Avery Farms

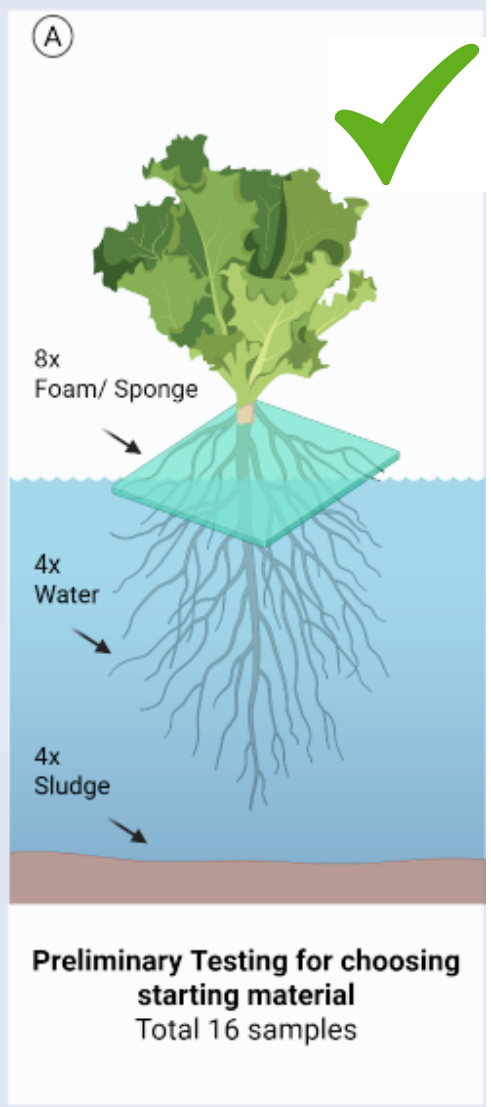
Monthly Update Meeting

2.3.2026

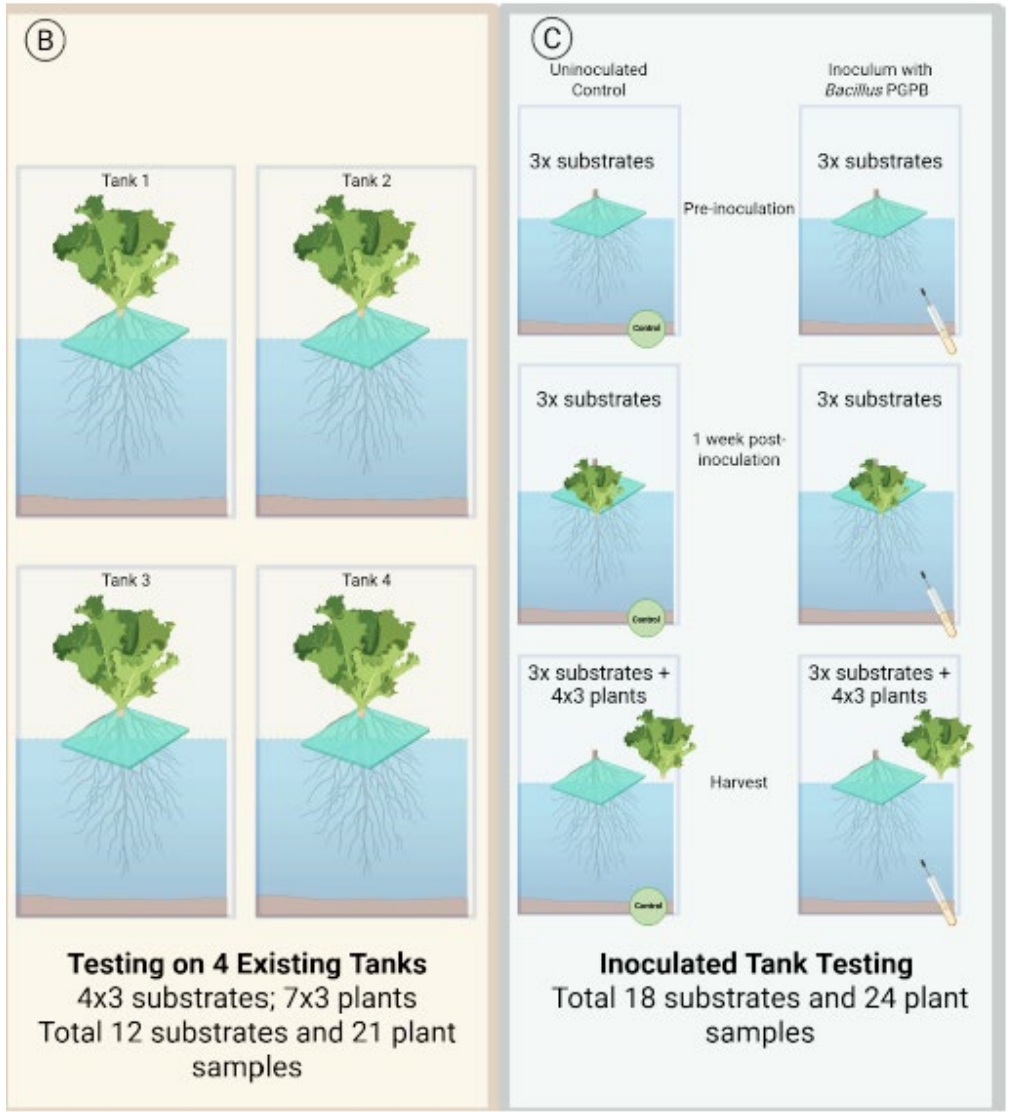
Agenda

(1)Sampling input
(2)Diversity metrics
(3)Bacteria of interest
(4)Summary and Next Steps

Expected Sampling Plan



Preliminary Testing for choosing starting material
Total 16 samples



Testing on 4 Existing Tanks
4x3 substrates; 7x3 plants
Total 12 substrates and 21 plant samples

Inoculated Tank Testing
Total 18 substrates and 24 plant samples

Current Data Digestion

Sponge	Tank Type			
Timepoint	Control	Inoculated	Existing	Total
Pre inoculation	3	3		
Post inoculation	3	3		
Harvest	3	3	15	
Control	1			34

Leaf	Tank Type			
Timepoint	Control	Inoculated	Existing	Total
Harvest	12	12	15	39

Analysis Goals

Current Analysis

Compare the **control** to the **inoculated** tanks throughout 3 different time points:

- **Pre-inoculation**
- **Post inoculation**
- **Harvest**

There are other factors we can look into such as

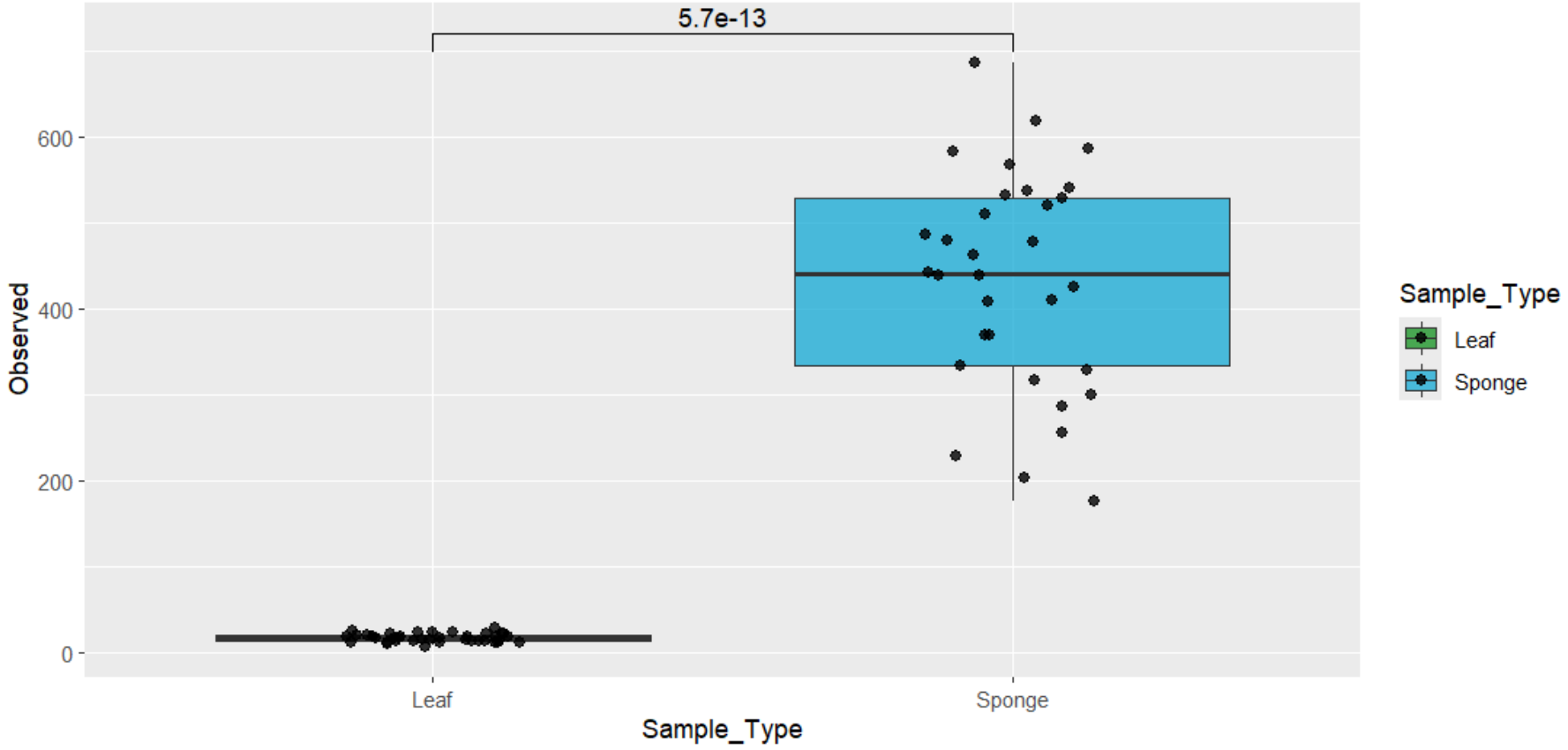
- **Lettuce type** (Red leaf vs Romaine vs Crispy leaf vs Sweet Leaf)
- **Tank Number** (Existing tank vs Experimental tank)

Alpha Observed – measures the number of unique sequences detected in sample type

Sponge samples exhibit **substantially higher richness** than leaf samples after rarefaction, with **a significant difference** detected using a Wilcoxon rank-sum test (**$p = 5.7 \times 10^{-13}$**).

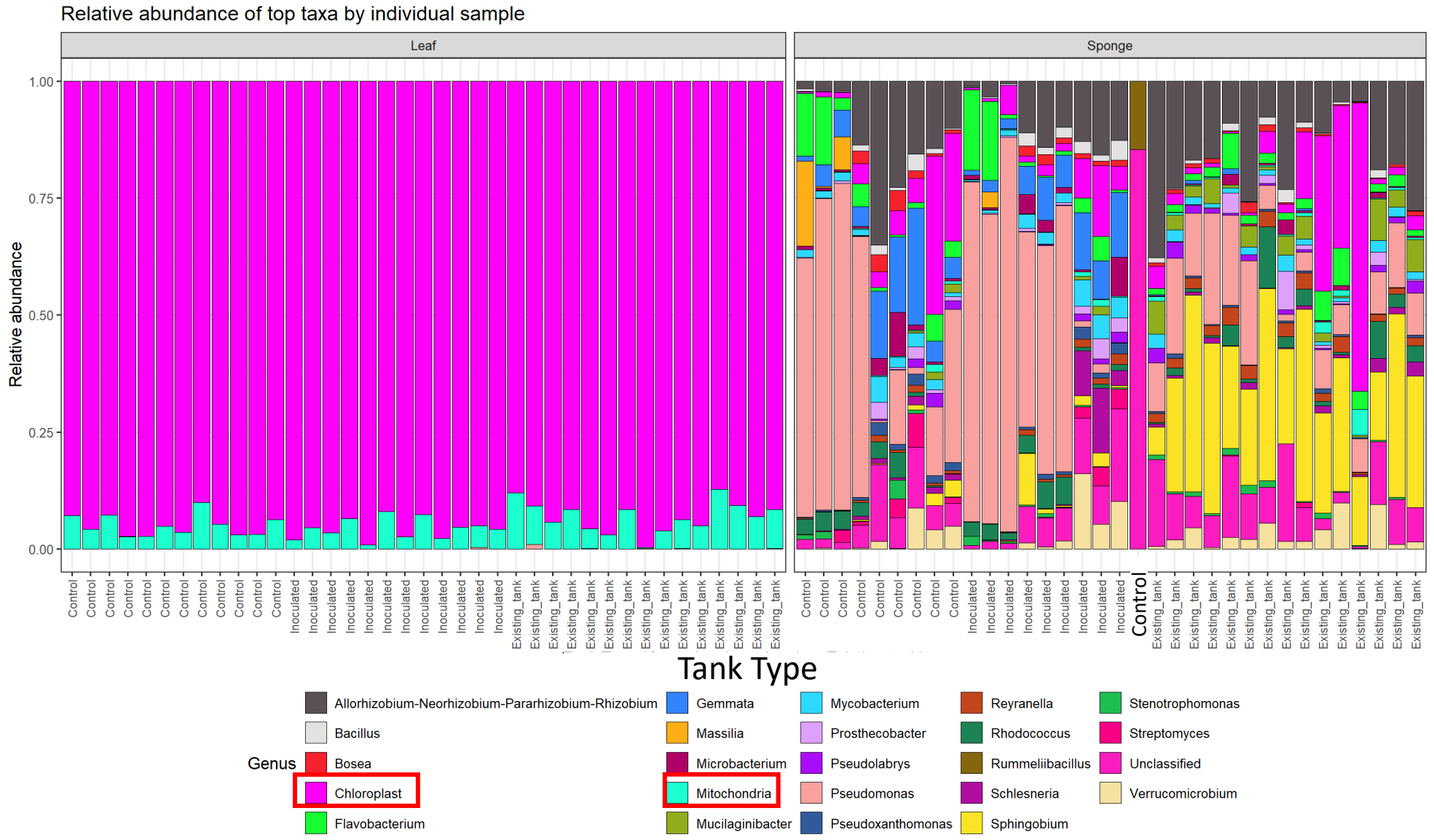
- There are a **higher number** of **unique sequences (ASVs)** detected **in sponge** compared to leaf

- This usually means that **in downstream analysis** – **sponge will have a higher number** of **unique bacterial classifications compared to leaf**



Relative abundance of Top Genus by Sample Type

Relative abundance of bacterial genera by sample type. Leaf samples are dominated by chloroplast-associated Cyanobacteria (~97% relative abundance), whereas sponge samples show substantially lower chloroplast contribution (~10%) and greater bacterial diversity



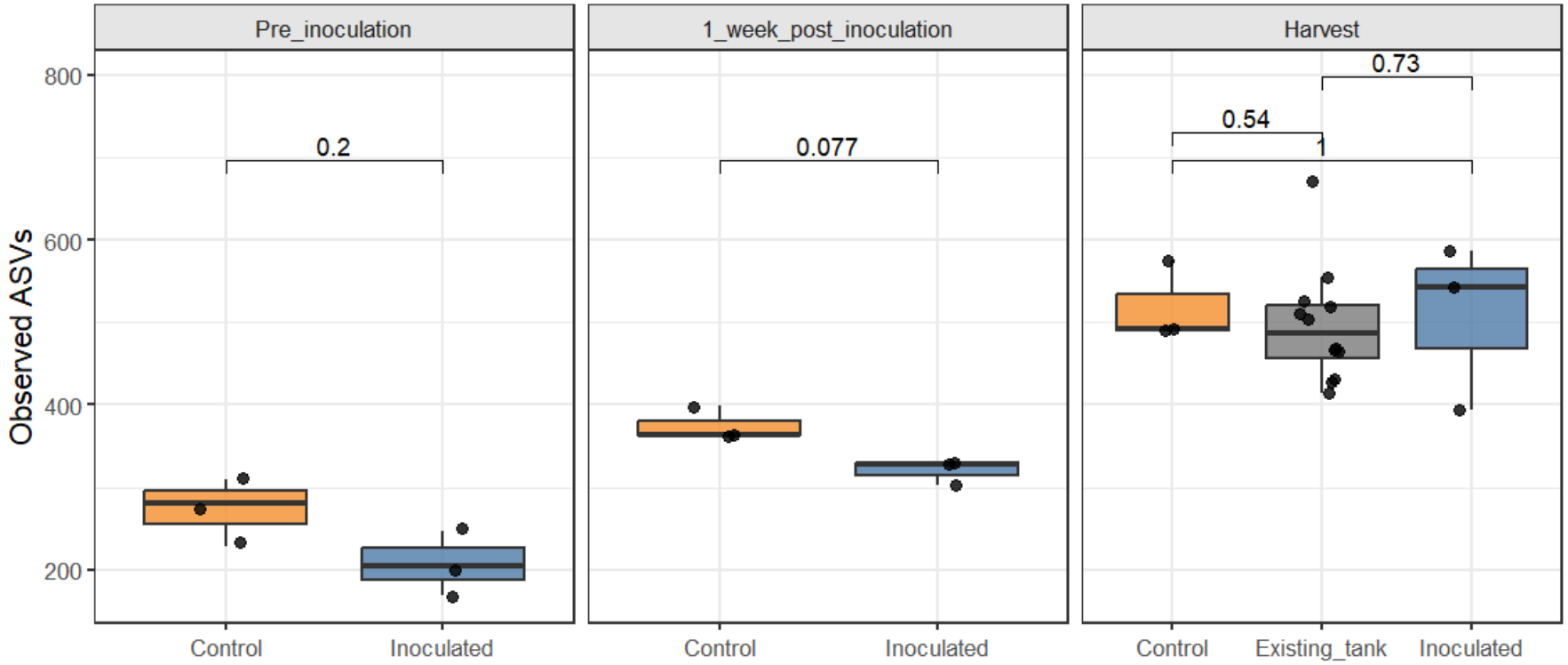
- 99% of reads in leaf samples belong to chloroplast taxonomic signals
- Leaf is dropped for subsequent analysis
- Sponge retains a high bacterial diversity

Control Sponge vs Inoculated Sponge across Time

Observed ASV richness in rarefied sponge samples across experimental timepoints. Richness is compared between control and inoculated treatments at pre-inoculation and one week post-inoculation, with existing tank samples included at harvest. Boxplots summarize the distribution of observed ASVs and points represent individual samples; pairwise differences within each timepoint were assessed using Wilcoxon rank-sum tests, with p-values shown on the plot.

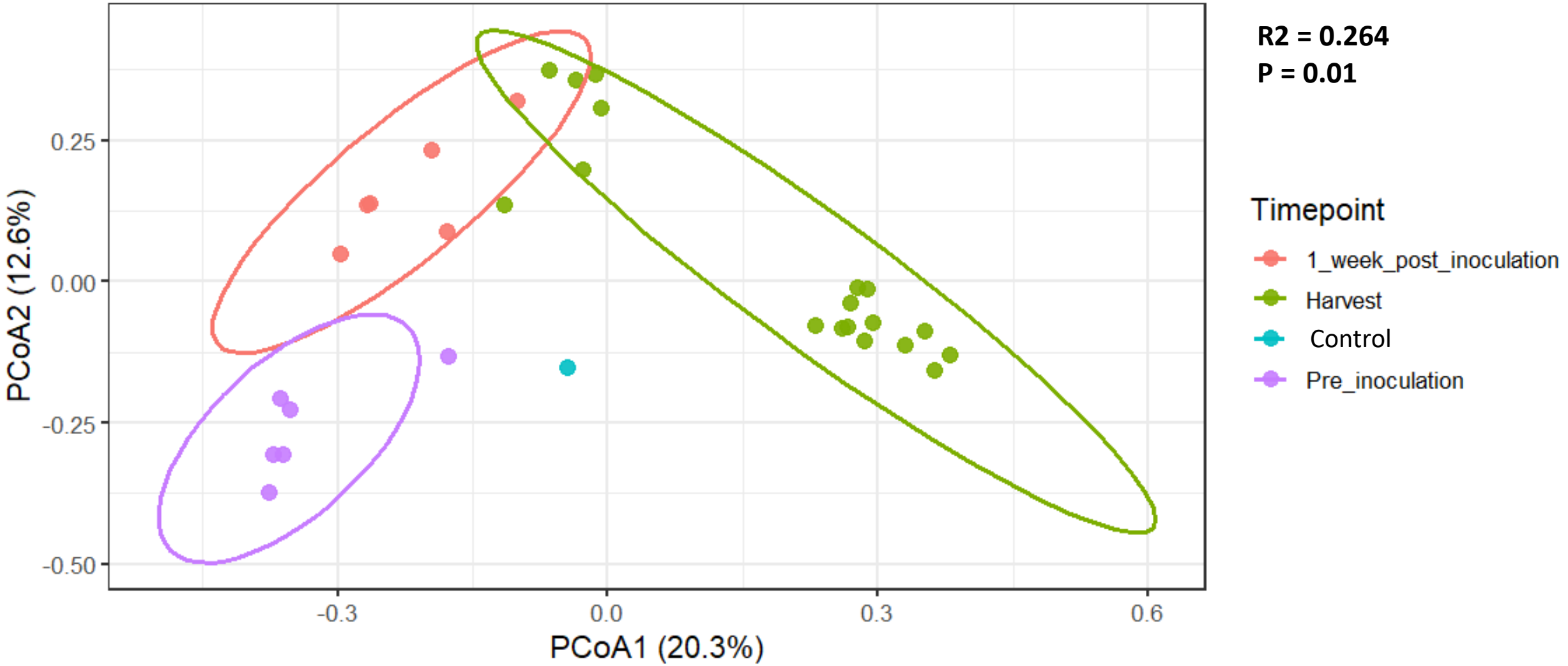
- The number of unique sequences increases (ASVs) over time

- Even though it looks like control has a slightly higher number of ASVs compared to inoculated – statistics do not support this notion



Beta Diversity – Jaccard Presence / Absence

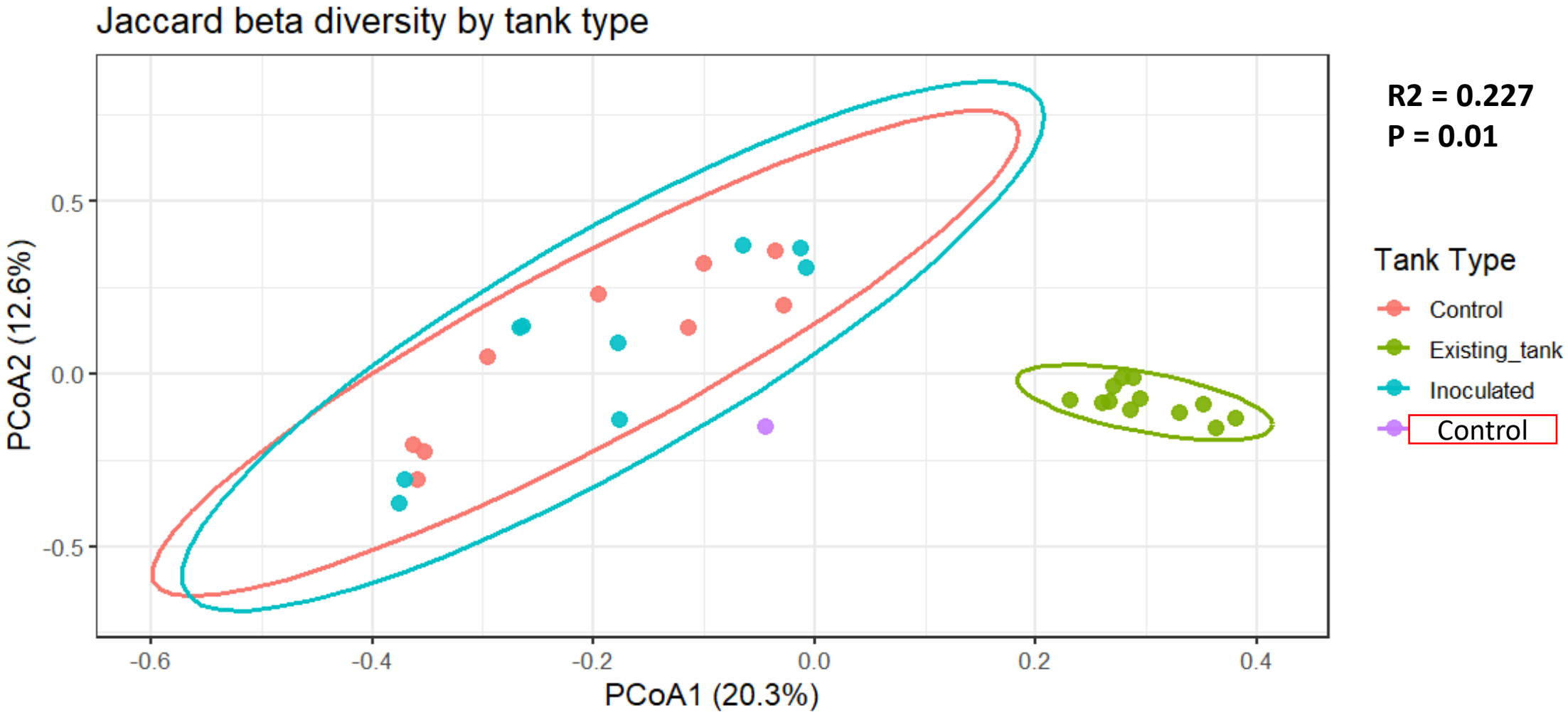
Jaccard quantifies differences between samples based on **presence or absence of taxa**



Samples **cluster by timepoint**, indicating that **microbial communities might change over time**. The ellipses show the spread of samples within each timepoint.

Beta Diversity – Jaccard Presence / Absence

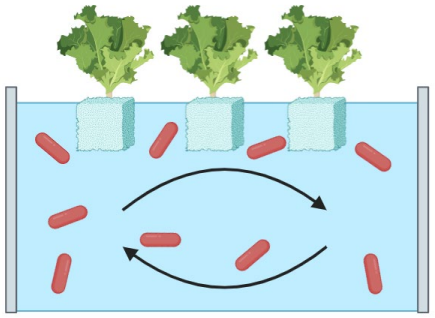
Jaccard quantifies differences between samples based on **presence or absence of taxa**, ignoring abundance



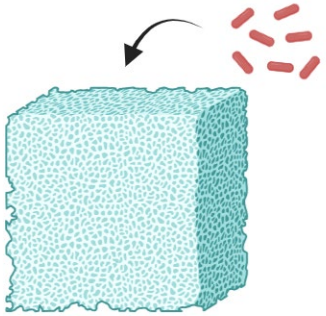
Control and **Inoculated** samples **cluster together** – suggests **no change** in microbial communities between these groups.
Existing tank samples **clusters away** – suggests existing tank's **microbial communities are different** from the

Inoculant – control sponge and in tank application

- Commercial product or pure *Bacillus?*
- Rate of application?
- Storage conditions?



Inoculated tank



Control sponge (with inoculant)

Bacteria of Interest

Bacteria of Interest	
Actinobacteria	*Bacillus pumilis*
Azospirillum brasilense	*Paenibacillus chitinolyticus*
Pseudomonas chlororaphis	*Pantoea dispersa*
Pseudomonas putida	*Enterobacter spp.*
Pseudomonas spp.	*Streptococcus spp.*
Bacillus subtilis	*Aeromonas sp.*
Bacillus amyloliquifaciens (inoculant)	*Klebsiella sp.*
Bacillus velezensis (inoculant)	*Streptomyces spp.*
Ralstonia spp.	

Other BOI

Actinobacteria Phylum
Streptococcus sp.

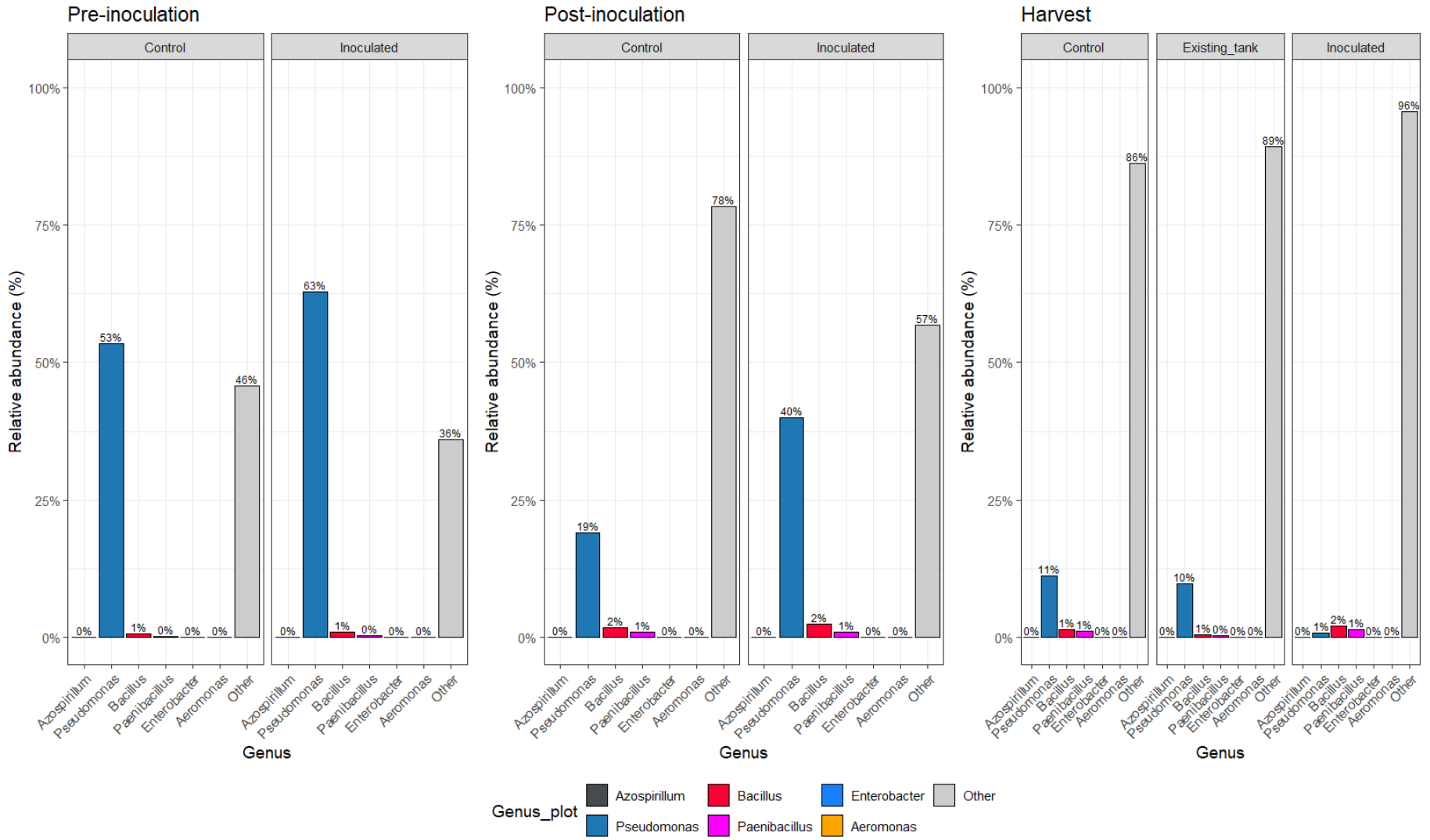
- These bacteria are of interest and are further highlighted in the downstream analysis

At Harvest – Pseudomonas drops to 1% in Inoculated tank

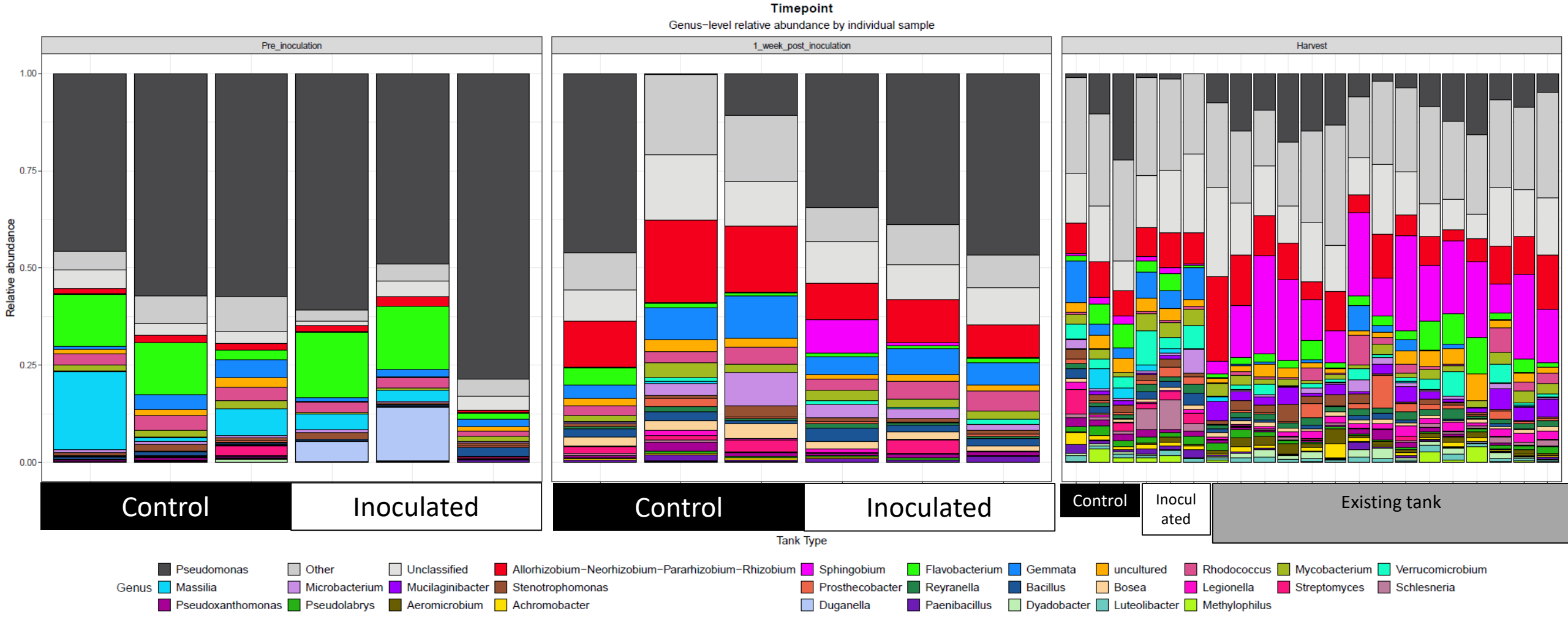
- ***Pseudomonas* relative abundance** is seen to **decrease over time** in **both the control** and **inoculated tank**

- ***Pseudomonas*** relative abundance in the Inoculated tank **drops to 1%**

- At **harvest inoculated tank** does show a **1% increase** in *Bacillus* relative abundance compared to the pre-inoculation



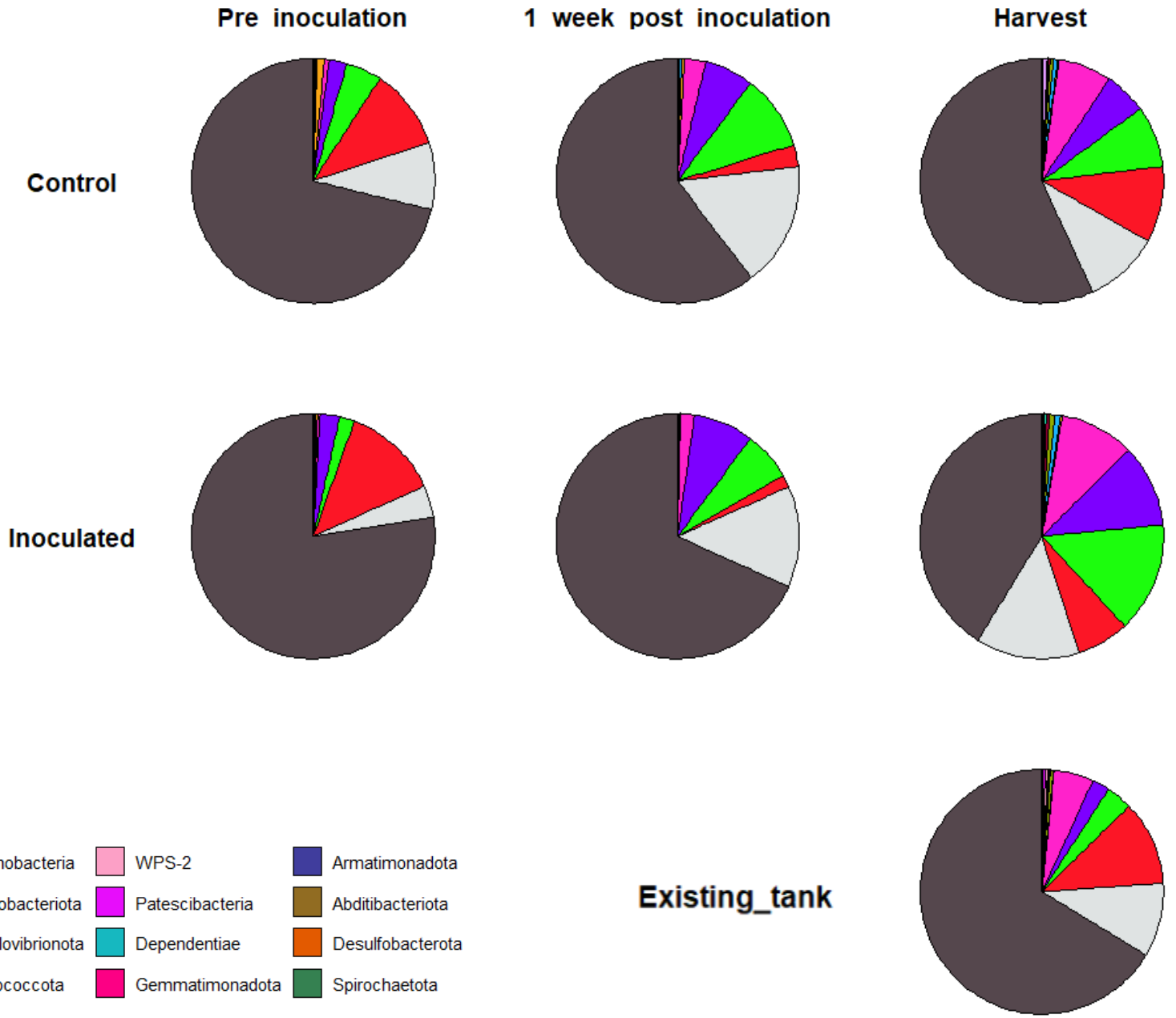
Relative abundance of top genus classifications



- *Pseudomonas* relative abundance drops at a higher rate in inoculated compared to control
- *Flavobacterium* relative abundance drops over time

At Harvest – Pseudomonas drops to 1% in Inoculated tank

- Inoculated vs control at harvest timepoint looks different
- Existing tank shows a different profile when compared to experimental tanks at harvest
- Low *Firmicutes* (potentially low ***Bacillus*** **genera** in existing tank)



Summary

1. **Control** vs **Inoculated does not seem** to show a statistical difference in alpha and beta diversity of sequence variants in all 3 timepoints

2. **Timepoints** and **Tank_Type (experimental vs existing)** seem to be most influential in bacterial community changes

3. ***Phylum*** and ***Genera*** **profile changes** are observed – further work is required to investigate patterns

Next steps

1. **Functional group** analysis
    I. Investigation of **functional bacterial groups**
        I. Nitrogen fixing bacteria, methane production etc.

2. **Investigate Lettuce Type** (Romaine vs Red Leaf vs Crispy Leaf vs Sweet Leaf) for any patterns or correlations.

3. **ITS is ongoing**, on track for sequencing.