

Microbiology of the EDEN ISS Greenhouse and crops during the 2021 overwintering season.

Mary Hummerick¹, Jennifer Gooden¹, Lashelle Spencer¹, Jess Bunchek¹, Jacob Torres¹, Christina Khodadad¹, Cory Sporn¹, Daniel Schubert², Paul Zabel², Vincent Vrakking², Markus Dorn², Ralph Fritsche³, Raymond Wheeler³

¹AMENTUM, SURA-LASSO, Kennedy Space Center, FL

²DLR, German Aerospace Center

³NASA, UB-A, Kennedy Space Center, FL

EDEN ISS



- EDEN ISS Greenhouse: A confined test facility at the Neumayer III station in Antarctica for testing methods for space crop production.
- A unique opportunity to evaluate the microbiology of plants grown in an ecologically isolated environment.
- Plants are cultivated in an aeroponic system in a potentially low microbial diversity environment.
- Like a space craft, microbes are introduced by the crew, water, air and other environmental factors.

Microbial samples

- Edible plant parts: Outredgeous lettuce (25), Waldmans green lettuce (15), Mizuna mustard (15), Amara mustard (5), Toscano Kale (5), Basil Dolly (5), Chives (3), Oregano, (3), Parsley Laura (10), Peppers: Chimayo (4), Espanola (4), Mimi Red (4), Red Skin (4), Joy Red tomato (5), Red Robin tomato (10), Radish leaves (5) and Radish (5).
- Environmental samples: Surface swabs and nutrient solution. Weekly for 10 weeks then monthly through 11th month.
- Root samples of the plants.

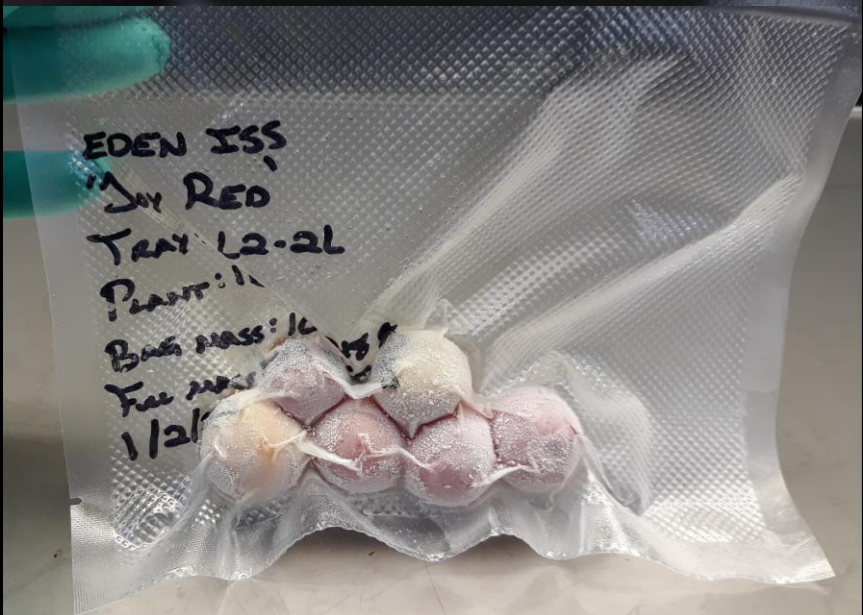
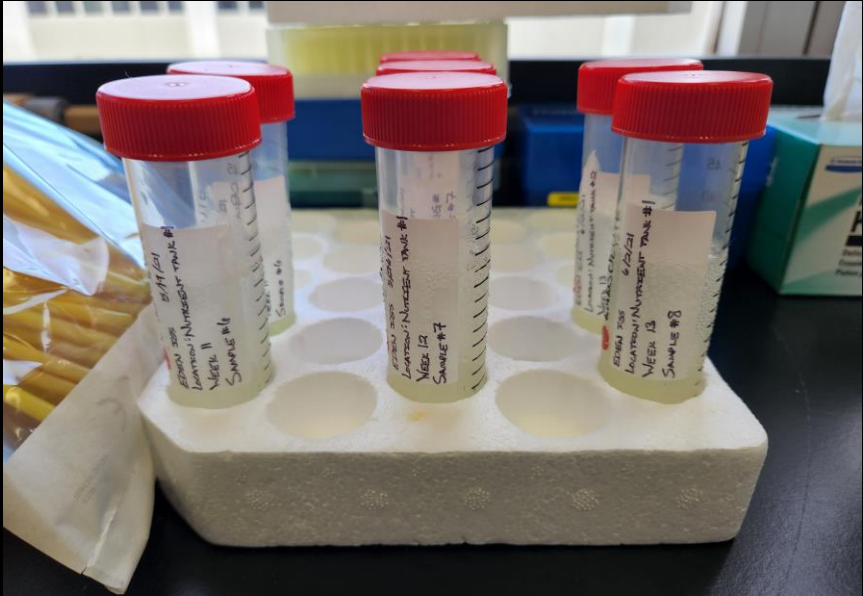
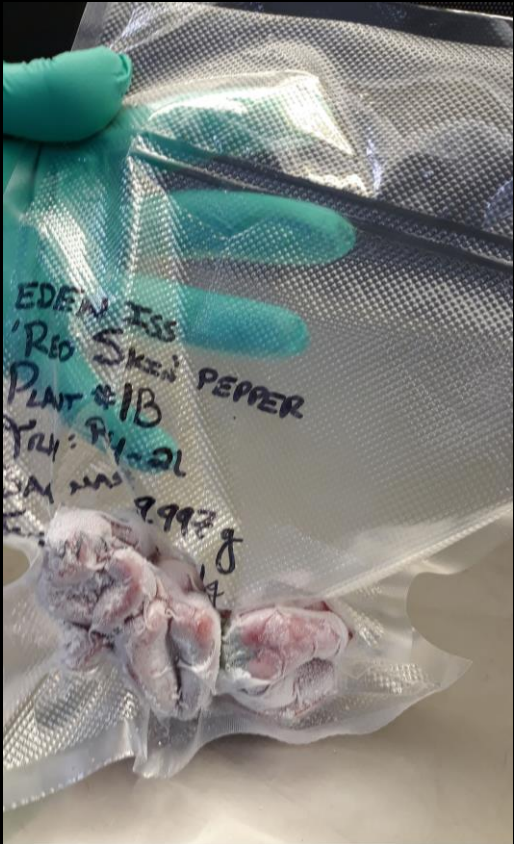


		Rack L1		Rack L2		Rack L3		Rack L4		
		L	R	L	R	L	R	L	R	
Left side	Level 4	Tomato – Red Robin 4P, 4M	Tomato – Red Robin 4P, 4M	Tomato – PAT orange 4P, 4M	Tomato – PAT orange 4P, 4M	Rosie 6P, 2M	Parsley – Laura 20P, 4M	Kale – Toscano 12P, 4M	Lettuce – Waldmann’s Green 6P, 2M	4
				Tomato – PAT orange 4P, 4M	Tomato – PAT orange 4P, 4M	Mizuna 12P, 4M	Rucola 36P, 4M	Lettuce – Othilie 12P, 4M	Nursery	3
	Level 2	Cauliflower – Hybrid Baby 5P, 4M	Broccoli – both varieties 5P, 4M	Tomato – Joy Red 4P, 4M	Tomato – Joy Red 4P, 4M	Swiss Chard – Bright lights 12P, 4M	Frizzy Lizzy 20P, 4M	Basil – Dolly 20P, 4M	Basil – Dolly 20P, 4M	2
						Radish – Raxe 36P, 4M	Mint 12P, 4M	Lettuce – Outredgeous 6P, 2M	Lettuce – Expertise 6P, 2M	1

		Rack R1		Rack R2		Rack R3		Rack R4		
		R	L	R	L	R	L	R	L	
Right side	Level 4	NASA plant cultivation system						Pepper – Mimi Red 4P, 4M	Pepper – Española 2P, 4M	4
	Level 2	Kohlrabi – Korist 5P, 4M	Kohlrabi – Korist 5P, 4M	Tomato – Amoroso 2P, 4M	Tomato – Amoroso 2P, 4M	Cucumber – Picowell 2P, 6M	Cucumber – Picowell 2P, 6M	Pepper – Chimayo 2P, 4M	Pepper – Redskin 2P, 4M	2

YES!! Samples are here. Cleared customs and still frozen!

Sample receipt. May 2022

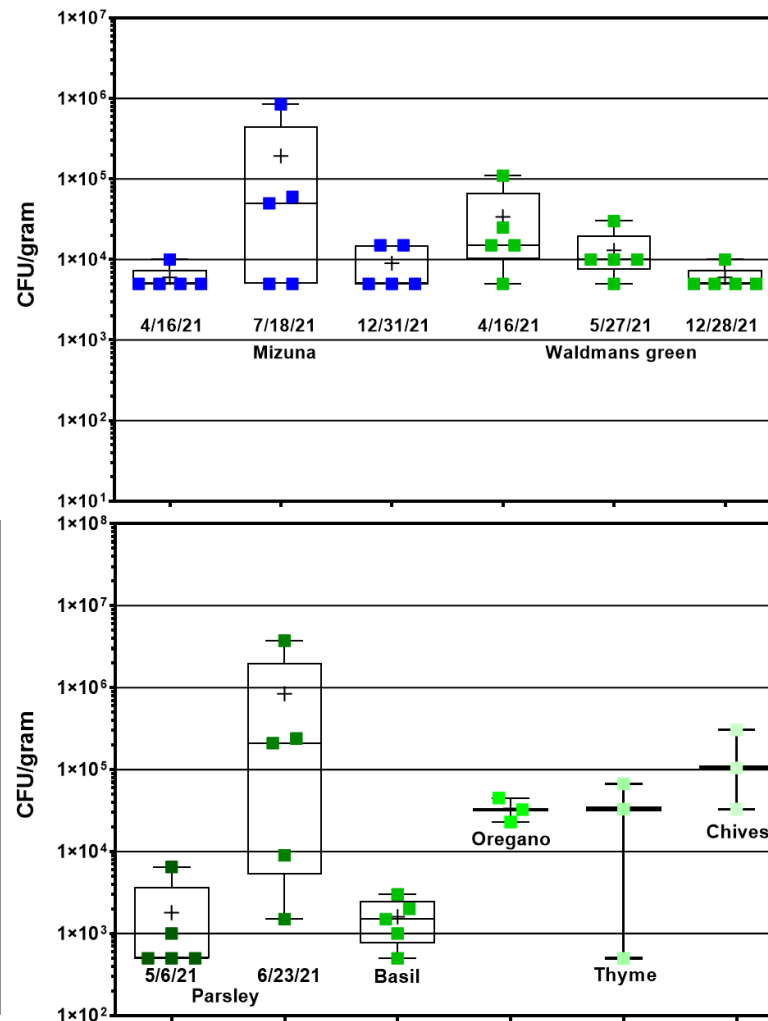
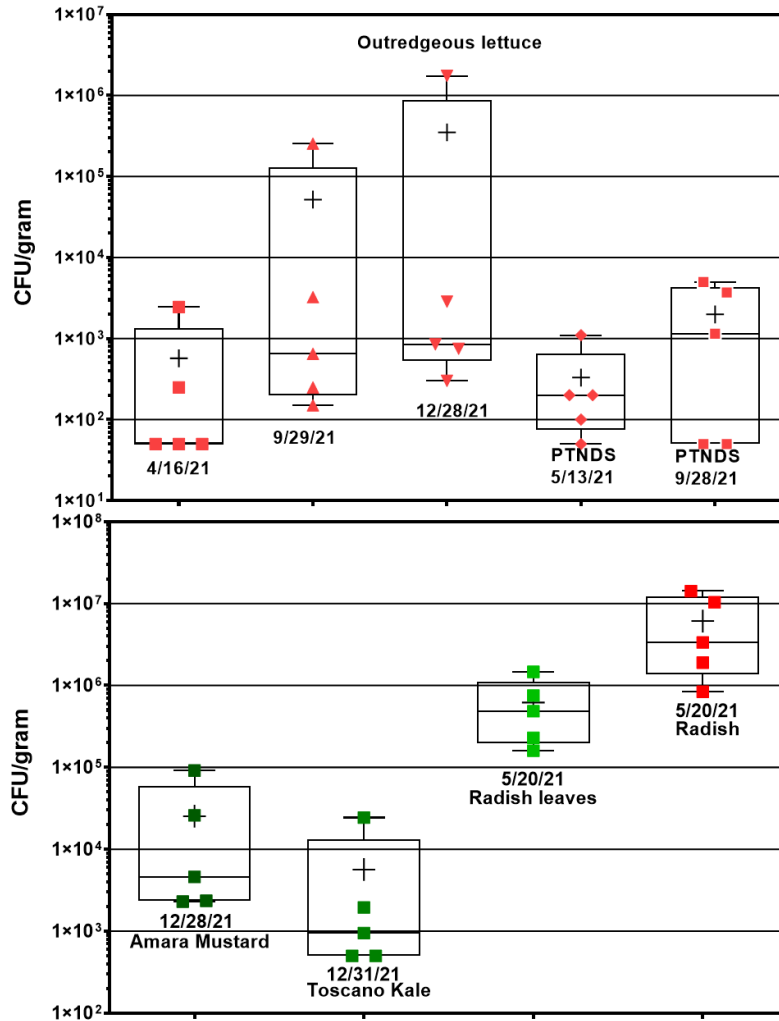




Microbiological analysis methods

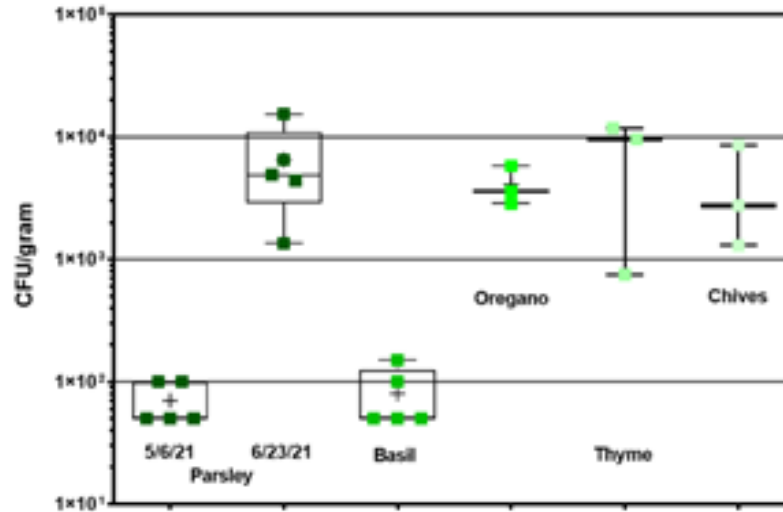
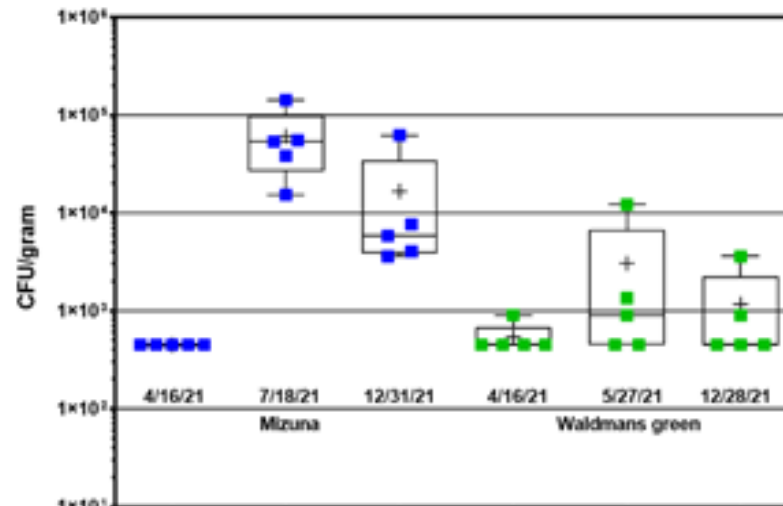
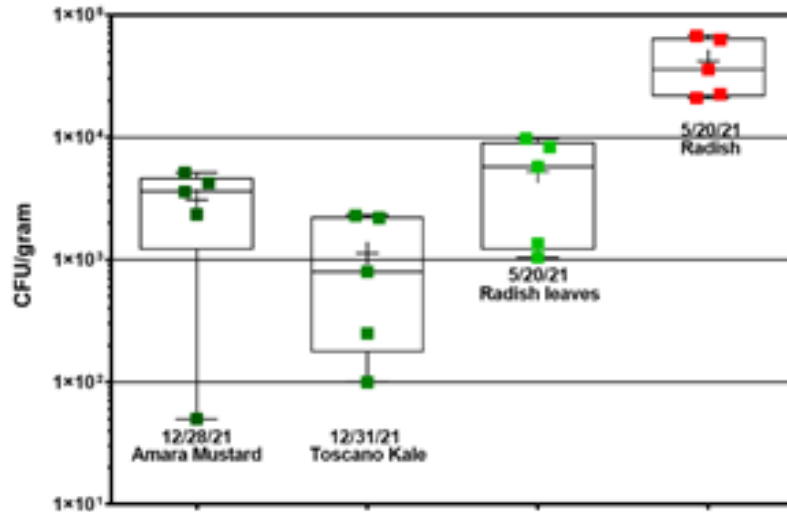
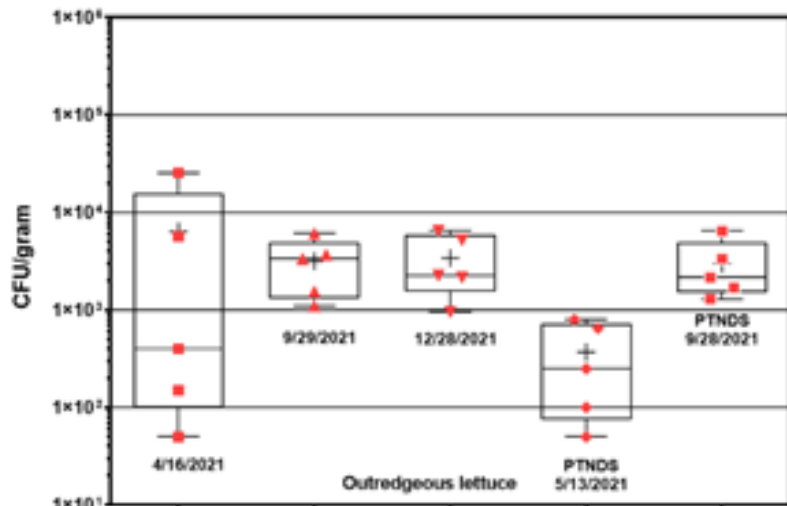
- Total Culturable Microorganisms
 - Aerobic Plate Counts (APC)/Bacteria
 - Total Yeast and Mold Count
- Specific pathogens
 - Generic *E. coli*/Coliforms (Petriefilm)
 - *Salmonella* sp. (enrichment/selective media)
 - *Staphylococcus aureus* (Petriefilm)
- Isolate Identification
 - Biolog MicroID system
 - MicroSeq fungal and bacteria identification

Bacterial counts



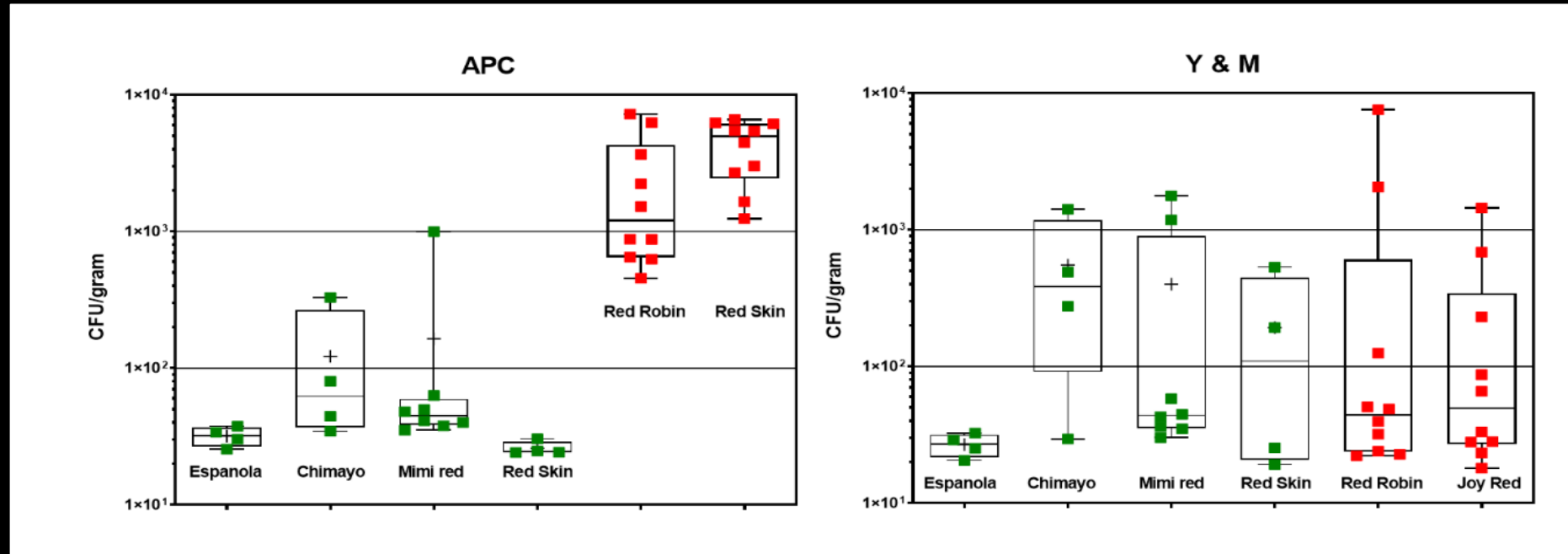
- Bacterial counts trended upward with each harvest of Outrageous lettuce ranging from ≤ 50 CFU/g to 3.5×10^5 CFU/g.
- 3/5 samples from the July harvest and 2 from the December harvest of Mizuna were above the NASA limit ranging from below detection limit to 8.5×10^5 CFU/g.
- Waldman's green counts were below detection to 1.1×10^5 CFU/g.
- Of the five herbs sampled, Parsley was the only one with a "cut and come again" harvest with an increase in both bacterial and fungal counts after the first harvest.

Fungal counts



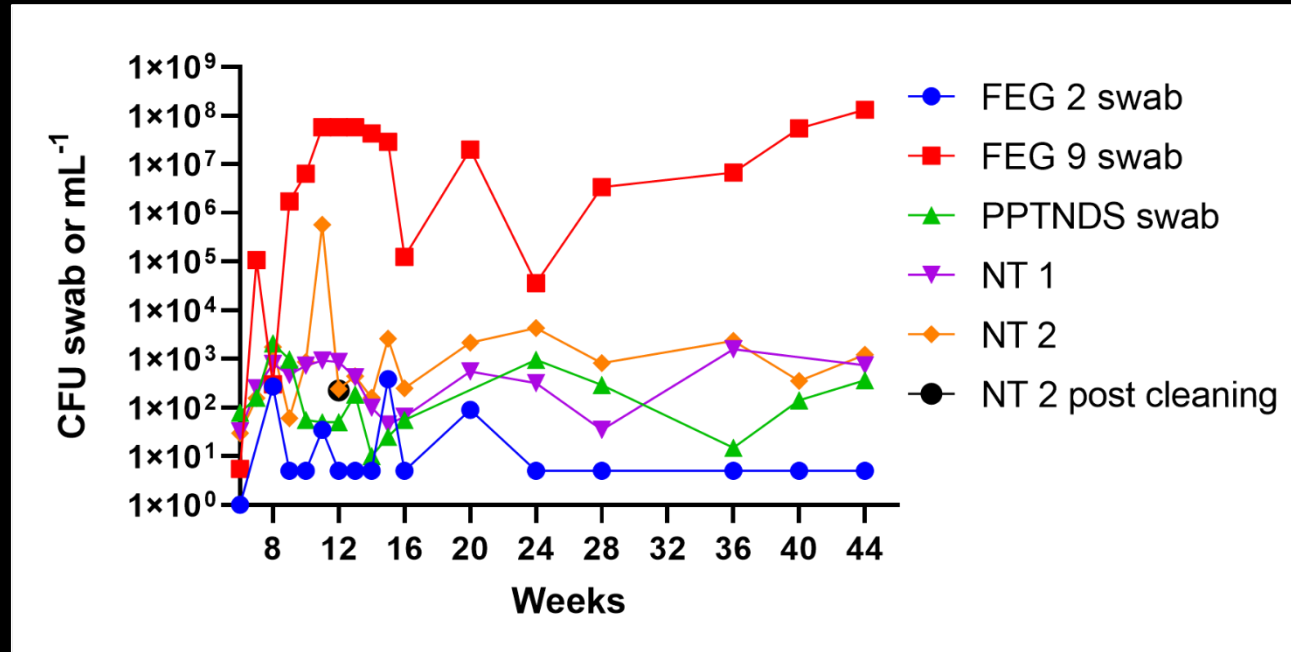
- A similar trend in increased fungal counts was seen with the consecutive harvests of Outredgeous lettuce, Mizuna and Waldmans green.
- The NASA standard for yeast and mold on non-thermostabilized food is 1×10^3 CFU/g. Several of the leafy greens and herbs exceeded this standard.

Tomato/pepper



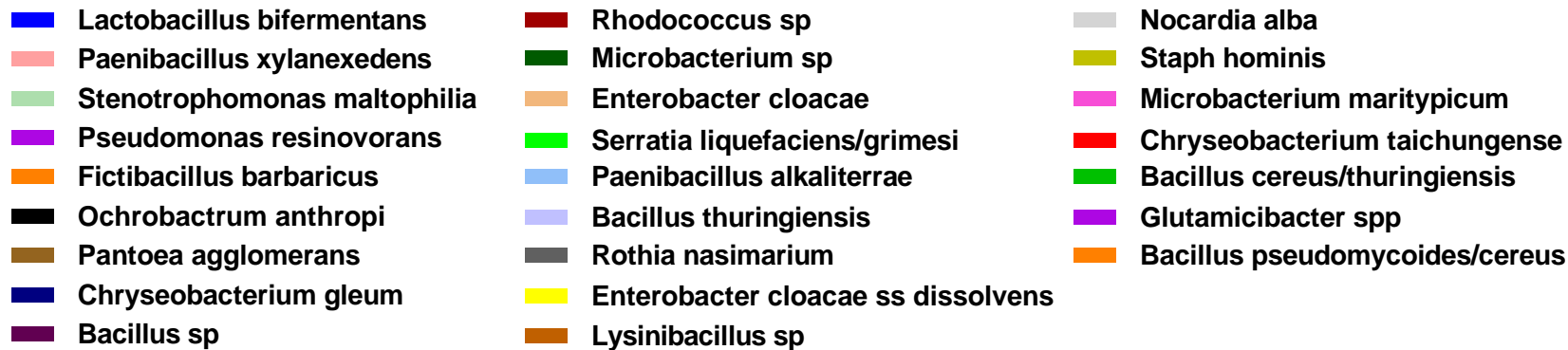
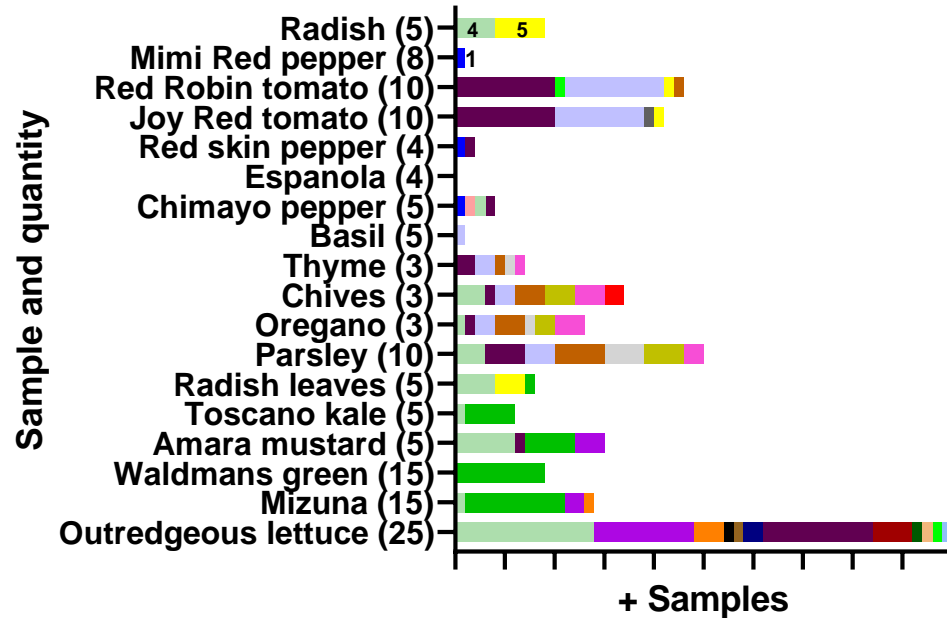
- The four varieties of peppers had low microbial counts overall.
- The bacterial counts were all below 1,000 CFU/g, Espanola and red skin were below or at detection limit.
- Red Robin and Joy Red tomatoes had bacterial counts all below 1×10^4 CFU/g.
- Fungal counts on Espanola peppers were very low < 50 CFU/g.
- The highest fungal count on the pepper varieties was 1.7×10^3 CFU/g on the Mimi Red pepper.

Environmental Samples



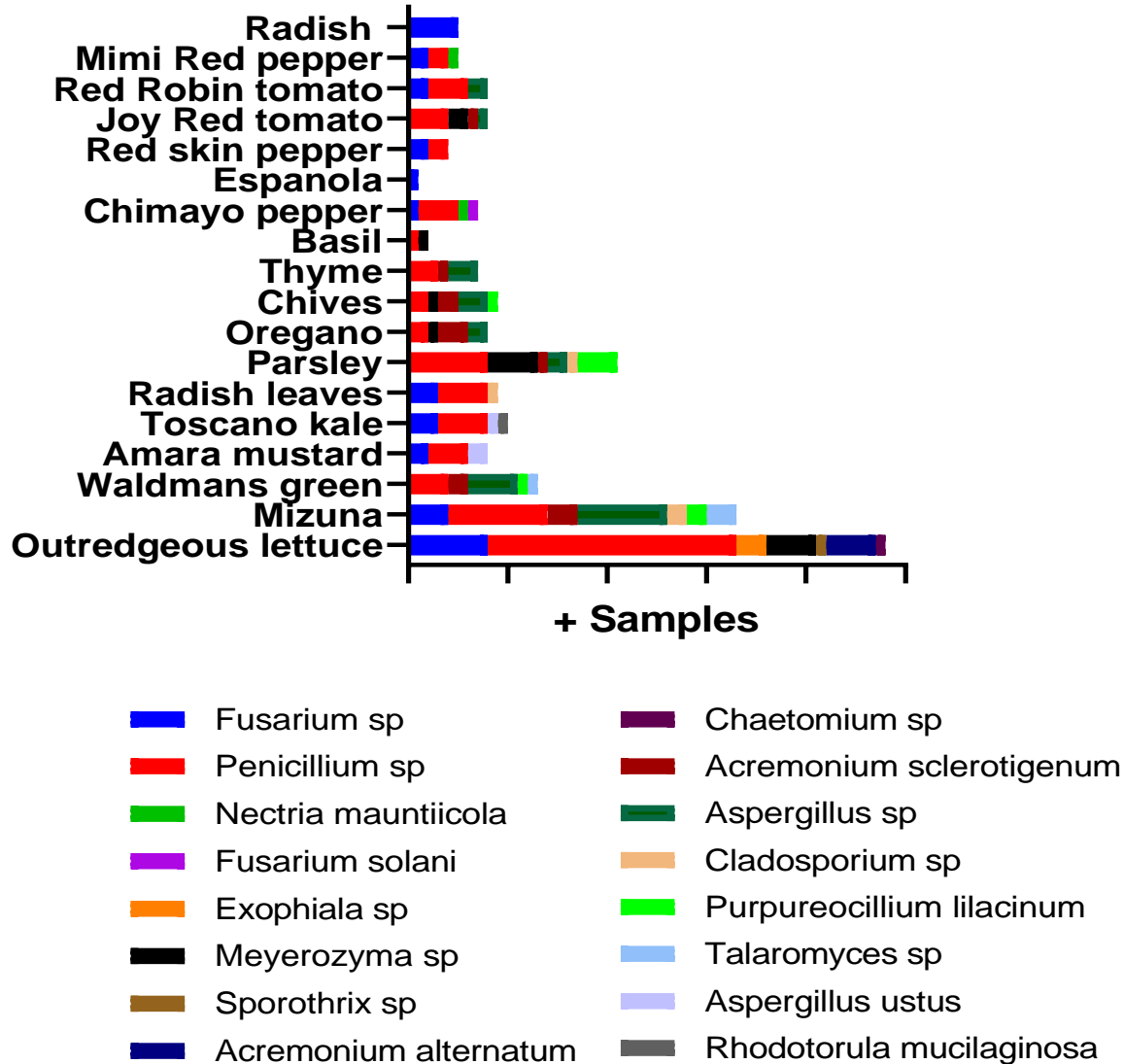
- FEG 2 (wall) was low in bacterial and fungal load, ranging from ≤ 1 CFU/25cm² to 385 CFU/25cm²
- FEG 9 (floor) had the highest bacteria load increasing over the first 10 weeks of sampling from week 6 to week 16
- The surface of the PPTNDS ranged from, detection limit to 2.1×10^3 CFU/cm².
- The nutrient tank levels fluctuated over the 11 month period. Nutrient tank 2 reached a peak of 5.7×10^5 bacteria CFU/mL at 11 weeks but the counts dramatically declined by 3 orders of magnitude after a cleaning event.
- Fungal counts over the 11 month sample period were low, most below detection limit with the exception of the floor sample.

Bacteria



- Eighteen genera identified.
- A total of 25 isolate different identifications. 12 on lettuce leaves, 4 on mizuna and 1 on Waldmann's green.
- *Stenotrophomonas maltophilia* was found on 10 types including the leafy greens and herbs, except Waldmann's green, basil and thyme.
- Except for the Chimayo pepper it was not identified on any of the tomatoes and peppers.

Fungi



- Sixteen different fungi were isolated and identified.
- Two fungi dominated most of the plant samples.
- *Penicillium* sp was found on 16 of the 18 sample groups followed by *Fusarium* on 11.
- *Fusarium solani*, isolated from the Chimayo pepper only, is a phytopathogen, its primary hosts being potato, pea, bean, and members of the curcubit family.

Bacterial isolates from environmental samples.

Bacteria	FEG 2	FEG 9	NT1	NT2	PPTNDS
<i>Acinetobacter beijerinickii</i>		+			
<i>Bacillus acidicola</i>			+		
<i>Bacillus cereus/thuringiensis</i>		+	+	+	
<i>Bacillus decolationis</i>			+	+	+
<i>Bacillus firmus</i>			+		+
<i>Bacillus macauensis</i>			+		
<i>Bacillus marisflavi</i>	+		+		
<i>Bacillus pseudomycooides/cereus</i>			+	+	
<i>Bacillus pumilis/safensis</i>		+			
<i>Bacillus scohaenanensis</i>			+	+	
<i>Brevibacterium casei</i>					+
<i>Brevundimonas diminuta</i>		+		+	
<i>Brevundimonas vesicularis</i>		+	+	+	
<i>Chryseobacterium gleum</i>			+		
<i>Chryseobacterium taeanense</i>		+	+		
<i>Clavibacter michiganensis ss insidiosus</i>		+			
<i>Curtobacterium herbarum</i>		+			
<i>Curtobacterium pusillum</i>		+			
<i>Exiguobacterium undae</i>		+	+		
<i>Fictibacillus arsenicus</i>			+	+	
<i>Leifsonia aquatica</i>		+	+	+	
<i>Leifsonia poae</i>		+			

Bacteria	FEG 2	FEG 9	NT1	NT2	PPTNDS
<i>Microbacterium dextranolyticum</i>		+			
<i>Microbacterium maritypicum</i>		+		+	+
<i>Micrococcus luteus E</i>				+	+
<i>Micrococcus yunnariensis</i>					+
<i>Mycobacterium smegmatis</i>		+			
<i>Paenibacillus agarexedens</i>		+	+		+
<i>Paenibacillus polymyxa</i>			+	+	
<i>Paenibacillus soli</i>			+	+	+
<i>Paenibacillus tarimensis</i>			+	+	
<i>Paenibacillus tundrae</i>		+	+	+	
<i>Paenibacillus xylanilyticus</i>			+	+	
<i>Paenibacillus massiliensis</i>			+	+	
<i>Paenibacillus xylanexedens</i>				+	
<i>Rathayibacter rathayi</i>			+	+	
<i>Rhodococcus erythropolis</i>			+		
<i>Sphingomonas paucimobilis</i>		+	+	+	+
<i>Staphylococcus intermedius</i>		+			
<i>Staphylococcus saprophyticus</i>		+	+		+
<i>Stenotrophomonas rhizophila</i>		+			

- Forty different species in 18 genera were found on surfaces and in nutrient tank solutions.
- These were also the sites with the highest bacterial load, FEG 9 and nutrient tanks.

Conclusions

- The bacterial and fungal isolation and identification done in this study are based on those microorganisms that can be cultured on the media utilized, therefore their absence only indicates that on certain samples they may have not grown or were too low to detect.
- Whole genome community sequencing to identify bacteria and fungi is a method that does not require cultivation and in the future could be utilized to expand the list of microbial community members in these samples.

Thank you!

