MICROBIOME OF PLANTS:

What Is on the Horizon?

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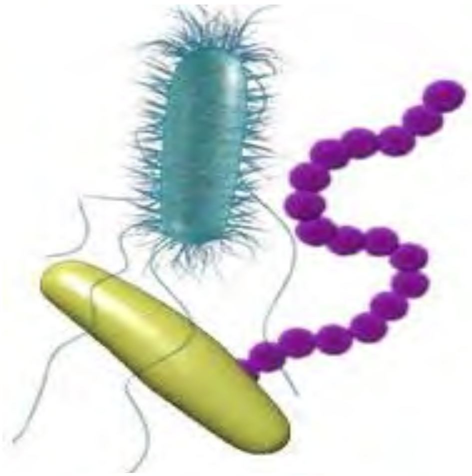
Microbes to MICROBIOMES

Microbome: Two Greek words. **"Micro"** (μικρος) = small and **"biome"** = (βιος) bios or life.



"Microbiome" is **"a** characteristic community of **microorganism"** in a well-defined habitat or a particular environment which has distinct physio-chemical properties as their **"theatre** of **activity"**.

Microbiome

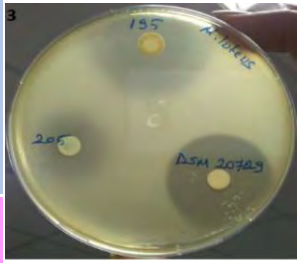
Microbes	+	Theatre of activity
❖ Algae ❖ Archaea ❖ Bacteria ❖ Fungi ❖ Protists		▪ DNA/RNA ▪ Proteins/peptides ▪ Toxins ▪ Polysaccharides ▪ Lipids ▪ Signal molecules

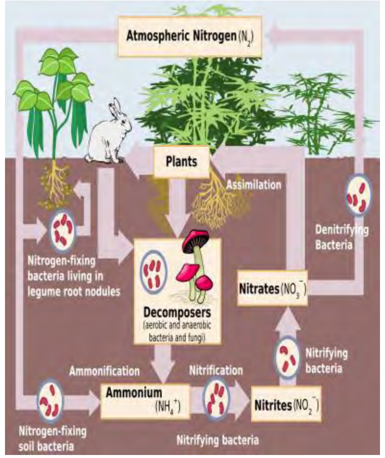
Crop Microbiome
Plant Microbiome
Soil Microbiome
} **Phytobiomes**

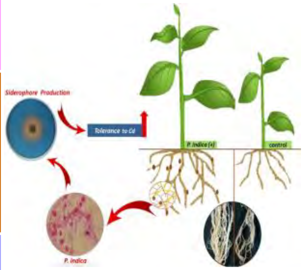
Berg et al. 2020. Microbiome 8:103.

MICROBIOMES’ Impacts on Plants

Interaction	Microbiome	Plant	Examples
Mutualism or Symbiosis	Benefit	Benefit	Rhizobacteria & mycorrhizae
Synergism	Benefit	Benefit	Nitrosomonas (oxidizes ammonia to nitrite) & Nitrobacter (oxidizes nitrite to nitrate)
Antagonism	Harmed	Benefit	Antibiotics, hormones etc. producing bacteria
Parasitism or Pathogenic	Benefit	Harmed	Compete for nutrients
Mycoparasitism	Benefit or harmed	Benefit	*Trichoderma* (fungus-fungus interactions) or fungus – nematode interactions







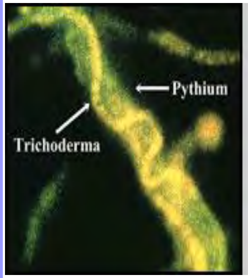
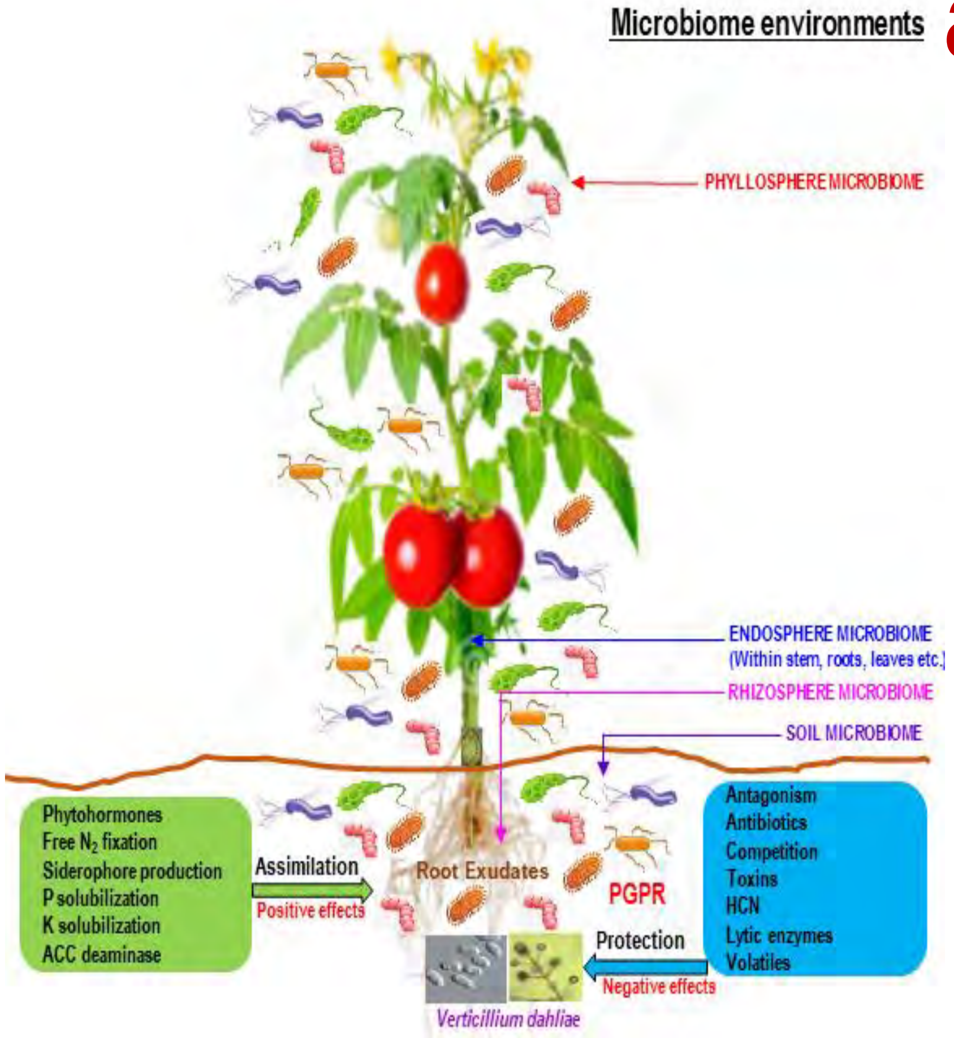


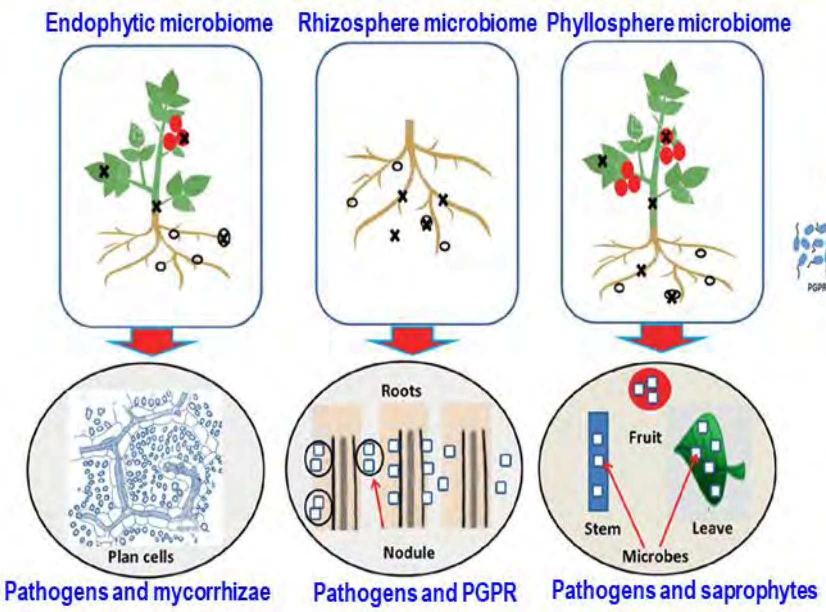
Fig 1: Symptoms of early blight with diagnostic concentric rings (L), near-complete defoliation of non-sprayed plants (M) and stem-end lesion on fruit (R).

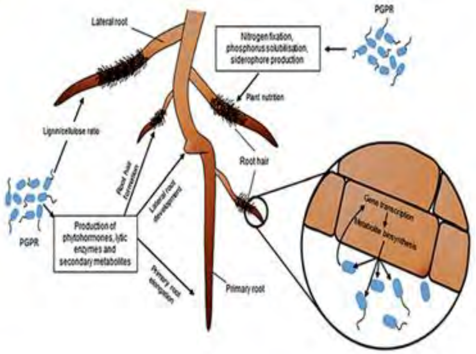
Microbial Ecology, 2021. Environmental Microbiology, 2005. Biotechnology Journal,2017.

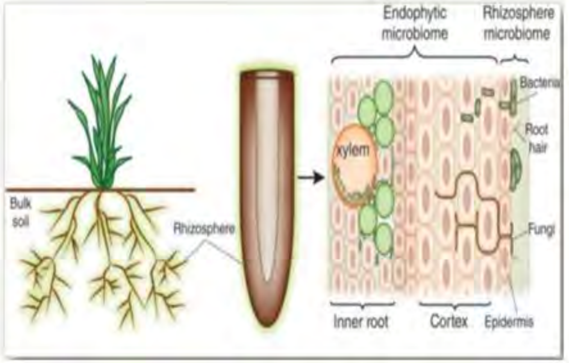
MICROBIOMES

Assemblages of microbes living in, on and around plants



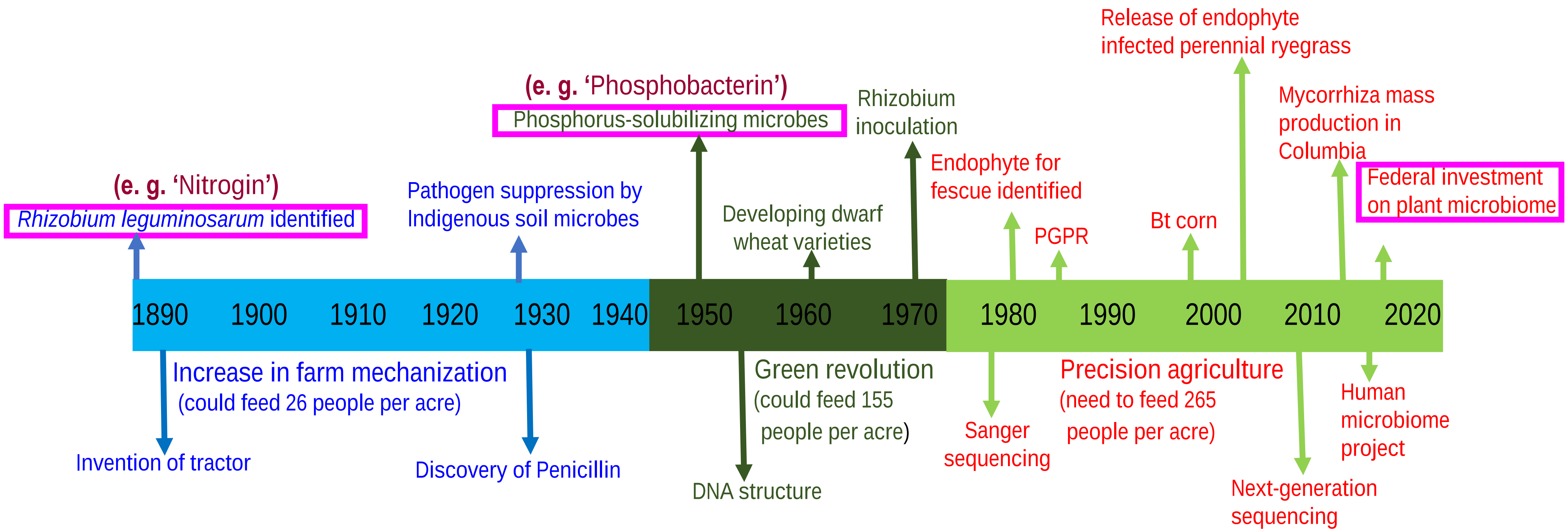






Acharya et al. 2021. Plants.
Hirsch & Mauchline . 2012. Nature.
Lundberg, D.S. et al. 2012. Nature.

Agricultural Revolution



Historical Use of Microbiomes to Improve Crop Yields

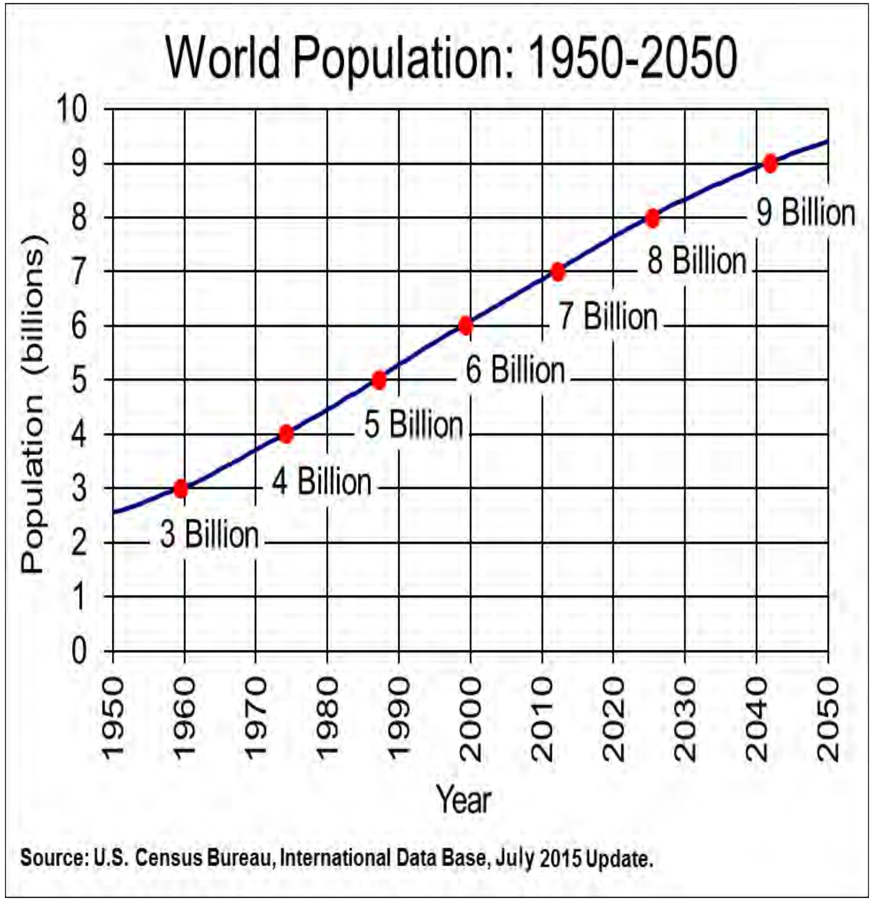
1910s: Use of nitrogen-fixing bacteria (Rhizobia) to soils.

2000s: Use of fungi and bacteria to protect plants from pathogens insects, nematodes, weeds, and drought.

CAST, 2019.

Global Demand and Consumption of Agricultural Crops for Food

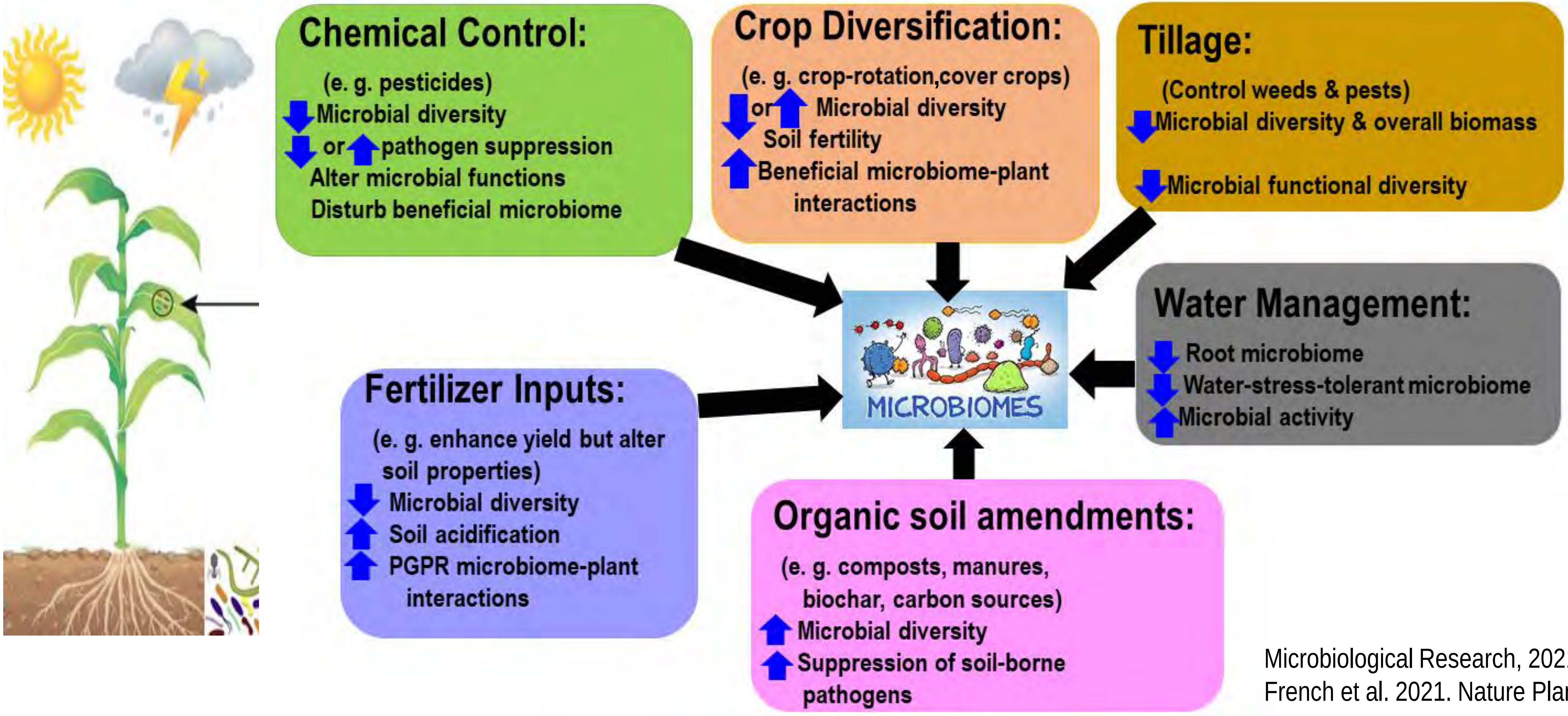
Increasing population growth ➡ Increasing global demands for food + Changing climate ➡ Increasing demand for plant productivity



Harnessing **plant microbiomes** has tremendous potential to improve plant production by improving nutrient uptake, enhancing tolerance to environmental stress, and providing protection against pests and diseases is the **next regenerative agriculture revolution.**

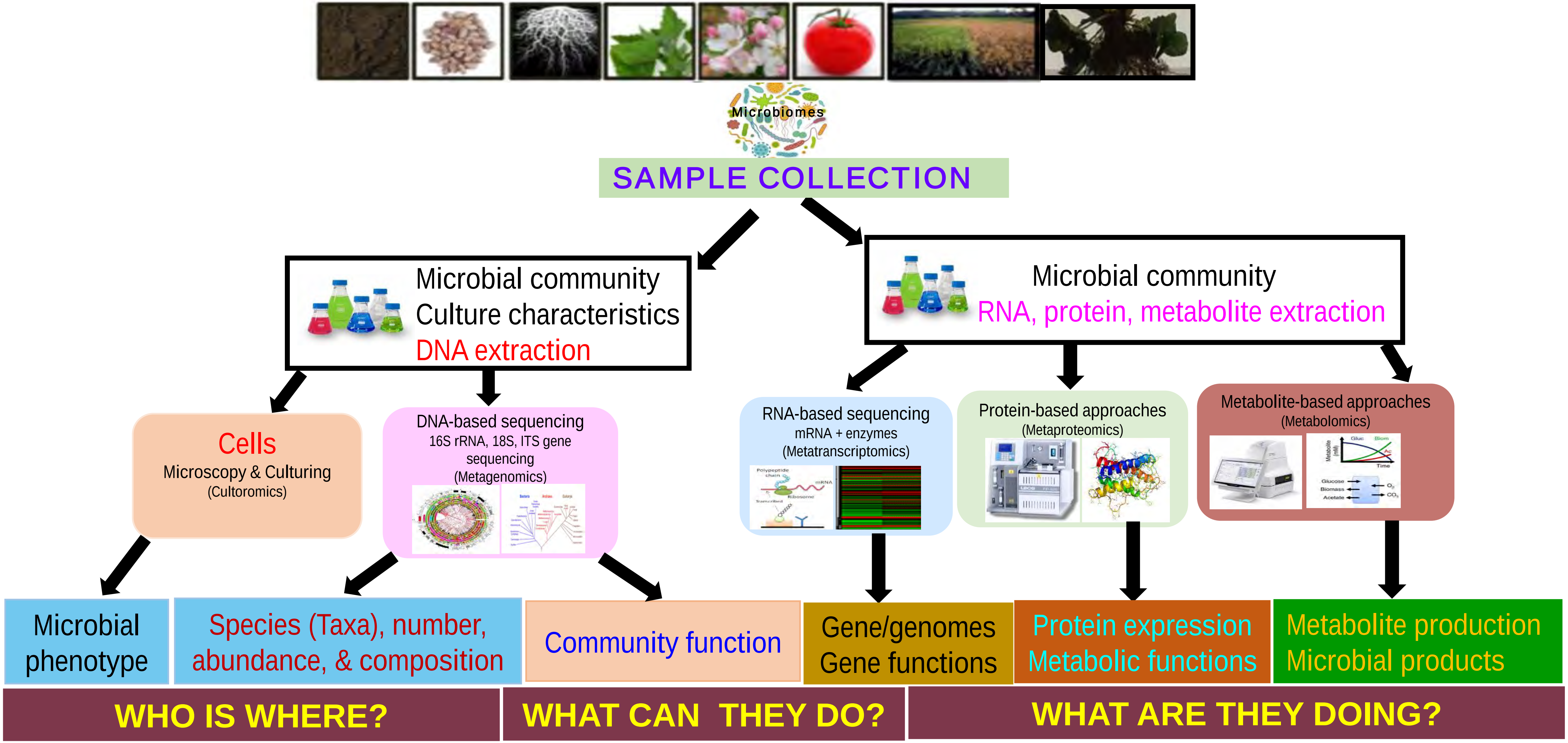
Phytobiomes Journal, 2021.

Effects of Management Practices on Plant MICROBIOMES



Microbiological Research, 2021.
French et al. 2021. Nature Plants.

How to Assess Plant MICROBIOMES?

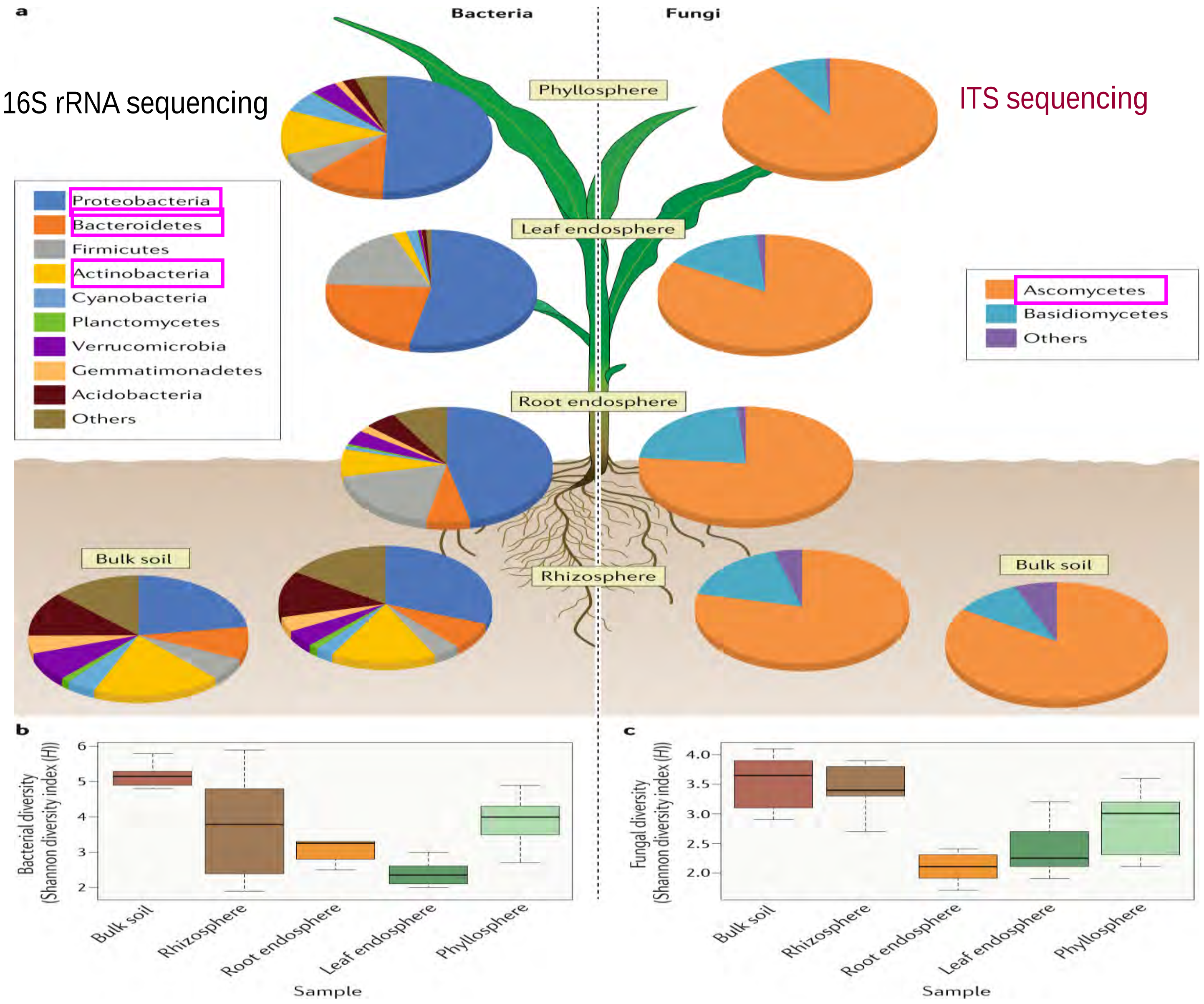


Acharya et al. 2021. Plants; Phytobiomes Journal. 2021. 5:249-262.

Who Are There ?

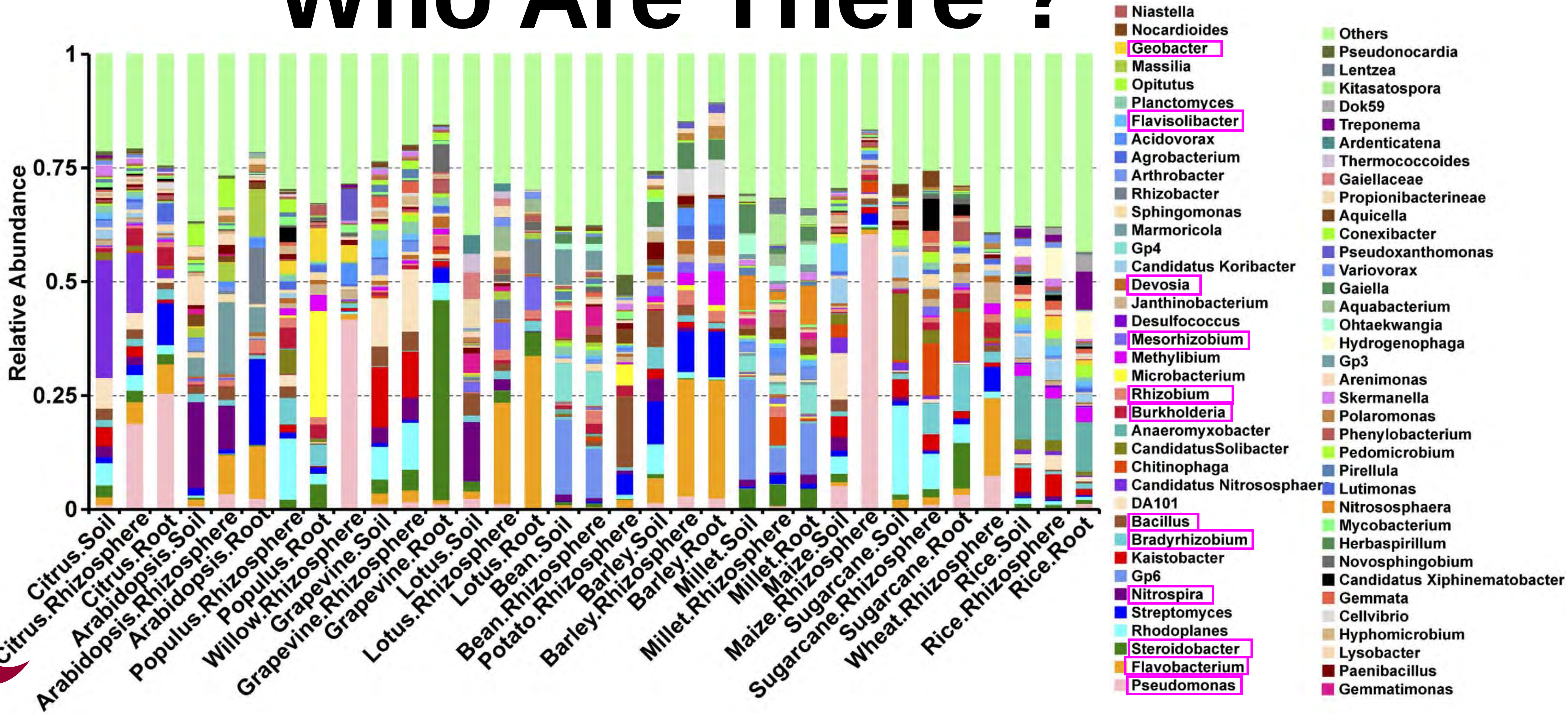
Bacterial & Fungal communities

Census of Microbial Communities on Different Tissues of Plant



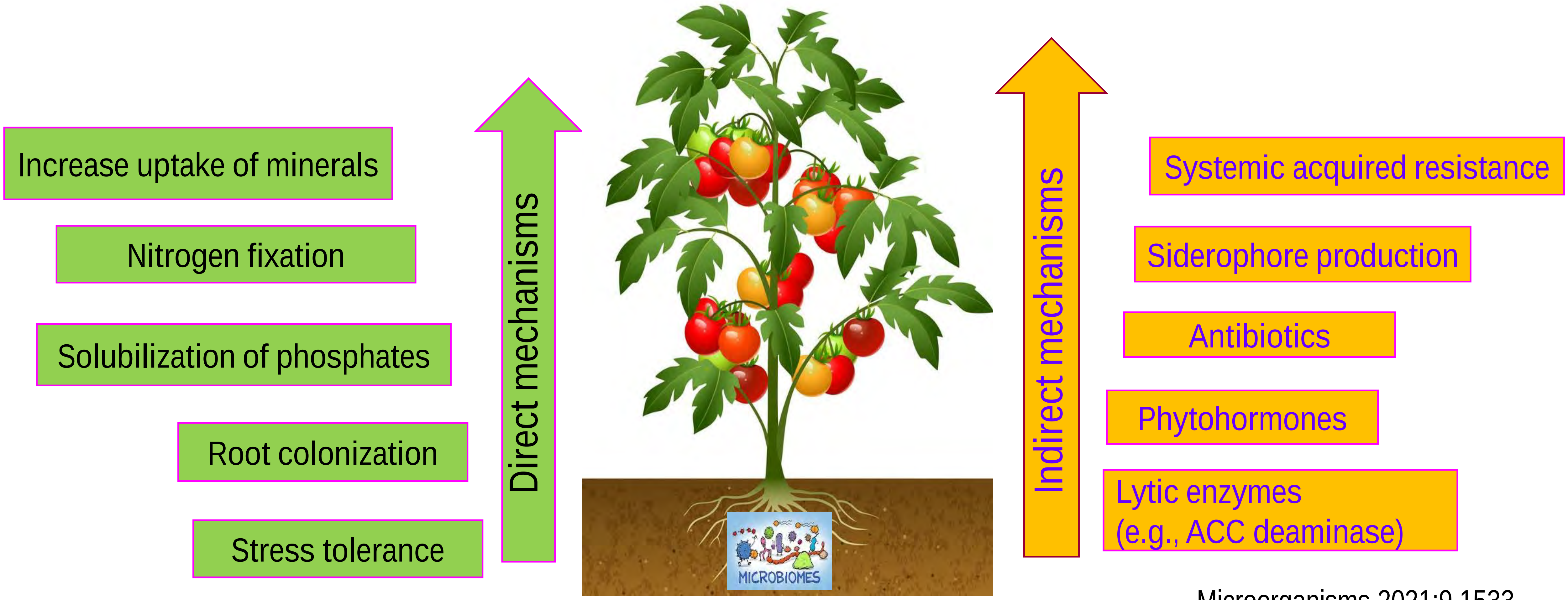
Nature Reviews Microbiology, 2020:18:607-621.

Who Are There ?



Taxonomic distribution of plant root-associated microbiomes of 15 plant species at the genus level estimated by 16S rDNA sequencing. Phytobiomes Journal. 2021. 5:249-262.

Direct and Indirect Beneficial Effects of MICROBIOMES on Plants



Microorganisms 2021:9,1533.

Endophytic Bacterial Communities

Can Plant Select **MICROBIOMES ?**

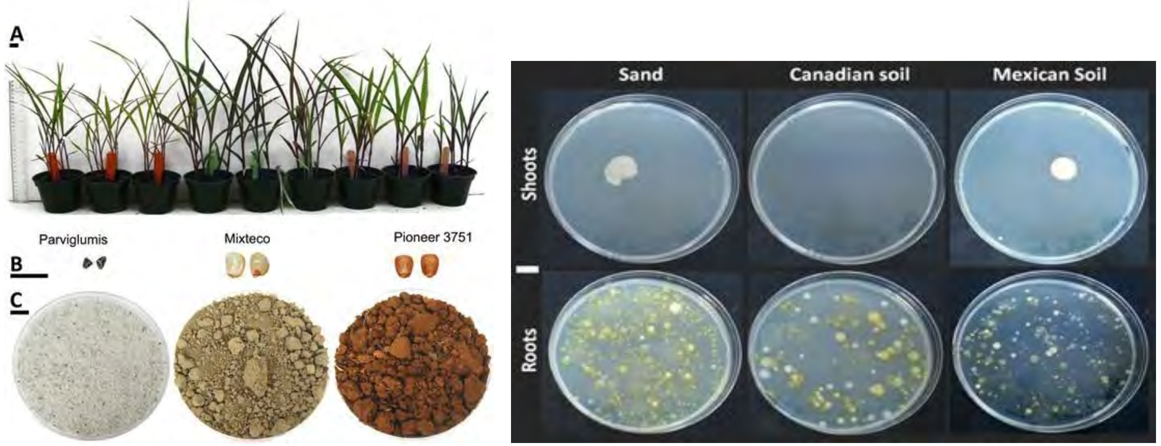
A. Three maize genotypes (pre-domesticated or wild, ancient landrace & modern hybrid).

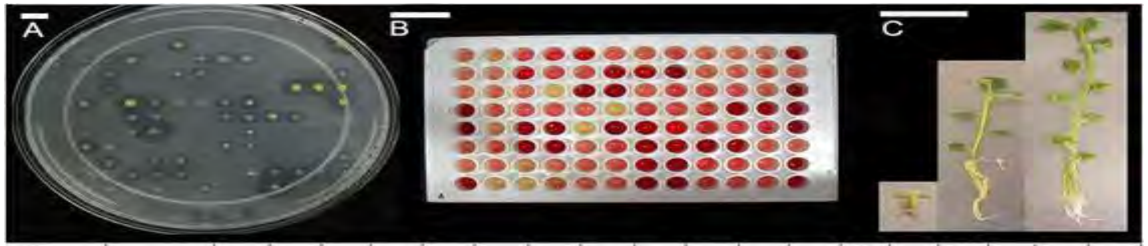
B. Seeds (Parviglumis, Mixteco & Pioneer 3751).

C. Soils (Sterilized sand, and non-sterilized Canadian soil & Mexican soil).

D. Analyzed 17 functional traits of 124 bacterial endophytes cultured from maize plants.

In Table, isolates were scored as either having activity (1) or not (0). Thus, numbers indicate the number of isolates that express the trait noted. Light yellow = <25% isolates exhibited; Deep yellow = 25-50%; Orange = 50-75%, and red = 75-100%.





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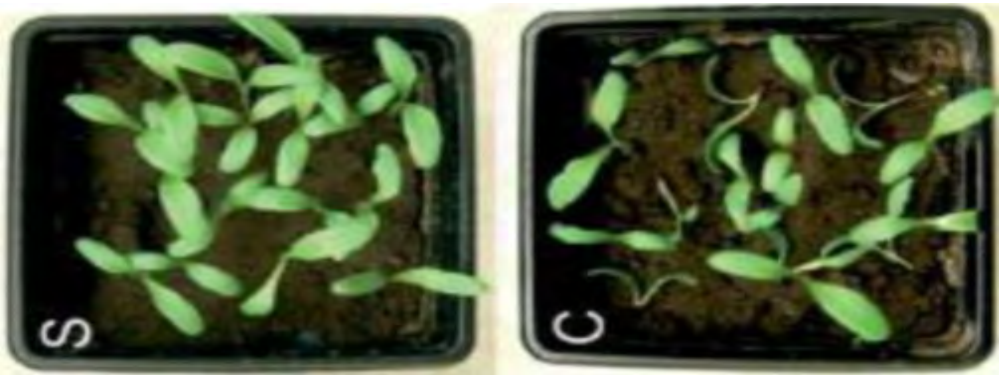
Soil Treatment	*Zea* seed type	Total Isolates Tested	Stimulates Shoots	Stimulates Roots	Stunts Shoot	Stunts Roots	Increases Root/Shoot	Decreases Root/Shoot	Growth on N Free Media	ACC Deaminase Activity	Solubilizes Phosphate	Secretes RNAse	Produces Acetoin	Produces Indolic Compounds	Secretes Siderophores	Secretes Pectinase	Secretes Cellulase	Antagonize *F. graminearum*	Antagonize *A. flavus*
Sand	Parviglumis	[illegible]	1	0	8	8	0	10	8	8	9	4	[illegible]	3	0	4	4	5	0
	Mixteco	[illegible]	3	1	3	4	0	3	8	5	8	2	[illegible]	2	0	0	3	0	3
	Pioneer	[illegible]	0	0	3	5	0	4	3	2	3	1	[illegible]	2	0	3	4	1	1
Canadian soil	Parviglumis	[illegible]	0	0	10	11	0	9	10	12	7	2	[illegible]	3	0	3	4	8	2
	Mixteco	[illegible]	0	0	1	2	0	1	0	0	0	3	[illegible]	0	0	0	1	0	0
	Pioneer	17	0	0	12	9	0	7	6	7	8	3	6	2	0	2	2	0	1
Mexican soil	Parviglumis	[illegible]	0	0	10	9	0	7	3	5	6	1	[illegible]	5	0	1	3	5	3
	Mixteco	[illegible]	3	0	7	6	0	7	4	4	4	2	[illegible]	2	0	2	2	4	0
	Pioneer	[illegible]	1	0	4	4	0	3	2	2	3	1	1	2	1	0	1	0	1
	Total	124	8	1	58	58	0	51	44	45	48	19	89	21	1	15	24	23	11

Johnston-Monje et al. 2014. BMC Plant Biology, 14:233.

MICROBIOMES Protect Plants against Pathogens and Pests

Plant are subject to infection by diverse plant pathogens, insects and nematodes.

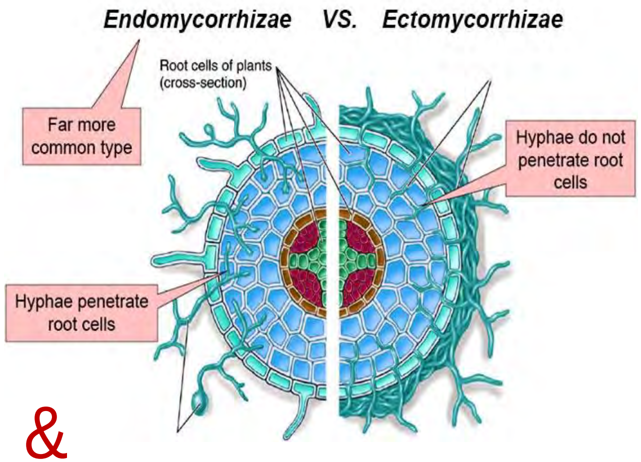


Microbial communities in soil can suppress diseases.

Mendes et al. 2011.

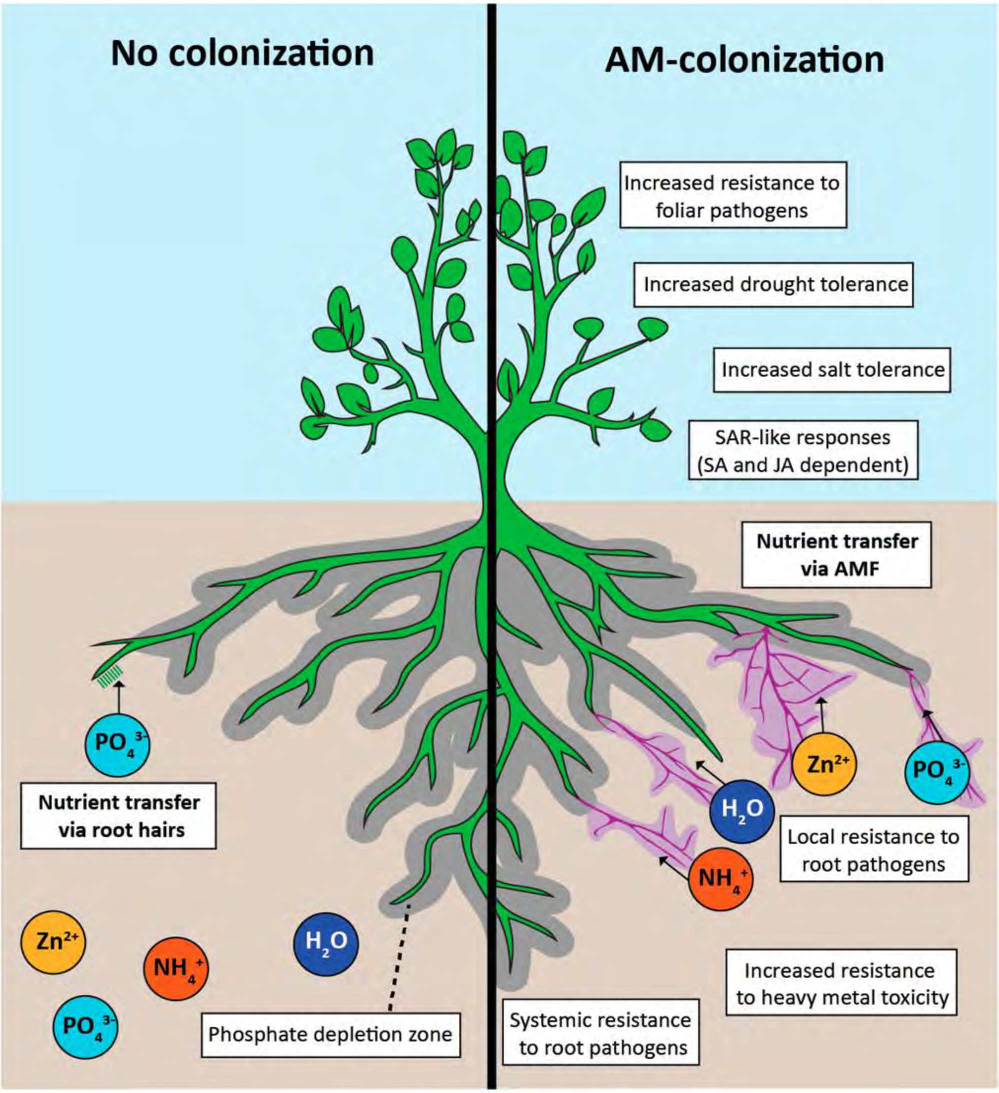
Vesicular Arbuscular Endomycorrhiza (VAM): Applications

- a bio-fertilizer, has the symbiotic association between plant roots and VAM.



- induces plant growth & makes resistance to abiotic stresses.

- increases the resistance to root-borne or soil-borne pathogens and nematodes.

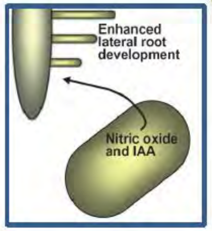


Jacott et al. 2017. Agronomy 7:75.

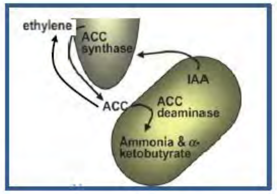
MICROBIOMES Reduce Abiotic Stresses (e.g., Drought, Salinity)

Microbes reduce effects of drought, flooding & salinity

- Microbes enhance root growth via phytohormone production
  → more and deeper roots

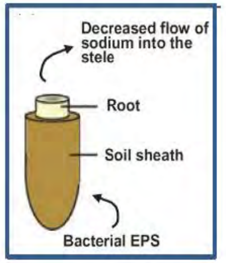


- Microbes minimize the inhibitory effect of ethylene on plant growth
  - produce ACC deaminase

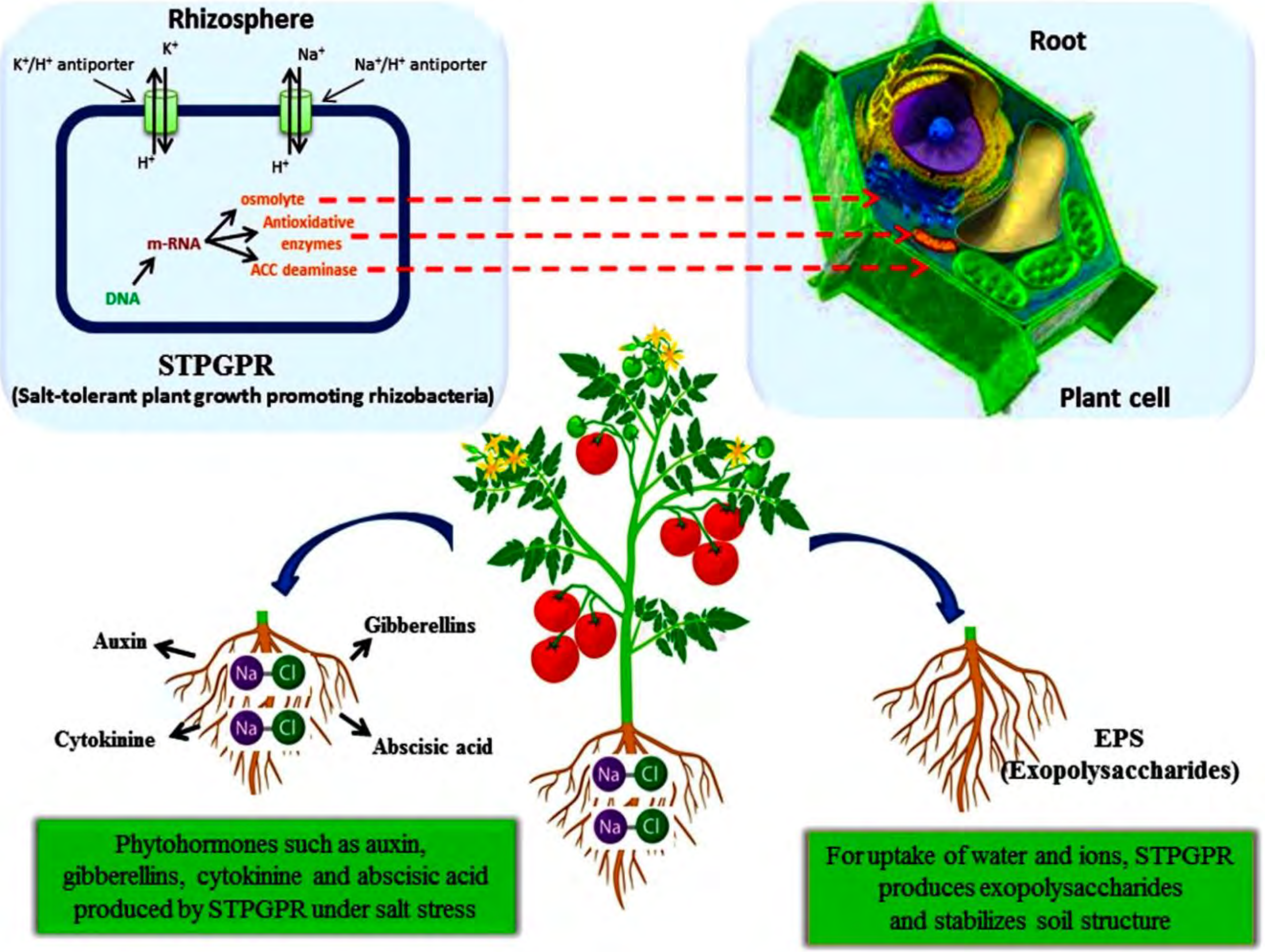


- Microbes form biofilms that reduce ion movement into the plant

(e.g., *Enterobacter*, *Bacillus*, *Pseudomonas*)



Dimkpa. 2009. Plant Cell Environ 32:1682



Chandran et al. 2021. Sustainability. 13:10986.

MICROBIOMES in Agriculture

Challenges:

- Increase efficacy tests to reduce the year-to-year and field-to-field variation.
- Improve the scale-up from small plot studies.
- Improve the formulations.

Opportunities: Microbiomes can positively impact crop production and food security.

- Soil is rich in bacteria and other microorganisms that colonize plants.
- Fungi comprise much of the microbial biomass in the soil.
- 1 gram of soil = >10 billion bacterial cells or 10,000 bacterial species.
- Soil properties (e. g., composition, soil pH or acidity, moisture content) impact the number and diversity of microbiomes and their interactions.

Improvement can be achieved by detailed understanding of the complex interactions of microbiomes and the use of current technological advances.

CAST, 2019.

MICROBIOMES Research Priorities for Sustainable Agriculture

1. Develop model host-microbiome systems for crop plants and non-crop plants with associated microbial culture collections and reference genomes.

2. Define core microbiomes and metagenomes in model host-microbiome systems.

3. Elucidate the rules of synthetic, functionally programmable microbiome assembly.

Future Plant Microbiome Research Priorities in Agriculture

4. Determine functional mechanisms of plant-microbiome interactions.

5. Characterize and refine plant genotype-environment-by-microbiome-by-management interactions.

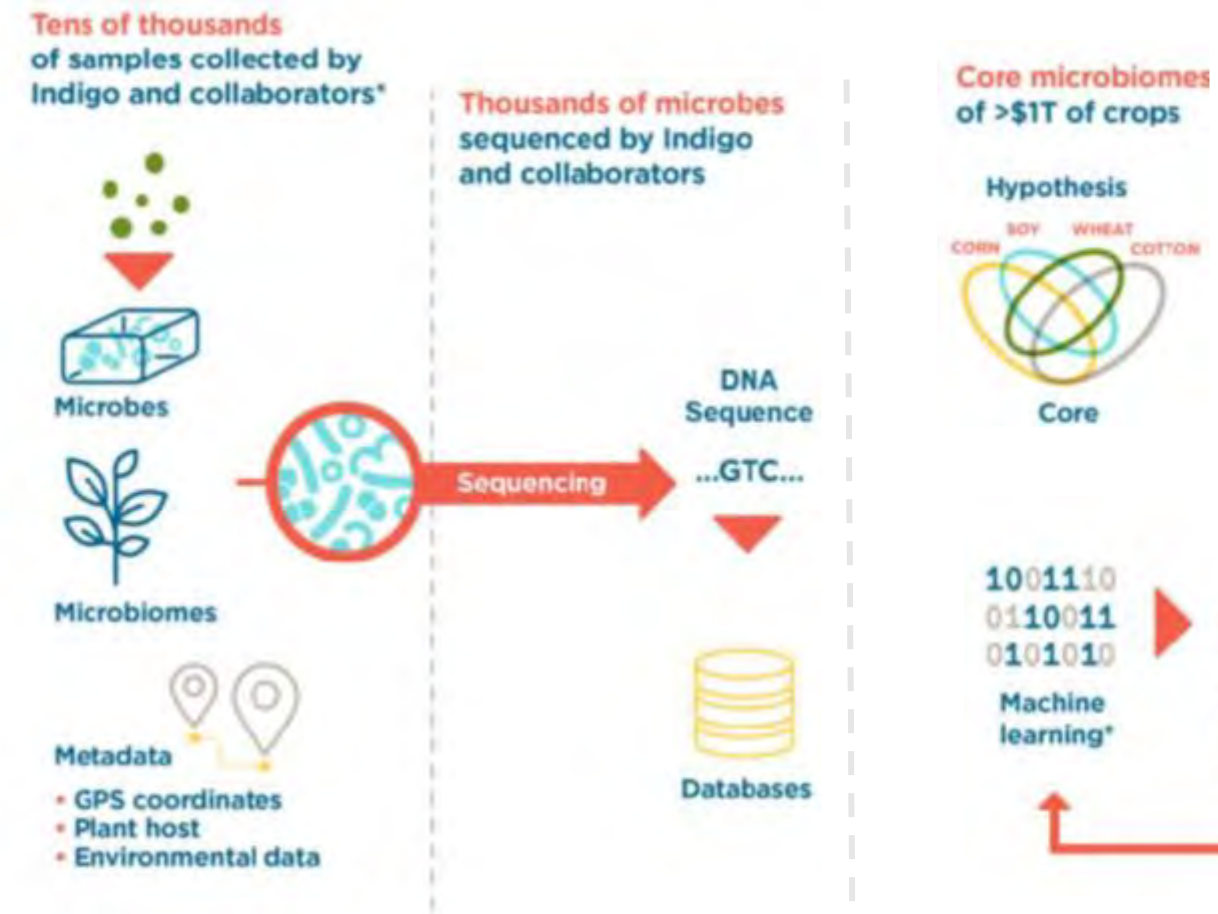
Busby et al. 2017. PLoS Biol.15: e2001793.

Commercialization of Microbial Product Research and Development

Considerations:

* Commercially viable product - Take long time
* Testing performance & efficacy - Complex
* Field trails – Costly *(cost-effective screening methods is required before performing a field trial)*.

By combining machine learning technologies and DNA sequencing, research & development of microbiome products can be achieved between 12 and 28 months. However, it takes 10-15 years to develop a new class of chemicals or crop genetics & traits.



Isolation | Analysis and Prediction | Screening, Refinement and Commercialization Lunch

CAST, 2019; Indigo Ag. Inc., 2017.

Partnerships Between University Researchers and Ag-Biotech Industry

Caveats:

* A patent may not guarantee a product.

Academic laboratory-based idea (University)

+

Large-scale field trials, market research, regulatory and production (Industry)

- Accelerated knowledge
- Patent
- License
- Commercial Product

Incentives:

* Allow graduate students to gain experience in industry labs.

University technology transfers offices can function as a critical bridge between university and industry

CAST, 2019.

For the Next Agricultural Revolution:

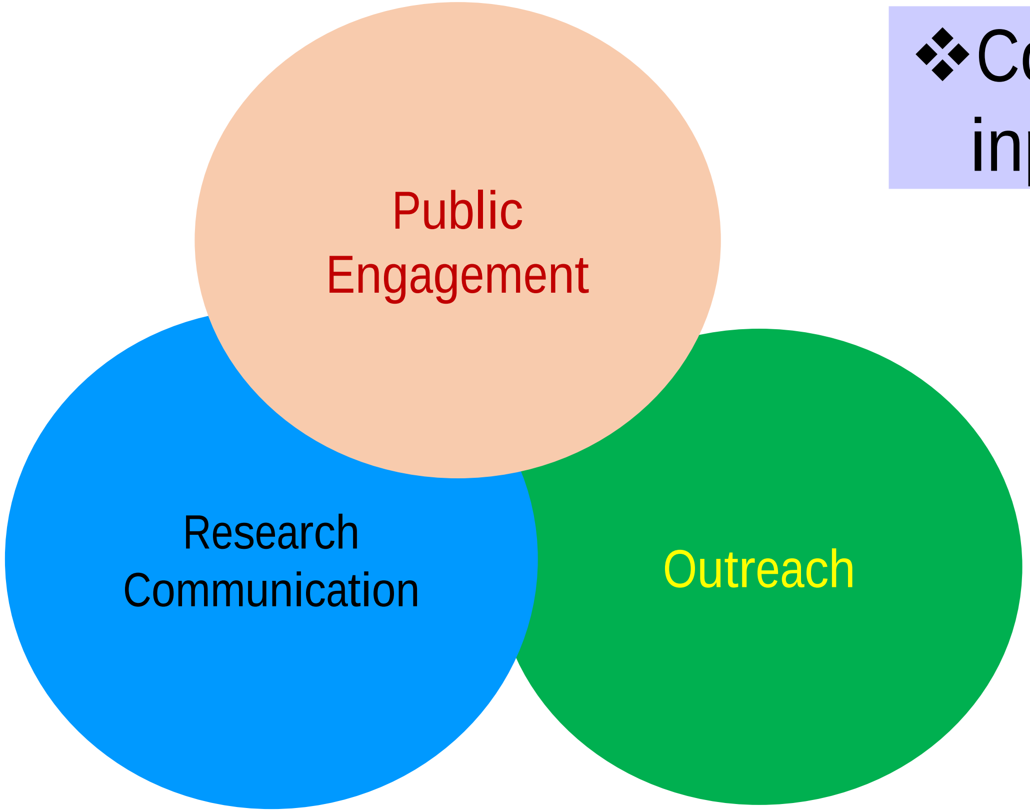
NCSU is Leading an International Collaboration on MICROBIOMES

Dr. Amy Grunden, William Neal Reynolds Distinguished Professor of Plant and Microbial Biology, is leading a six-year, $30 million study on the wheat microbiome to make the staple more resilient. The project is supported by the Novo Nordisk Foundation, NC State University and Novozymes.

Amy Grunden, NCSU

"**Goal**: To improve plant productivity in the face of climate change and emerging pathogens and pests by leveraging microbiomes to help plants avoid stresses while acquiring nutrients to reduce fertilizer, pesticide and **irrigation**".

Communication for Successful Commercialization



❖Consumer pressure to reduce chemical inputs drives interest in microbial products.

❖Regulatory policy is informed by public opinion.

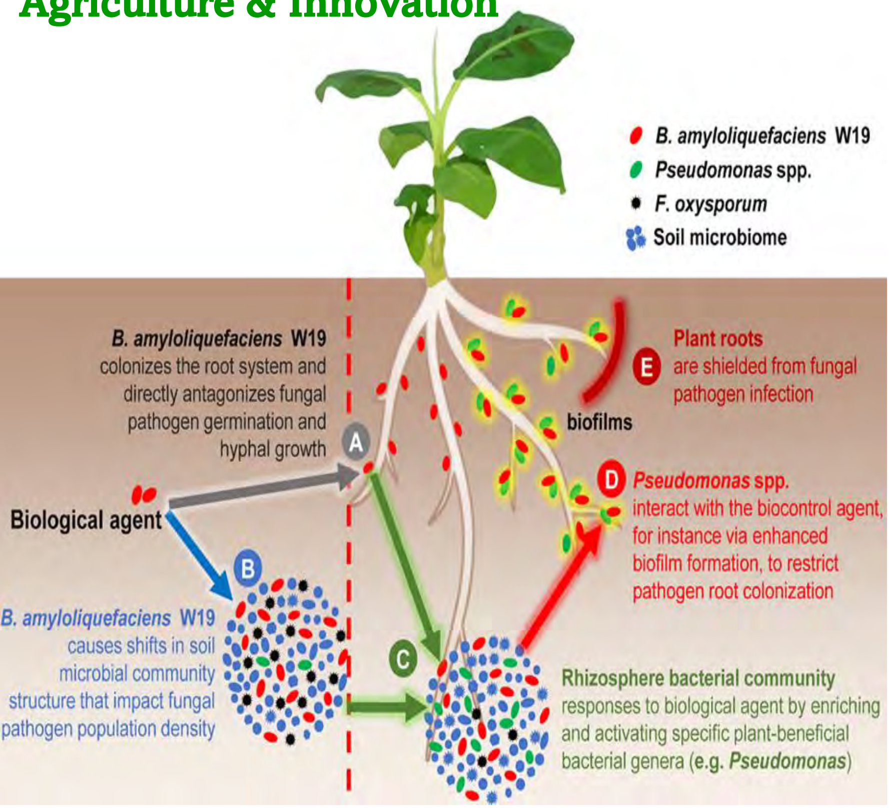
❖Microbial products must be effectively communicated to public audiences.

Communicate directly with growers, buyers, and consumers to accelerate the adoption process of new technologies.

CAST, 2019.

MICROBIOMES-mediated Biological Fertilizers and Related Groups

Agriculture & Innovation



Microbiome. 2020. Plant Science, 2018.

Effects	Microbiome Genera
N2 fixing biofertilizers and symbiotic.	*Azotobacter, Rhizobacterium, Azospirillium, Frankia, Mesorhizobium, Sinorhizzobium Pseudomonas.*
P solubilizing and mobilizing biofertilizers.	*Bacillus, Pseudomonas, Serratia, Mycorrhizae.*
Mono-nutrients and silicate and zinc solubilizing biofertilizers.	*Bacillus, Pseudomonas, Serratia Burkholderia* spp.
Phytohormones and siderophore	*Pseudomonas, Rhizobium, Bacillus, Azotobacter*
Bio-control antifungal.	*Streptomyces, Bacillus, Pseudomonas.*

Next Generation Biologicals

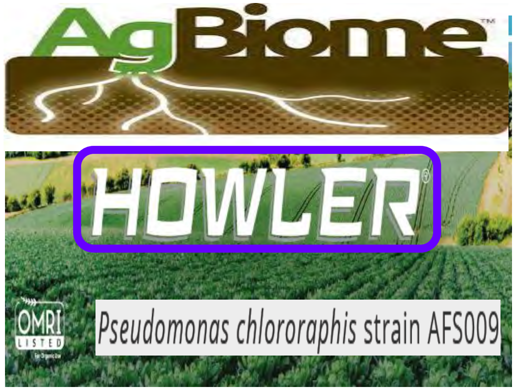
For Grower Yield, Sustainability and Commercial Outcomes in the United States

Biotrinsic™ is microbial seed inoculant for corn, soybean, wheat, small grains, cotton rice etc.

Companion® WP is a broad-spectrum bio-fungicide suppresses diseases, enhances crop fertility, promotes plant growth, and stress resistance.

Serenade® for soil and foliar bacterial and fungal diseases.

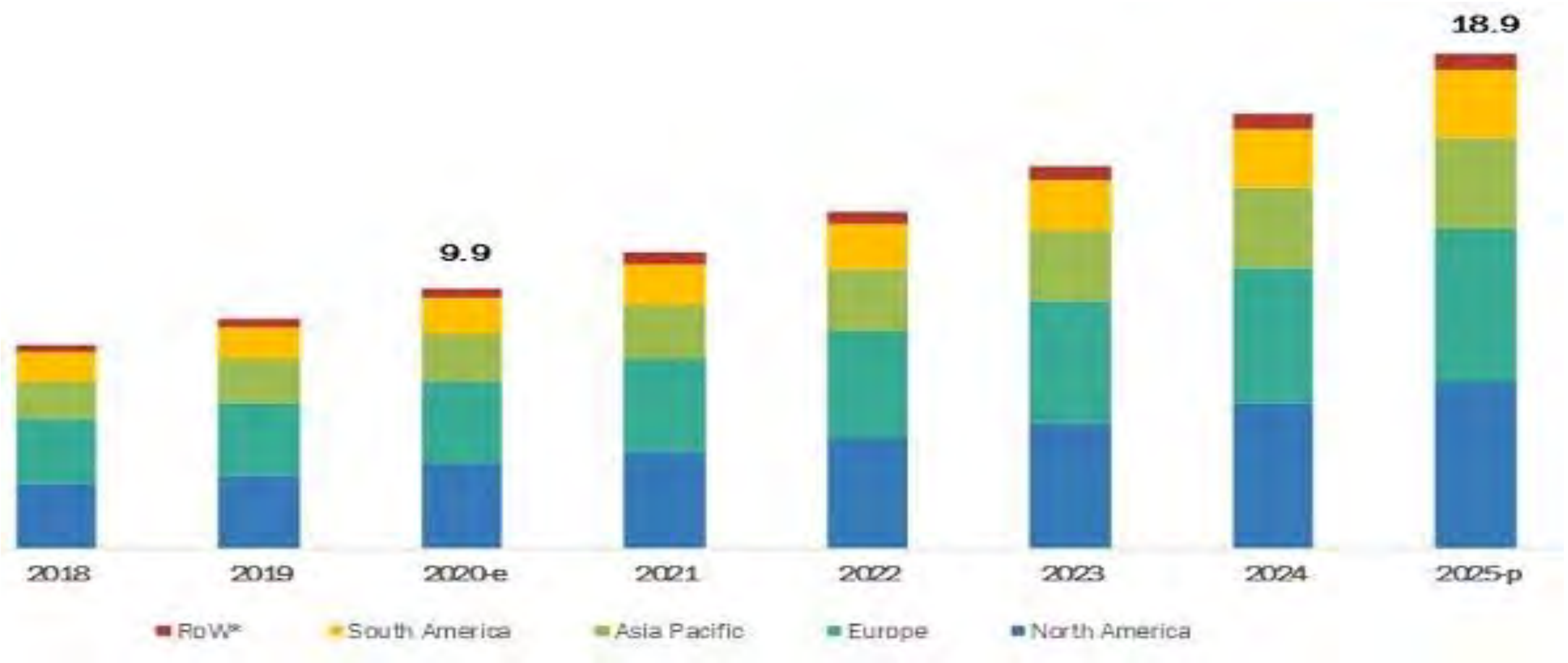
BioniQ®, JumpStart®, TagTeam®, Optimize® ST, Optimize® LV, QuickRoots® for seed treatment for several crops.



Utrisha™ is a nutrient efficiency optimizer.

BioSolution: Poncho® Votivo® 2.0 for seed treatment.

Agricultural Biological Markets by Region (US $ Billion)



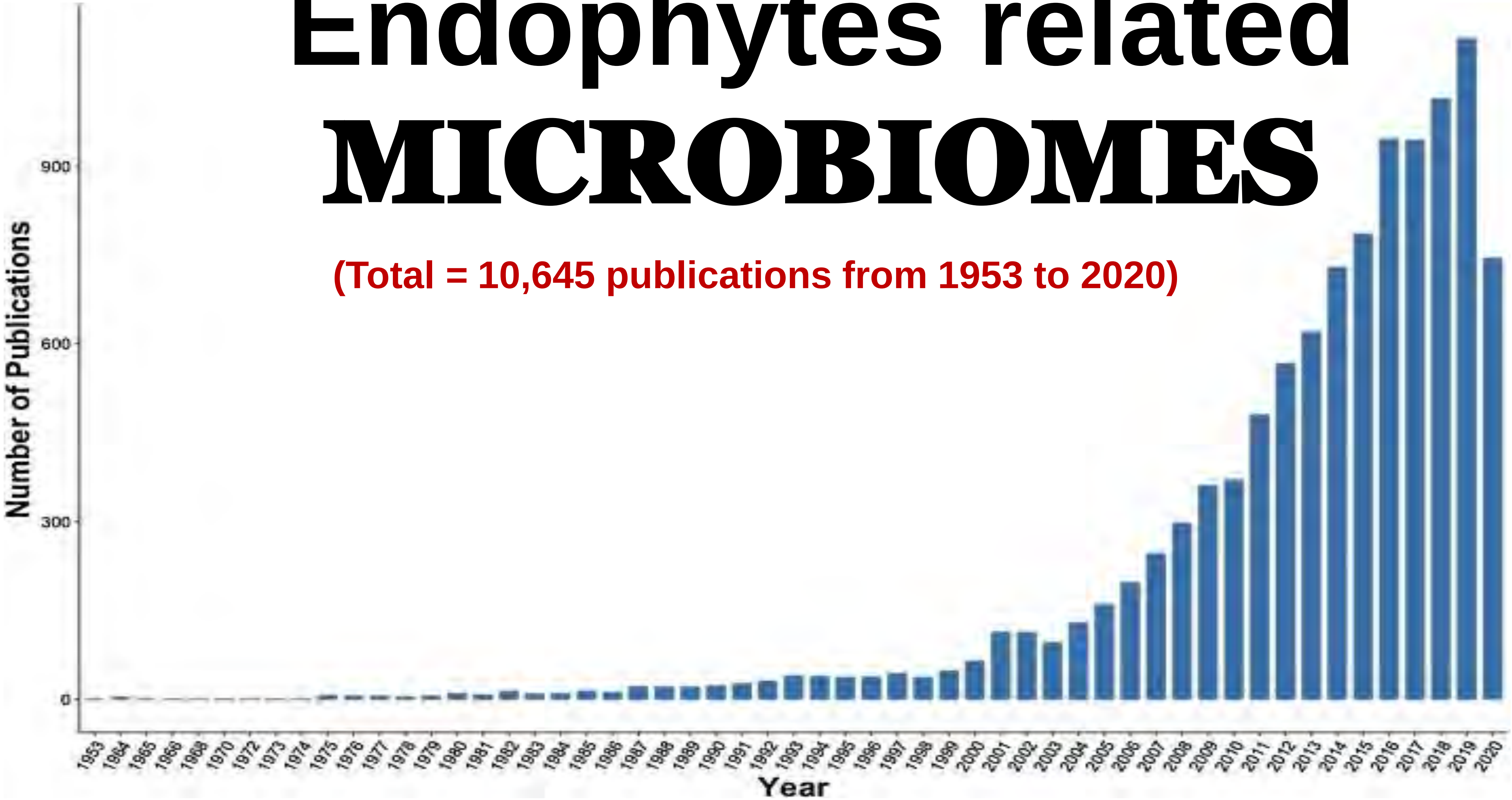
*RoW = Middle East & Africa.
e = Estimated; p = Projected.

Sources: Government publications, Company annual reports

Publication Trends of Plant Endophytes related

MICROBIOMES

(Total = 10,645 publications from 1953 to 2020)



Microbiol. Res. 2021. 245:126691.

SUMMARY: MICROBIOMES for Sustainable Agriculture

Microbiome-mediated beneficial effects on (1) plant health, (2) pest or pathogen management and (3) yield improvement

Approach

Plant Breeding:
Microbe-optimized plants

Microbiome Engineering:
Plant-optimized microbiomes

Biologicals:
Optimized microbial biofertilizers & biopesticides

Deliverable

- Increased productivity
- Disease resistance
- Abiotic stress tolerance
- Improved plant-water-soil relationship
- Phytoremediation

Microbial Biotechnology. 2017.10:999-1003.

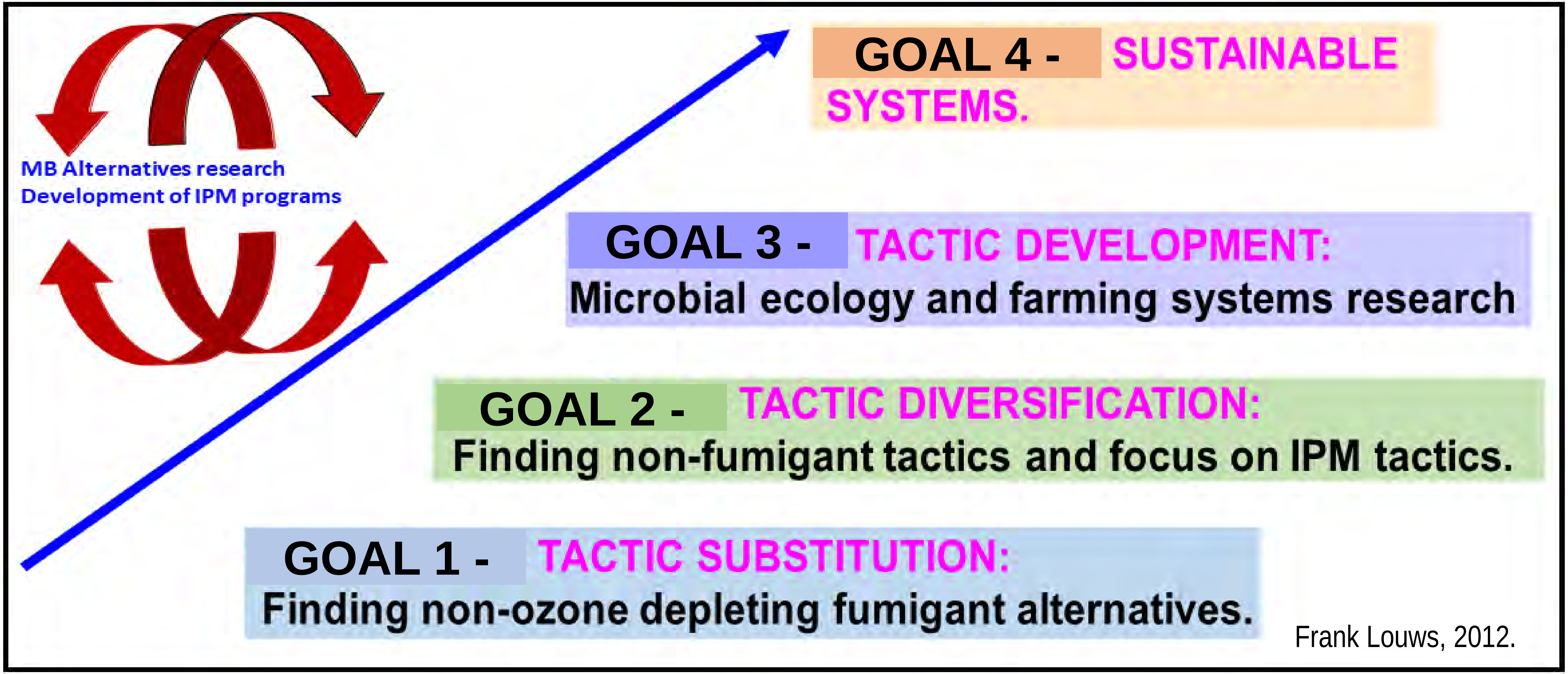
The Future: MICROBIOMES

Doctors turn to good microbes to fight disease. The same strategy will work with crops:

- ❖Management strategies that create disease-suppressive microbial communities.
- ❖Plants that select for and maintain beneficial microbiomes.

Coombs, A. 2013. The Scientist.

Methyl Bromide Alternatives for Soil-borne Pathogens of Strawberry



Research Objectives

- To determine the effects of anaerobic soil disinfestation (ASD) with different carbon sources enhancing soil microbial communities.

- To evaluate the efficacy of ASD in comparison with soil fumigation on marketable fruit yield.

OUR FUTURE GOALS:

- ✓ **Create disease-suppressive microbial communities.**
- ✓ **Treat diseases/destroy soil-borne pathogens.**

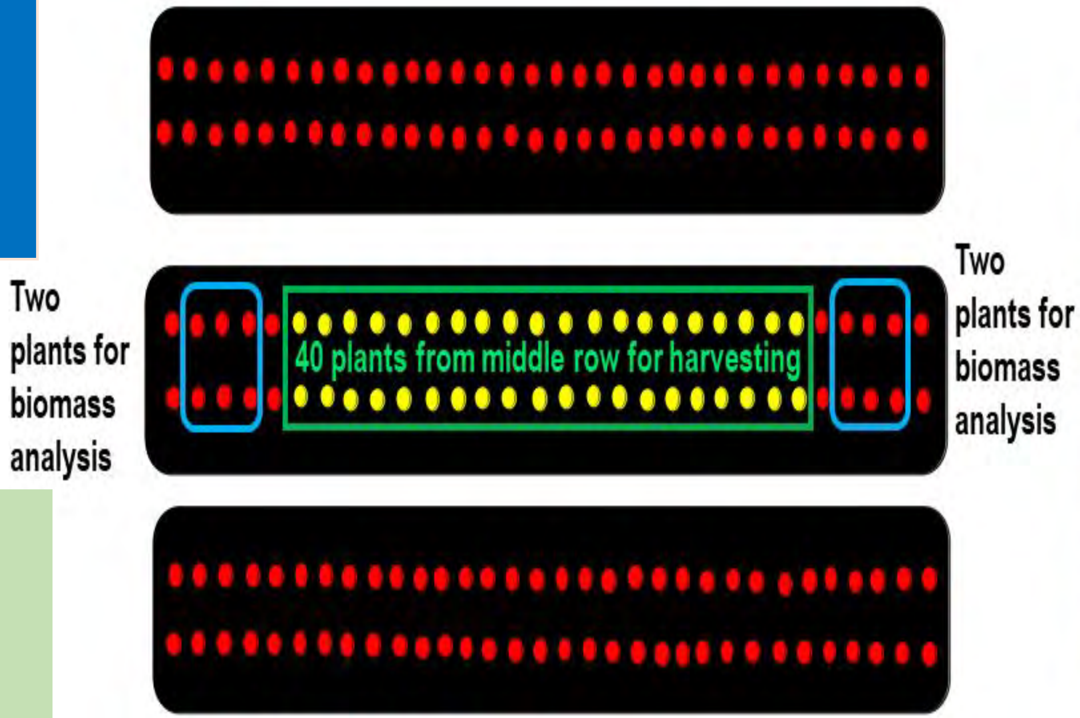
Treatments 2019-2022

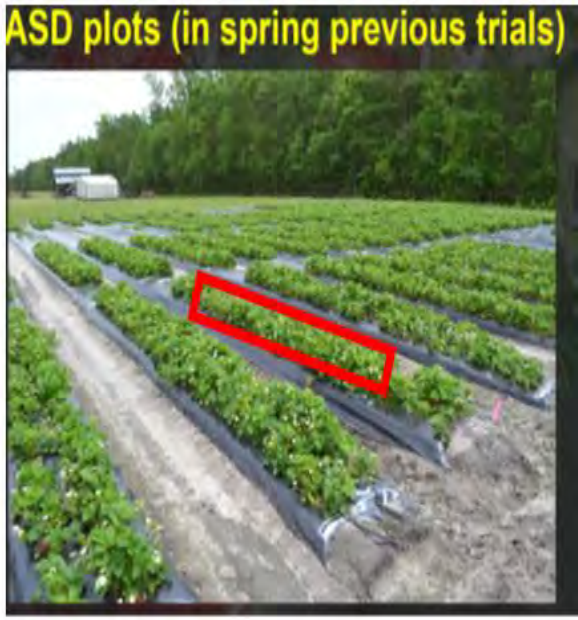
S. #	Treatments	Treatment Description	Rate
1	PicClor-60	Fumigation control	175 lbs/A
2	No fumigation		None
3	Cover crop + compost	Cowpea : Pearl millet + Compost	(cowpea: pearl millet 100:10 lbs./A) + Compost (12 tons/A)
4	ASD carbon source 1	Molasses full rate	5000 lbs/A
5	ASD carbon source 2	Molasses half rate	2500 lbs/A
6	ASD carbon source 3	Mustard meal half rate	1000 lbs/A
7	ASD clear plastic carbon source 1	Molasses full rate	5000 lbs/A
8	Clear plastic	No fumigation	None
9	Mustard meal	Mustard meal full rate	2000 lbs/A
10	Must meal 1/2+ Carbon source ½	Mustard meal half rate + molasses half rate	1000 lbs + 2500 lbs/A

Land Preparation at Castle Hayne Research Station, NC 2019-2022

Sampling Strategy for Soil Analysis and Fruit Yield

Design: RCBD
Replications: 4
Treatments: 10
Plot size: 5’ W x 30’ L with 3 rows





Pic-Clor60:175 lbs in row
Molasses: 5000 lbs/acre
Mustard meal: 2000 lbs/acre
Cover crops: Cowpea 100 lbs/acre
 Pear millet: 10 lbs/acre

Data Collection (2019-2022)

- Soil health analysis (3x):
  Before fumigation, pre-planting & harvesting.

- Pathogens (e. g., *Pythium* and *Rhizoctonia*) detection and quantification:
  Quantitative real-time PCR assays before fumigation, pre-planting & harvesting.

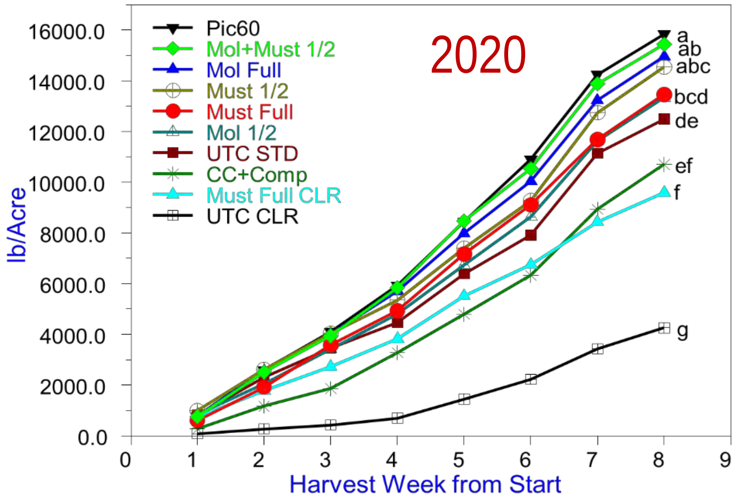
- Nematode population counts (3x)

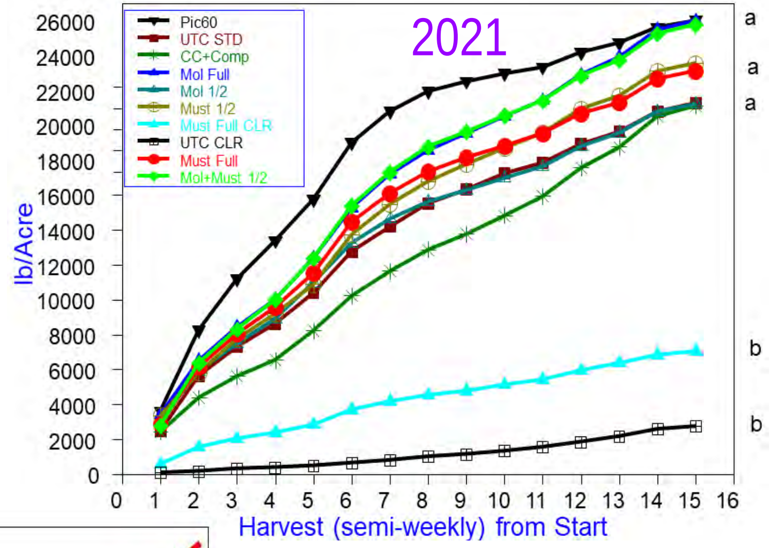
- Weed seed counts (3x)

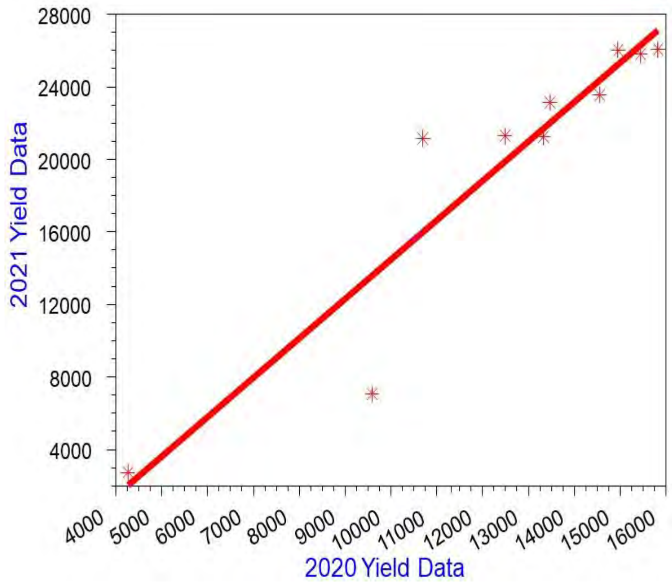
- Microbiome Analyses (4x) using 16S rDNA (for bacteria) & ITS (for fungi) sequencing (Metagenomic analysis is in progress):
  Pre-planting, 2019, Harvesting 2020, Harvesting 2021 & Strawberry Roots, 2021.

- Marketable yield data (1-2x/week)

Cumulative Marketable Yield Progress Curves for 2020 and 2021







Spearman's correlation coefficient (0.98; *P* = 0.01) between the 2020 and 2021 yield data

ACKNOWLEDGEMENTS

USDA NIFA

United States Department of Agriculture
National Institute of Food and Agriculture

the Southern Region small fruit consortium

THANK YOU!

North American Strawberry Growers Association

North Carolina Strawberry Association