

WGS SERIES: Whole Genome Sequencing in the Industry

**Metagenomics and
Beyond**

May 28, 2020



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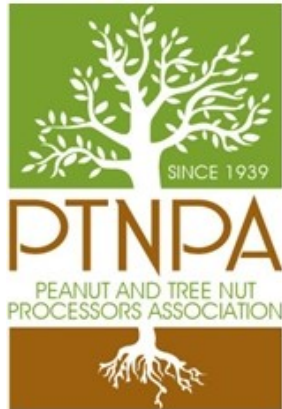
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Webinar Logistics

- Everyone is muted
- Questions will be addressed during the Q&A session at the end of the presentation
- The presentation is being recorded
- The recording/slides will be distributed following the presentation
- Adjourn (60 minutes)
- There will be 3 important survey questions at the conclusion of this webinar. Your response is appreciated

Speaker

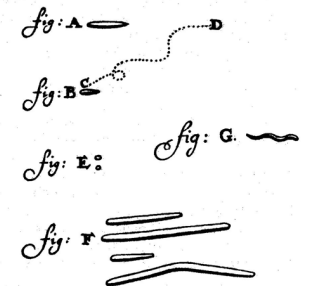


Dr. Pablo Carrion



Microbial Community Profiling of a Low Moisture Food Manufacturing Environment

PLATE XXIV



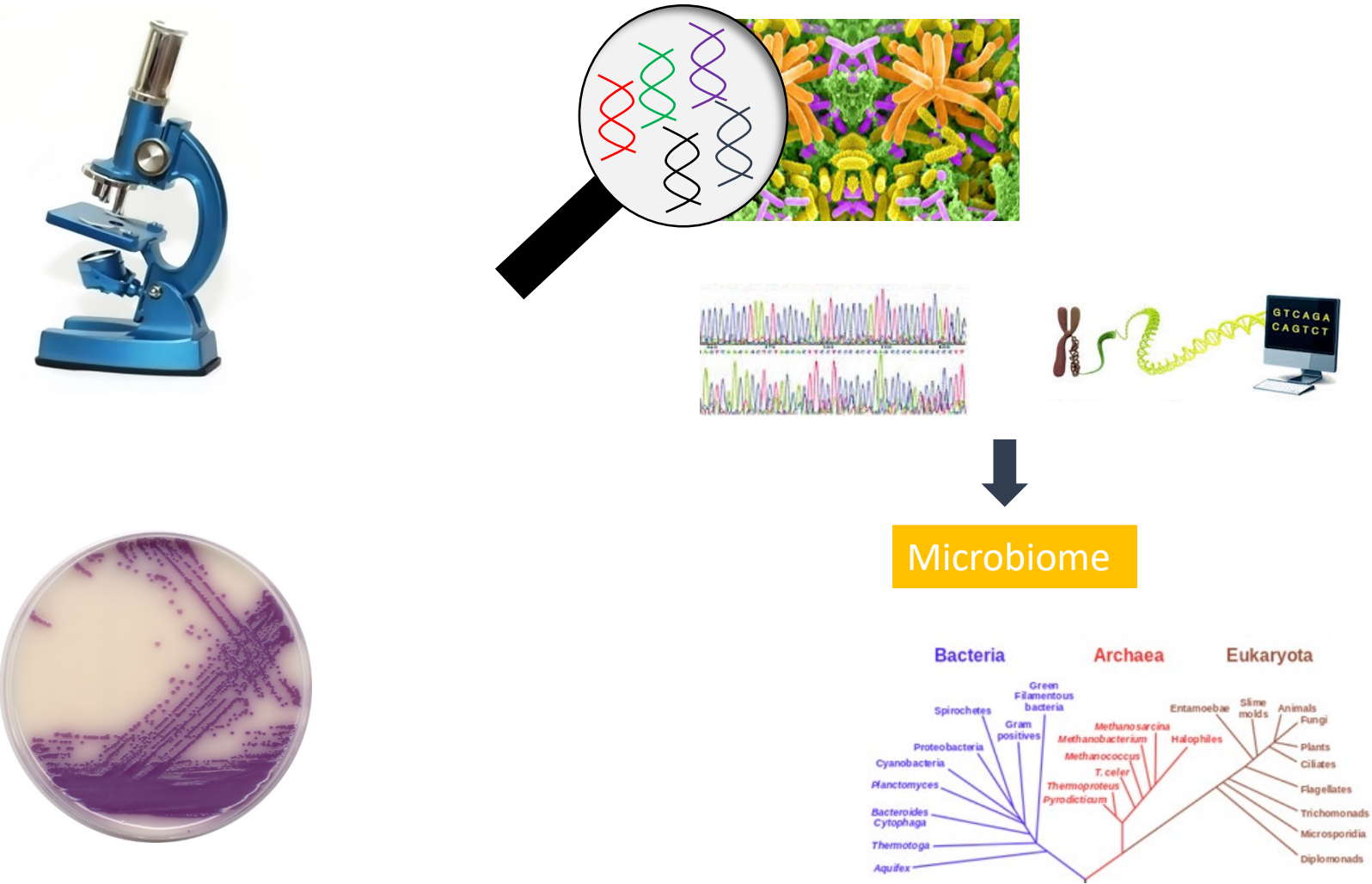
Need:

Low moisture foods ($a_w < 0.7$ or 16% moisture) are shelf stable foods. Manufacturing usually has an early CCP and much further processing. A hygienic environment is vital to avoid re-contamination.

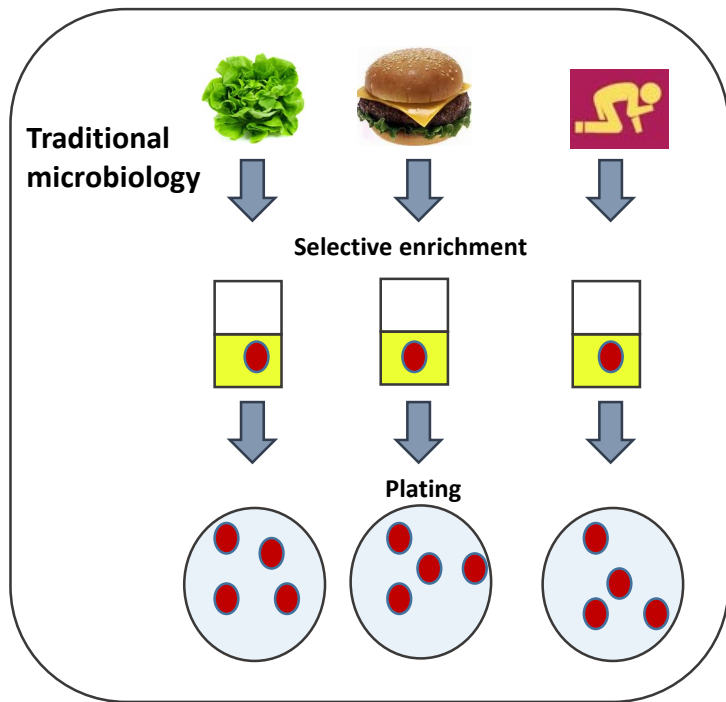
Research question:

Can food safety be improved by gaining deeper insight into the microbial community structure of food and/or food associated environment?

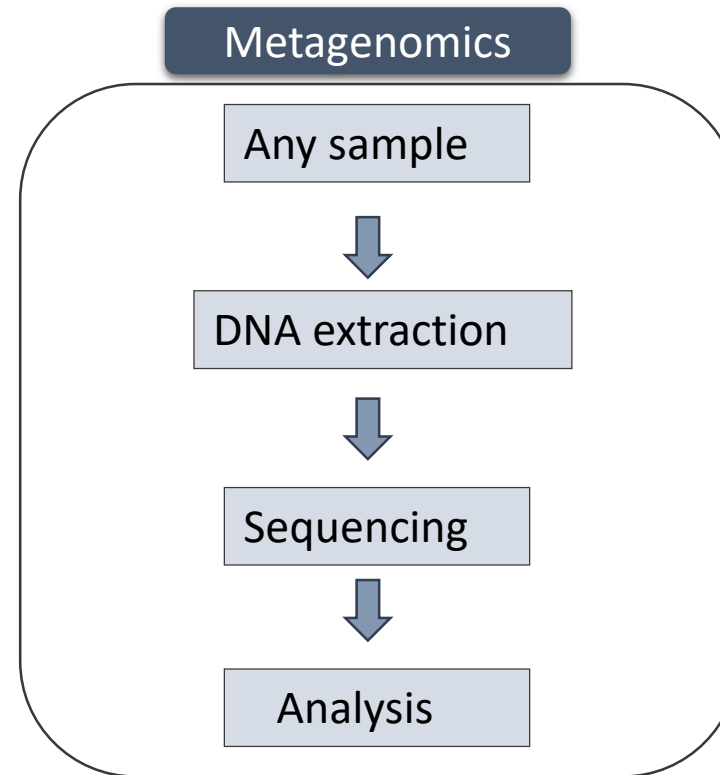
What is metagenomics ?



Single colony versus community



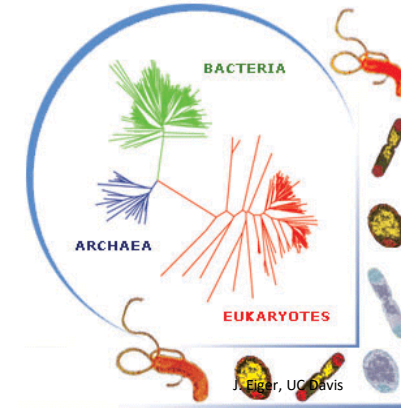
Targeted



Untargeted

Metagenomics applications/ approaches

Metagenetics/ metabarcoding – 16s & 18s rRNA gene

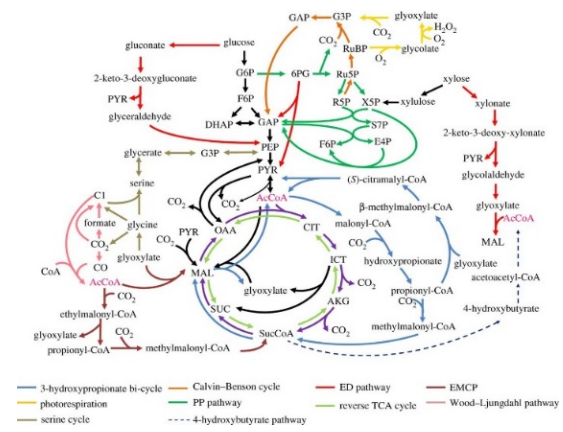


Taxonomic profiling

NGS (community DNA - metagenomics) «Shot Gun»



NGS (community RNA – metatranscriptomics)



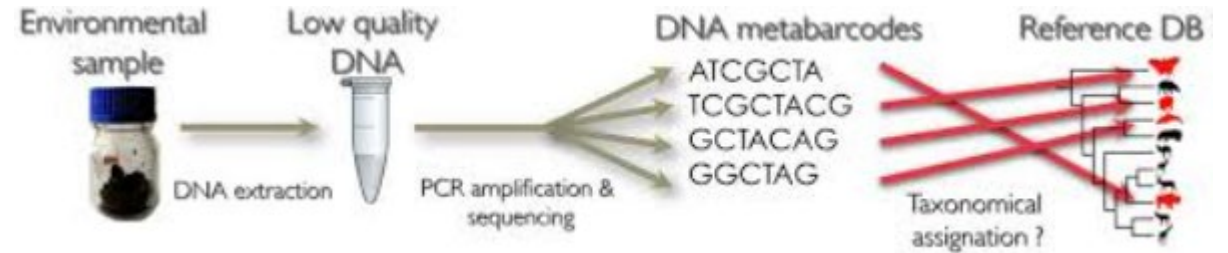
Functional capability

Project Outline

3 production environments
were sampled 3 times in a year



DNA was extracted and bacteria present identified
via 16S rDNA Metabarcoding technique



DNA isolation was performed using the PowerLyzer PowerSoil
DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA)
following the modified protocol suggested by the manufacturer
for low-biomass samples.

Paired samples were also cultured for EB and *Salmonella*

Metadata – information collected with each sample

We then analyzed the data by comparing Biomes at each Metadata Category

Meta data groupings	Meta data categories per grouping			
Factory PMP Priority Rating	Line	P1	P2	P3
Hygienic Zone (factory designation)	Basic	Medium		
Cleaning Type (factory designation)	Dry	Wet	Controlled wet	
Visual Area Cleanliness at time of sample collection	Dry	Wet	Clean	Fines*
Climate	Hot	Cold	High Relative Humidity**	Low Relative Humidity**

* This category was latter combined with “dusty”. ** These categories were not used as part of the Meta data due to subjectivity of the observation.

Questions that were asked?

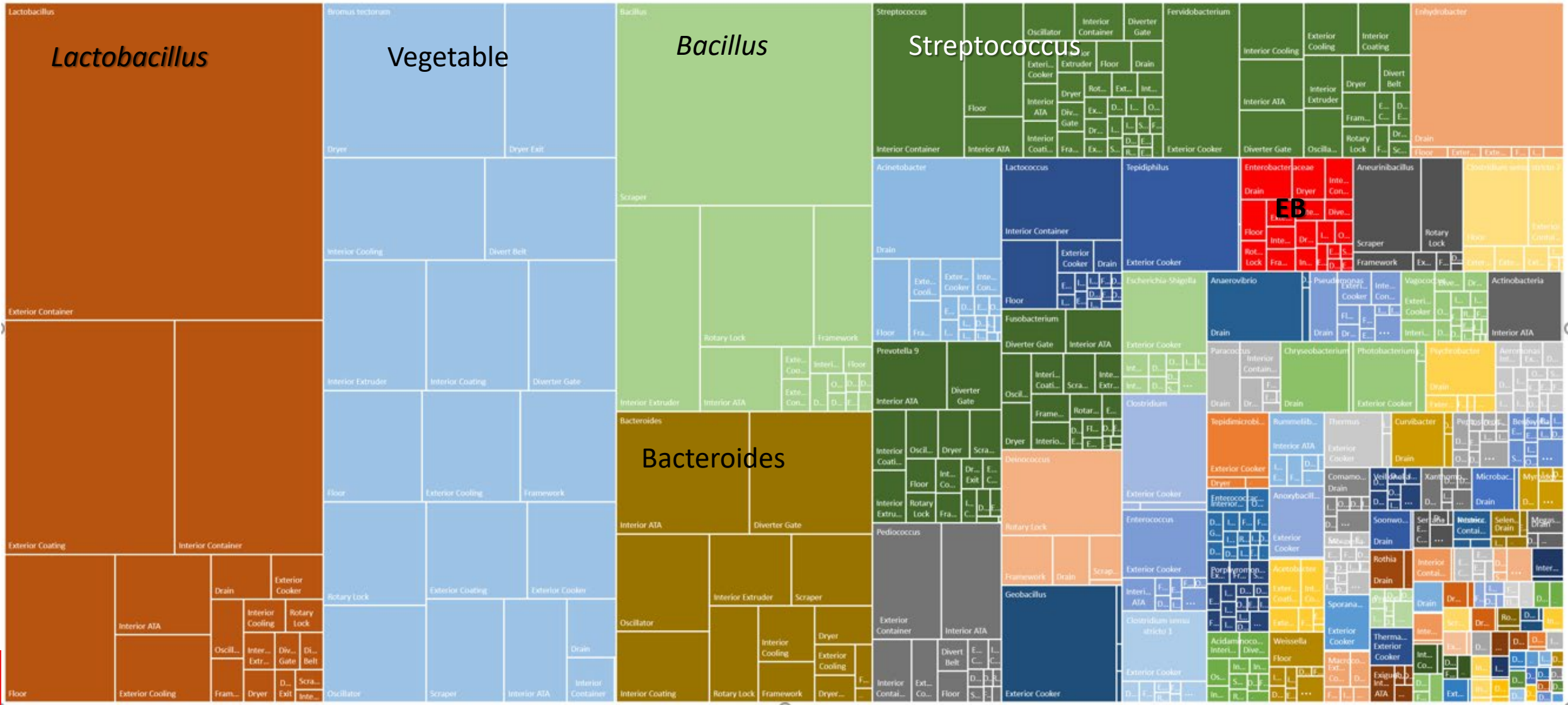
- 1) Is the microbiome the same across sites?
- 2) Does the microbiome change with season?
- 3) Does internal zoning change the microbiome?
- 4) Does cleaning method alter the microbiome?
- 5) Can *risk* be predicted by the resident flora?

Microbial Diversity

The Factory Biome

Family versus location

■ Bifidobacterium
 ■ Corynebacteriaceae
 ■ Gordonia
 ■ Rhodococcus
 ■ Microbacterium
 ■ Kocuria
 ■ Nesterenkonia
 ■ Rothia
 ■ Actinobacteria
 ■ Bacteroides
 ■ Porphyromonas
 ■ Prevotella 9
 ■ uncultured bacterium
 ■ Lunatimonas
 ■ Mongolitalea
 ■ Negadavieja
 ■ Bergiella
 ■ Chryseobacterium
 ■ Cloacibacterium
 ■ Empedobacter



Alpha Diversity

The different genetic lineages in a sample by accounting for the total number different species.

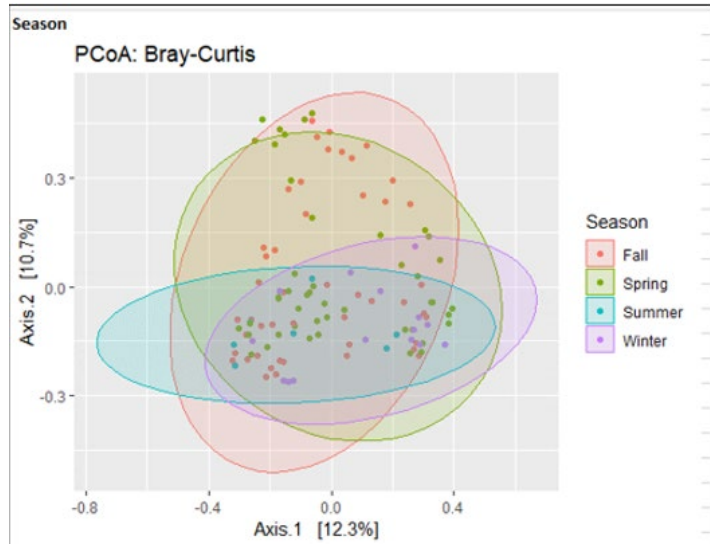
In other words, how many discrete genetic families exist in a sample?



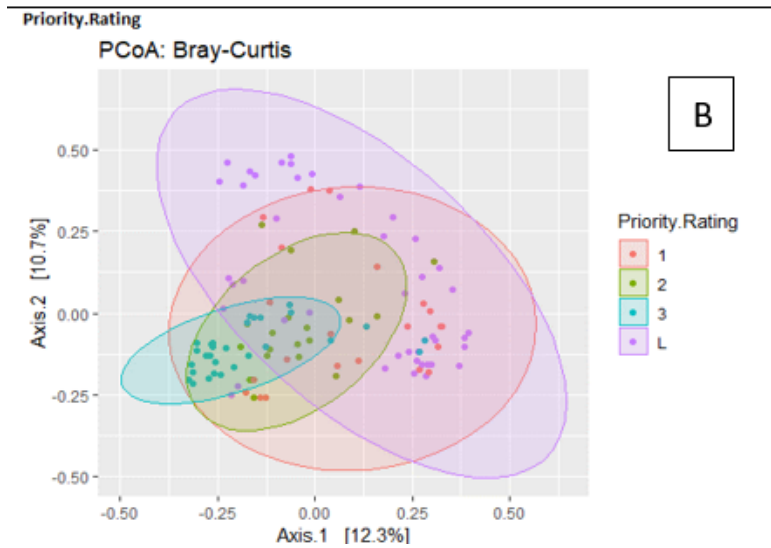
Beta Diversity:

How similar microbiomes are by comparing shared taxa among samples versus missing taxa among these same samples, i.e. equality in species abundance

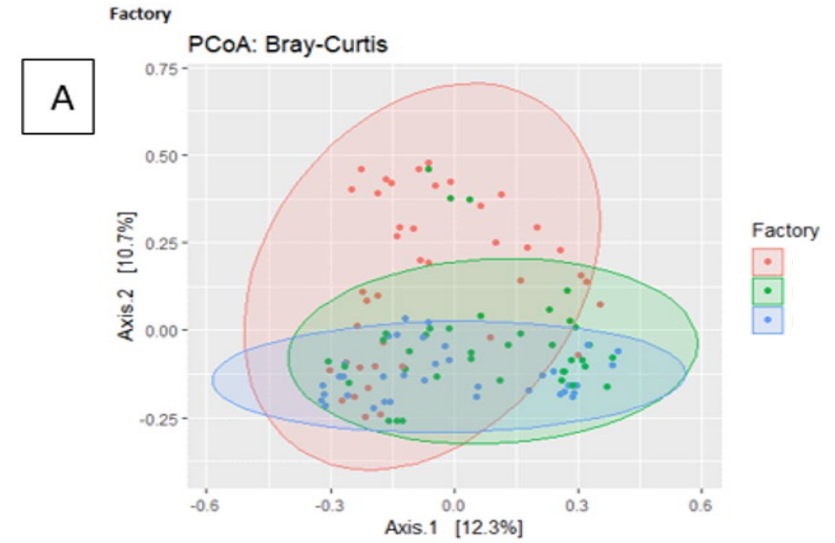
season



priority area



factory

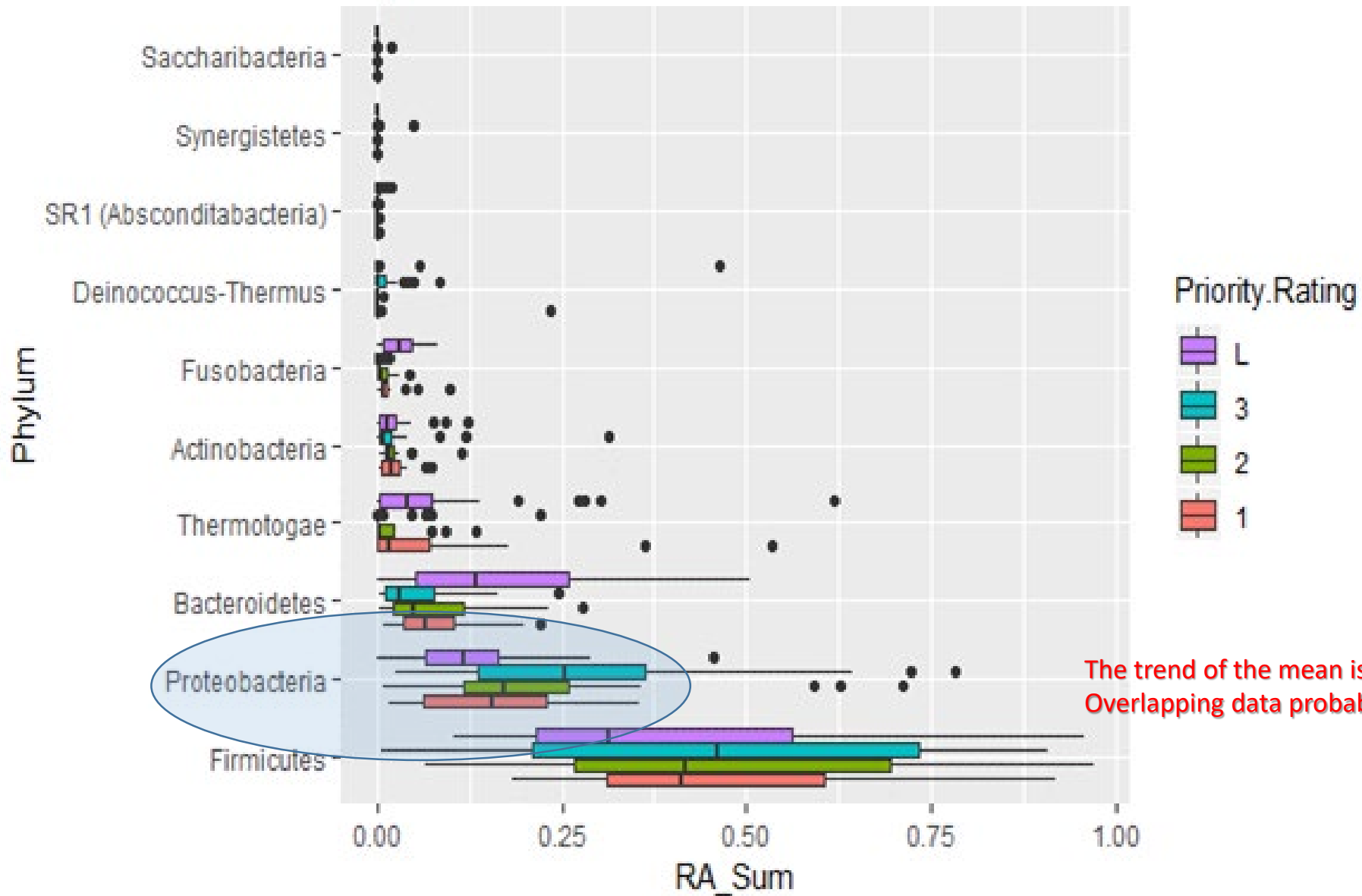


LEfSe Analysis between Factories

LEfSe (Linear discriminant analysis Effect Size) determines the organisms most likely to explain differences between factory biomes

Family	1	2	3	Gram	Biology
	Order of Median DNA concentration from high (1) to low (9)				
<i>Bacillaceae</i>	6			+	Environmental, sporulated
<i>Bacteriodales</i>	1*			-	Enteric mammalian anaerobe
<i>Enterococcaceae</i>	7	8		+	Enteric, lactic acid producers
<i>Enterobacteriaceae</i>	8	2	2*	-	Enteric , Indicator for risk of Salmonella
<i>Fervidobacteriacea</i>	4	1*		-	Thermophilic anaerobic bacteria
<i>Flavobacteraceae (Bacteriodetes)</i>			3	-	Environmental bacteria
<i>Fusobacteriaceae</i>	5	7	6	-	Enteric mammalian anaerobe
<i>Lactobacillaceae</i>	9	3*	1*	+	Environmental, lactic acid producers
<i>Moraxellaceae</i>		5	5	-	Commensals of mammals occur in water or soil
<i>Prevotellaceae (Bacteriodales)</i>	2			-	Rumen and gut of cattle and sheep
<i>Porfyromonaceae (Bacteriodales)</i>		6	4	-	Oral anaerobic flora of mammals
<i>Streptococcaceae</i>	3	4	7	+	Fecal, lactic acid producers

Top 20 Bacteria



Number of Different Genera (% DNA) on Line, Near Line, Near-Near Line, & General Surfaces

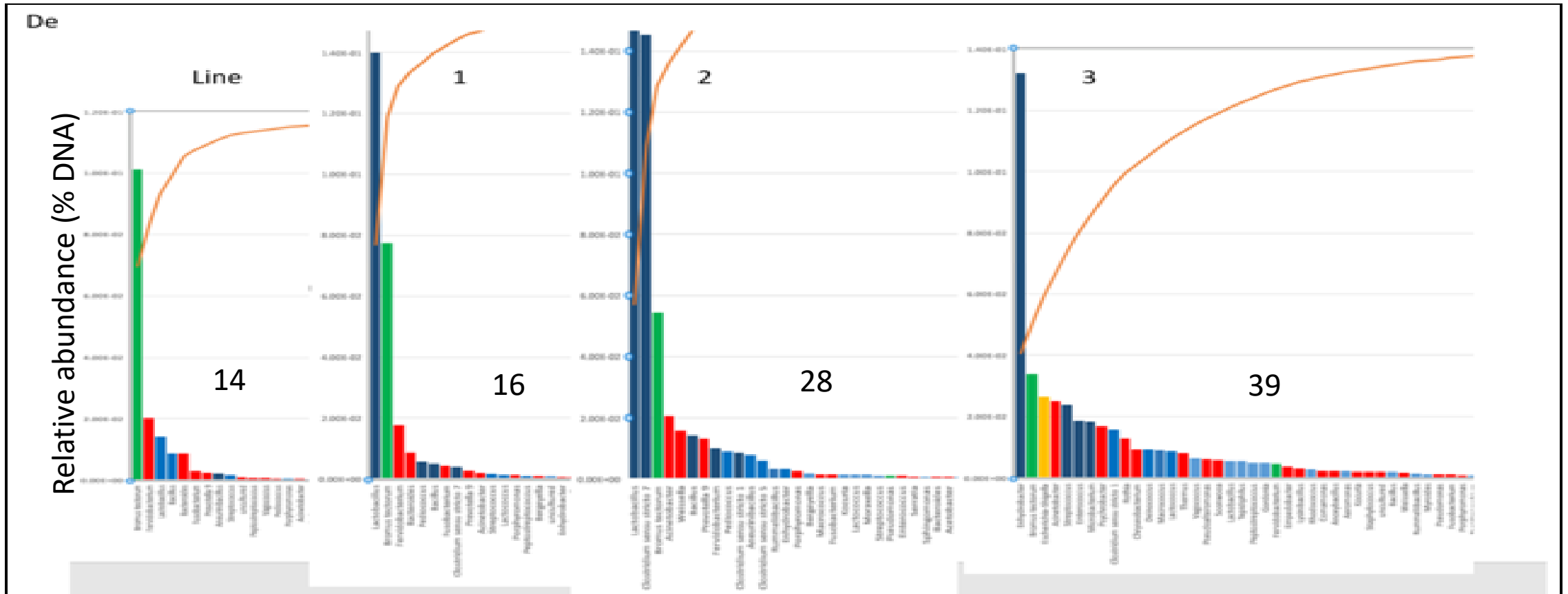
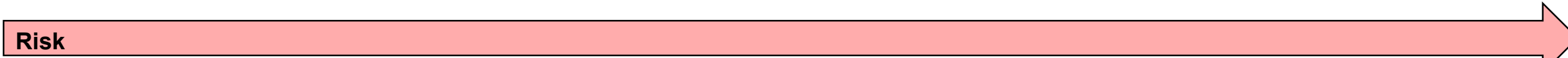


Figure 11. *16S rDNA Biome of product contact surfaces at a factory showing a progressive increase in the number of microorganisms with high relative abundance as we move away from the Line to Zones P1, P2 and P3. Green bar denotes plant DNA, Red bar denotes gram negative bacteria, and Blue bar denotes Gram positive bacteria and Yellow line denote Enterobacteriaceae. Y scale is % DNA and has been adjusted for equivalence between graphs.*

LEfSe: Differentiators between PMP Priority Areas

Risk 			
Line	M1	M2	M3
Bacteroidales	Pediococcus	Acinetobacter	Enhydrobacter
Bacillus	Lactobacillus	Aquaspirillum	Moraxellaceae
	Achromobacter	Rummeliibacillus	Gammaproteobacteria
	Alcaligenes	Lactobacillus	Pseudomonadales
		Leuconostocaceae	Oxalobacteraceae
			Streptococcus
			Lactococcus

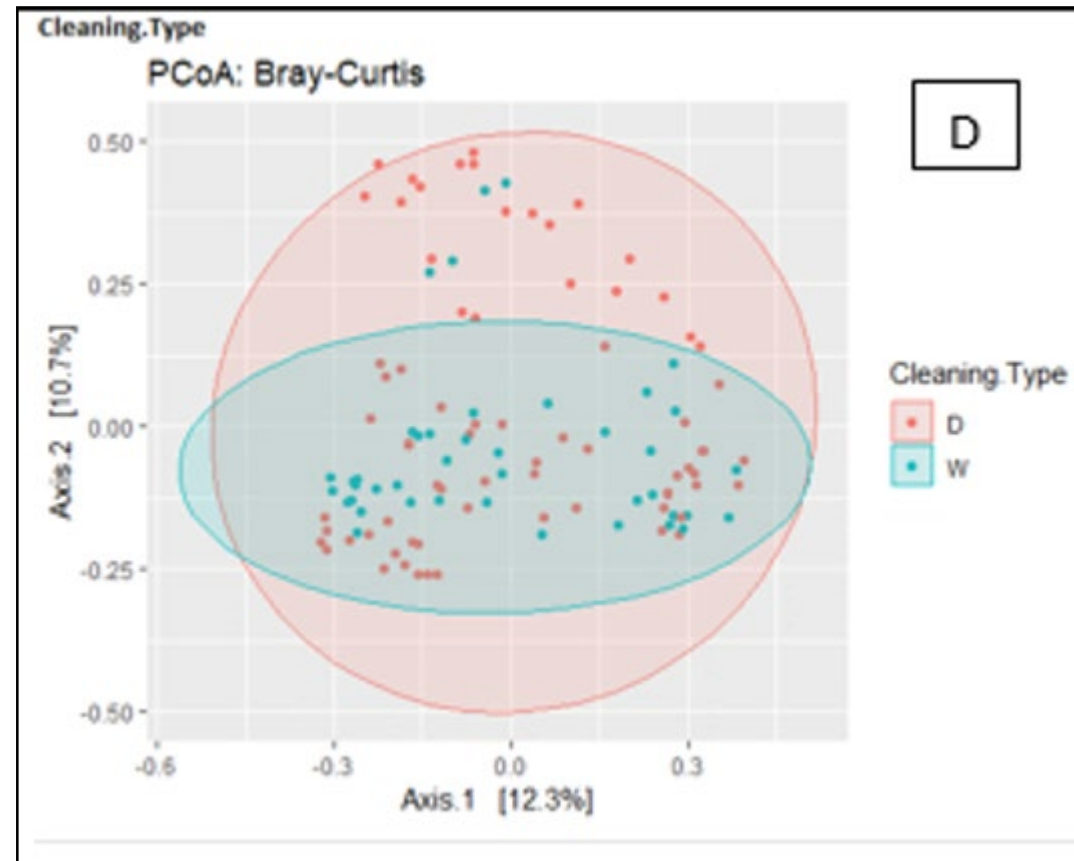
Conclusion: Diversity

- Alpha diversity can discriminate between samples of factory hygienic zoning
- Beta Diversity less so...
- Bacteroidetes, Proteobacteria and Firmicutes make up the vast majority of surface DNA
- The number of microbial lineages is higher in surfaces distant to the Line

Assessing Surface Hygiene

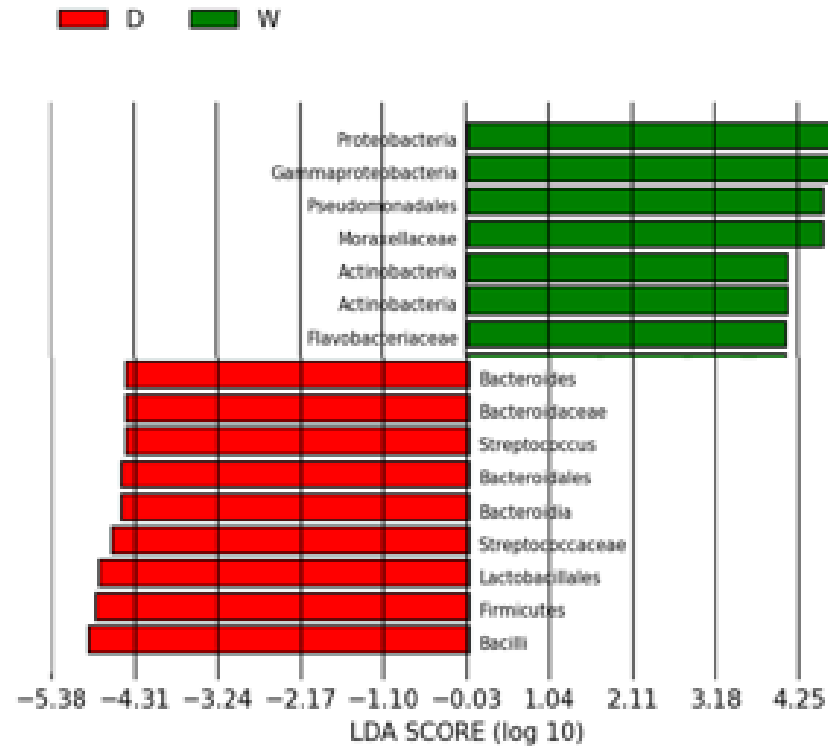
Dry Cleaned vs Wet Cleaned Surfaces

Beta Diversity



Major Differences are Seen at Phylum Level Between Dry Cleaned and Wet Cleaned Equipment Surfaces

Figure 5 LEfSe analysis of dry clean versus wet cleaned surface biomes. Red color represent data from dry surfaces. Green color represents data from wet surfaces. The category wet encompasses data from a surface that is wet often, sometimes and rarely.

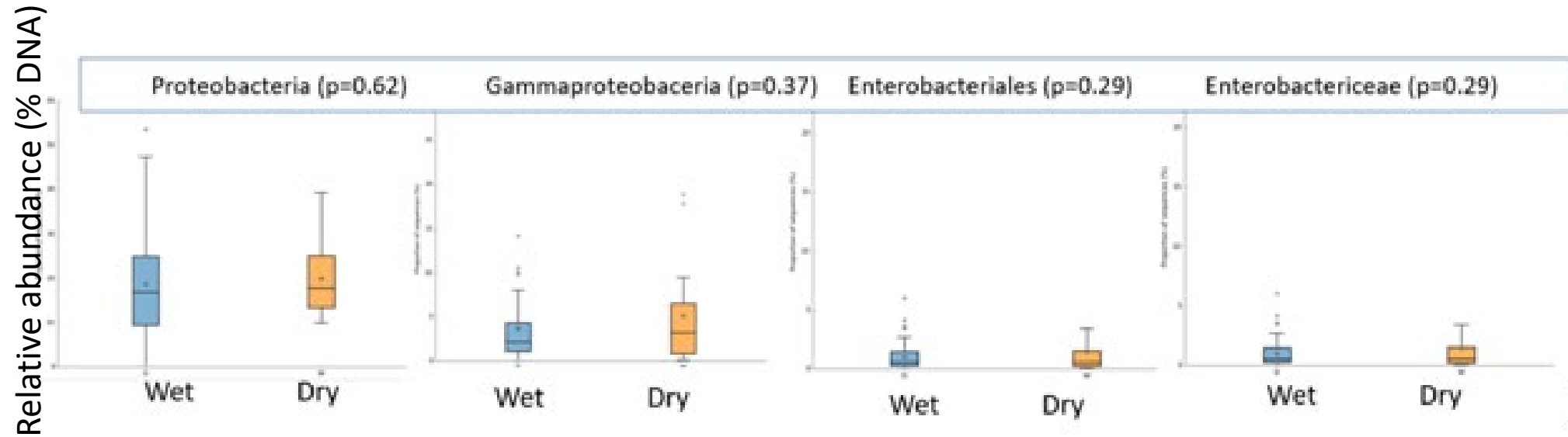


Gamma-proteobacteria

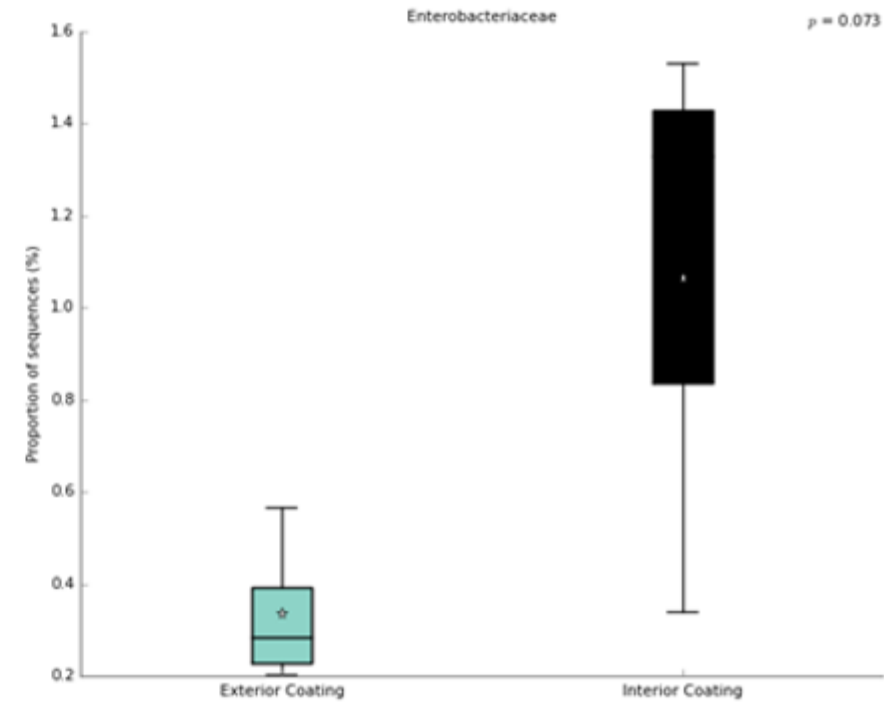
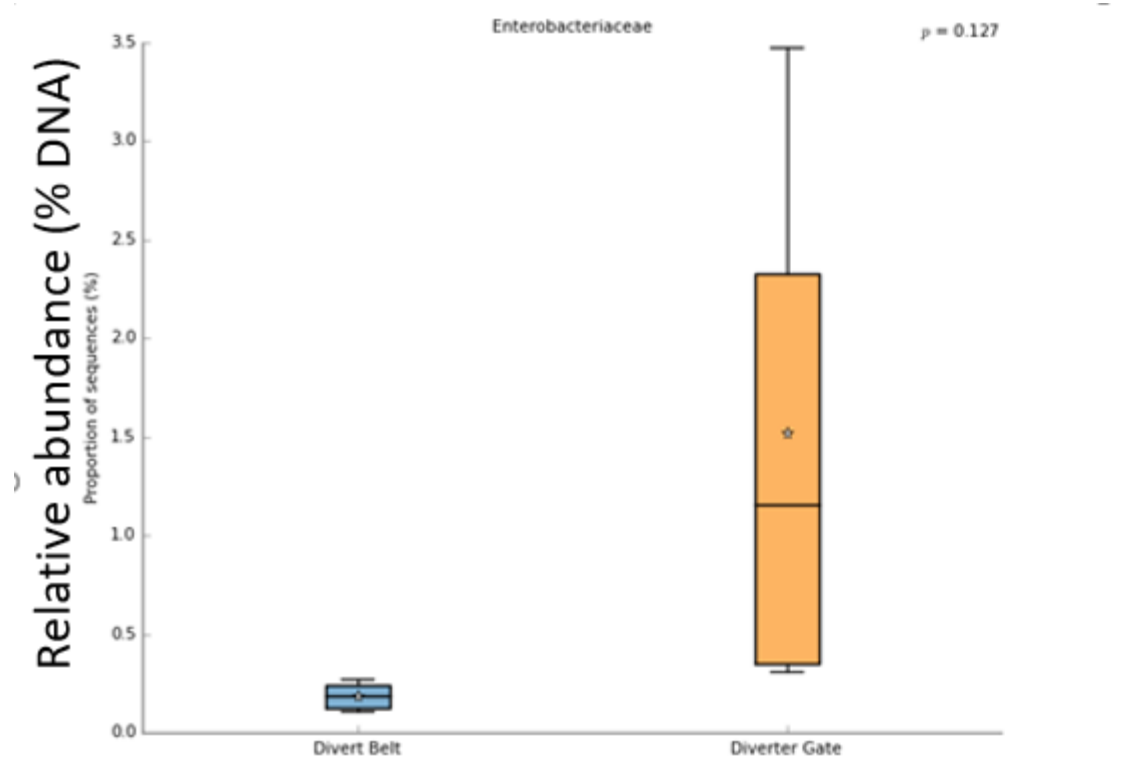
Firmicutes & Bacteroidetes

Enterobacteriaceae by Cleaning Type

Presence or absence of DNA does not discriminate between dry and wet surfaces



Differences between dry and moist surfaces



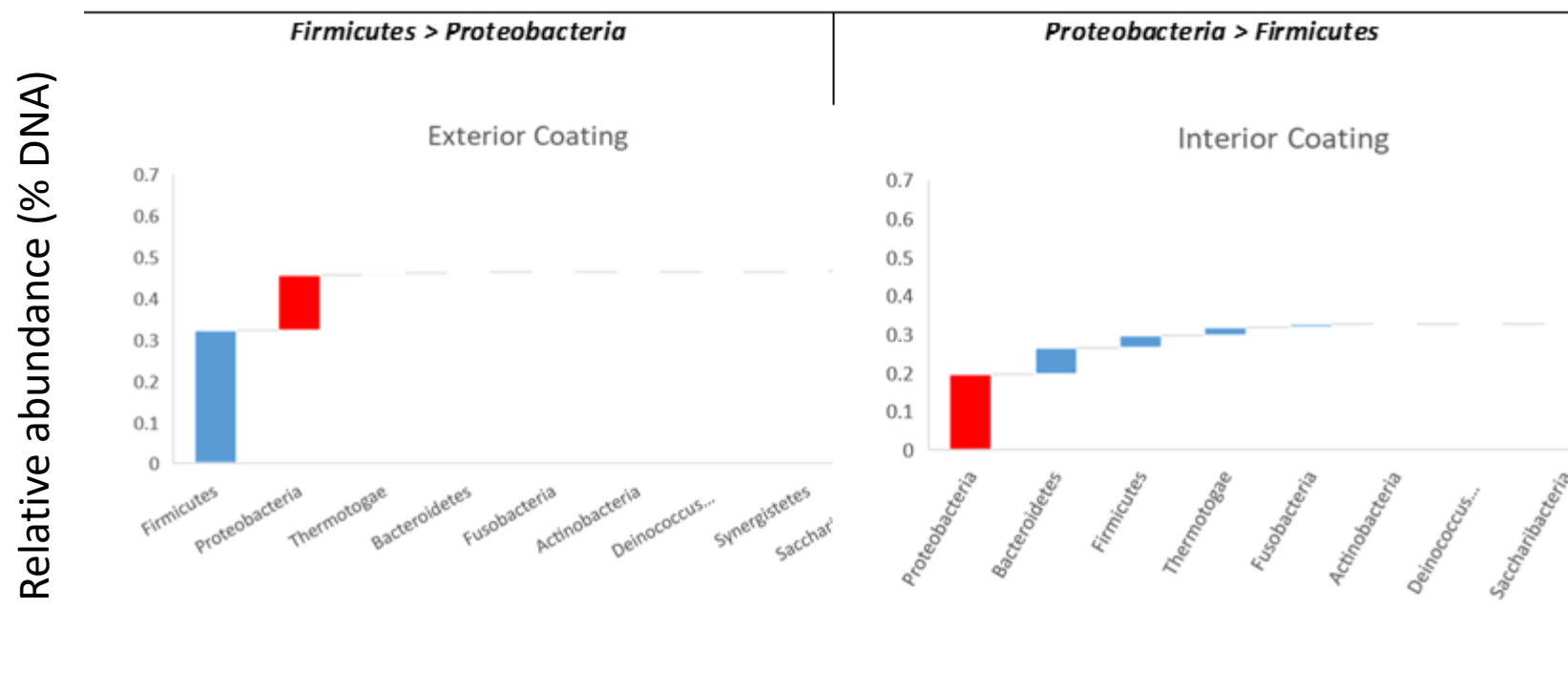
Conclusion: Cleaning Methods

The flora from wet and dry cleaned surfaces is similar. The proportion of bacterial groups varies:

- The dry cleaned equipment show a preponderance of Gram positive bacteria i.e. *Bacillus* & *Lactobacillus*.
- Wet Cleaned equipment show a wide variety of gram negative bacteria including EB, Moraxellaceae (e.g. Moraxella and Acinetobacter).
- EB is present in both types of surfaces with wet cleaned surfaces having proportionally higher numbers.

Data to Assess Hygiene Status

Phylum Level Analysis



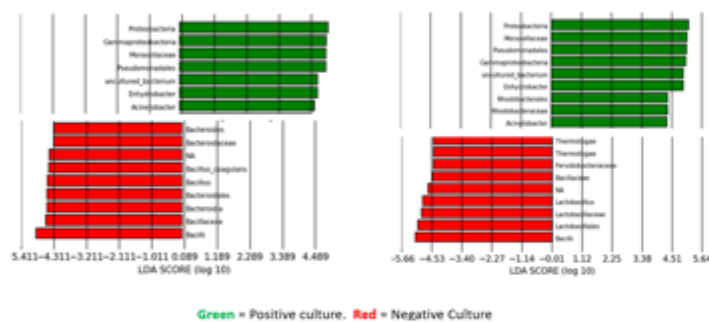
Conclusion: Hygiene evaluation

- When comparing the flora of surfaces at a high taxonomic level, low risk surfaces will show a higher prevalence of Firmicutes versus Proteobacteria. This pattern is consistent with moisture availability in the area.
- This high level pattern can be used to identify areas of increased risk and determine management approaches to reduce the risk.
- This assessment can be done to verify the effectiveness of hygienic design.

Microbiome of Monitoring Swabs

LEfSe: Analysis of positive and negative swabs

PCA: Analysis of *Enterobacteriaceae* and *Salmonella* positive and negative swabs



EB was not a significant differentiator
 Prevalence of Proteobacteria in
 EB+ samples
 Prevalence of Bacillus/Lactobacillus
 EB – samples

EB + (n=93)	EB – (n=22)
Proteobacteria	Bacilli
Gamma-Proteobacteria	Bacteriodales
Moraxecellaceae	<i>Bacillus coagulans</i>
Pseudomonades	
Enhydrobacter	
Acinetobacter	

Are we detecting mixed species biofilms?

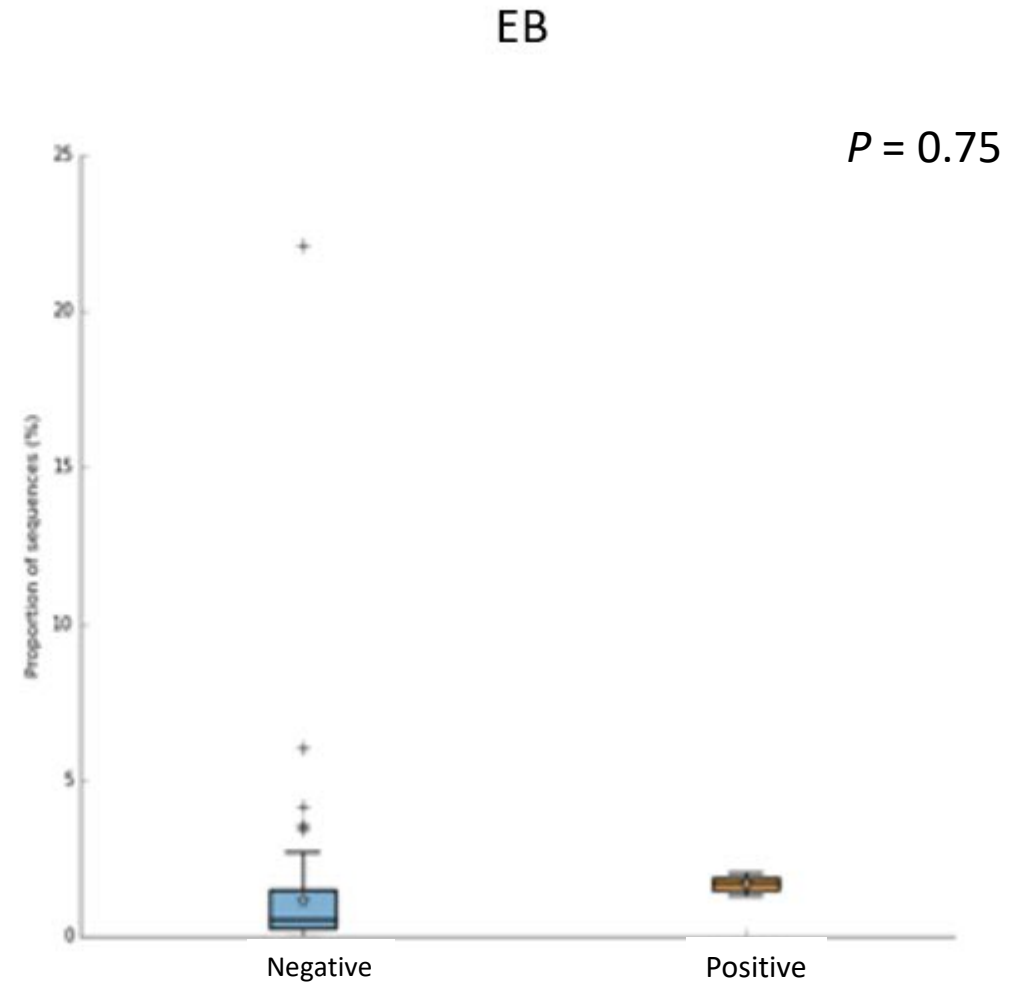
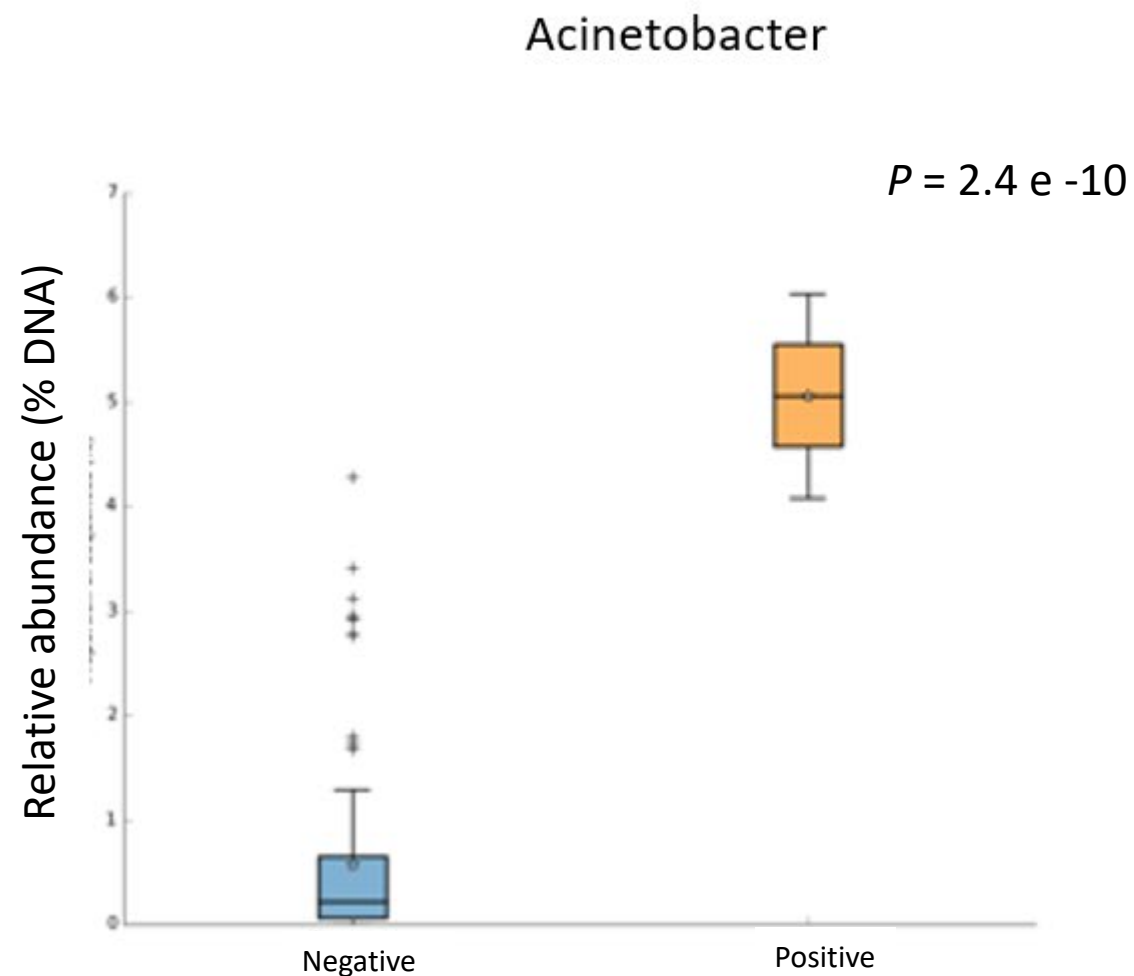
Pareto analysis of positive and negative swabs



EB +	EB -
Moraxellaceae	Lactobacillaceae
Enterobacteriaceae	Enterobacteriaceae
Lactobacillaceae	Streptococcaceae
Enterococcaceae	Porfyromonaceae*
Streptococcaceae	Moraxecellaceae
Flavobacteraceae*	Enterococcaceae
Pseudomonadaceae	Fervidobactereaceae**
Porfyromonaceae*	Flavobacteaceae*
Micrococcaceae	Fusobacteriaceae
Staphylococcaceae	Prevotellaceae
Comamonadaceae	Peptpstreptococcaceae
Planococcaceae	Bacillaceae
Aeromonadaceae	Microbacteraceae

Is there a better predictor of risk?

EB Culture Positive and Negative samples



Conclusion: Determining Risk in Low Moisture Food Manufacturing Environments

- EB DNA is widely distributed, even in EB negative samples.
- EB + samples have a wide variety of Gram negative bacteria whereas the EB- samples have few lineages and these are mostly Gram positive.
- *Moraxellaceae* (e.g. *Moraxella* and *Acinetobacter*) DNA is better at discriminating between samples with viable and non-viable EB than EB DNA and may point to a better indicator Microorganism strategy, research pending.

Next Steps

- Compare DNA extraction methods for efficiency
- Compare Metabarcoding with Shotgun
- Determine whether *Moraxcellaceae* is a better indicator MO than EB
- Determine whether Metabarcoding results can be continuously modeled to identify areas where hygiene improvement is required without detecting pathogens

**Thank
You**



Q & A



If your question wasn't answered...

Please contact Scott Nichols at snichols@wga.com or one of the trade organization representatives and we would be happy to respond.

Thank you.

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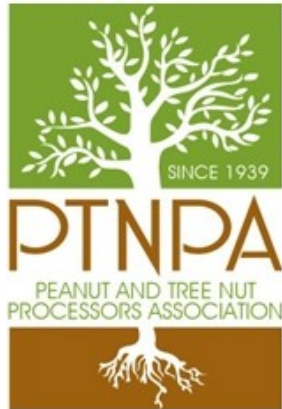
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