

# Leveraging carbon-based fertilizers and microbes mediated processes to move toward a circular citriculture

**Deborah Pagliaccia**

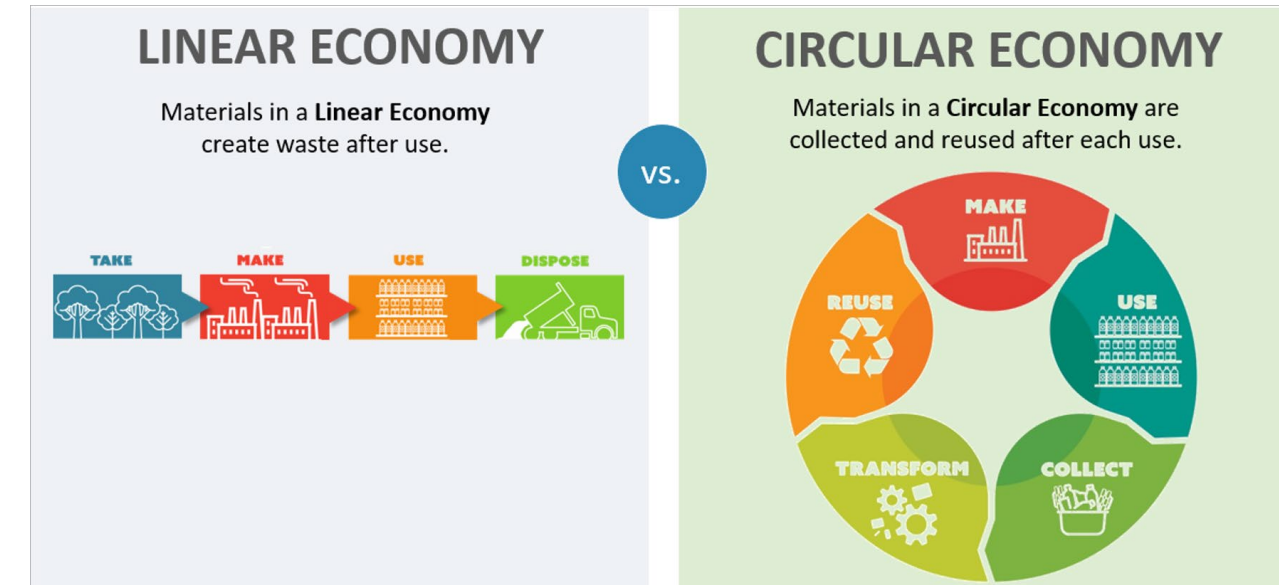
UC Riverside

&

California Citrus Nursery Society

Norman Ellstrand UCR  
Samantha Ying UCR  
Georgios Vidalakis UCR

James Borneman UCR  
Ashraf El-Kereamy UCR



Jonathan D. Kaplan SSU



SACRAMENTO STATE

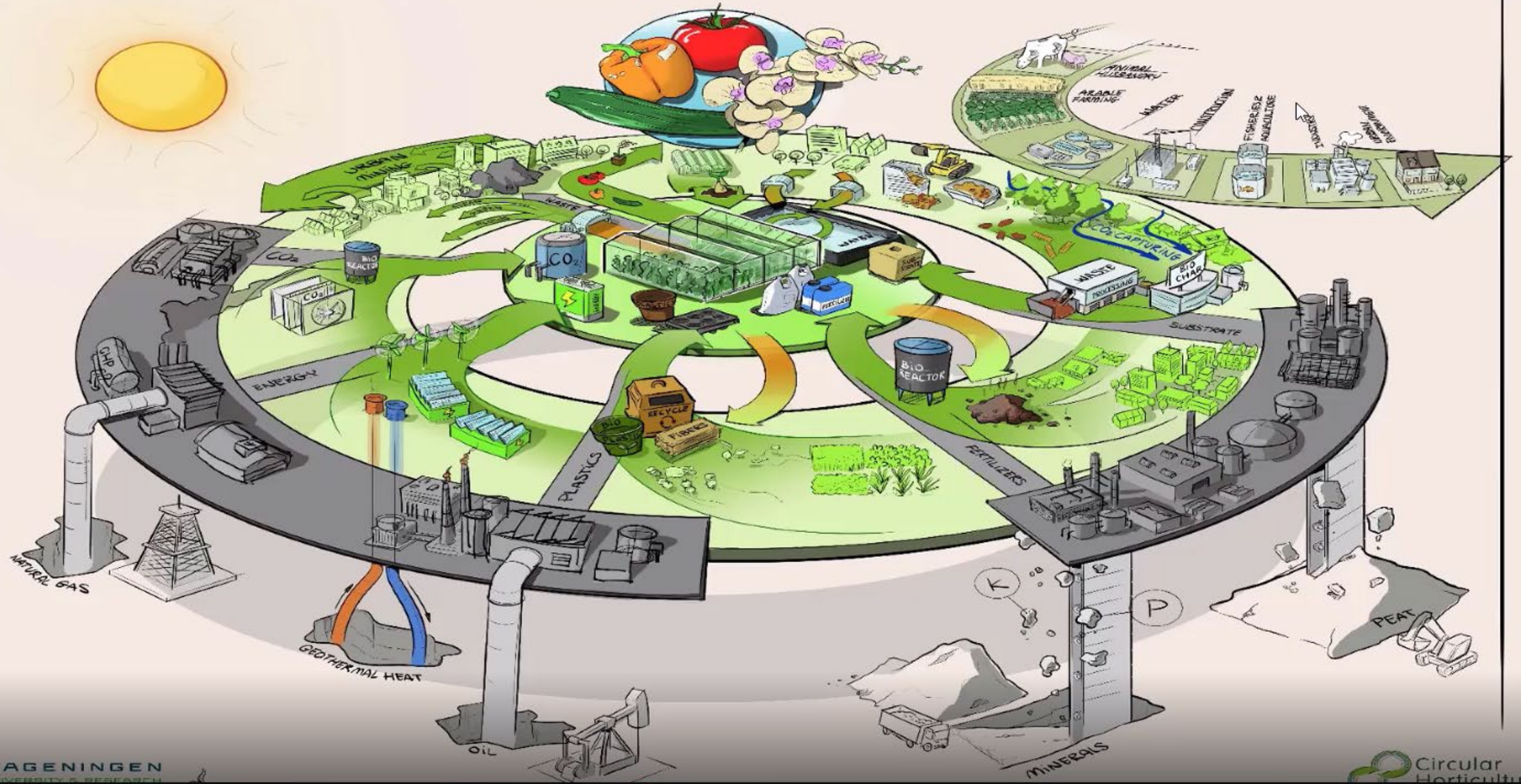
**Mike Woelk,**  
Co-founder and CEO





# GREENHOUSE HORTICULTURE IN THE CIRCULAR ECONOMY

EFFICIENT, CLEAN AND CONNECTED



# Today presentation summary



**Brief introduction & Project Goals**

**Benefit for plant production**

**PHASE 1 Results– Anaerobic FERMENTATION of citrus waste – Day 1 to 14**

- C & N
- MICROBIOME

**PHASE 2 Results– Aerobic COMPOSTING of by-products with nursery soil – Day 15 to 28/42**

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**PHASE 3 Results - Greenhouse TRIALS using AMENDED SOIL MIXES – from Day 30**

- Effect of **Almond waste derived biochar** on soil mixes and plant
- Effect of **Liquid Citrus waste derived Bokashi by-products** on soil mixes and plant
- Effect of **Solid Citrus waste derived Bokashi by-products** on soil mixes and plant

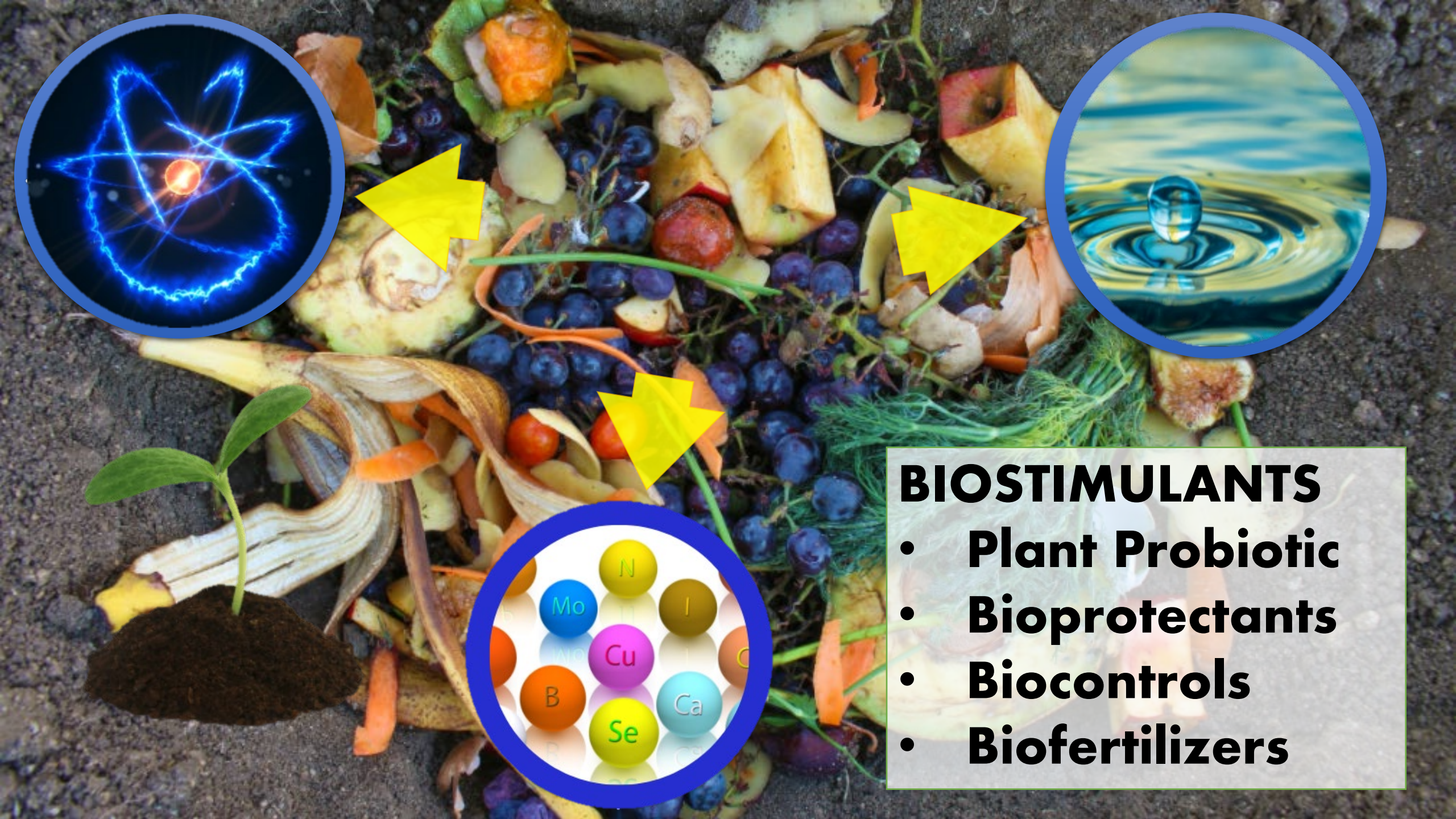
**Drawbacks & Opportunities**

**Next step.....scaling up**









## **BIOSTIMULANTS**

- **Plant Probiotic**
- **Bioprotectants**
- **Biocontrols**
- **Biofertilizers**



**Almond  
shells**



**PYROLYSIS**



# **Pyrolysis of Plant Biomass**

**BY-  
PRODUCTS**

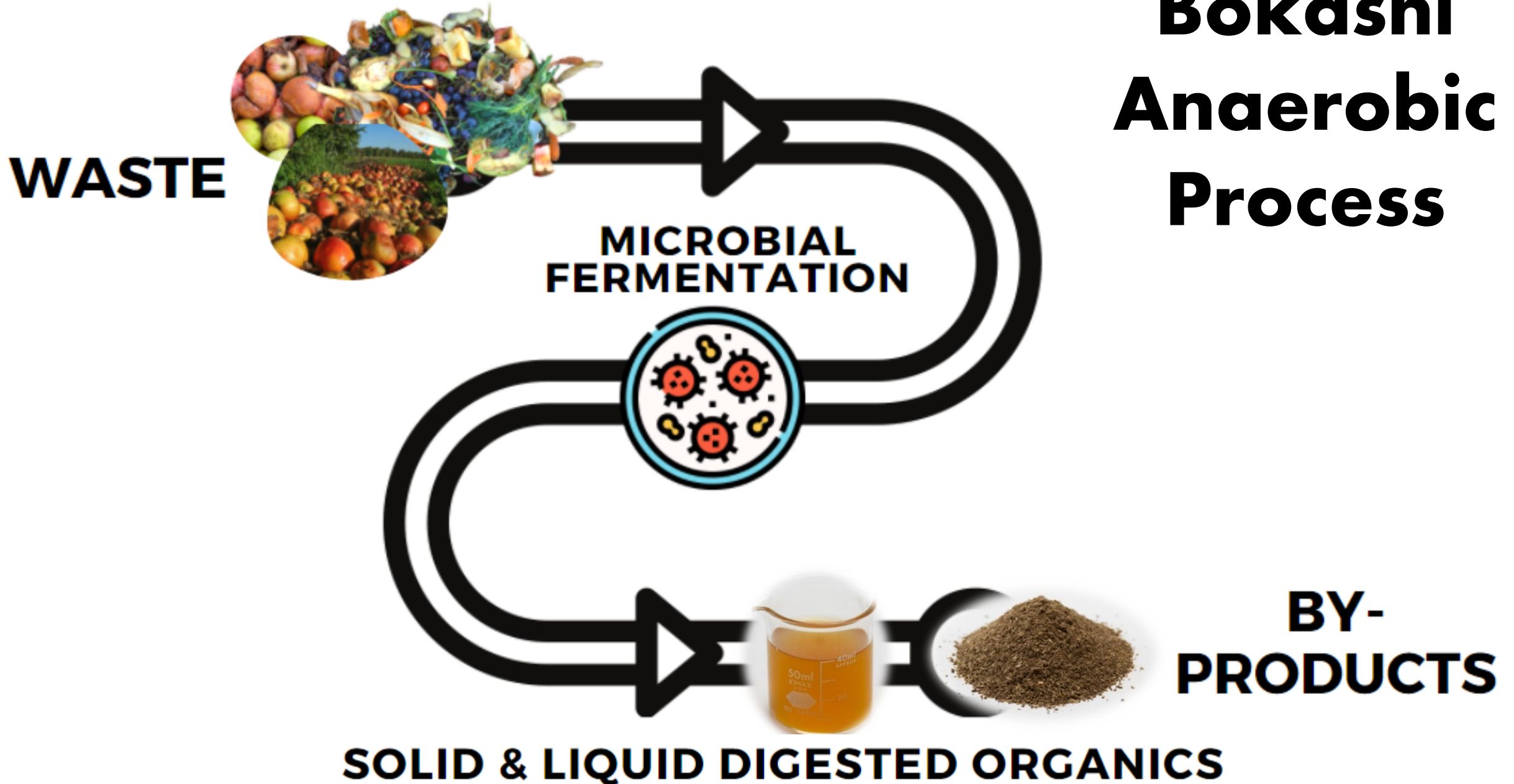


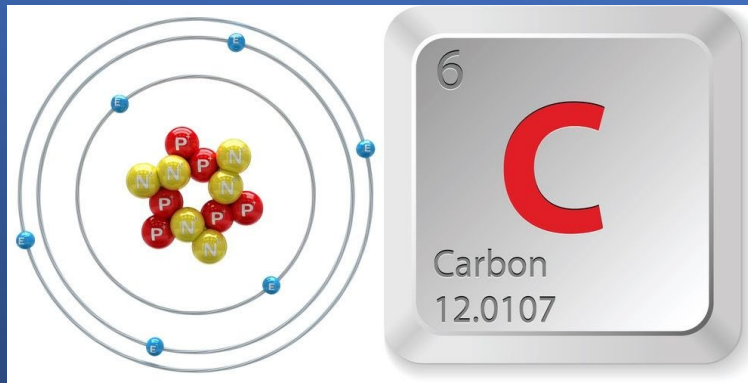
**BIOCHAR & LIQUID  
WOOD VINEGAR  
(PYROLIGNEOUS ACID)**

- PA cost is \$40/Gallon
- Biochar \$600/ton bone dry and \$100/Cubic Meter (m<sup>3</sup>)



# Bokashi Anaerobic Process





***Johannes- Soil organic matter is one of the, if not the, foundation of soil functioning, soil fertility, soil everything. It's the foundation of soil health and how carbon and nutrients cycle in soil. So understanding soil organic matter is really fundamental to understanding soils.***

That's

***Johannes Lehmann I'm a professor of soil science at Cornell University.***

**Priming for production: A podcast on soil health**

[https://projects.sare.org/sare\\_project/one15-251/](https://projects.sare.org/sare_project/one15-251/)



Our research projects aims to demonstrate the beneficial use of Ag and food waste byproducts, to

- Enhance beneficial native citrus microbiome
- Impact soil/potting soil carbon, nitrogen, and improve nutrient dynamics

**WHY?** Because play a crucial role in plant growth, health, and stress resilience.



# Summary

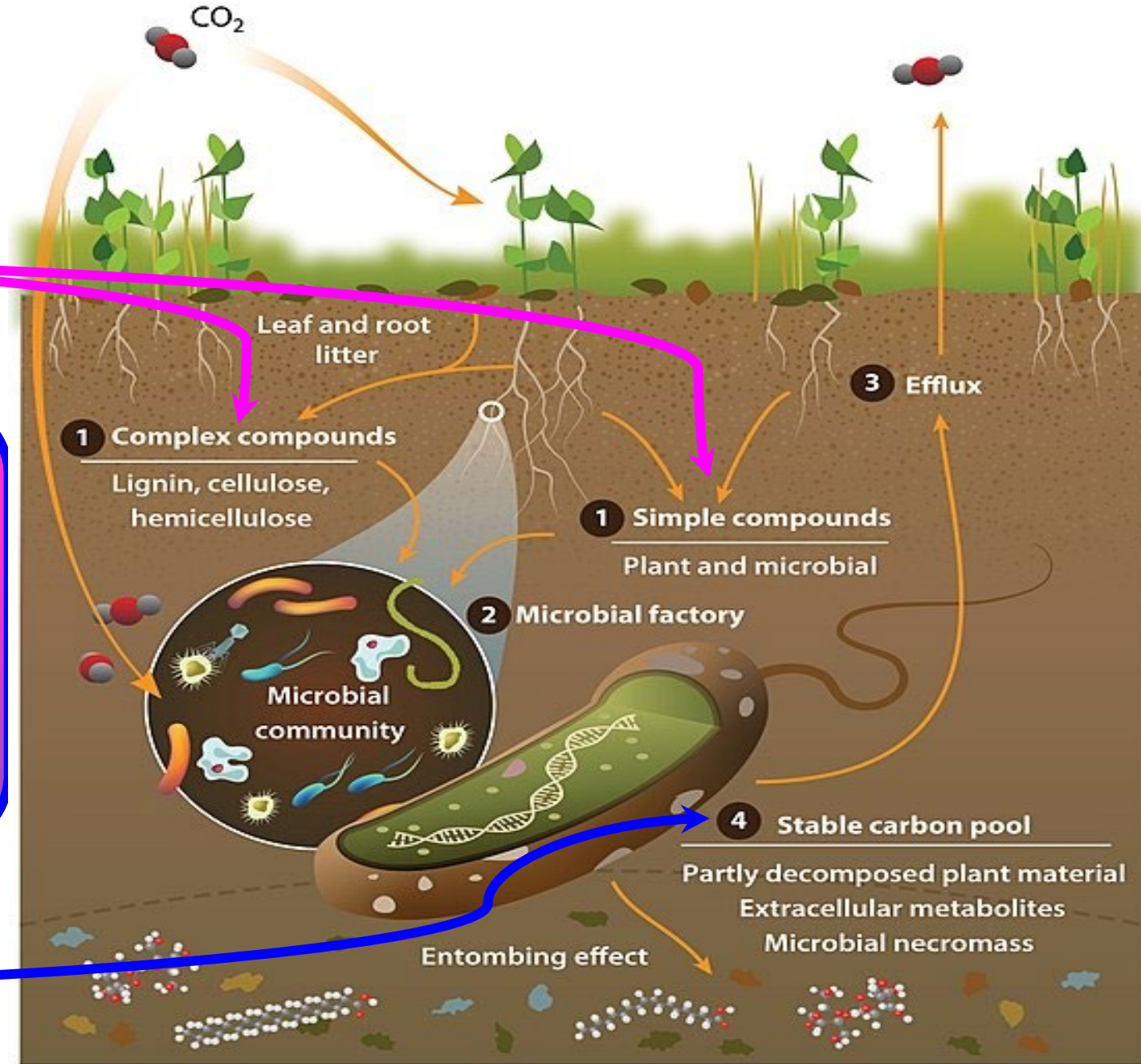


Nutrient-rich  
organic matter

Manage C to create a  
dynamics flow of nutrient  
availability and cycling to  
improve soil fertility,  
microbiome and plant health



Add stable  
**CARBON**





# Summary



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organic matter

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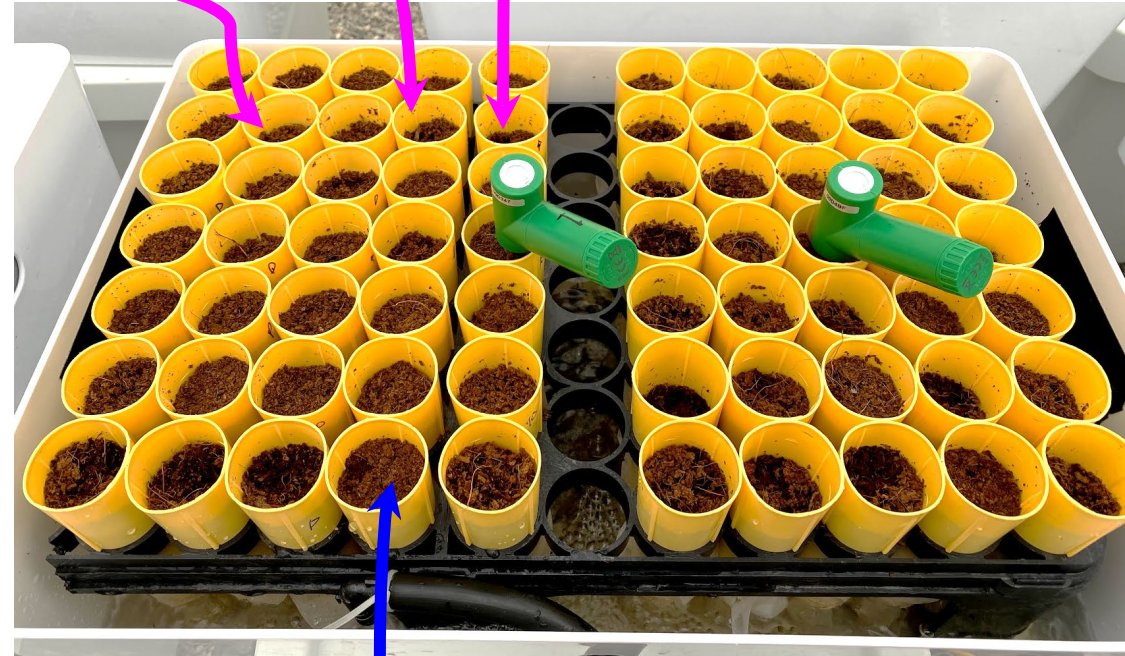


Add stable  
CARBON



The liquid  
MAINTAIN

The solid  
BUILD



Biochar helps to BUILD & MAINTAIN!



## Brief introduction & Project Goals

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## Drawbacks & Opportunities

Next step.....







Waste

PHASE 1

WASTE

MICROBIAL  
FERMENTATION

By-products



PHASE 2



PHASE 3



PHASE 2

Almond  
shells

PYROLYSIS







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## Drawbacks & Opportunities

Next step.....



For the **1st anaerobic phase** of the Bokashi fermentation process we are investigating:

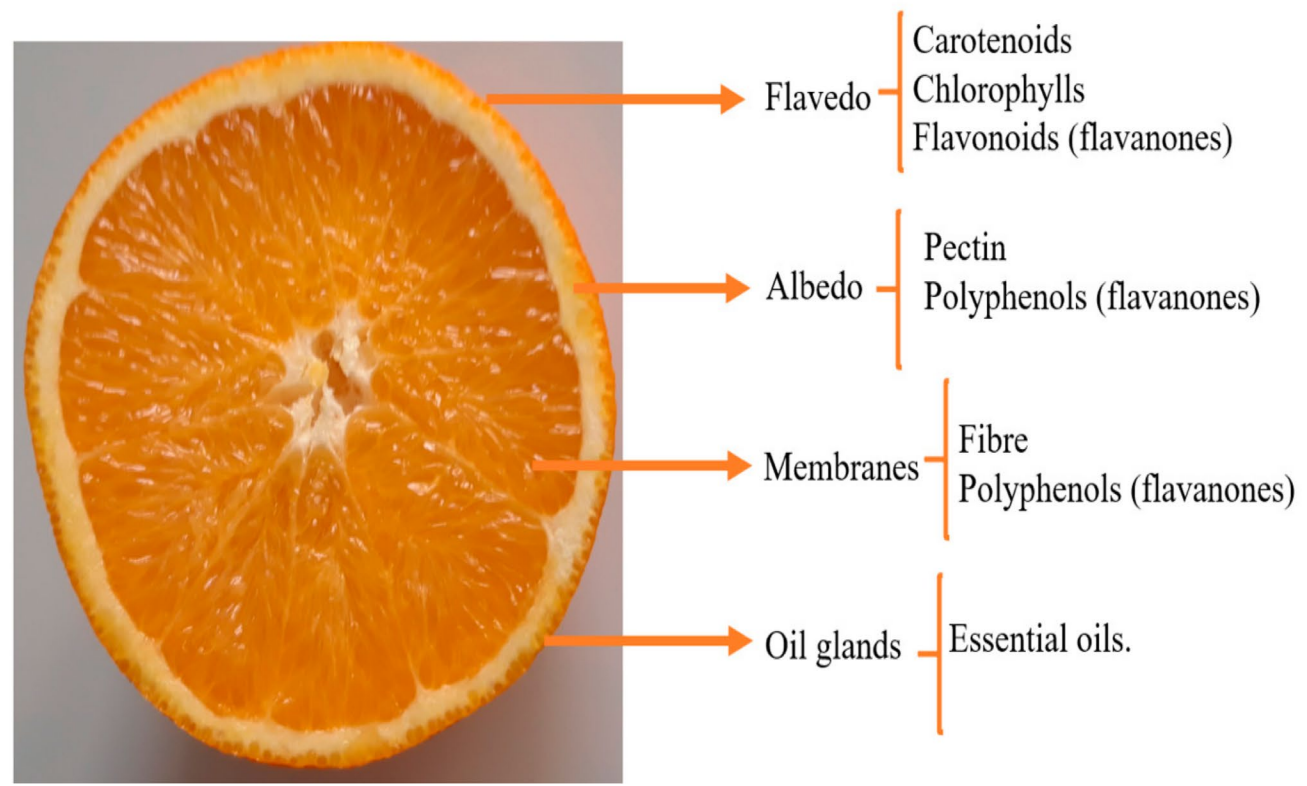
1. A & B. Nutrient composition of row and bokashi by-product material
2. C, N, and C/N ratio changes during fermentation
3. Microbiome changes over time during the 1st phase.
4. Understand GHG reduction (carbon dioxide ( $\text{CO}_2$ ), methane ( $\text{CH}_4$ ), and Nitrous oxide ( $\text{NO}_2$ ), during the 1st phase of the Bokashi fermentation process.



Soluble sugars (mainly fructose, glucose and sucrose), and structural polysaccharides, such as cellulose, hemicelluloses and pectin, including lignin-like compounds, such as flavonoids, and essential oils



1A. What's the nutrient composition of row citrus waste material?



**Time 0**

**Citrus solid**

pH 4.0-5.0

TC 40.3

TN 1.1

T C/N 36.85





Two Food Waste By-Products Selectively Stimulate Beneficial Resident Citrus Host-Associated Microbes in a Zero-Runoff Indoor Plant Production System



Parameter	Amount	
	Beer Mash	Food Waste
Soluble Salts (Electrical Conductivity)	8.7 dS/m	29 dS/m
pH	3.8	5.7
Chloride (Cl)	0.05%	0.16%
Organic Matter	3.07%	2.16%
C:N Ratio	8:1	3:1
Nitrate-N	<0.02 %	<0.02 %
Ammonium-N	0.03%	0.22%
Organic N	0.19%	0.14%
Water Insoluble N	0.01%	0.01%
Water Insoluble Organic N	<0.01 %	0.01%
Total Nitrogen	0.22%	0.36%
Phosphorus (P)	823 ppm	323 ppm
Potassium (K)	446 ppm	3268 ppm
Sulfur (S)	137 ppm	231 ppm
Magnesium (Mg)	385 ppm	351 ppm
Calcium (Ca)	392 ppm	1215 ppm
Sodium (Na)	165 ppm	1661 ppm
Iron (Fe)	14 ppm	18 ppm
Aluminum (Al)	1 ppm	6 ppm
Manganese (Mn)	5 ppm	2 ppm
Copper (Cu)	0.01 ppm	0.42 ppm
Zinc (Zn)	9 ppm	8 ppm
Boron (B)	0.18 ppm	3 ppm



1B. What’s the nutrient composition of the bokashi by-products material?

Time 14 - END

Citrus Solid Bokashi

pH 3.5  
C 49  
N 1.63  
C/N 29  
Nitrate-N 0.07  
Ammonium-N 0.14





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## Drawbacks & Opportunities

Next step.....



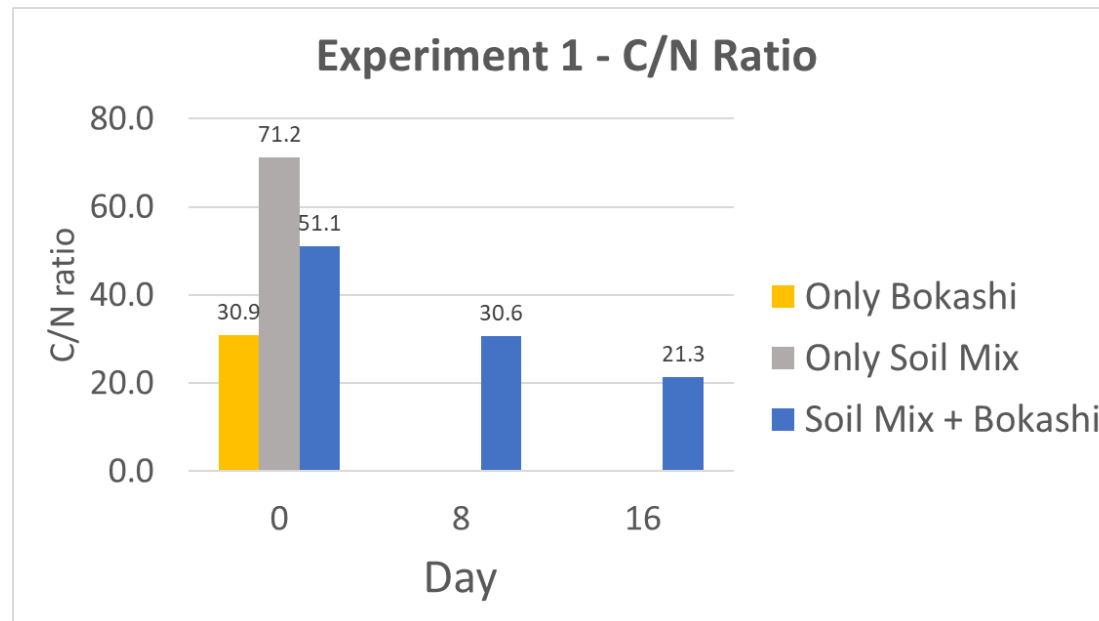
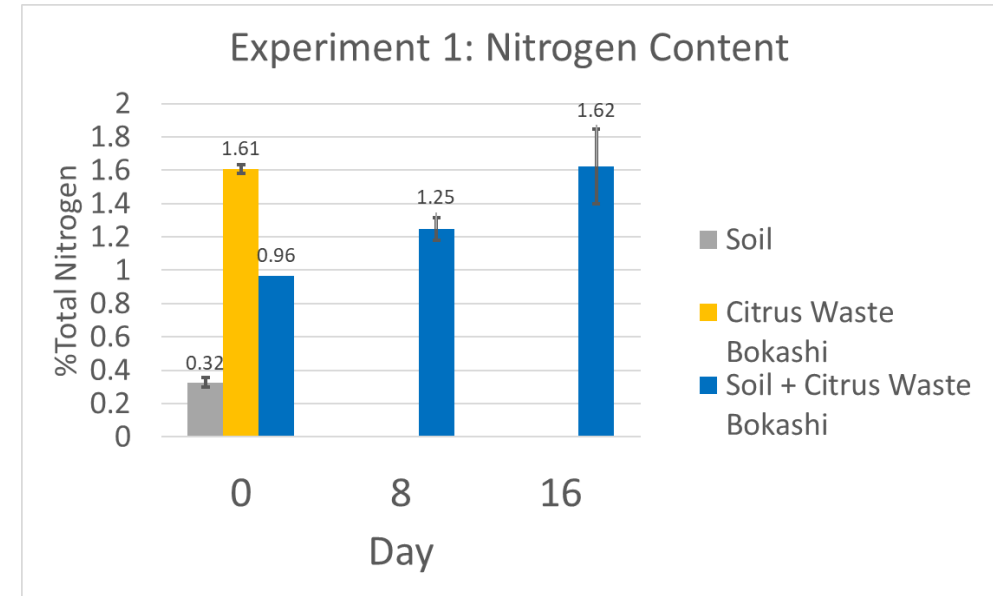
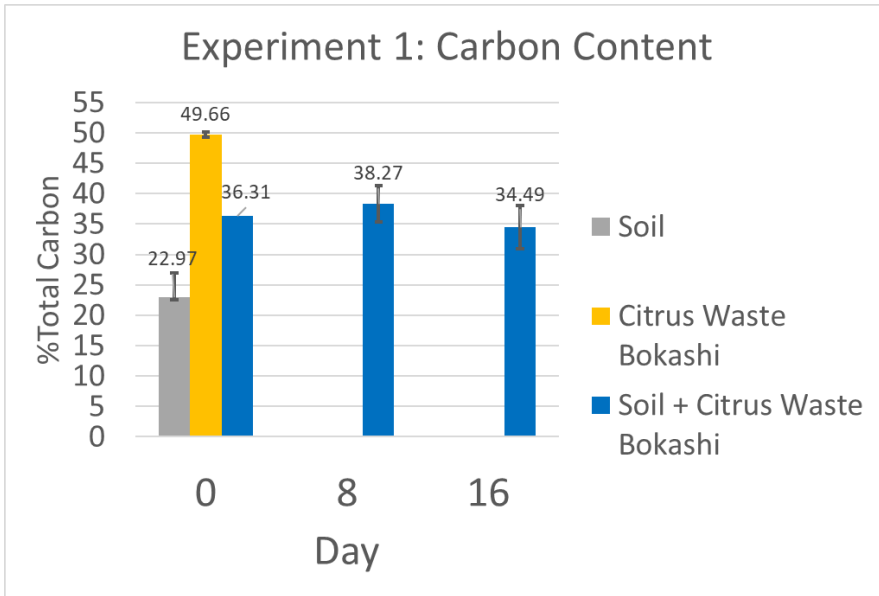
In the 2<sup>nd</sup> **aerobic phase** of the process, we are investigating:

1. C, N, and C/N ratio changes during 2<sup>nd</sup> PHASE
2. Microbiome changes in soil mixes time during 2<sup>nd</sup> PHASE
3. Understand GHG reduction (carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), and Nitrous oxide (NO<sub>2</sub>), during the 1st phase of the Bokashi fermentation process.

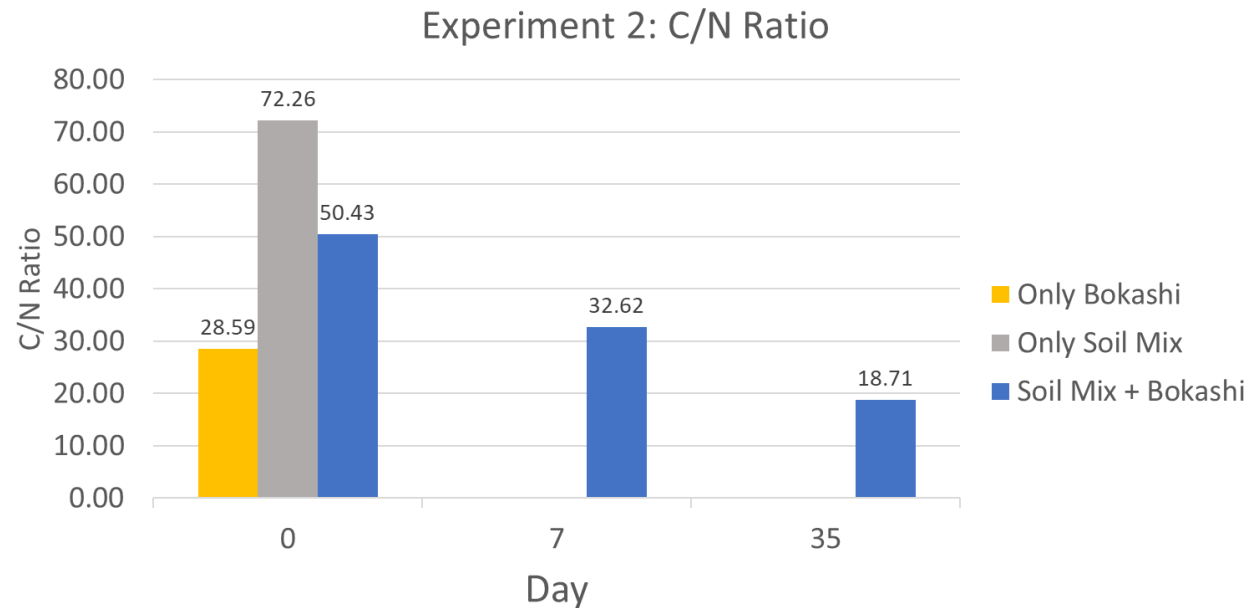
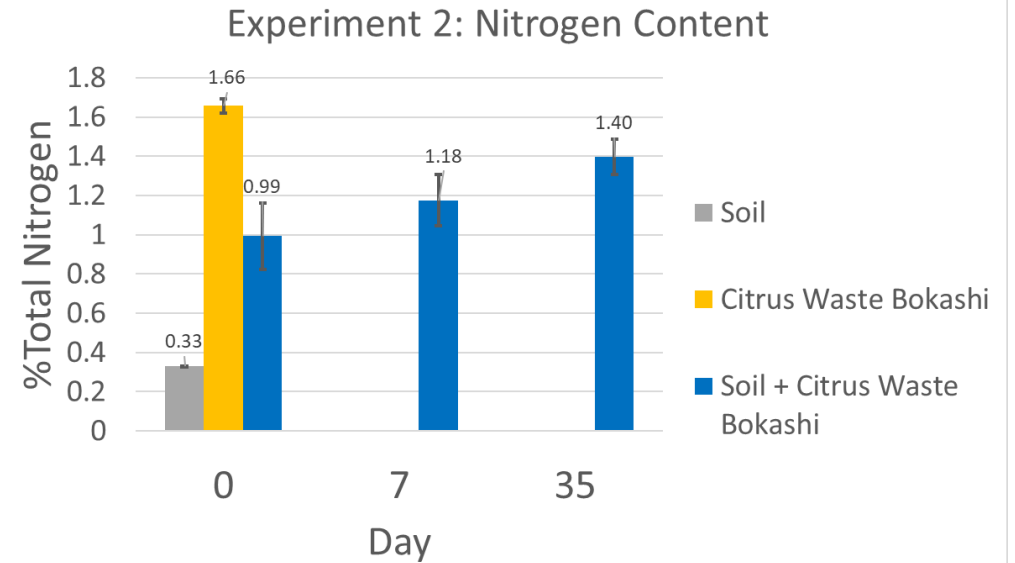
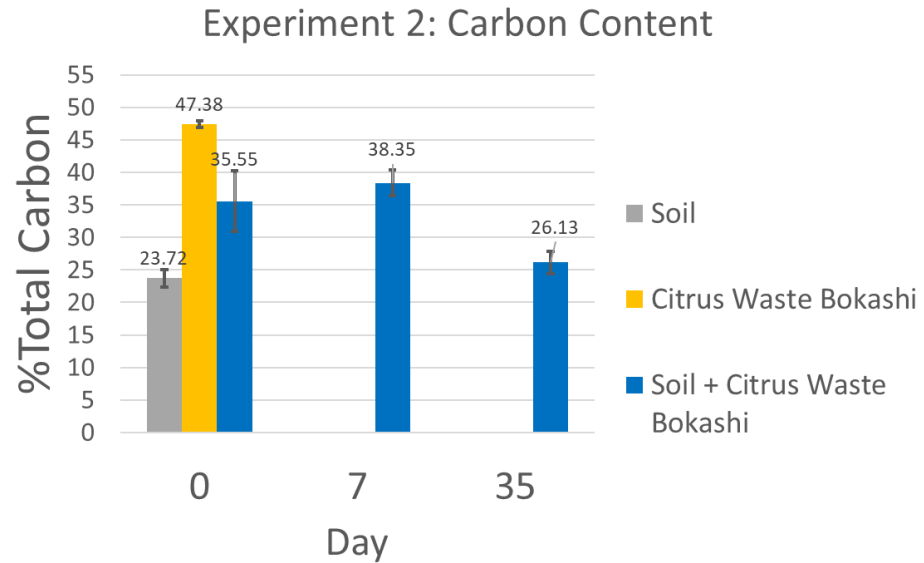


## 1st Experiment - OUR SOIL MIX & Bokashi Living

Mixed equal volume citrus solid bokashi with soil mix (made of equal parts perlite, peat moss and coco coir)



## 2nd Experiment - OUR SOIL MIX & Bokashi Living



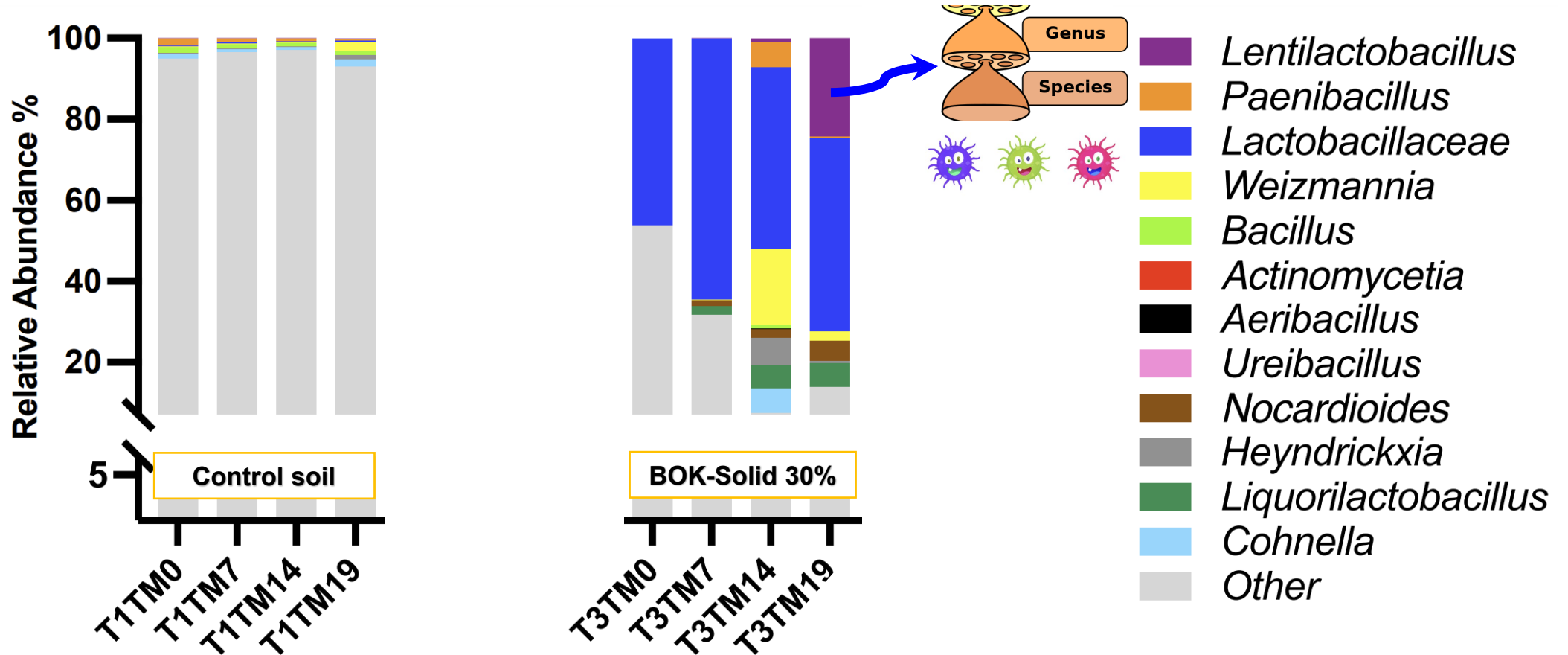


### 3rd Experiment - OUR SOIL MIX & Bokashi Living

We mixed 50% volume of citrus solid bokashi with 50% volume soil mix (made of equal parts perlite, peat moss and coco coir). We took the samples at TIME 0, 4, 7, 11, 14, 18, 21 up to 42 days and placed them in the -80c freezer for NGS microbiome and 4C for C/N sample processing.



		N Average	C Average	C/N
Time 0	Only Soil Mix	0.3	19.5	60.5
Time 0	Only Bokashi	1.5	43.0	29.1
Time 0	Soil Mix + Bokashi	0.9	31.2	44.8
Time 7	Soil Mix + Bokashi	1.0	29.5	28.6
Time 14	Soil Mix + Bokashi	1.1	29.1	25.4
Time 21	Soil Mix + Bokashi	1.1	26.4	24.5
Time 28	Soil Mix + Bokashi	1.2	26.6	22.4
Time 35	Soil Mix + Bokashi	1.1	24.1	21.0
Time 42	Soil Mix + Bokashi	1.2	25.0	21.3
Time 26	Only Soil Mix	0.4	23.5	58.1







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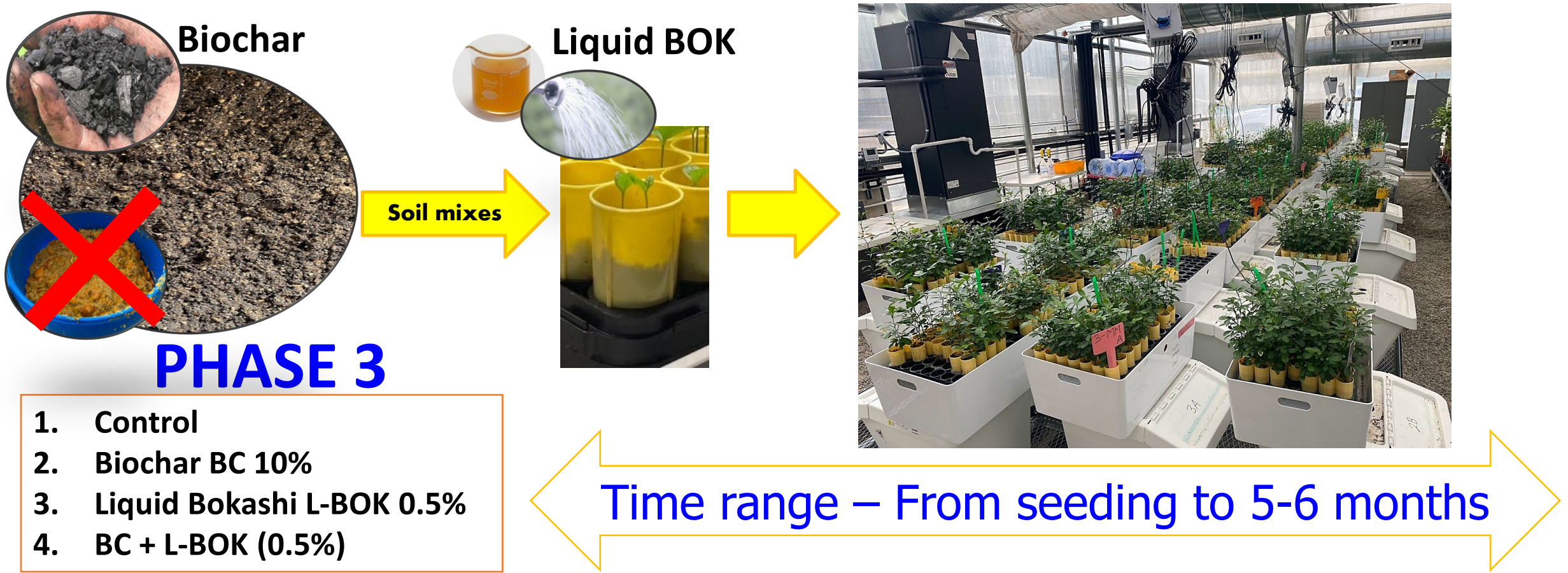
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## Drawbacks & Opportunities

Next step.....



For the **3<sup>rd</sup> phase** we are investigating under greenhouse conditions:

- |  |  |
|--|--|
|  | 1. C, N, and C/N ratio dynamics.   |
|  | 2. Microbiome changes over time during in soil and root in the first 6 months.                         |
|  | 3. Impact on plant growth parameters, including plant height, seeds germinations, weight, and caliper. |



# CARBON, NUTRIENTS, AND BACTERIAL DYNAMICS IN SOIL

Is soil C & N building up over time?

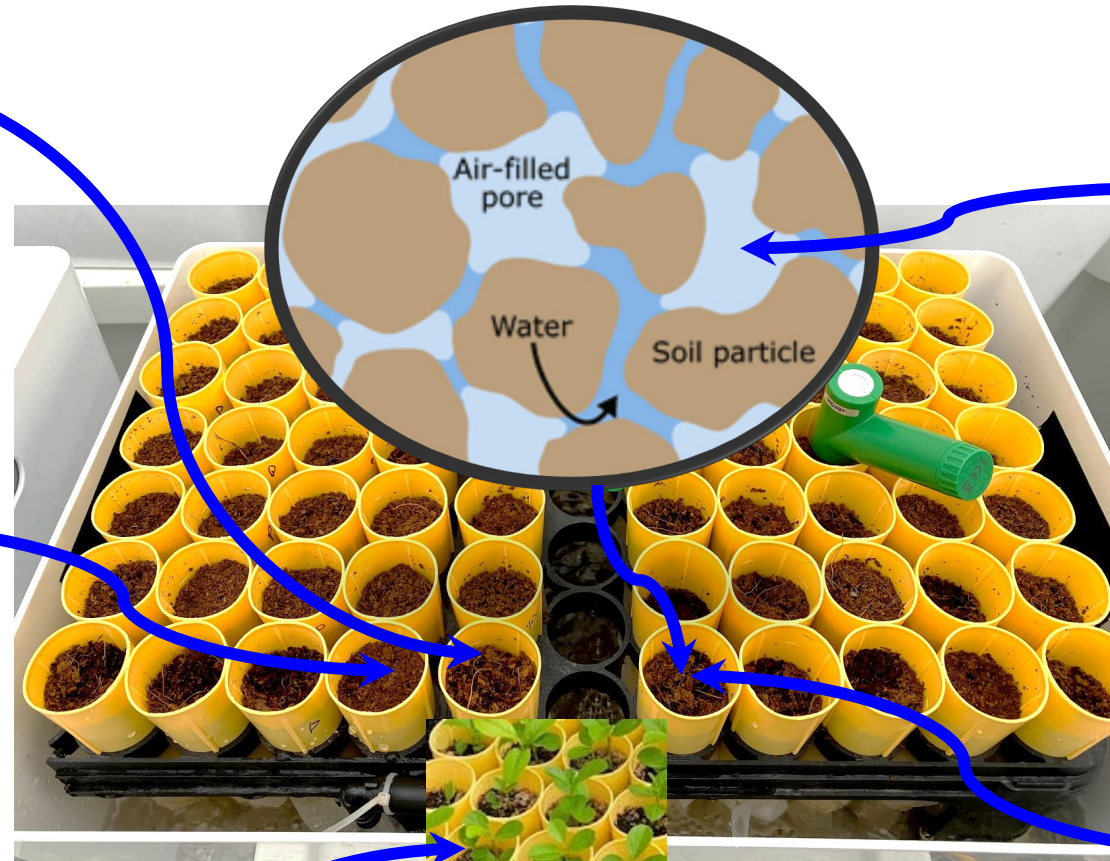
Costech ECS 4010 CHNSO elemental analyzer

Is there a change in total elemental composition in soils after repeat application?

X-ray Fluorescence Spectrometry (XRF)

Is there a + impact on plant growth parameters?

Plant height, seeds germinations, weight, and caliper

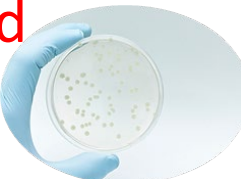


How do dissolved organic carbon concentrations change over time?

Water extractable organic carbon (WEOC) and nitrogen (WEON) concentrations

How does the microbial population changes in soil and root ?

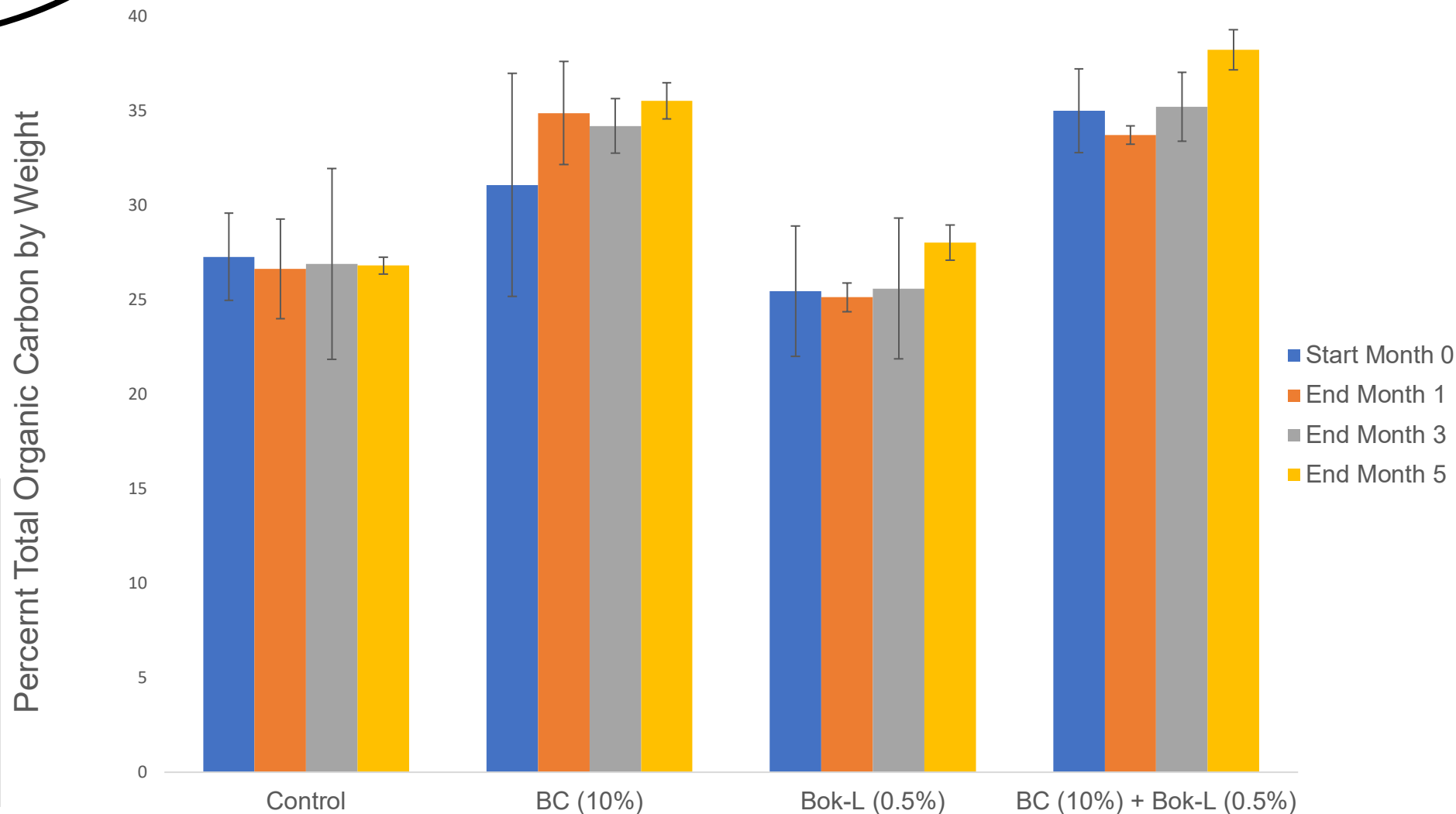
Plating and NGS



Is soil C & N building up over time?

Costech ECS 4010 CHNSO  
elemental analyzer

# Results: Change in Total Organic Carbon Over Time



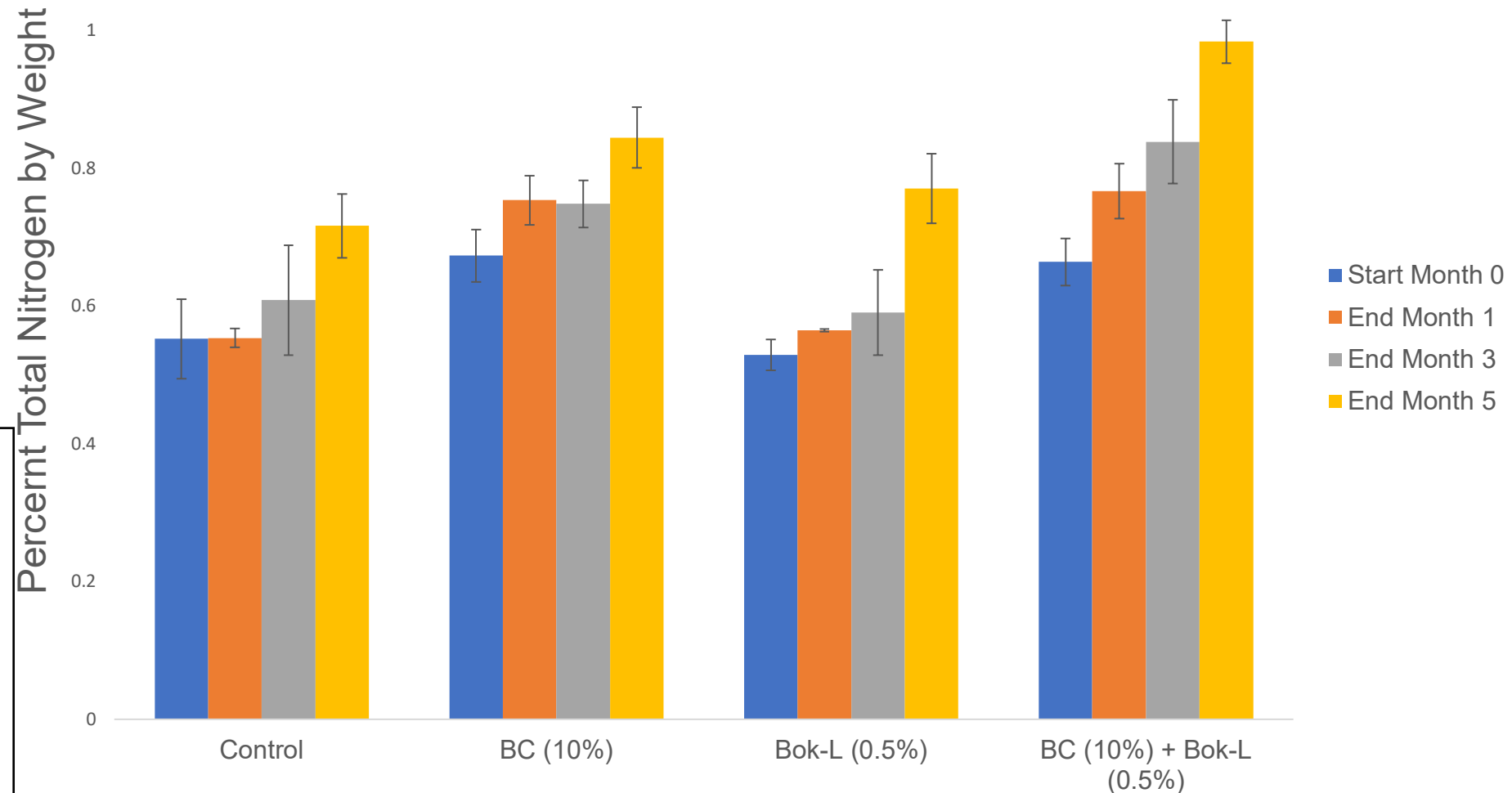


Is soil C & N building  
up over time?

Costech ECS 4010 CHNSO  
elemental analyzer

1.2

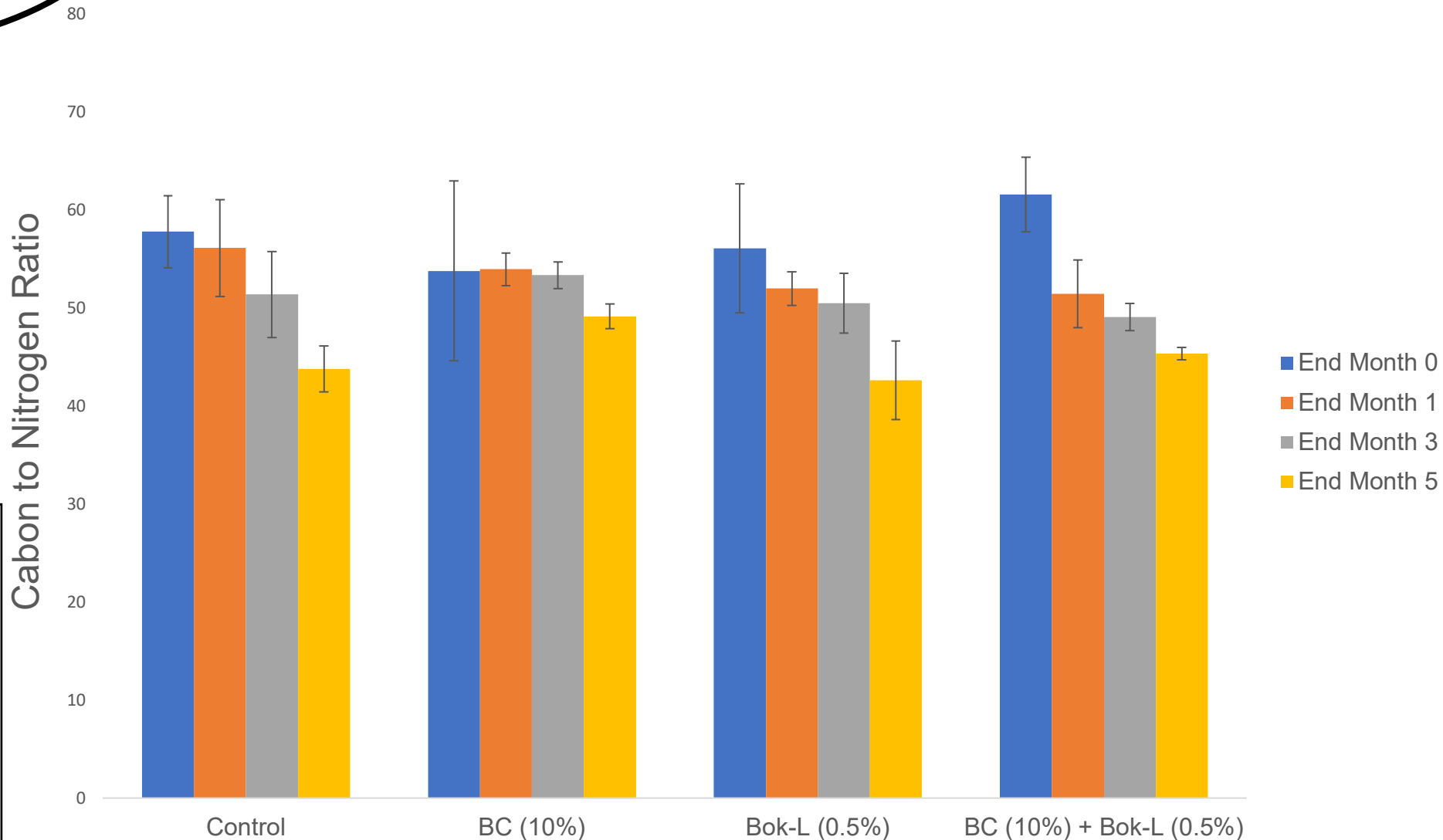
# Results: Change in Total Nitrogen Over Time



Is soil C & N building  
up over time?

Costech ECS 4010 CHNSO  
elemental analyzer

# Results: Organic Carbon to Nitrogen Ratio Over Time





## 5TM Soil Moisture and Temp Sensor

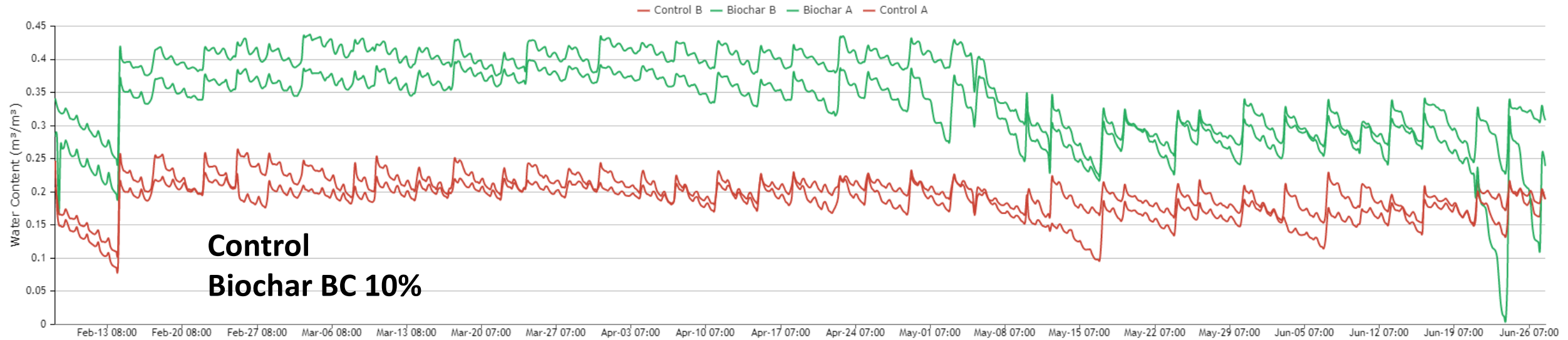
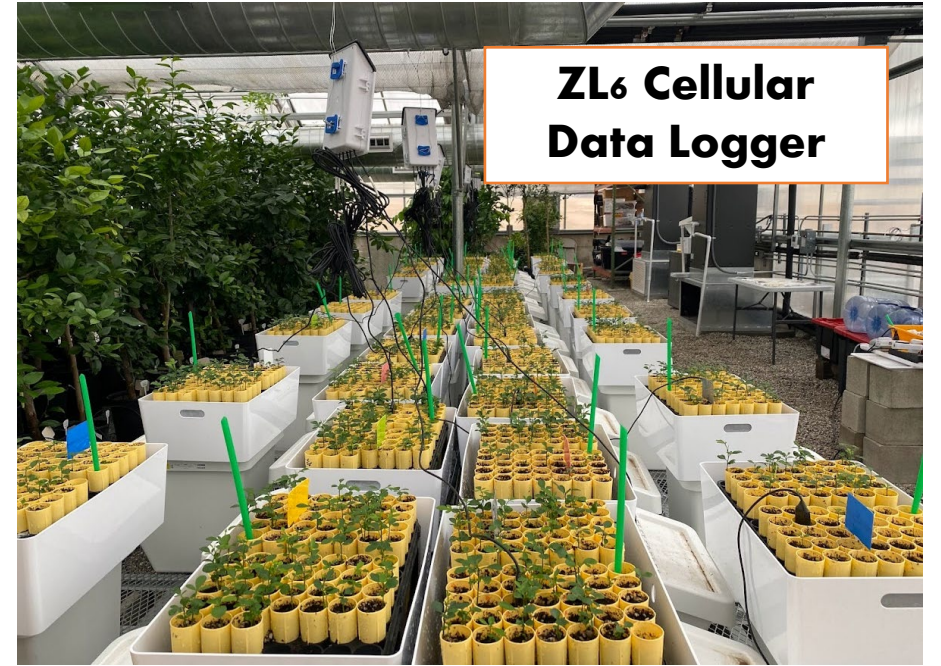


METER  
ZENTRA Cloud

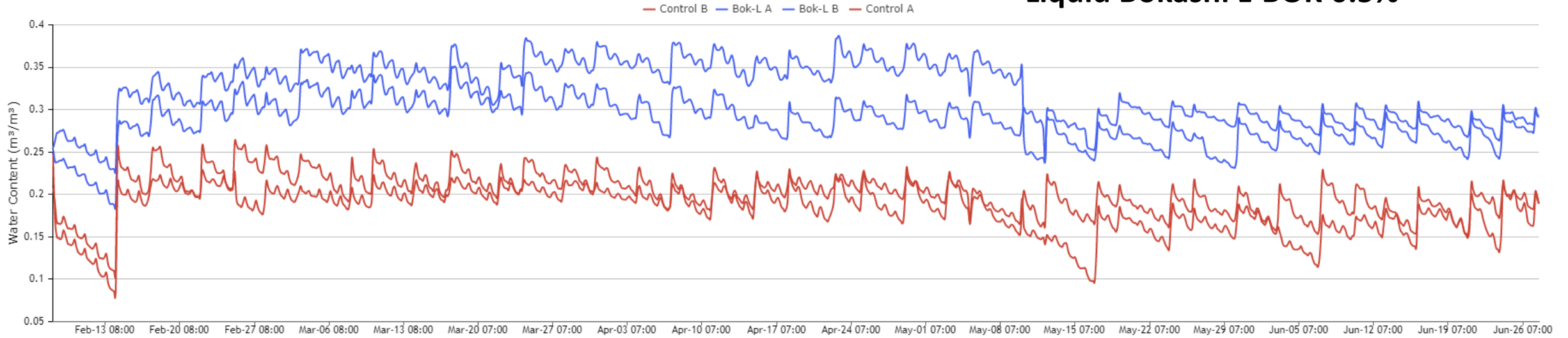
ZENTRA Cloud



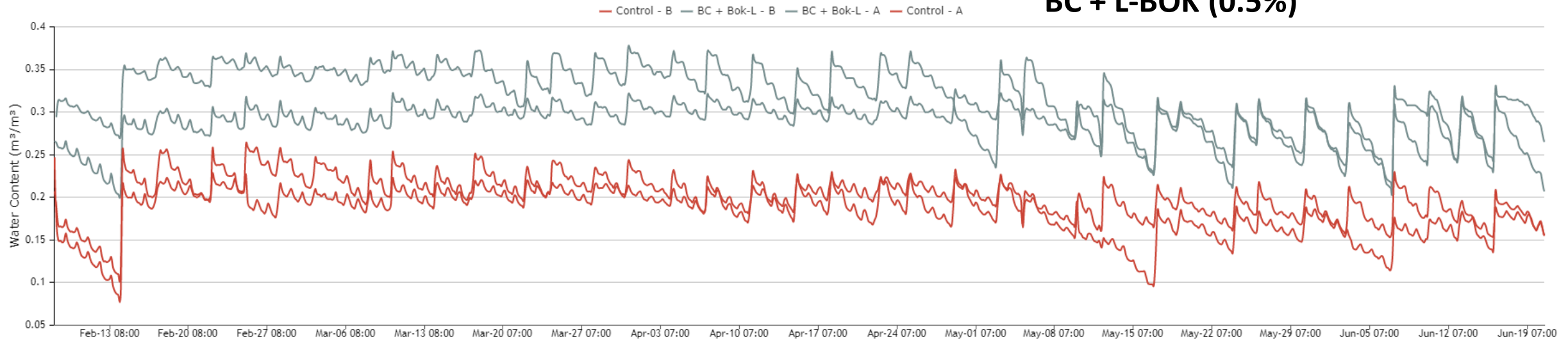
## ZL6 Cellular Data Logger



## Control Liquid Bokashi L-BOK 0.5%

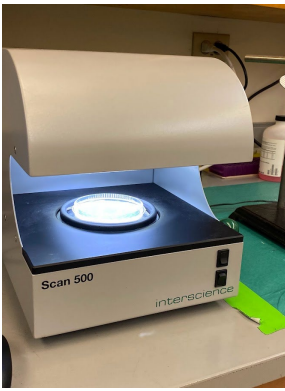
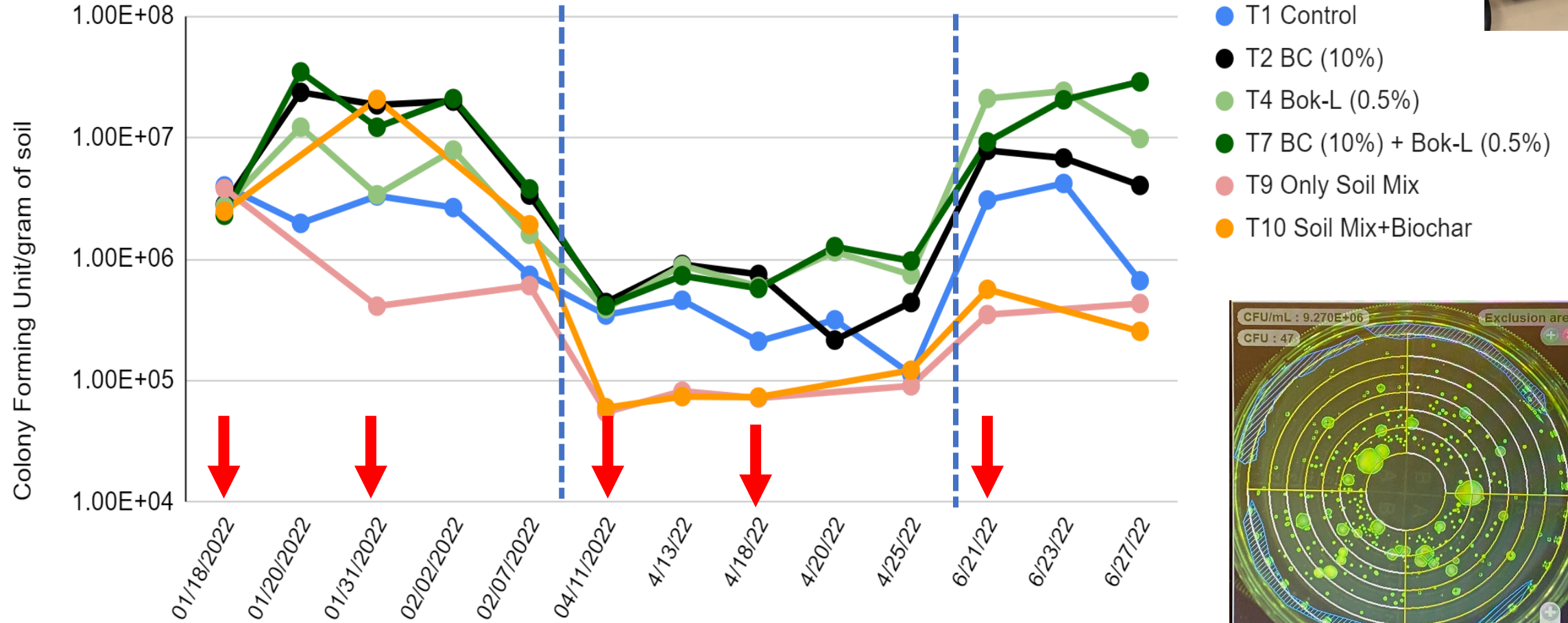


## Control BC + L-BOK (0.5%)

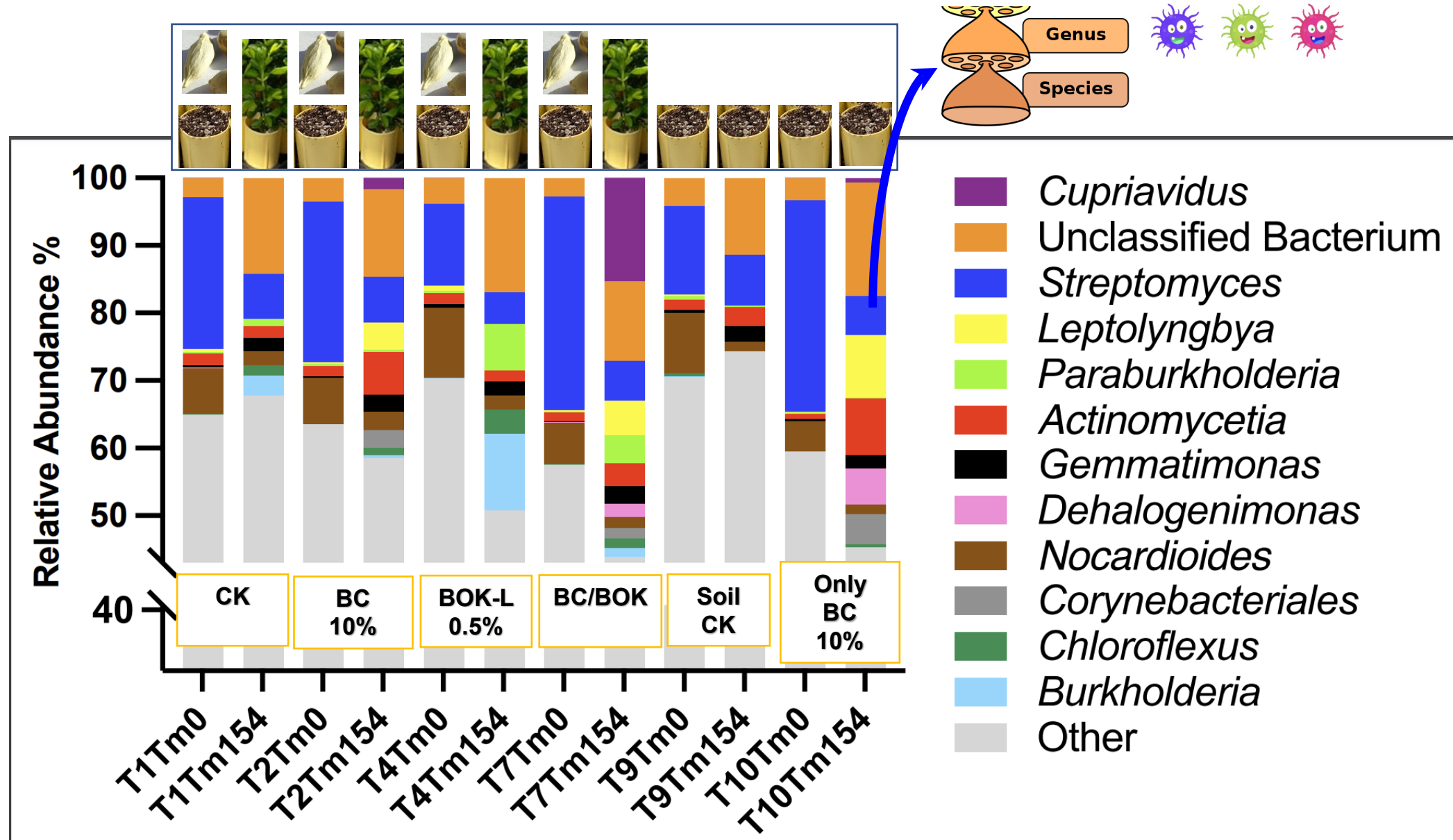




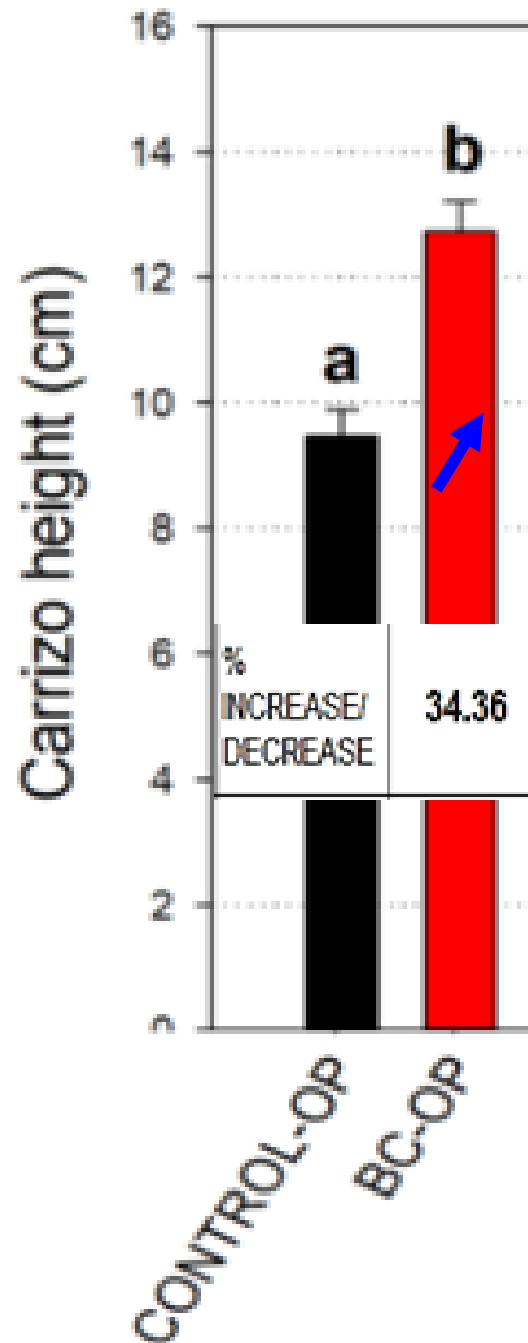
## All CULTURABLE Soil Bacteria Experiment 1



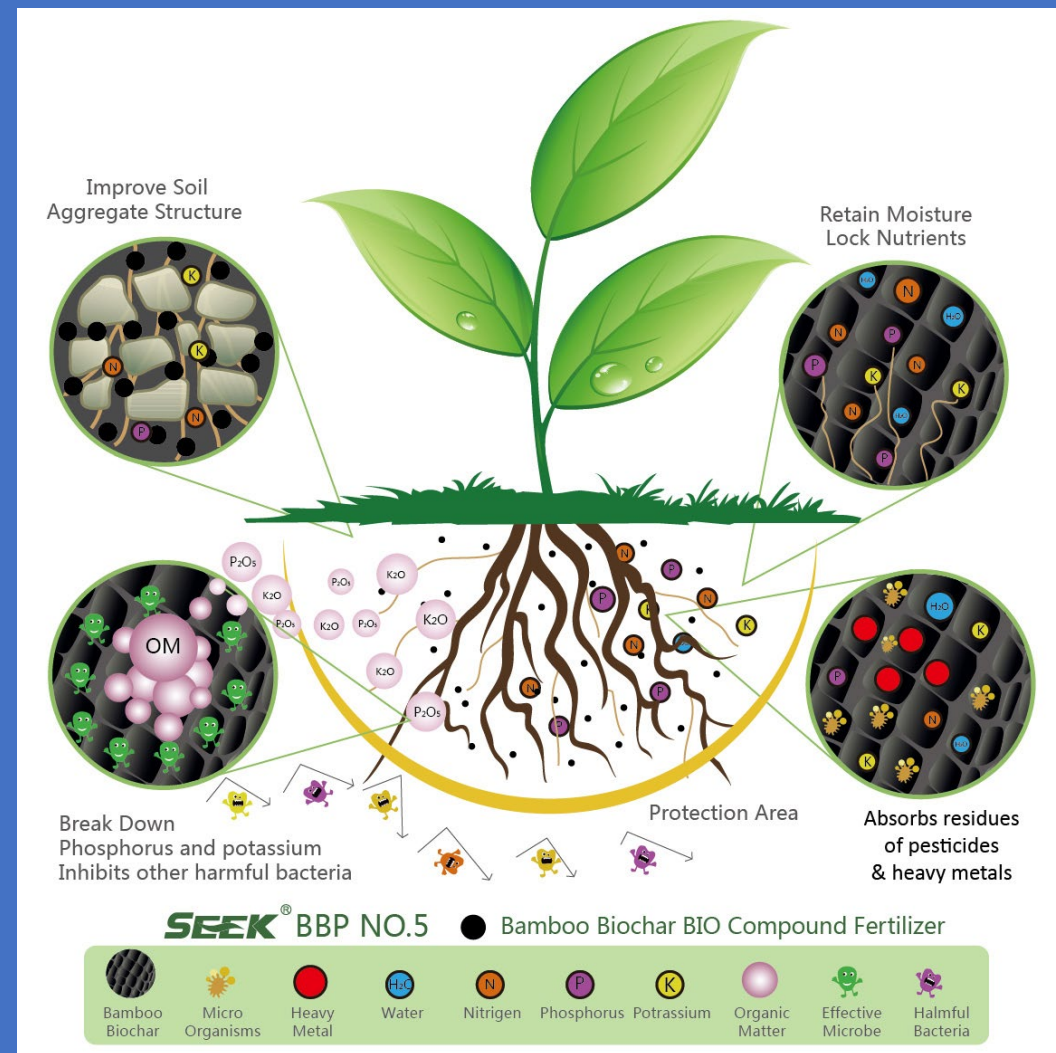
# BACTERIAL DYNAMICS ON SOIL SEEDED WITH CARRIZO





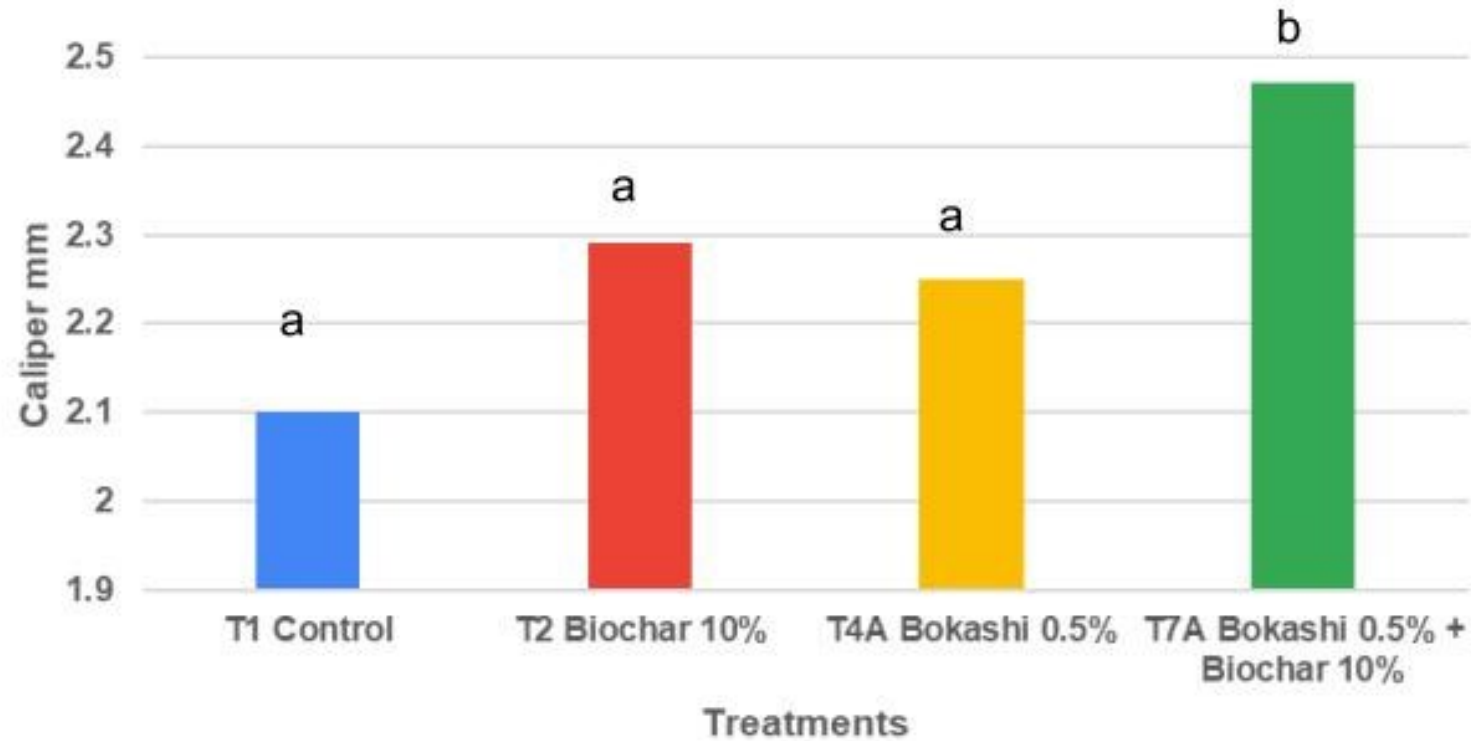


10% biochar alone has a significant positive effect on many plant growth parameters, including plant height, seeds germinations, and caliper



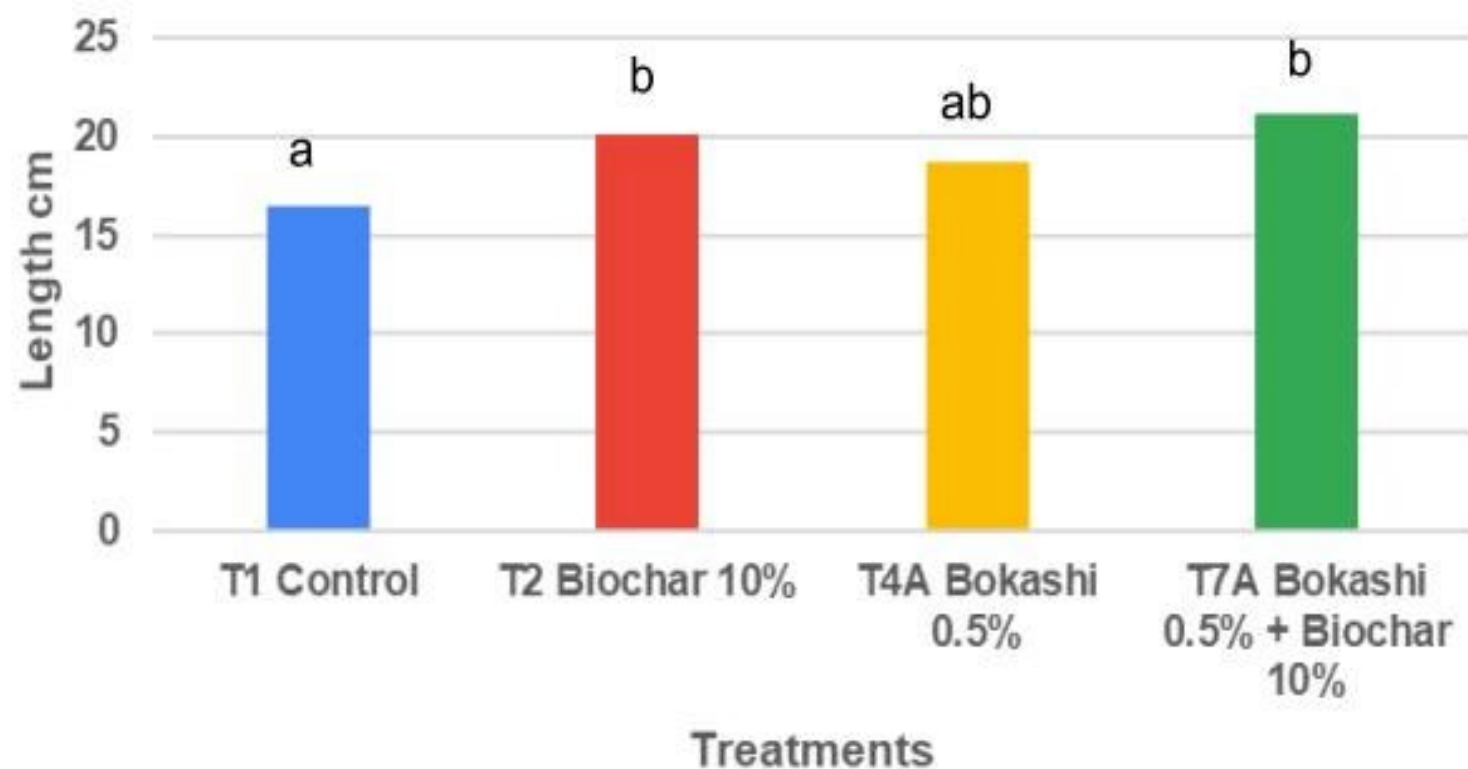
<https://biochar.co.nz/images/BBP-NO.5-Diagram.pdf>

## January 18, 2022 Experiment 1 Stem Diameter CAR Tm 168 Days



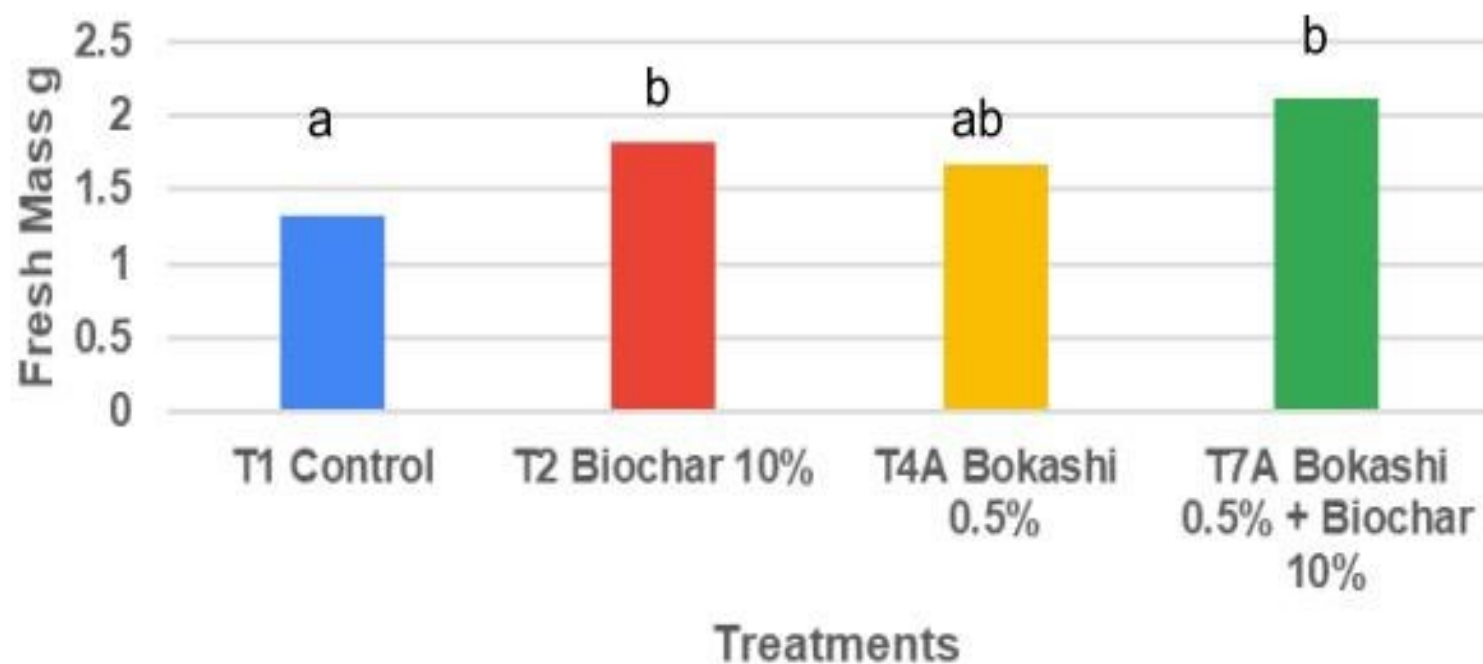


# January 18, 2022 Experiment 1 Top Length CAR Tm 168 Days



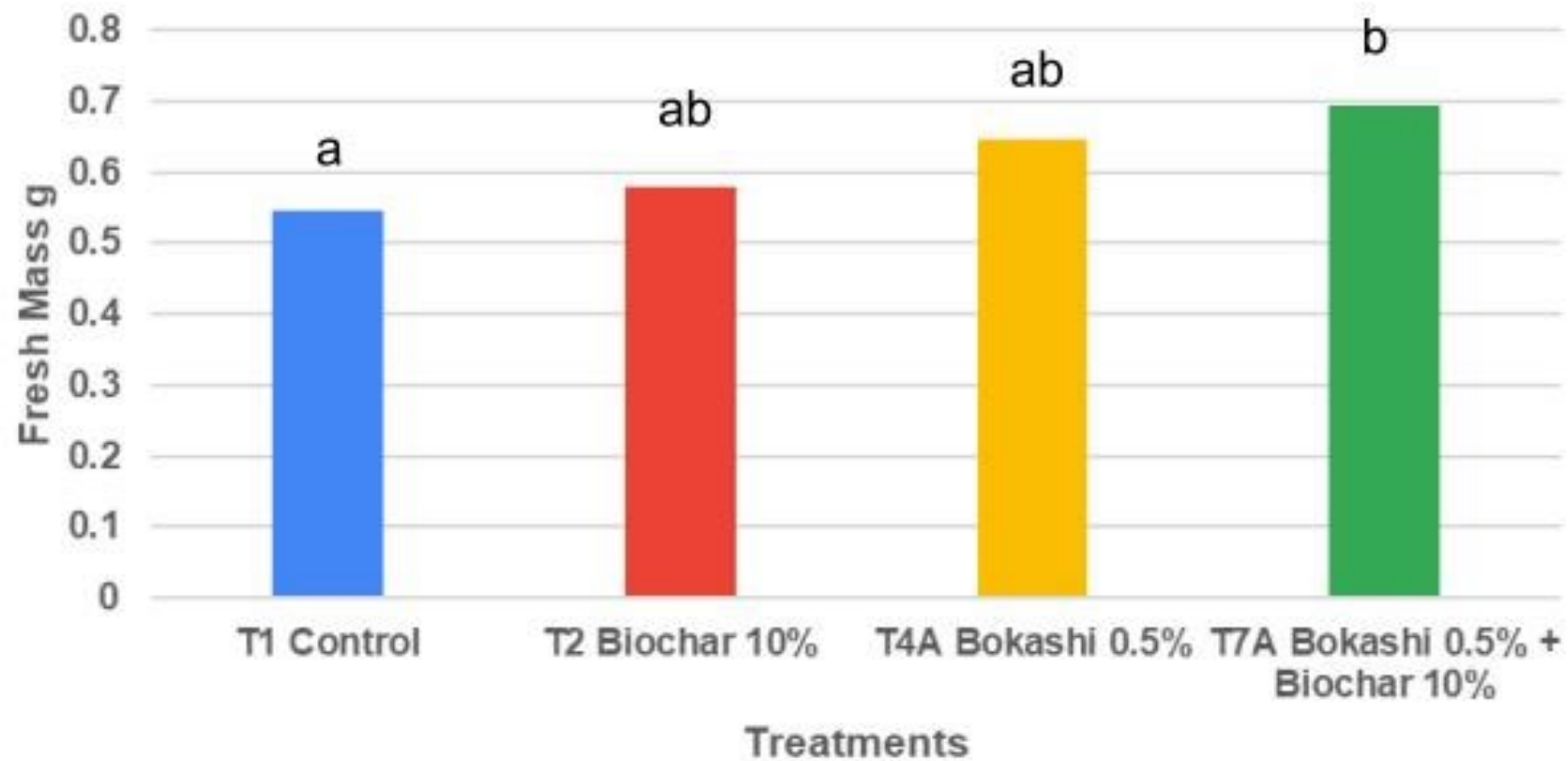
# January 18, 2022 Experiment 1

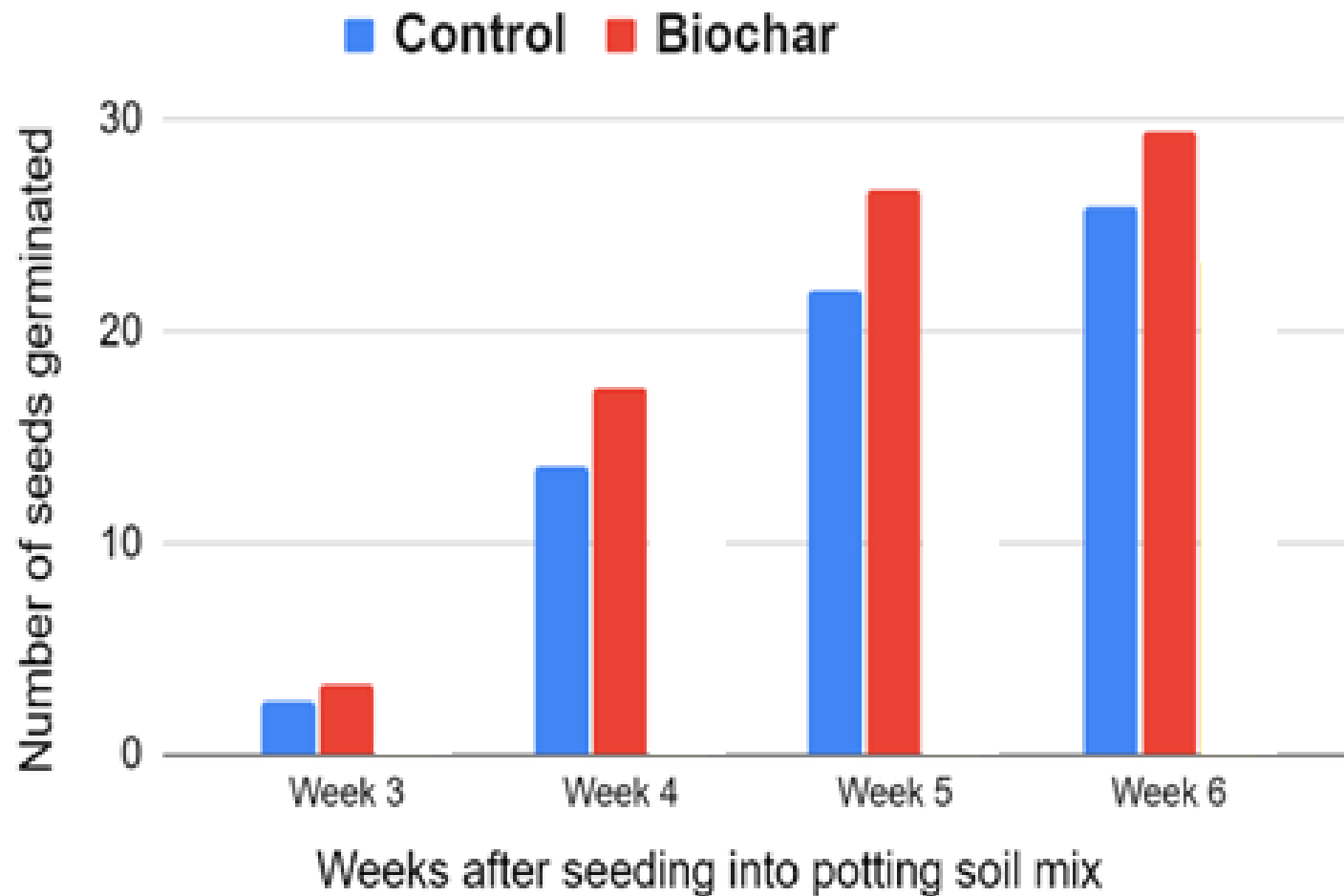
## Fresh Top Weight CAR Tm 168 Days





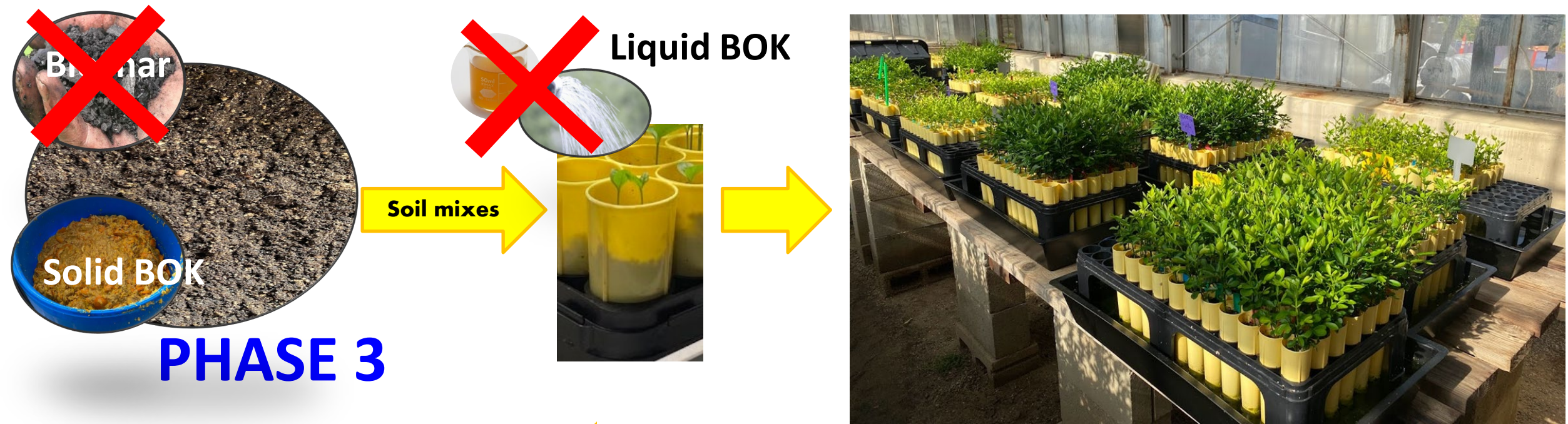
# January 18, 2022 Experiment 1 Fresh Root Weight CAR Tm 168 Days





10% biochar has a positive effect on many plant growth parameters including seeds germinations





For the **3<sup>rd</sup> phase** we are investigating under greenhouse conditions:

1. C, N, and C/N ratio dynamics.
2. Microbiome changes over time during in soil and root in the first 3 months.
3. Impact on plant growth parameters and stress resilience



Use deionized water	TREATMENTS	OPEN IRRIGATION	Cones per Rep	# Replicates (i.e. RACKS A, B, C)
Full Fertilizer dose 1400 uS/cm	Control	T1	<div>Wed, May 25 University of California, Riverside</div>	
Full Fertilizer dose 1400 uS/cm	Bokashi sprinkles directly	T2		
Full Fertilizer dose 1400 uS/cm	Bok Solid 6.25%	T3		
Full Fertilizer dose 1400 uS/cm	Bok Solid 12.5%	T4		
Full Fertilizer dose 1400 uS/cm	Bok Solid 25%	T5		
Half fertilizer dose 700 uS/cm	Control	T6		
Half fertilizer dose 700 uS/cm	Bokashi sprinkles directly	T7		
Half fertilizer dose 700 uS/cm	Bok Solid 6.25%	T8		
Half fertilizer dose 700 uS/cm	Bok Solid 12.5%	T9		
Half fertilizer dose 700 uS/cm	Bok Solid 25%	T10		
Zero fertilizer dose 0 uS/cm	Control	T11		
Zero fertilizer dose 0 uS/cm	Bokashi sprinkles directly	T12		
Zero fertilizer dose 0 uS/cm	Bok Solid 6.25%	T13		
Zero fertilizer dose 0 uS/cm	Bok Solid 12.5%	T14		
Zero fertilizer dose 0 uS/cm	Bok Solid 25%	T15		





Thu, Jun 9 University of California, Riverside



Zero Fert

Full Fert

$\frac{1}{2}$  Fert



Full Fert

Zero Fert

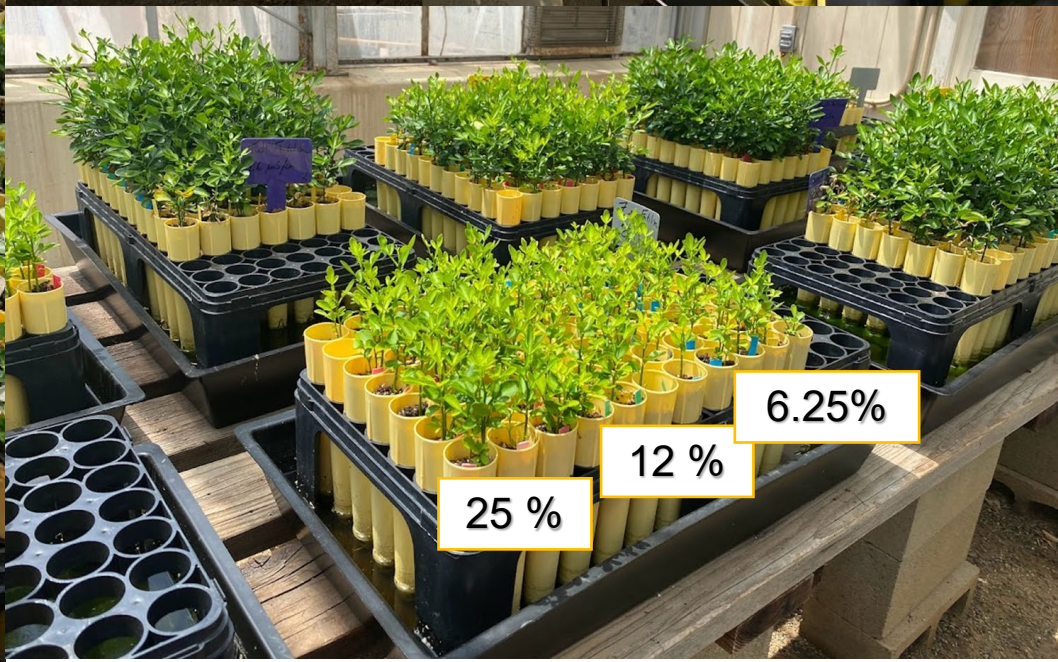
Full Fert

$\frac{1}{2}$  Fert

0%



Mon, Jul 18 Riverside, CA



6.25%

12 %

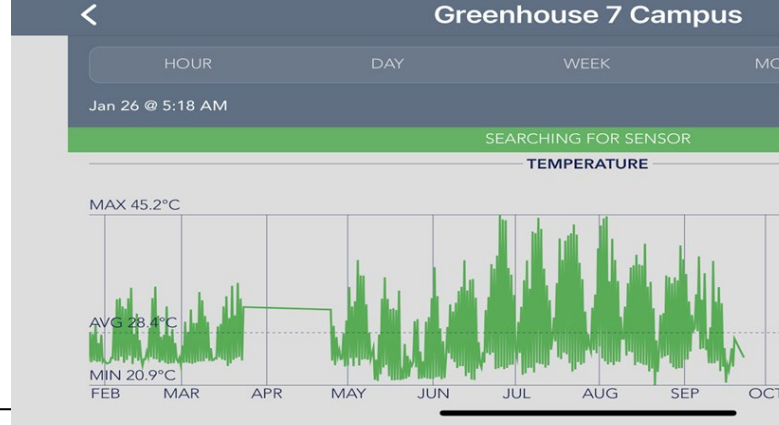
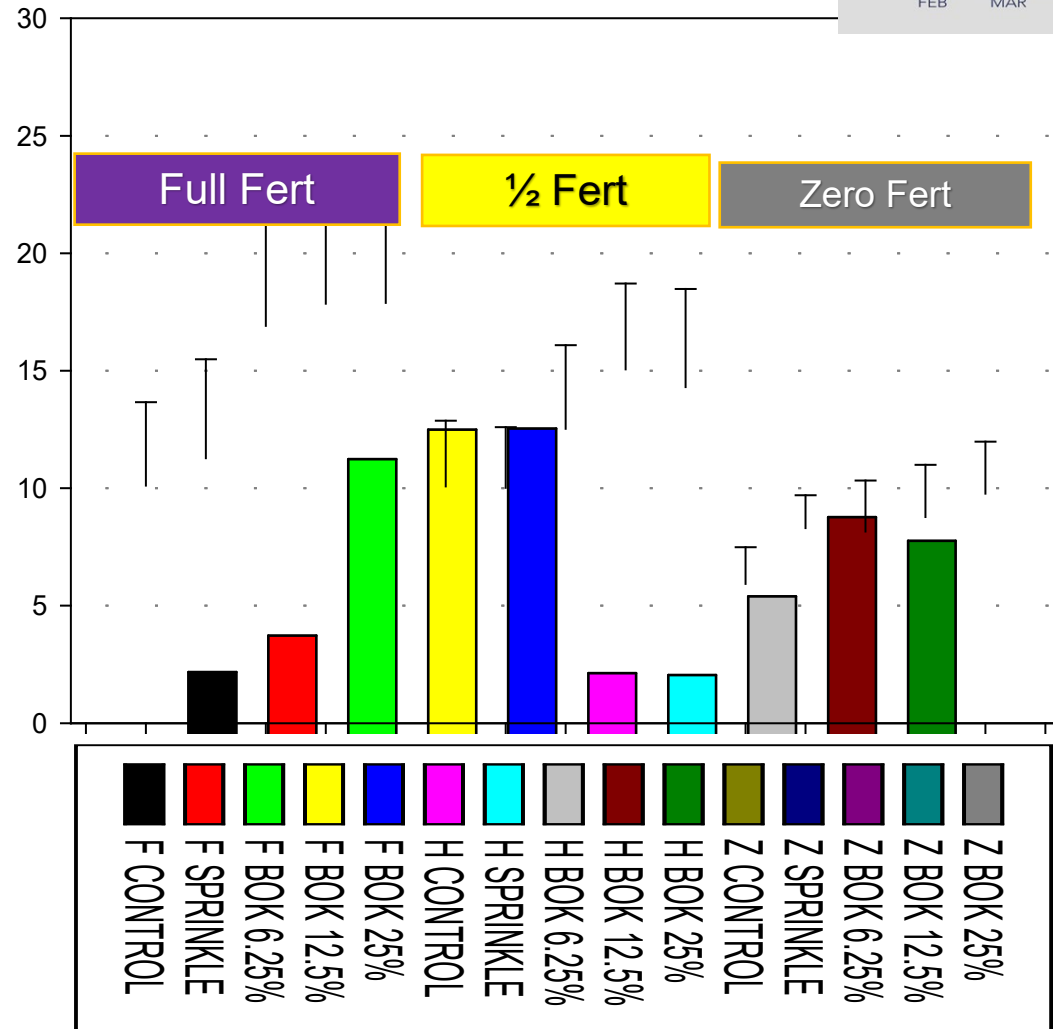
25 %



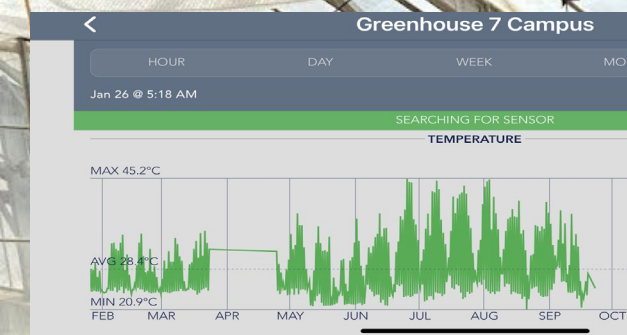
## Bokashi improved

1. plant stress resilience
2. Plant growth compared to control
3.  $\frac{1}{2}$  fertilizer with Bok 12.5% +++

100 Days after seeding







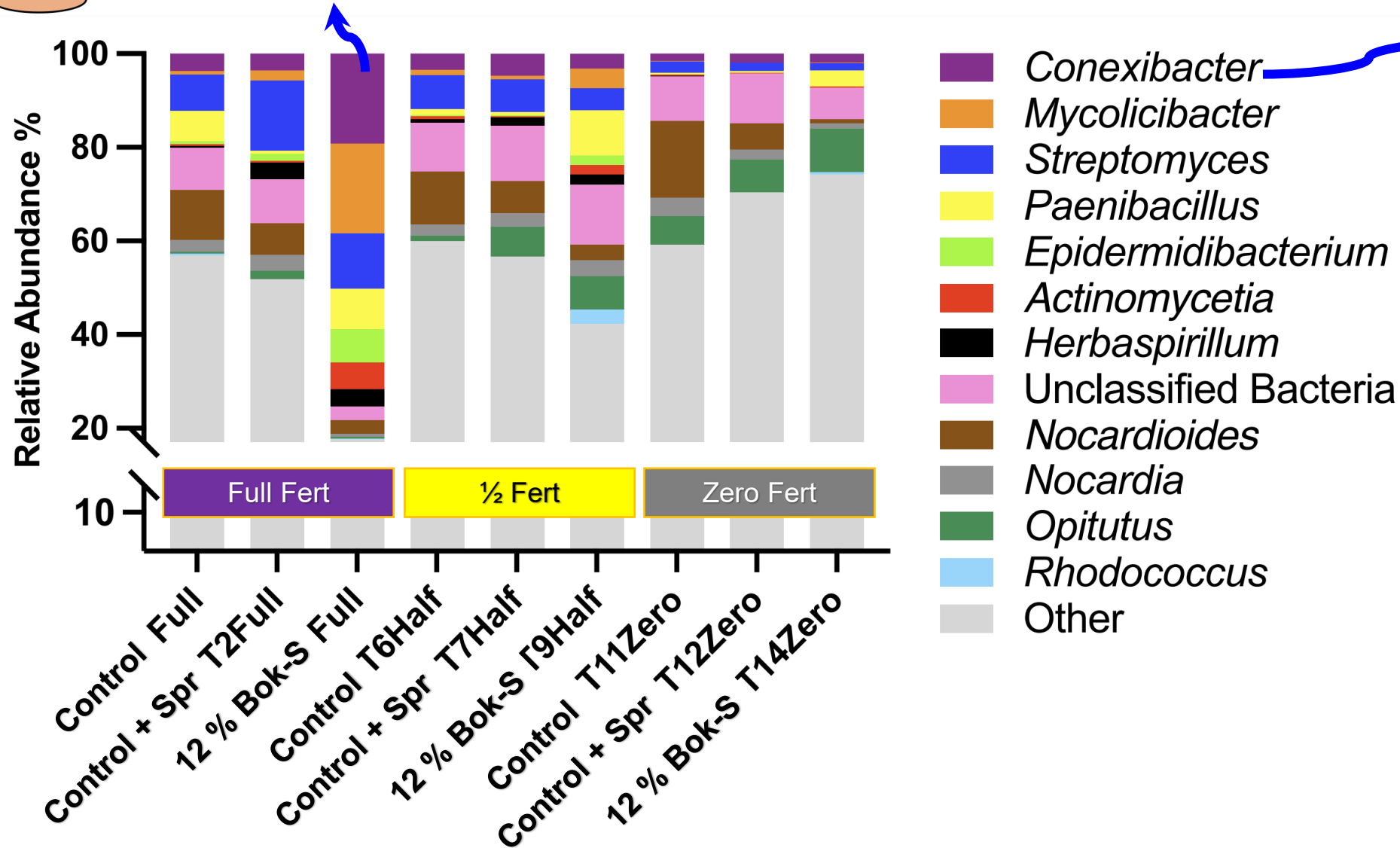
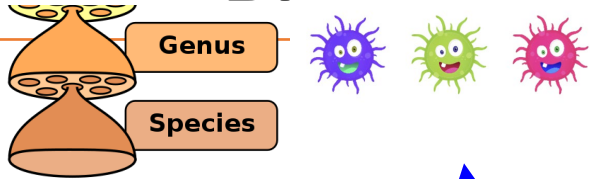
Bokashi improved plant growth

- For at least the first 2 months
- After that additional carbon sources needs to be amended.

Fri, Aug 12 Riverside, CA & 1 more ▾



# BACTERIAL DYNAMICS ON SOIL SEEDED WITH CARRIZO



They contribute to carbon cycling in soil environments and have the potential to also be contributing to nitrogen cycling.

# BACTERIAL DYNAMICS ON CARRIZO ROOT

**edgeRootCarSolid  
Treatments3rdPhasePL2  
Congress**

**COMING SOON!**



NO BIOCHAR

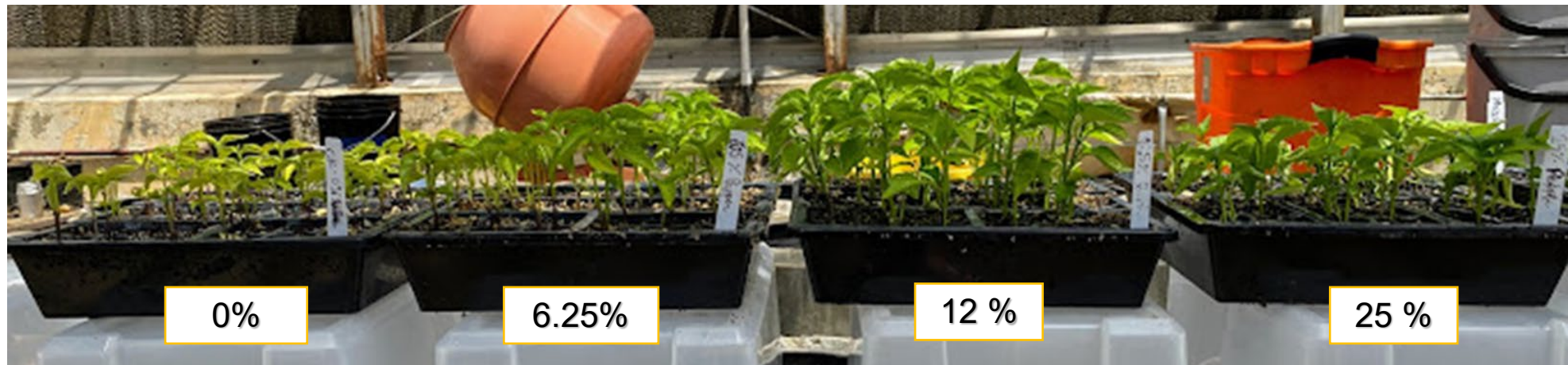


BIOCHAR 10%



Same results on other crops where Bokashi improved plant growth

- BIOCHAR has synergistic effect
- Amendment at around 10-12% seems to have best results.





# Going in the right direction....



Nutrient-rich  
organic matter

Manage C to create a  
dynamics flow of nutrient  
availability and cycling to  
improve soil fertility,  
microbiome and plant health

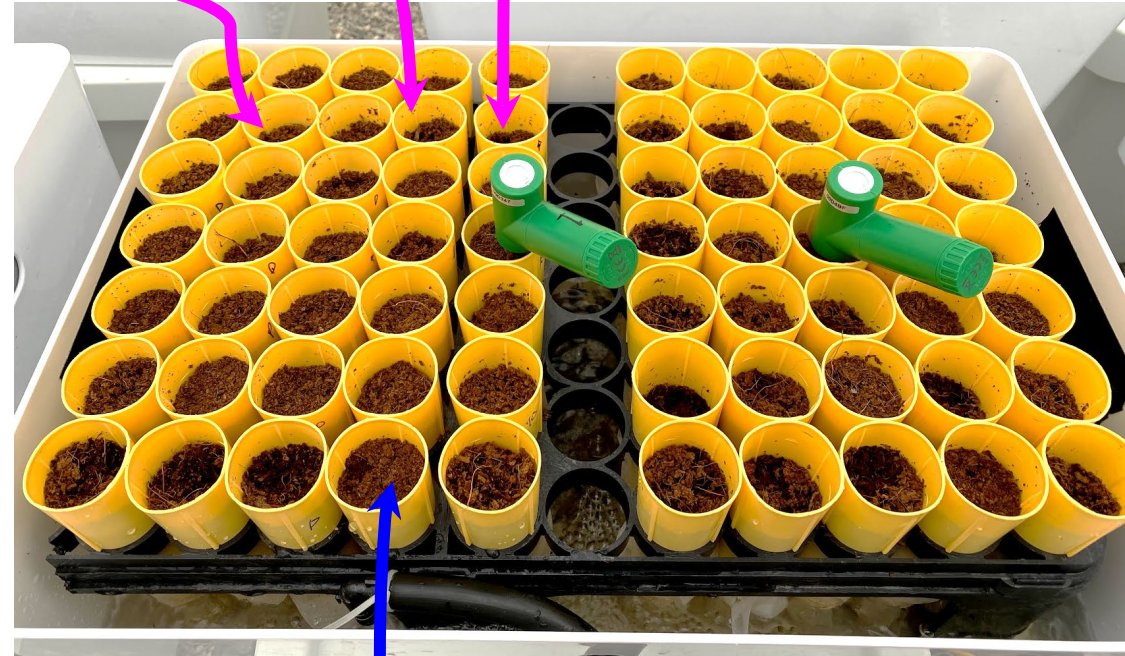


Add stable  
CARBON



The liquid  
MAINTAIN

The solid  
BUILD



Biochar helps to BUILD & MAINTAIN!

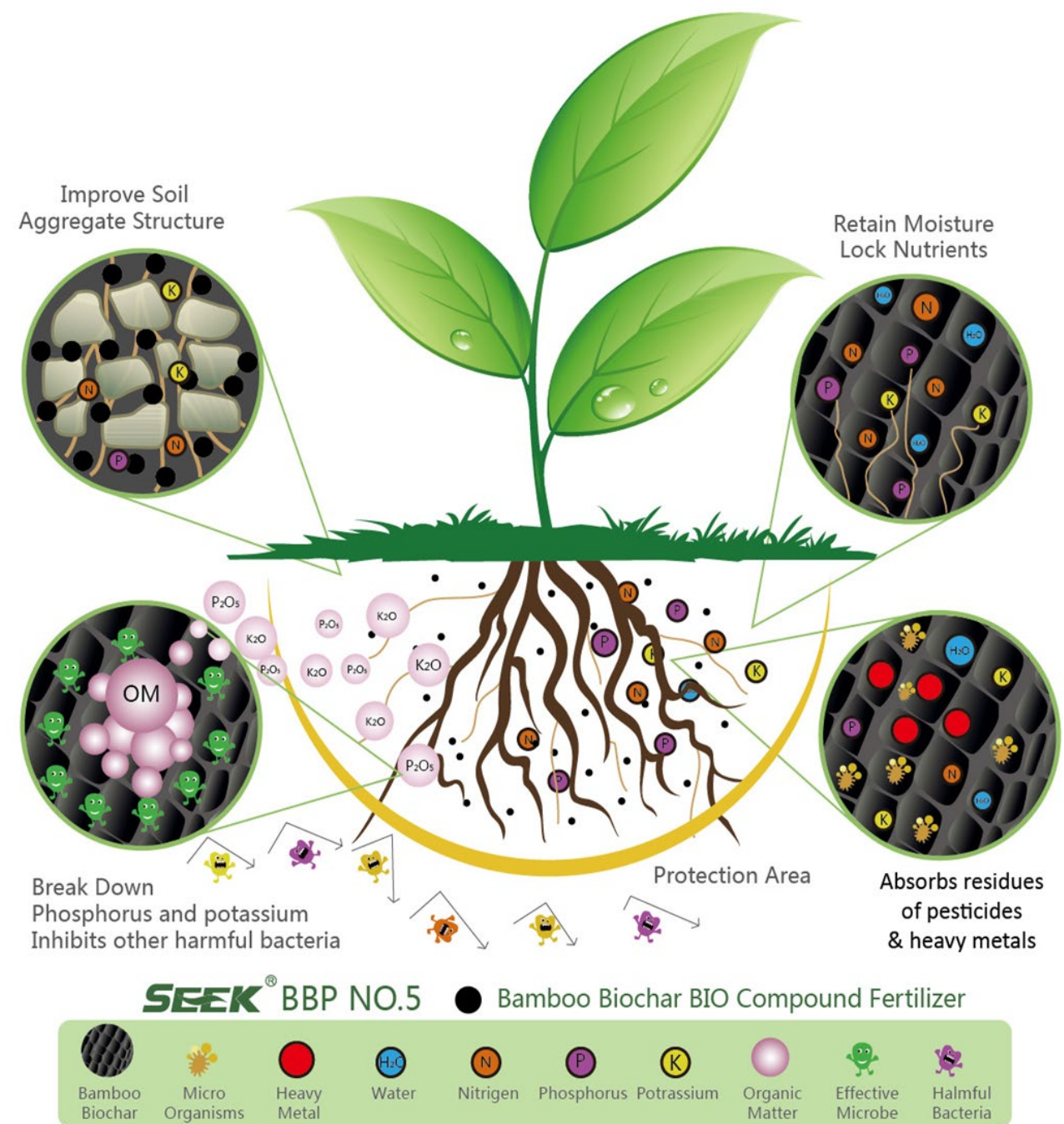


# Drawbacks & Opportunity

- Very promising use of
  - **Biochar at 10% &**
  - **Bokashi at around 10-15%**
- **However, both % can be reduced!**
- **Co-composting**
  - Composting substantially increases the concentration of nutrients in biochar
  - Organic carbon (OC)
  - Microbial biomass carbon ( $C_{mic}$ )
  - Cation exchange capacity (CEC)

Cleaner Run-Off

Greater Fertilizer Utilization Efficiency







Animal wastes



Municipal solid wastes



Sewage sludge



Agricultural residues



Food wastes

Review

The roles of co-composted biochar (COMBI) in improving soil quality, crop productivity, and toxic metal amelioration ☆

Jolo A. Antonangelo <sup>a, B</sup>, Xiao Sun <sup>B</sup>, Hailin Zhang <sup>a</sup>

Thermochemical  
pyrolysis



Biochar

Composting

Co-  
composting

**COMBI:**

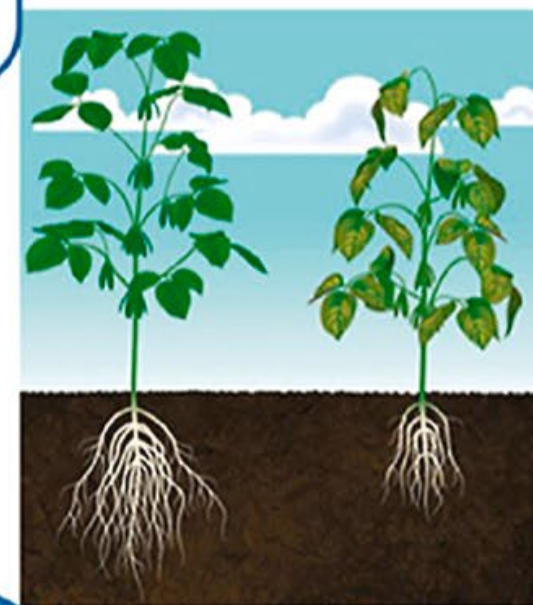
- Enhanced nitrogen content
- Immobilized heavy metals
- Bioavailable minerals
- Reduced  $\text{NH}_3$ ,  $\text{H}_2\text{S}$ ,  $\text{CH}_4$
- Improved microbial activity



Co-composted biochar  
(COMBI)

**Biochar:**

- High surface area
- Porous structures
- Functional groups
- Cation exchange capacity
- Buffering capacity
- Water holding capacity

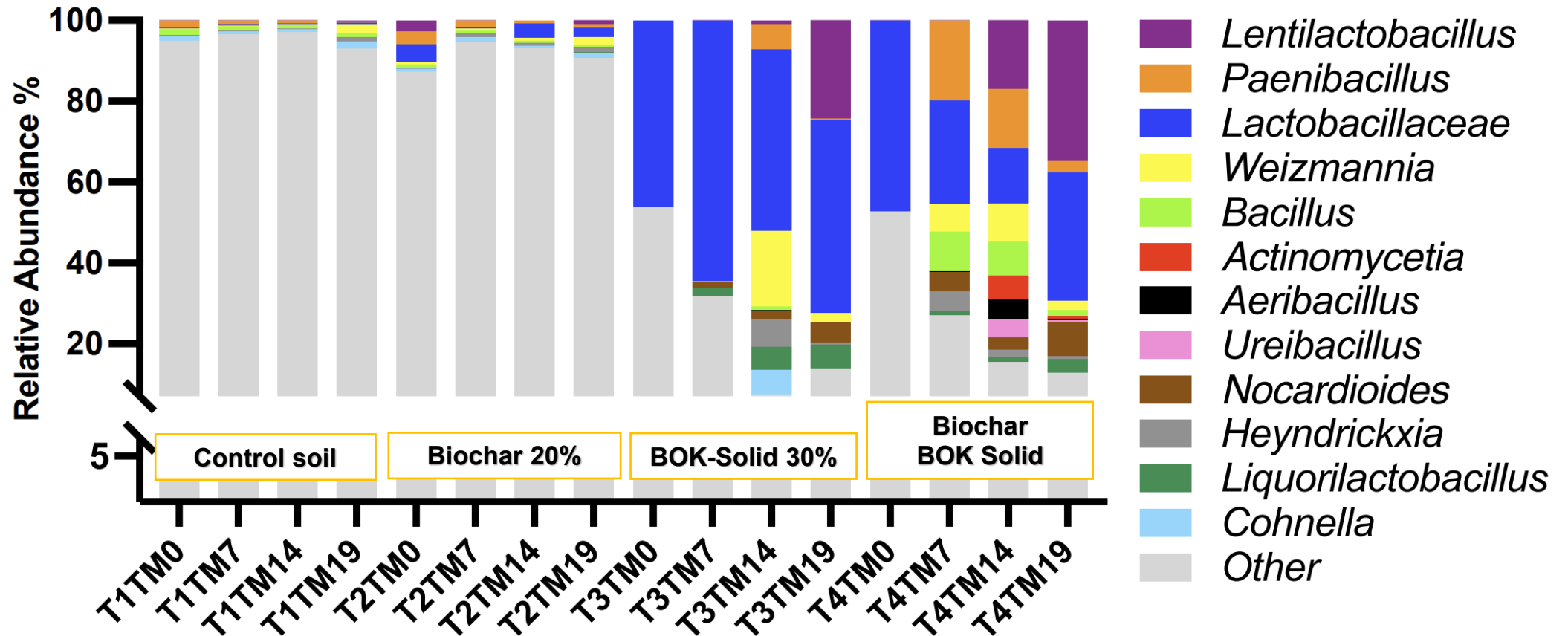


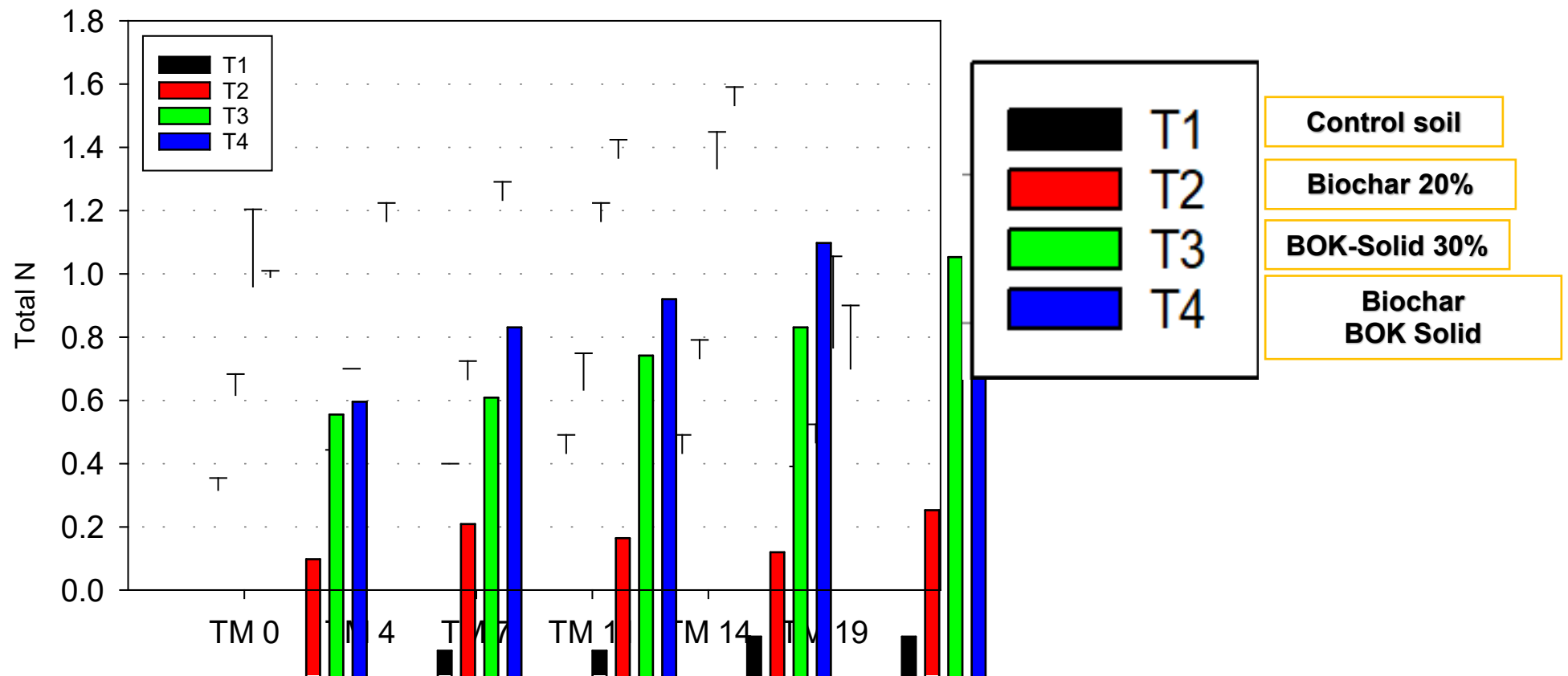
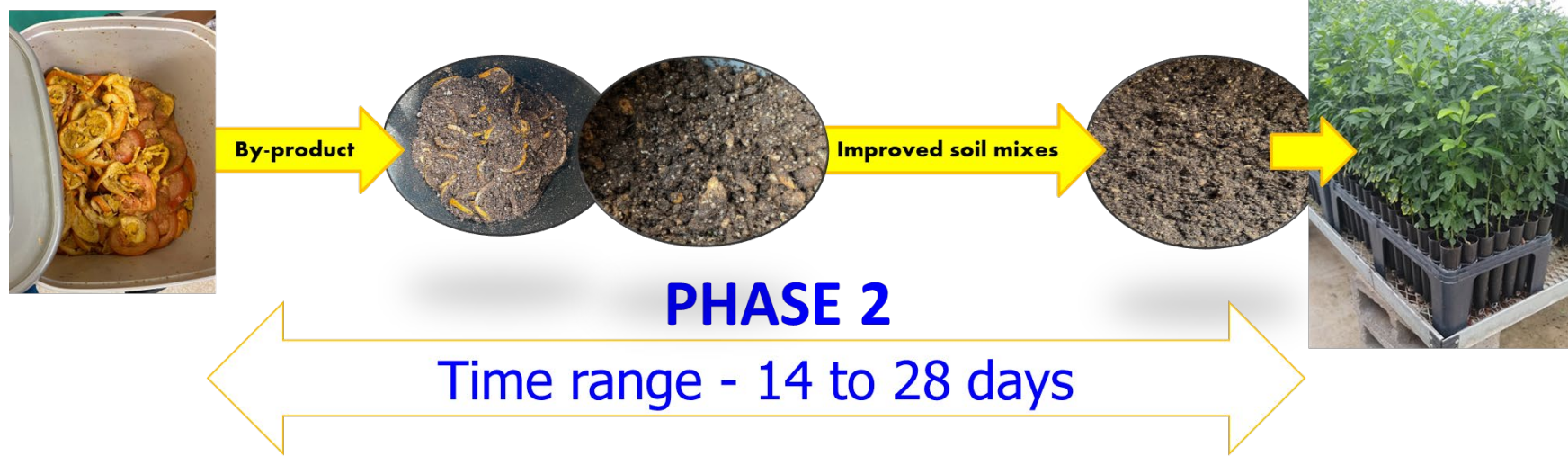
Soil application

**Soil application:**

- Improved soil microbial community
- Improved mineral uptake by plants
- Reduced toxicity from heavy metals
- Improved crop productivity
- Buffering capacity









# Future research on COMBI

Review  
The roles of co-composted biochar (COMBI) in improving soil quality, crop productivity, and toxic metal amelioration ☆

João A. Antonangelo <sup>a</sup>, 吳 健, Xiao Sun <sup>b</sup>, Hailin Zhang <sup>a</sup>

Stability of  
COMBI in soil



COMBI stability is affected by soil type, soil properties, climate, temperature, humidity, precipitation, etc. Their effects on COMBI stability should be further explored.

PTMs  
immobilization  
by COMBI



PTMs immobilization is mainly affected by properties of COMBI: type and mixing ratio of composting materials. Mechanisms of immobilization should be explored.

Production  
optimization  
for COMBI



COMBI quality is affected by biochar feedstock, biochar producing method, particle size, loading rate, etc. These factors should be further optimized for use in soil.

Overall  
benefits to soil  
with COMBI



Soil health and fertility, plant growth and productivity, heavy metal reduction, risk to food chain after COMBI application should be further evaluated.

Comparison of  
COMBI with  
other fertilizer



COMBI should be compared with traditional organic fertilizer (e.g. animal manure) and chemical fertilizer to evaluate its pros and cons.

Environmental  
assessment for  
long-term use  
of COMBI



Long term use of COMBI may build up elements such as N and P in soil, which could negatively impact water bodies. Its potential risk with long-term utilization should be evaluated.



# Scaling up



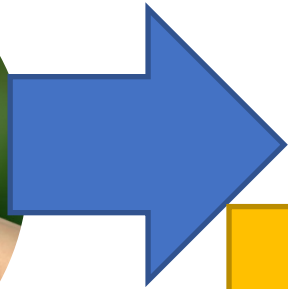




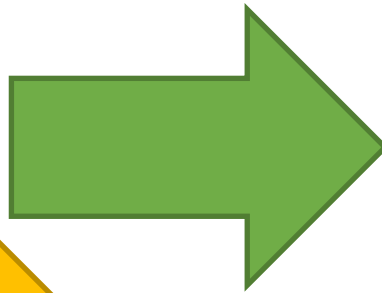
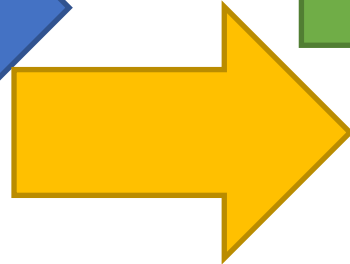
## Short- and long-term benefits for citrus nurseries and growers



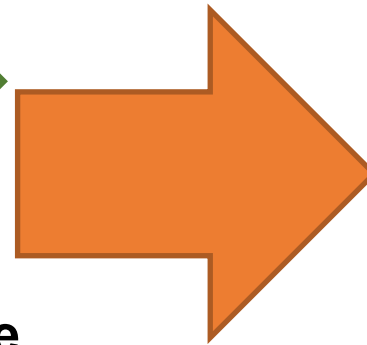
**Biochar &  
Closed  
irrigation  
system save  
water &  
fertilizer**



**Food waste by-  
product improve  
soil organic  
matter- carbon  
and nitrogen  
content, &  
biostimulant**



**Food waste  
by-product  
increase of  
beneficial  
naturally  
occurring  
microbes**



**Plant  
growth  
parameters  
are  
improved**



**Combining biochar, Food  
waste by-products will  
improve nursery production  
of Field-Transplant Ready  
Citrus Plants**



# Thank You

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# Thank You!



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