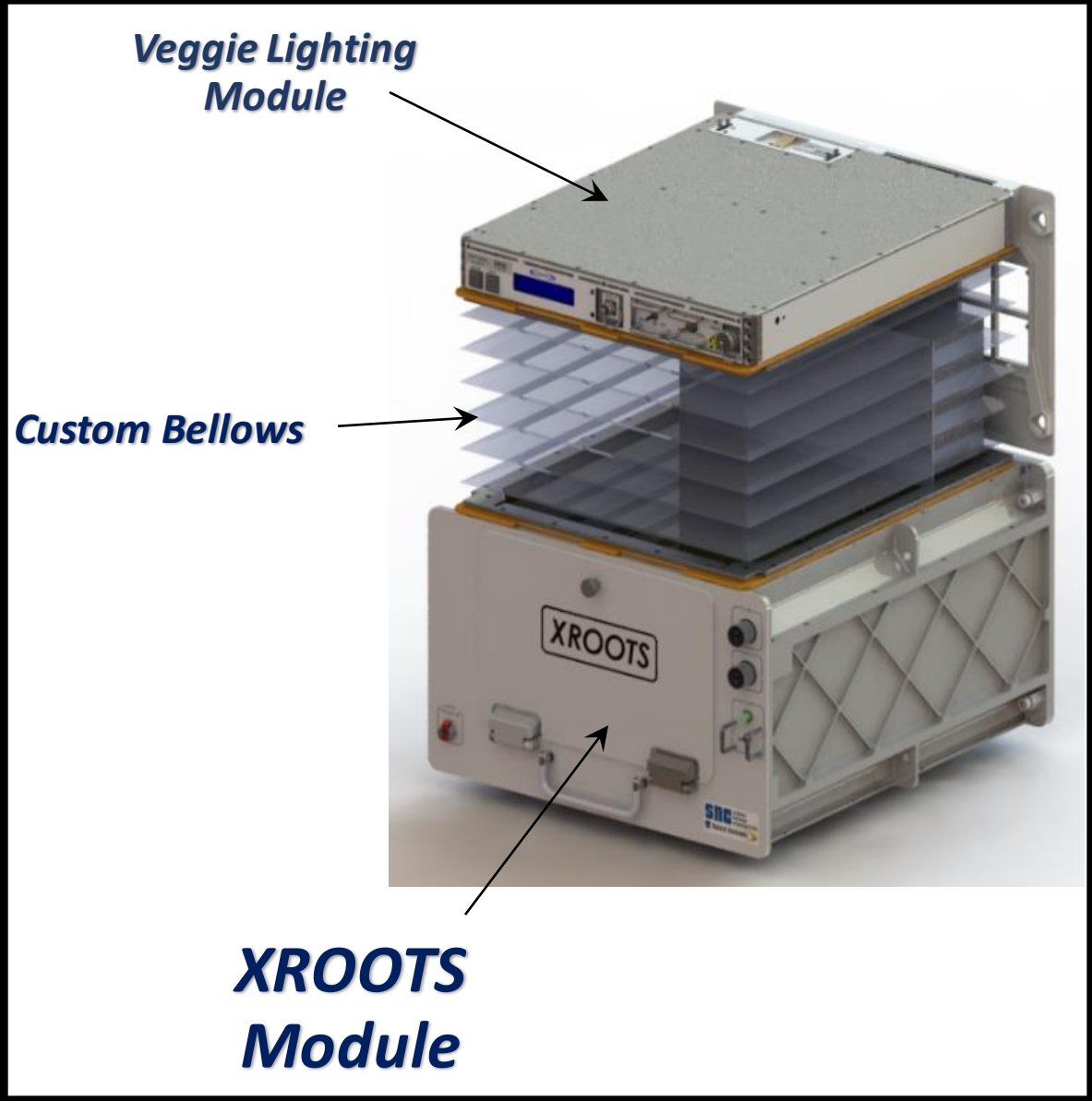
Introduction

- eXposed Root On-Orbit Test System
- Tech Demo: Hydroponic-aeroponic
- Launched in February 2022 (NG-17)
- Short/long-term crops such as leafy greens, tomato, radish and pea
- Grow outs on ISS: June 2022 – October 2022
- KSC sample processing: January 2023- February 2023



Back
1 2
3 4
Front

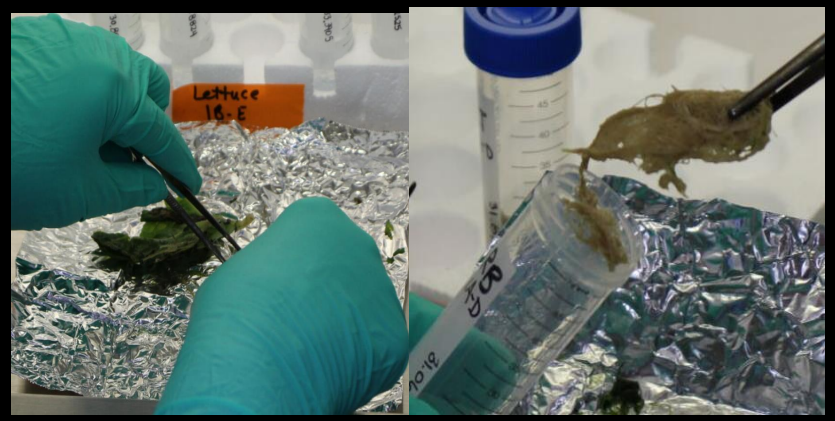
Leaf Top (LT)

Leaf Bottom (LB)

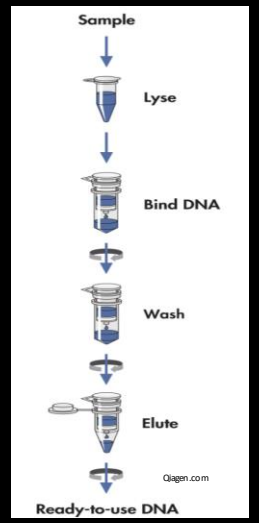
Root Top (RT)

Root Bottom (RB)

XROOTS Molecular Workflow



Sample processing



DNA Extraction

Polymerase Chain Reaction (PCR)

Illumina MiSeq

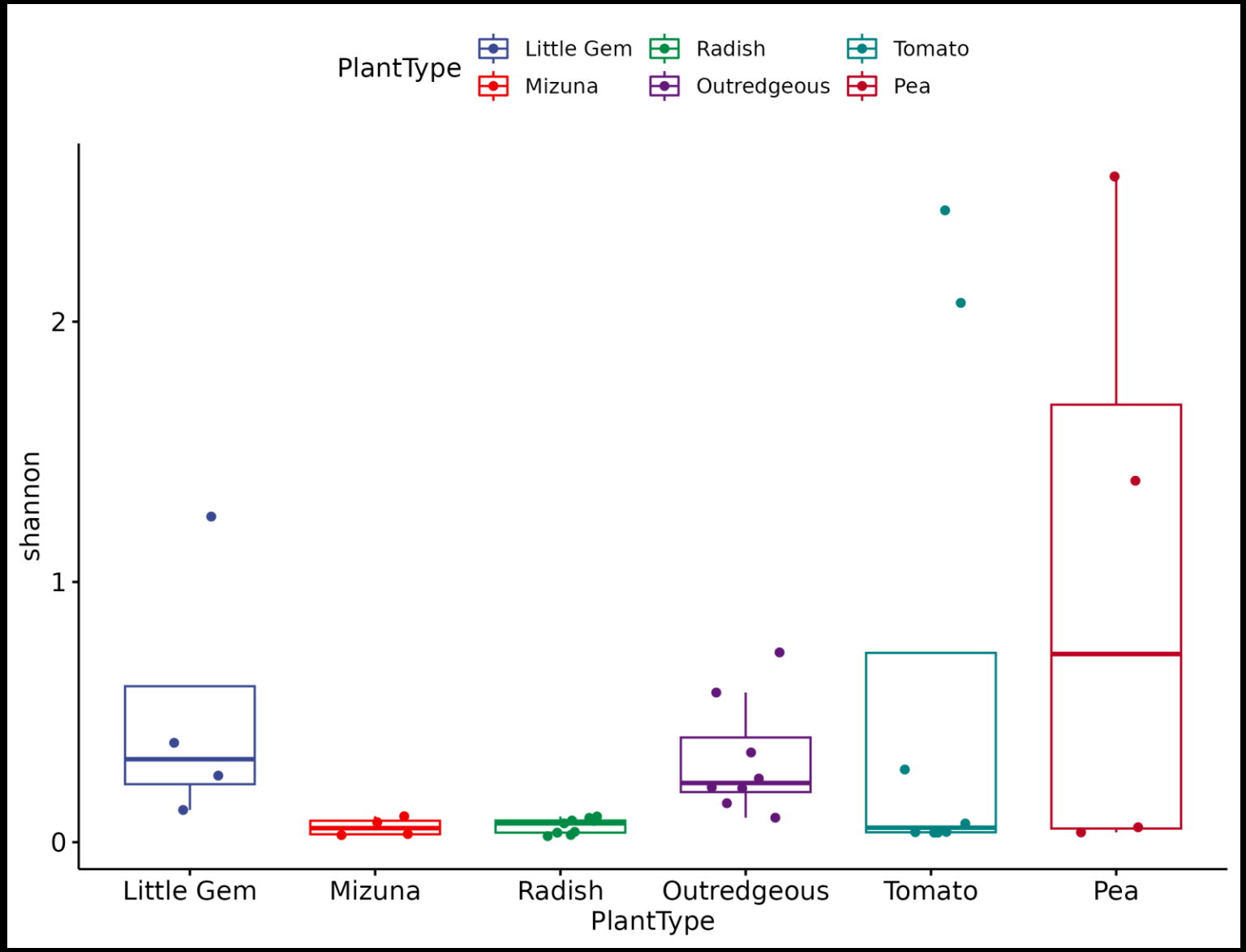
Fungi – Internal transcribed spacer (ITS)

Bacteria – 16S rRNA region (16s)

Kraken 2 – UNITE

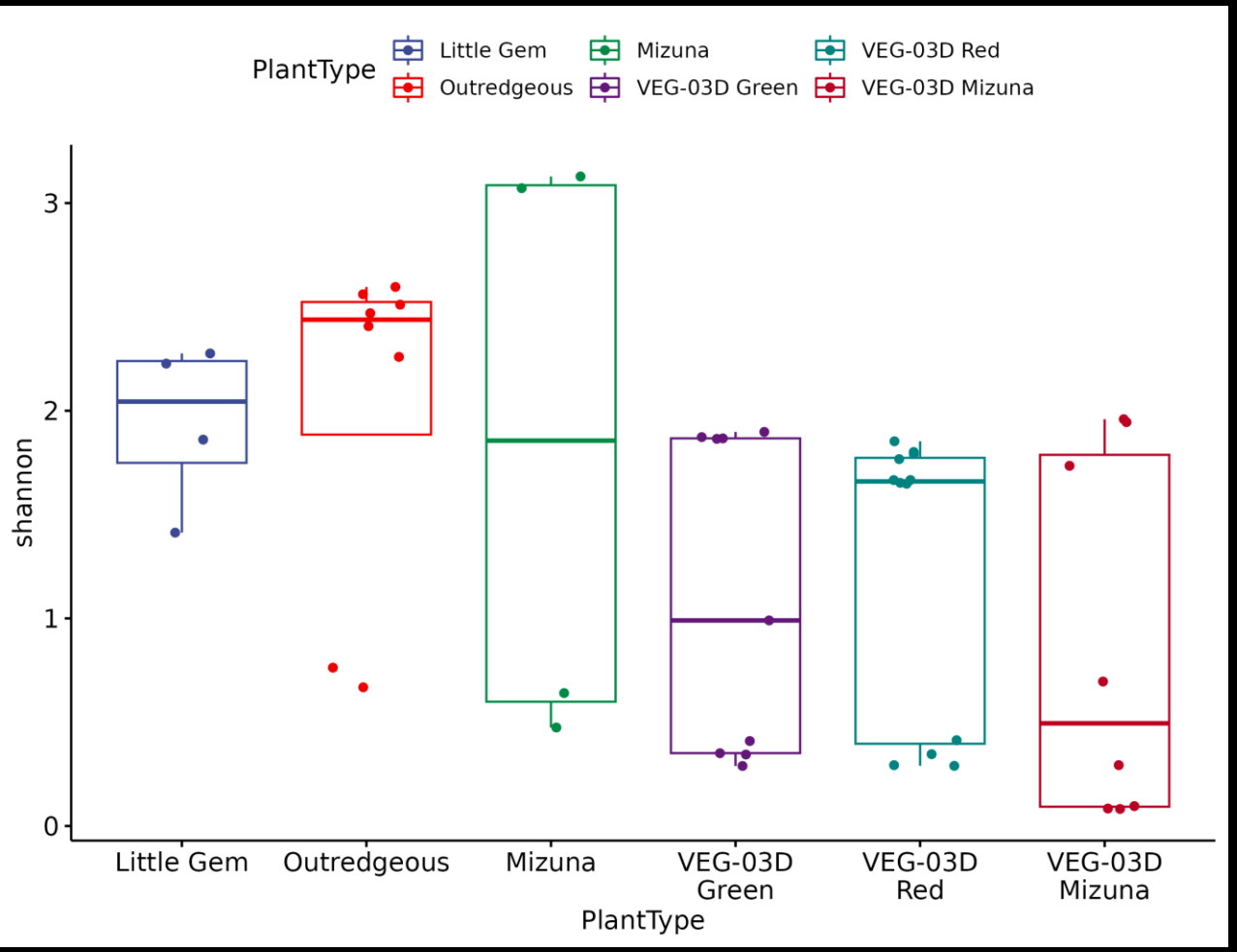
QIIME2 – Silva

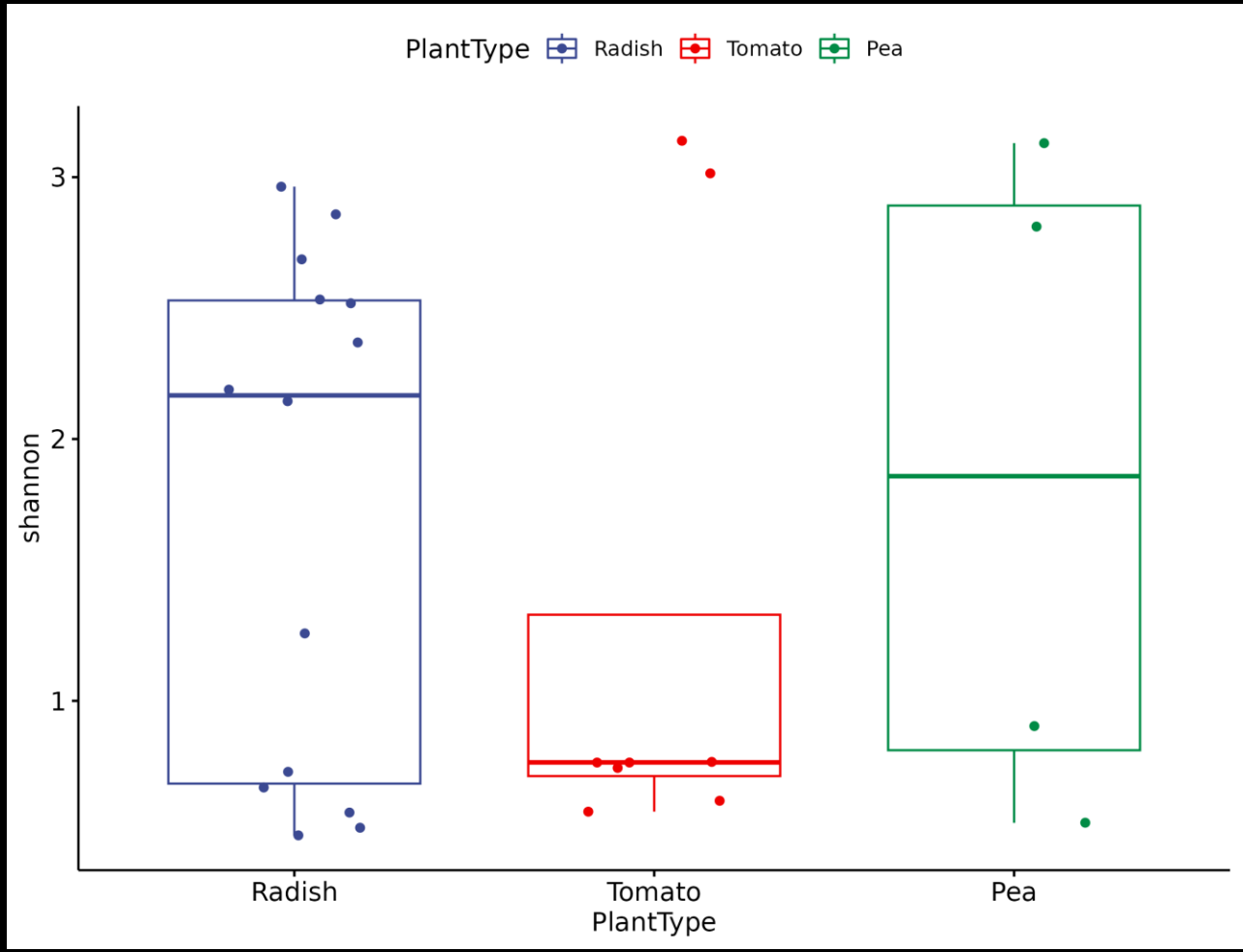
Alpha Diversity of Fungi



Dominant Fungal Genera:
Fusarium, Penicillium, Aspergillus, Cystobasidium

Alpha Diversity of Bacteria



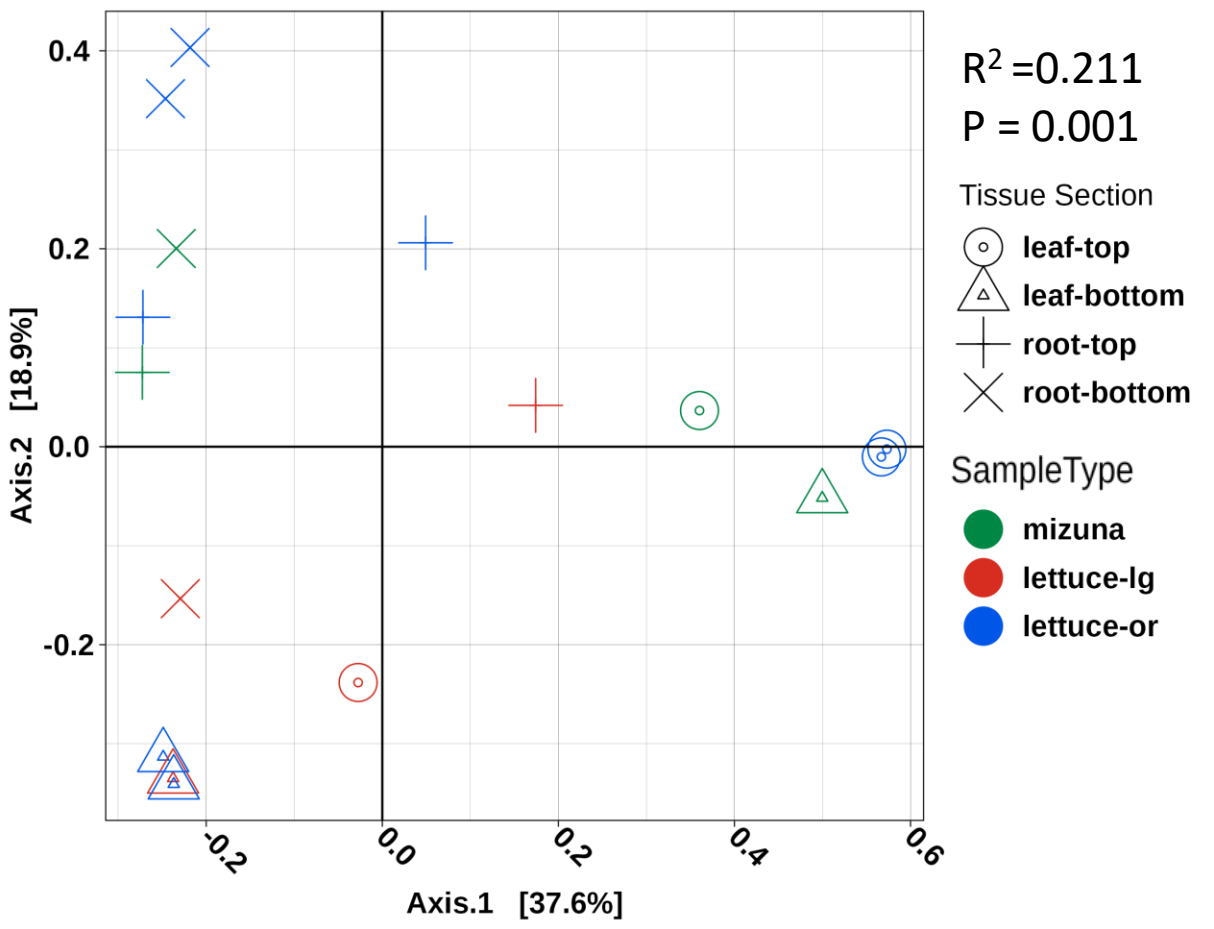


Dominant Bacterial Genera:

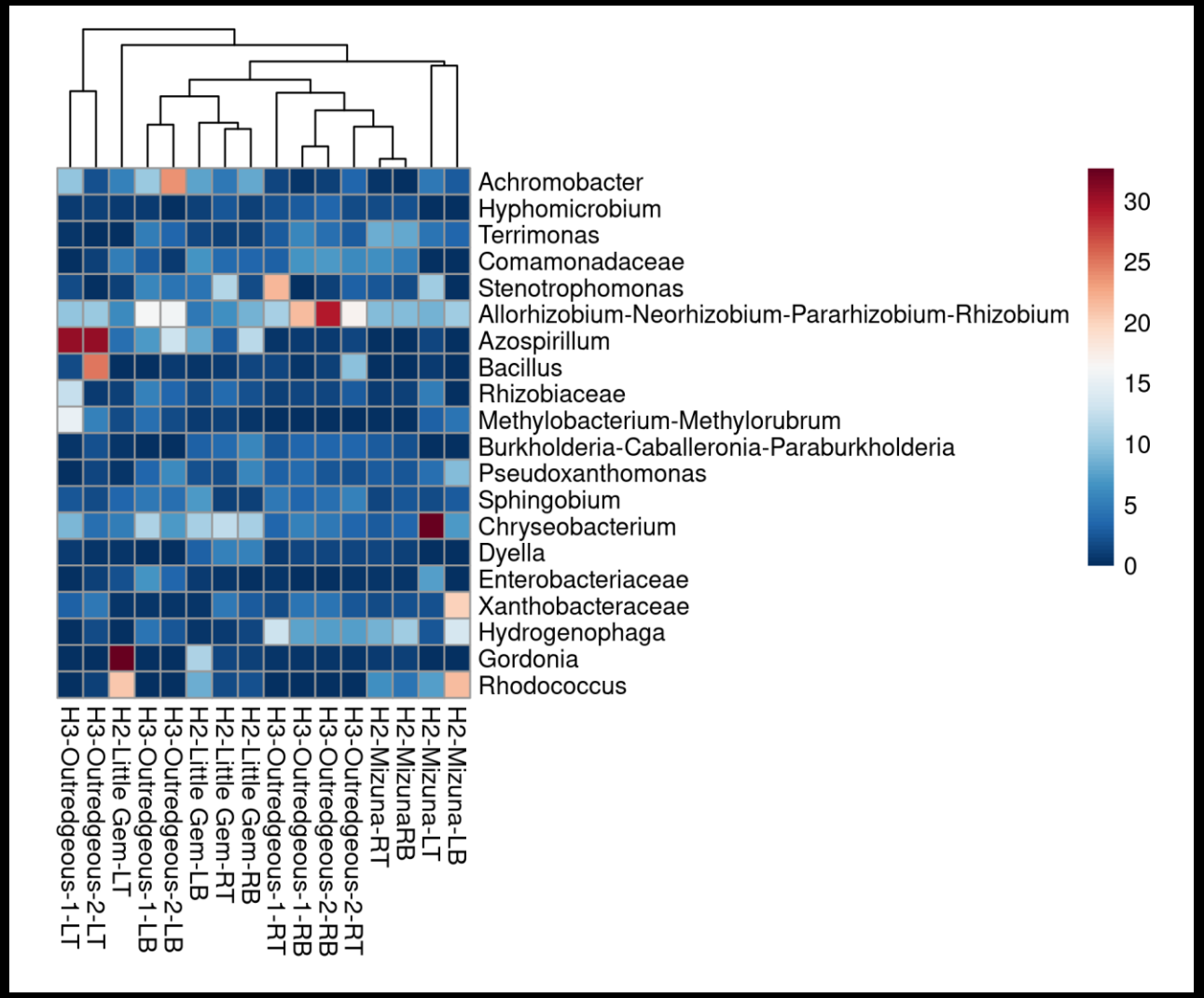
Pseudomonas, Chryseobacterium, Rhodococcus, Pseudoxanthomonas, Mycobacterium

Leafy Greens (16S)

Mizuna (*Brassica rapa var. nipposinica* n = 1)
Little Gem (*Lactuca sativa cv* Little Gem n = 1)
'Outredgeous' (*Lactuca sativa cv.* 'Outredgeous' n = 2)

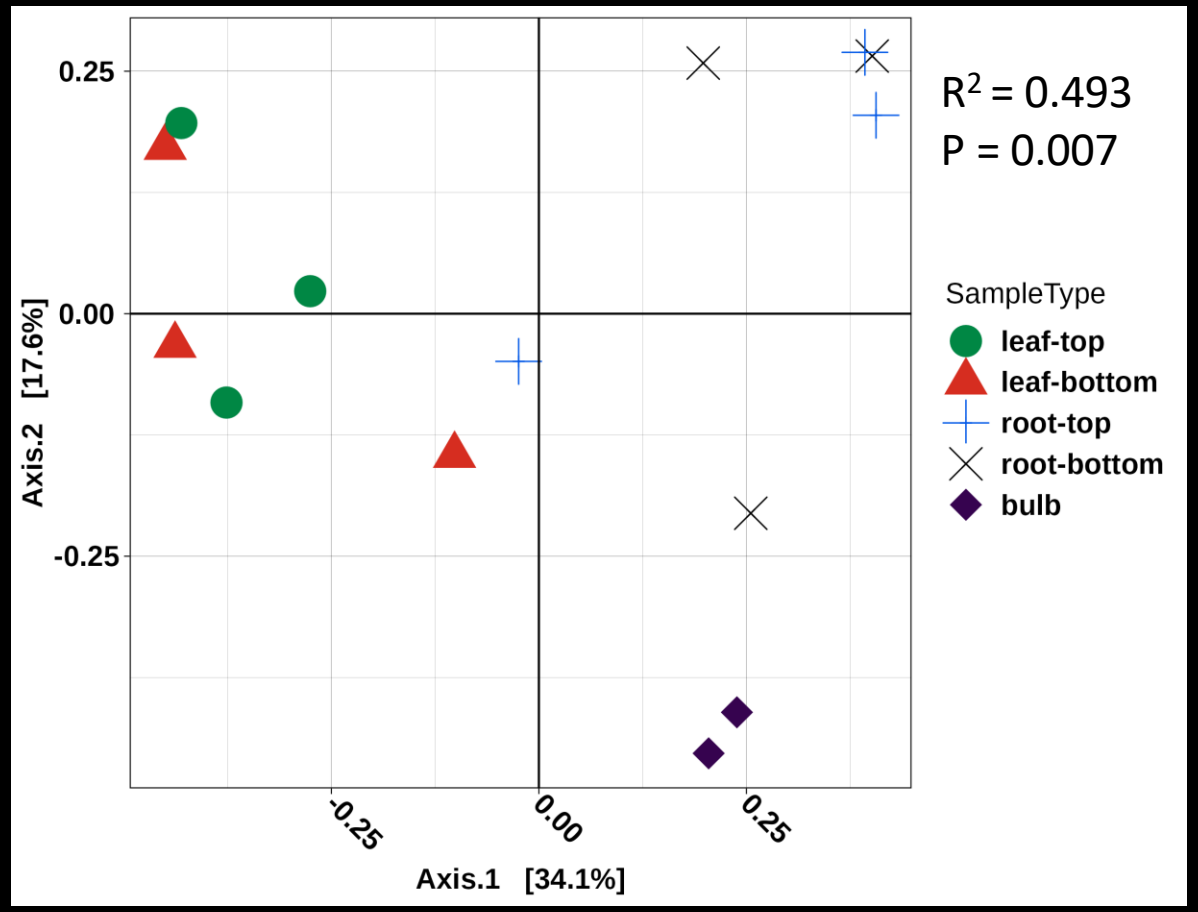


Principle Coordinate Analysis - Beta Diversity

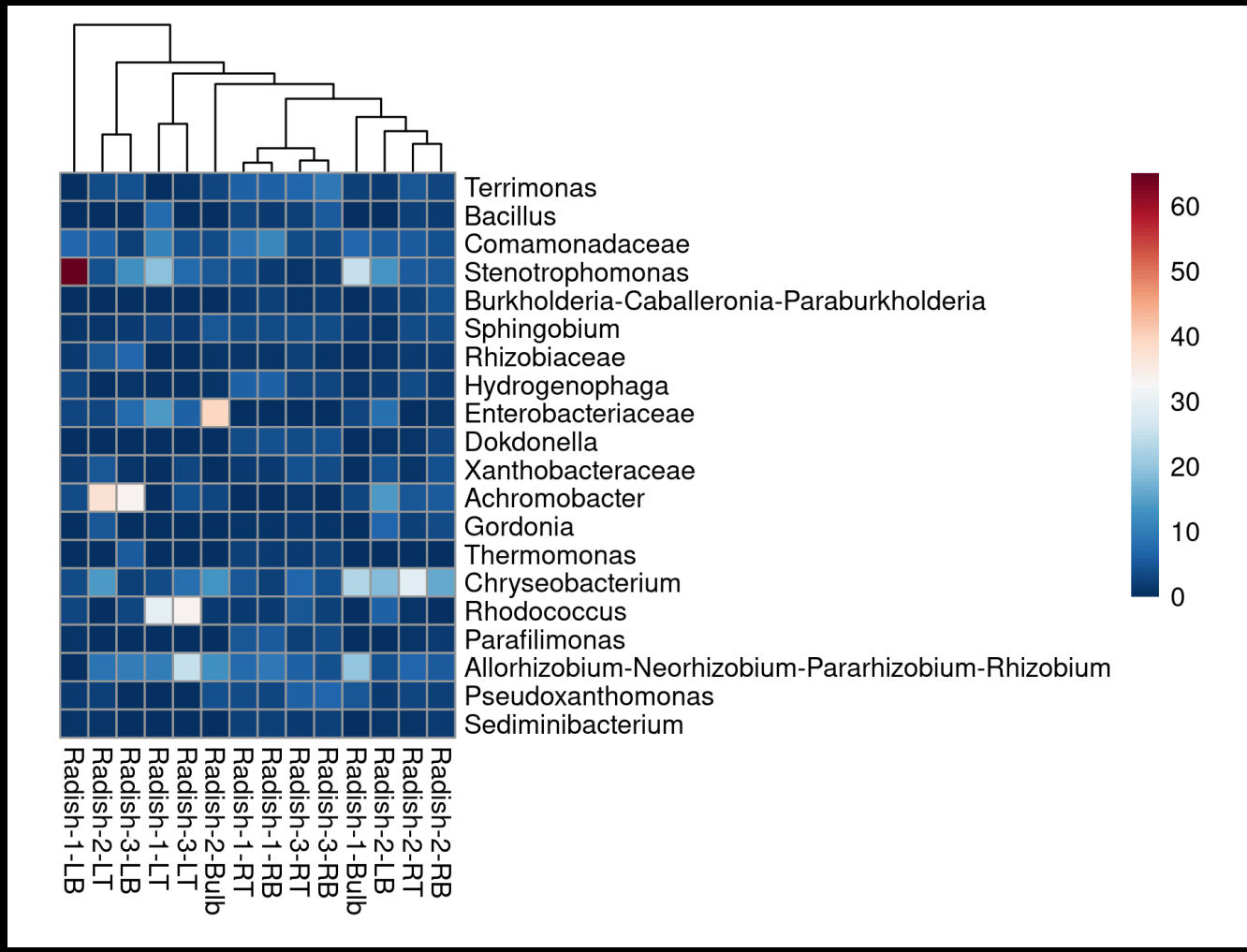


Heatmap showing Percent Read Abundance of top 20 Genera (Pseudomonas removed)

Radish (16S) – *Raphanus sativus* cv. Cherry Belle (n = 3)

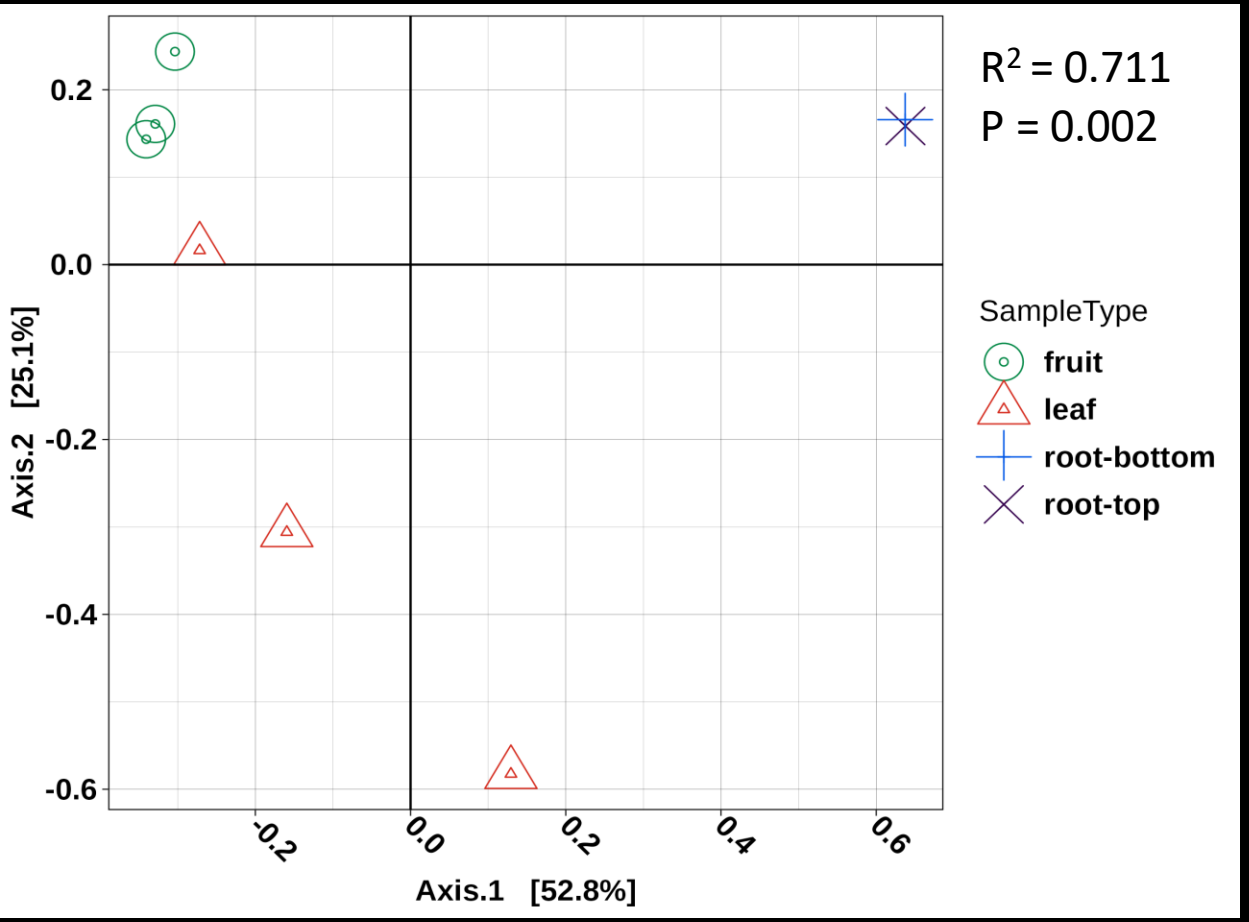


Principle Coordinate Analysis - Beta Diversity

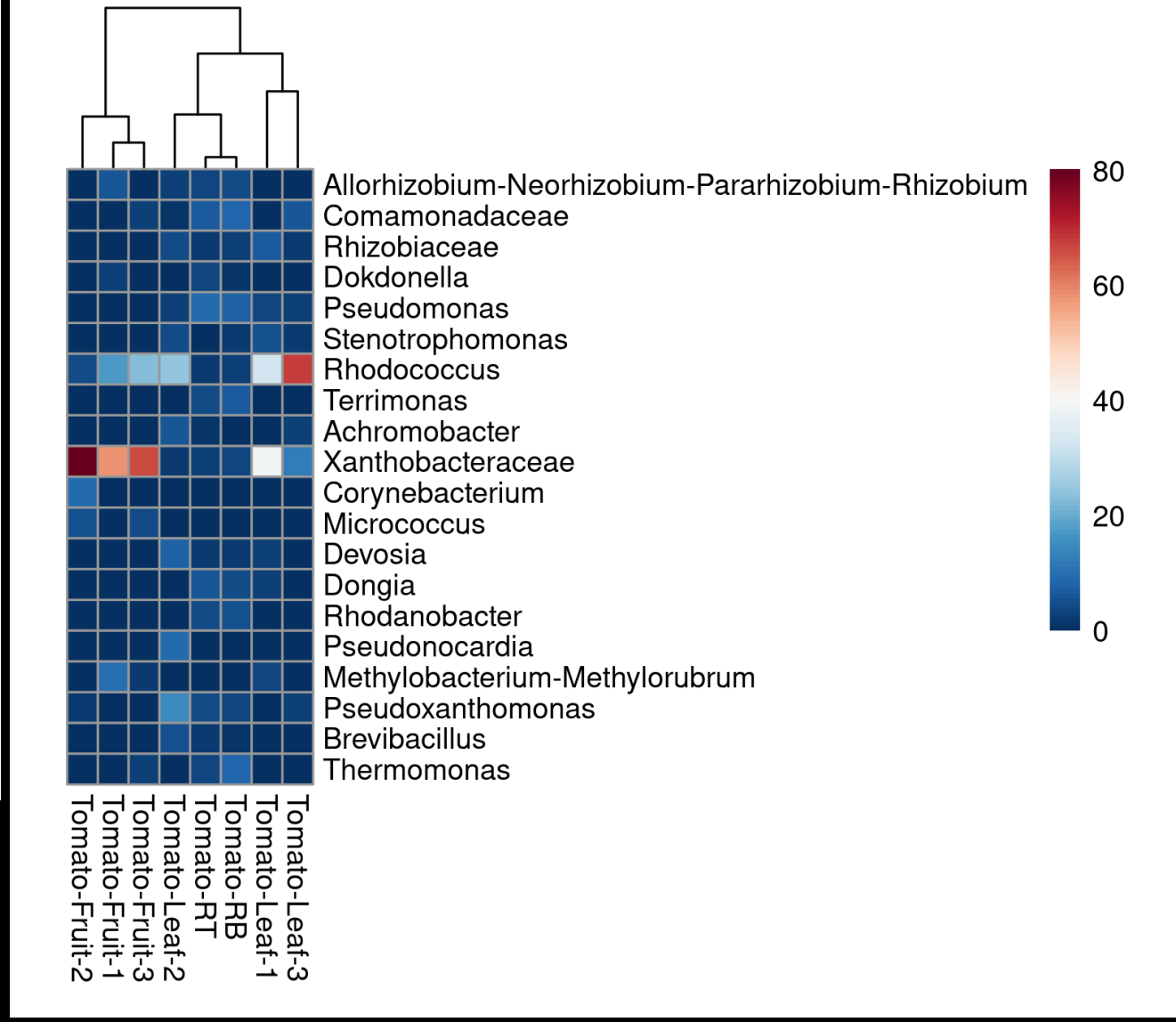


Heatmap showing Percent Read Abundance of top 20 Genera (Pseudomonas removed)

Tomato (16S) – *Solanum lycopersicum* L. cultivar-Micro-Tina (n = 1)

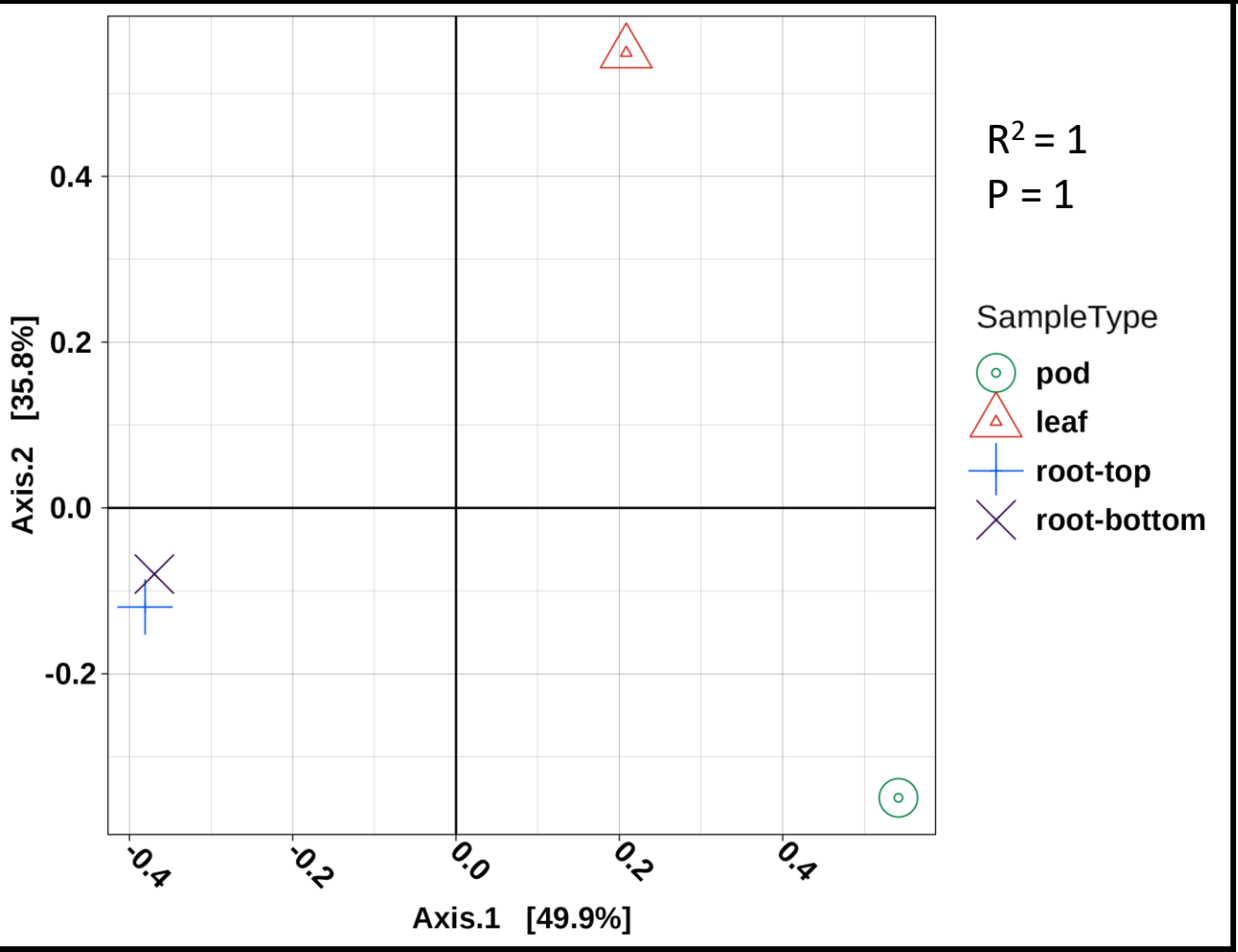


Principle Coordinate Analysis - Beta Diversity

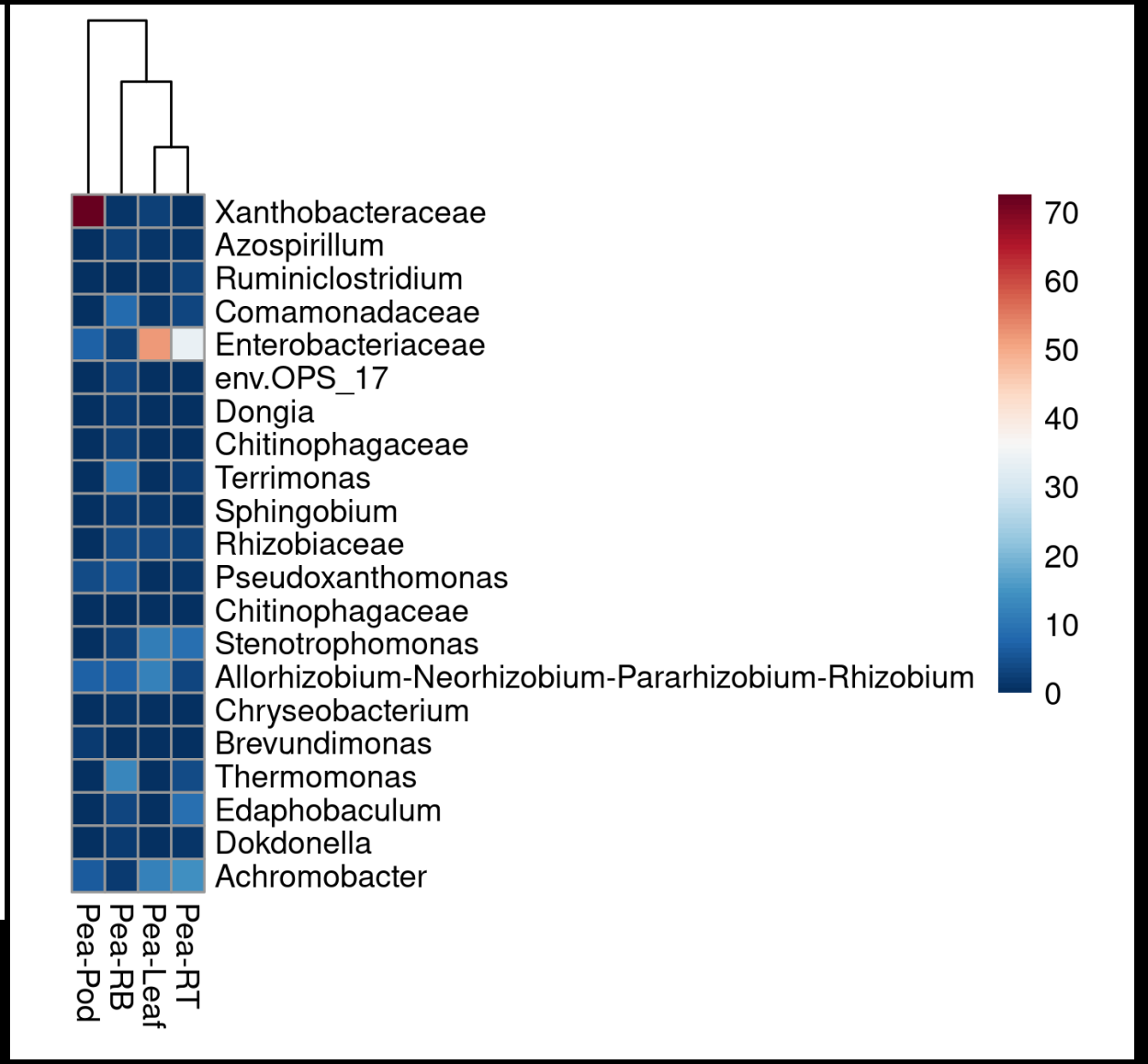


Heatmap showing Percent Read Abundance of top 20 Genera (Pseudomonas removed)

Pea (16S) – *Pisum sativum cv.* Earligreen (n = 1)



Principle Coordinate Analysis - Beta Diversity



Heatmap showing Percent Read Abundance of top 20 Genera (Pseudomonas removed)

Conclusions

- Tech demo successful: plants were grown in hydroponic/aeroponic system
- No human pathogens were detected
- Alpha diversity for bacteria was slightly higher than previous tech demos in Veggie (Leafy Greens).
- Tomato alpha diversity was lower than radish or pea.
- This study helps with a fundamental understanding of plant microbiome research on the International Space Station.

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Questions?

Sample processing

Harvest 2

Red Romaine Lettuce

Mizuna

Radish

Harvest 3

Red Romaine Lettuce

Wheat (cv. Apogee)

Radish

Harvest 4

Tomato plant and fruit

Pea plant and pod