3rd training school "Plasmas for plant and food processing"

Effect of cold plasma treatment on seed microbiome and plant-microbial interactions

CA19110

cost
European Cooperation in Science and Technology

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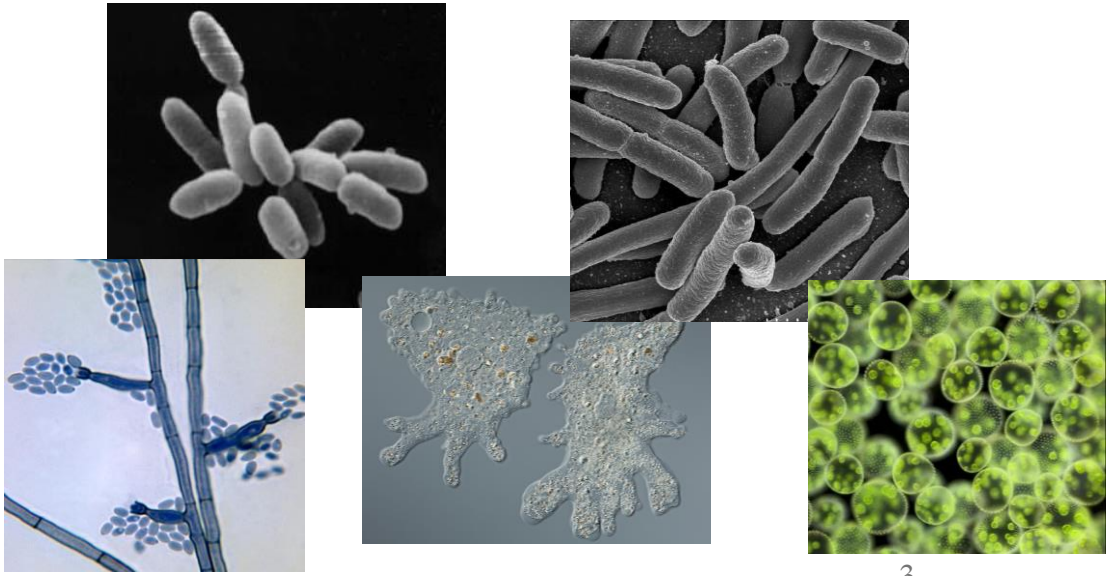
Lithuanian Research Centre for Agriculture and Forestry

13th June, 2024

- Microbiota and plant microbiome
- Plant seed microbiome
- Non-thermal plasma effect on seed and plant microbiome

- **Microbiota** is the assemblage of living microorganisms present in a defined environment

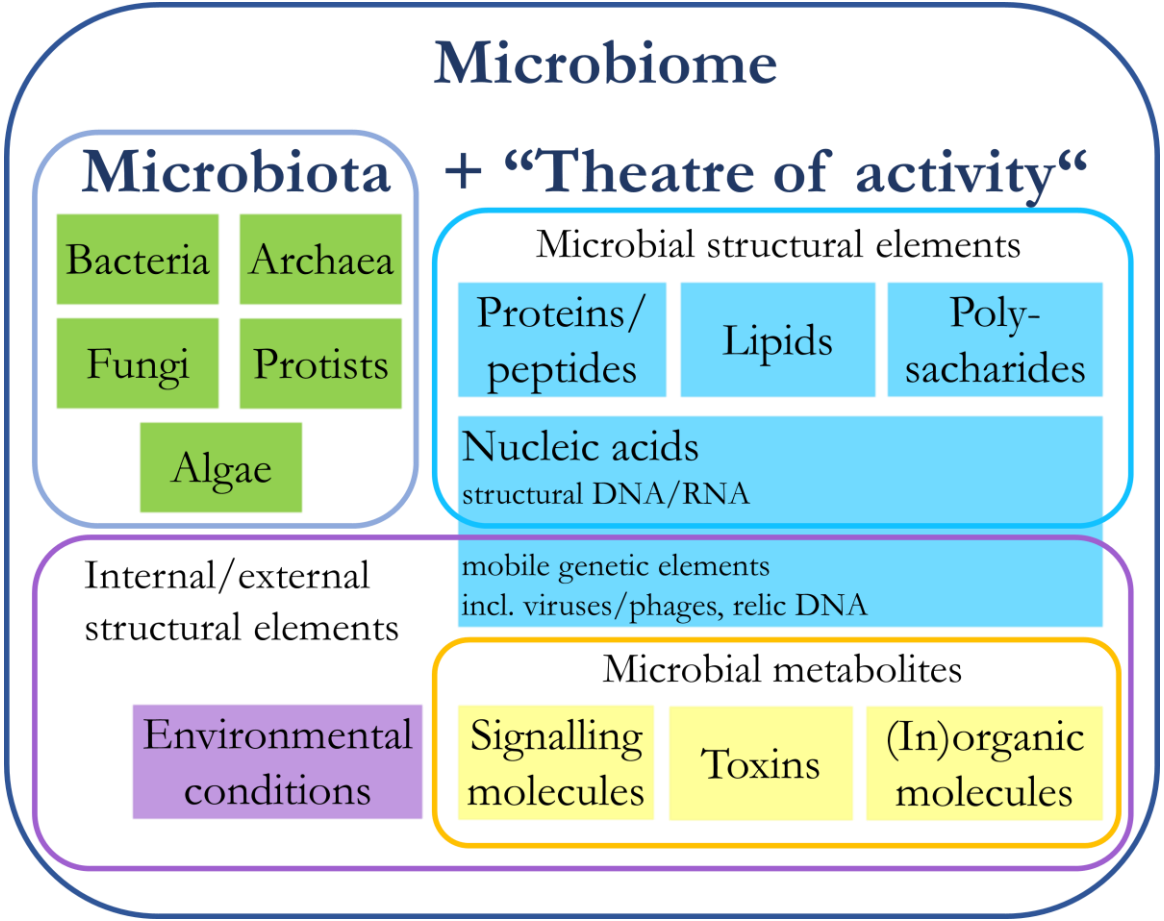


Microbiota

- Bacteria
- Archaea
- Fungi
- Protists
- Algae

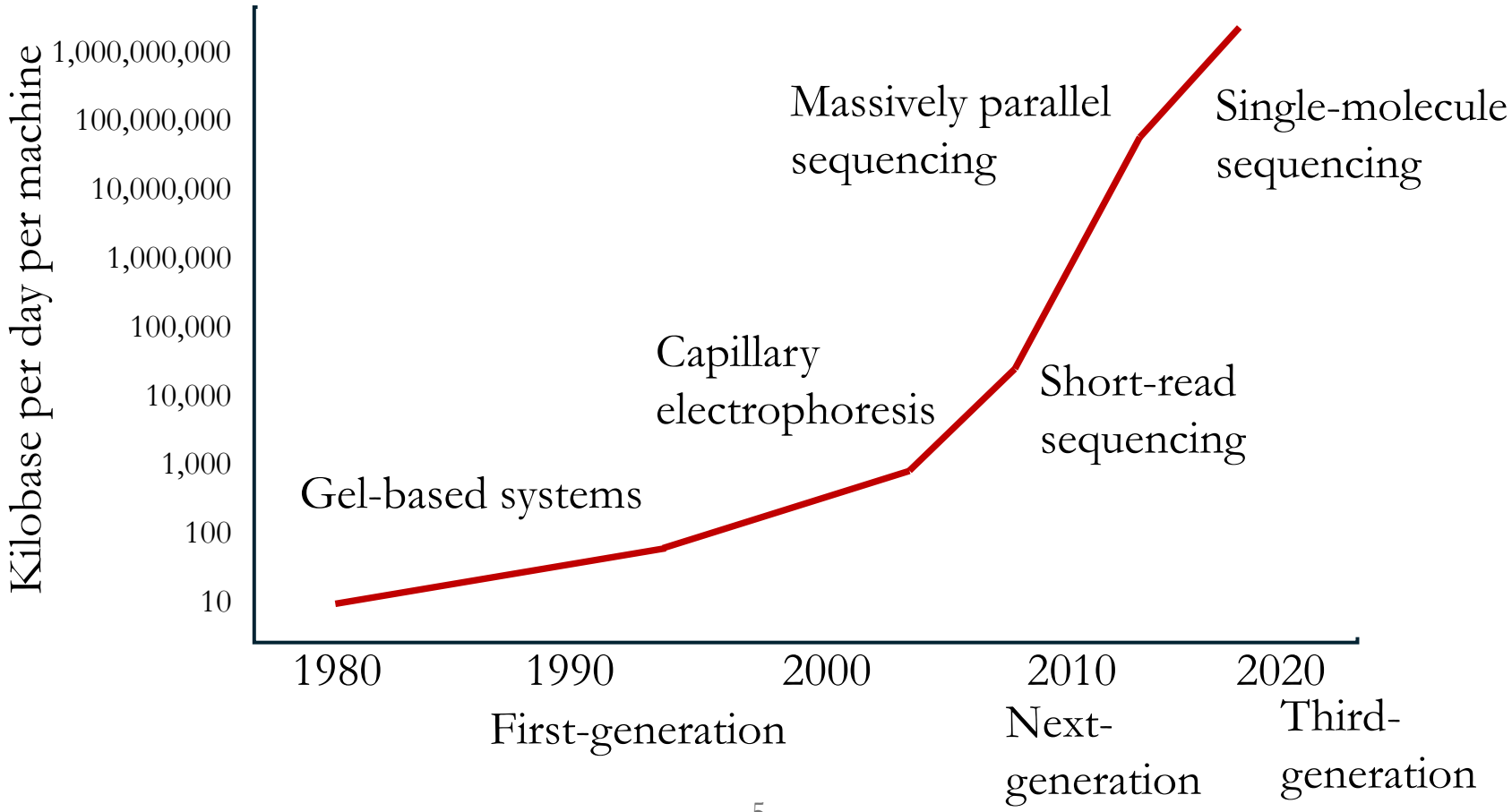
- **Microbiome** is a microbial community occupying a reasonable well-defined habitat which has distinct physio-chemical properties.

- The microbiome not only refers to the microorganisms involved but also encompass their theatre of activity, which results in the formation of specific ecological niches.



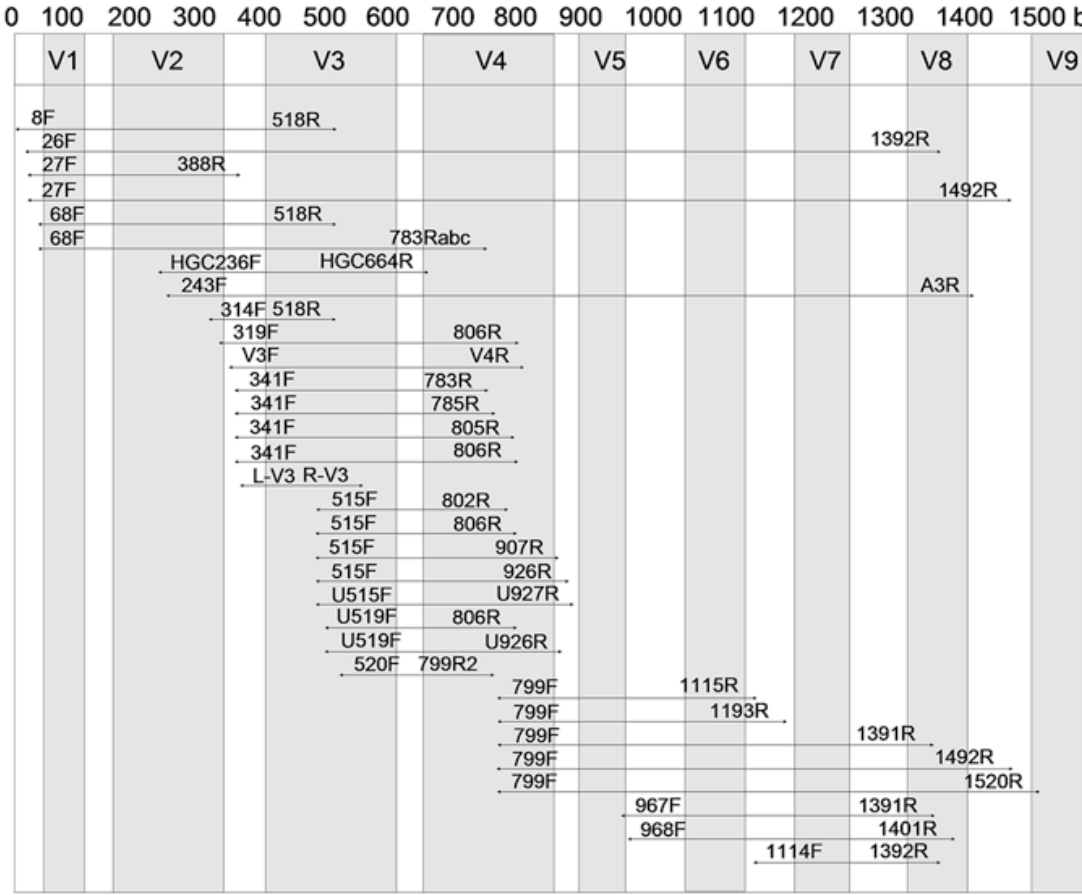
Adapted from Berg et al. (2020)

DNA sequence analysis



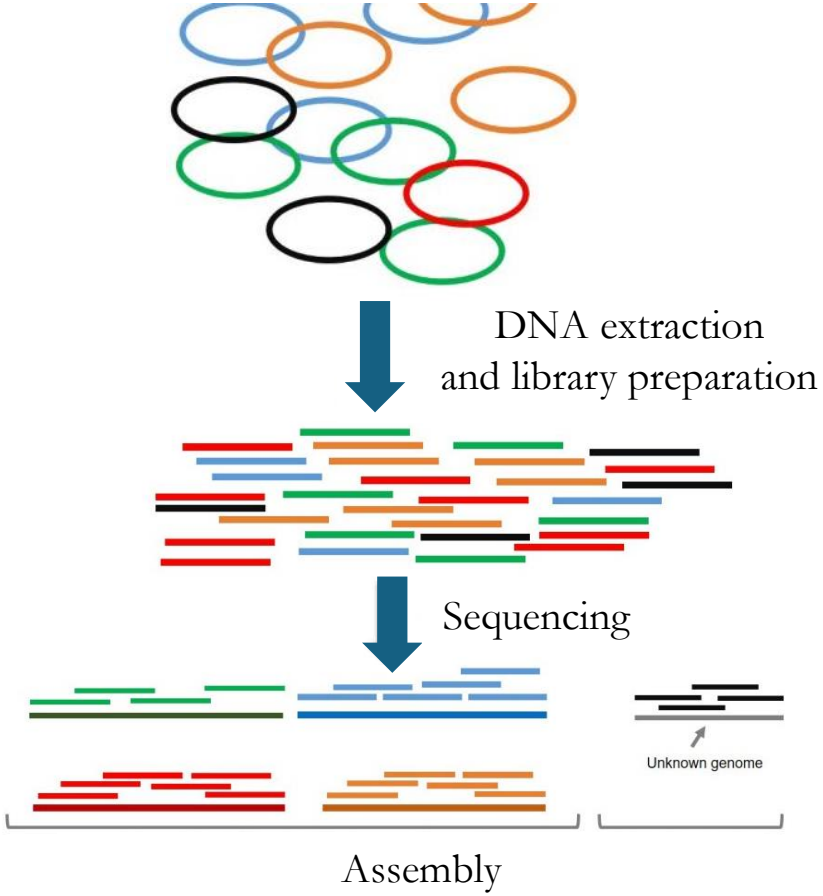
16S rRNA gene amplicon sequencing

- **Metataxonomic analysis** used high-throughput sequencing, primarily short amplicons of 16S rRNA gene sequence, to identify microorganisms within a complex mixture.



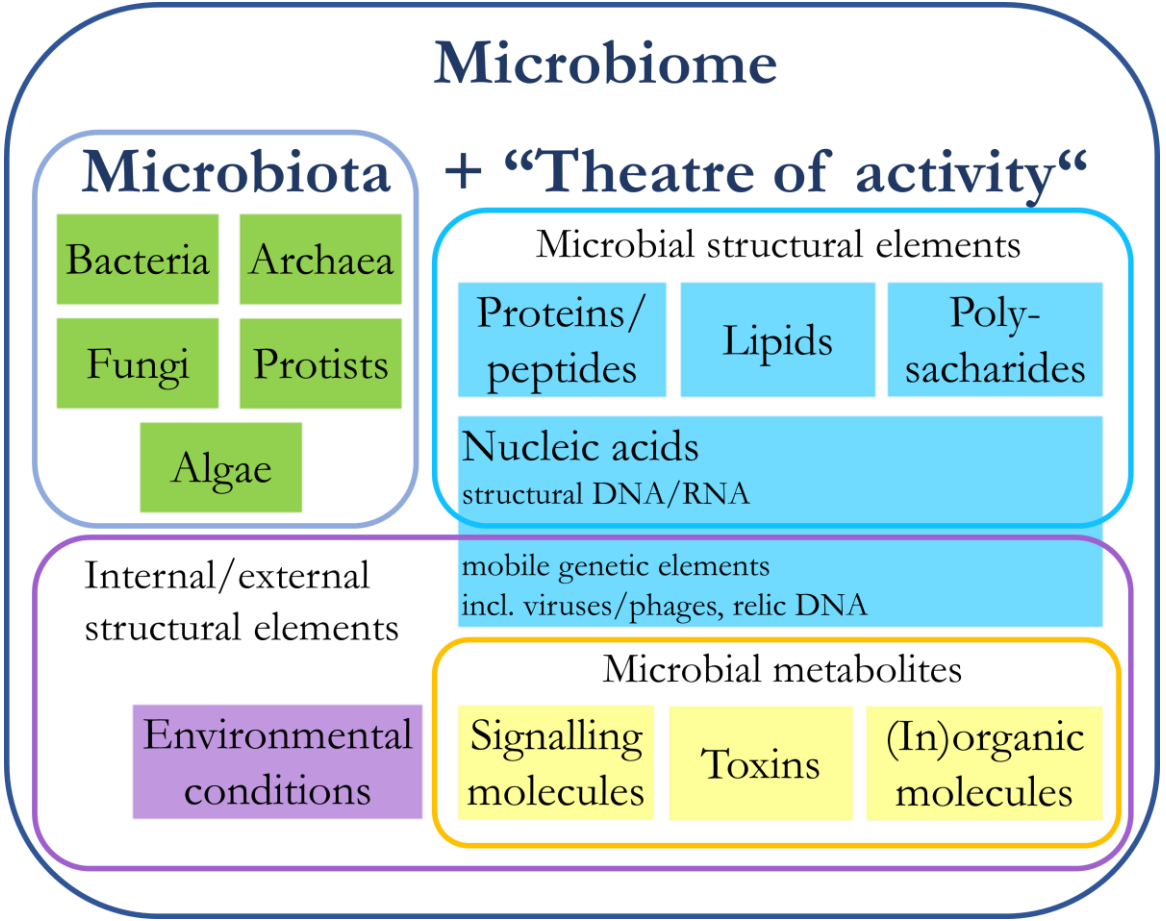
- **Metagenomics** is the study of the structure and function of entire nucleotide sequences isolated and analysed from all the microorganism in a bulk sample.

- MICROBI–OME or MICRO–BIOME?



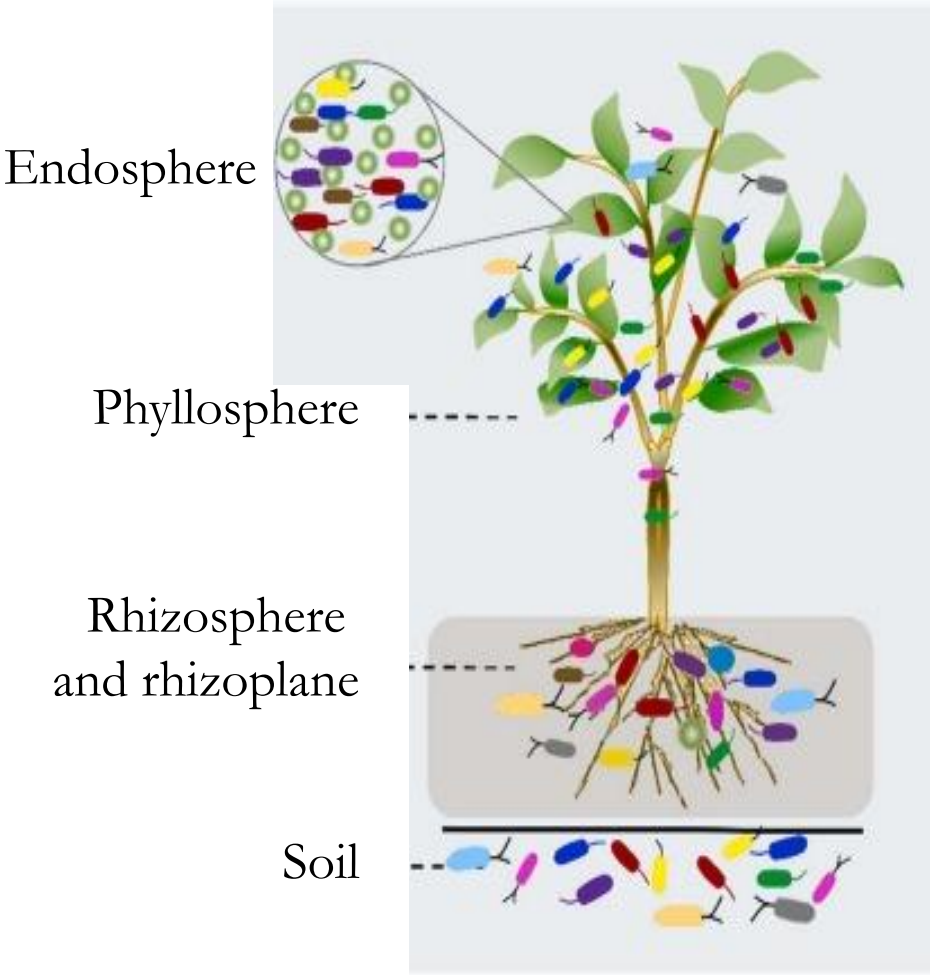
- The microbiome is integrated in macro-ecosystems including eukaryotic hosts, and here crucial for their functioning and health.

- Eukaryotes are meta-organisms and must be considered together with their microbiota as an inseparable functional unit



Adapted from Berg et al. (2020)

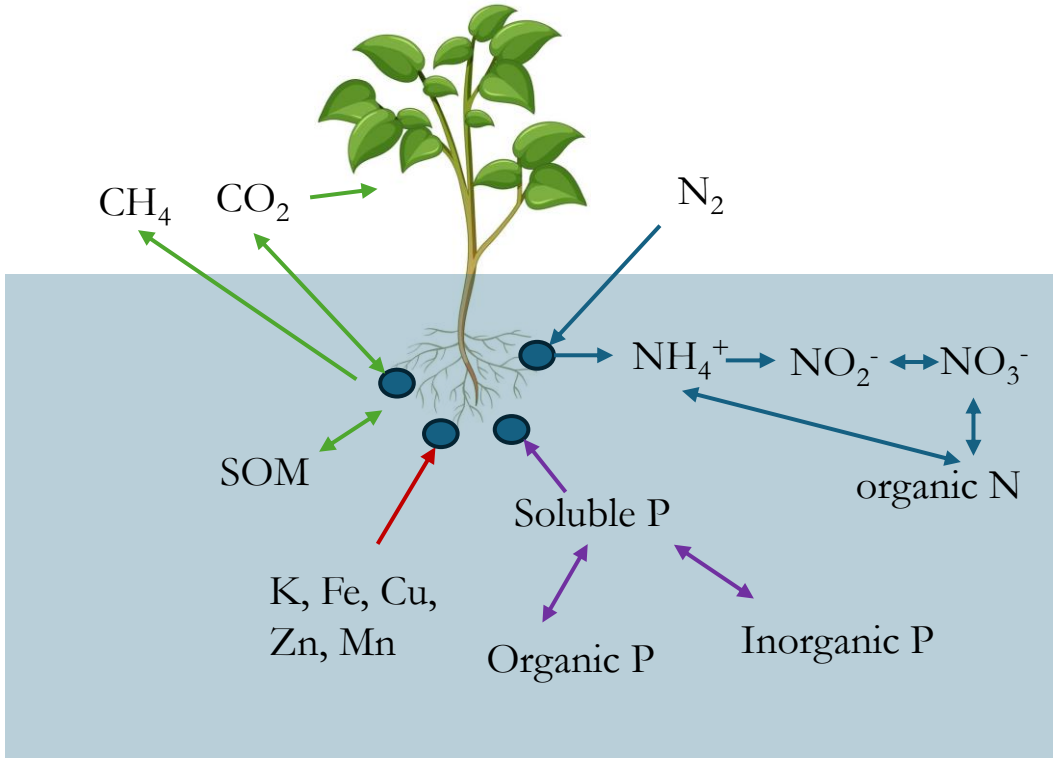
- **Plant microbiome** or **phytomicrobiome** is microbial community inhabiting the rhizosphere and plant tissues which establishes complex and dynamic interactions with the host plant.



adapted from Afridi et al. (2022)

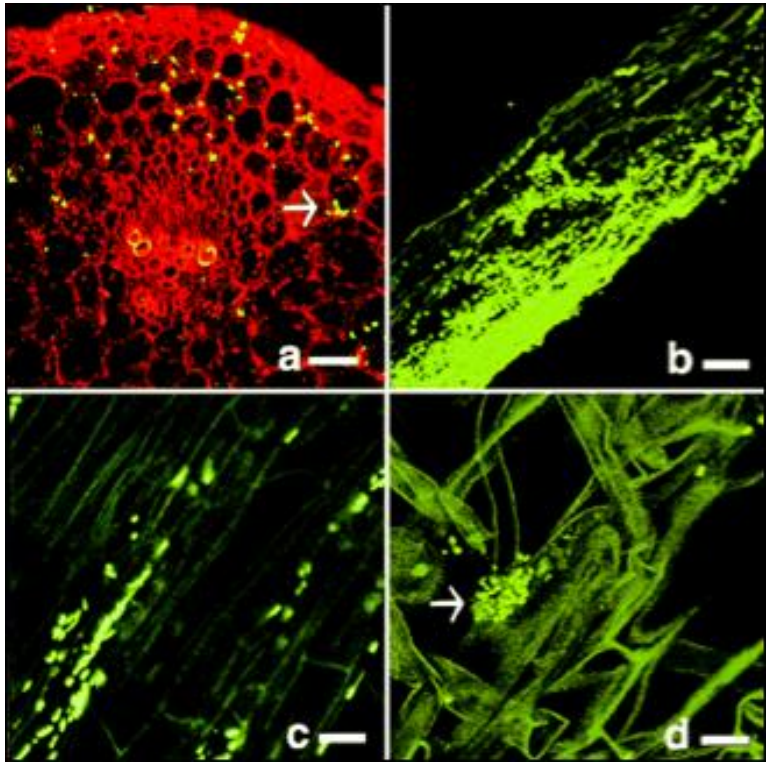
Rhizosphere is a “trading market”

- Carbon
- Nitrogen
- Inorganic and organic phosphorus
- Potassium, iron and other minerals (Cu, Zn, Mn)



Endophytes

- Endophytic bacteria reside within an intercellular space of plant tissues for at least part of their lives
- Endophytes do not cause any visible disease symptoms.

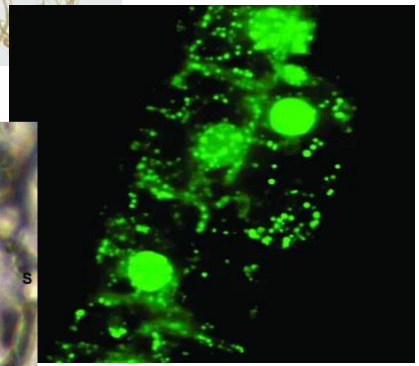


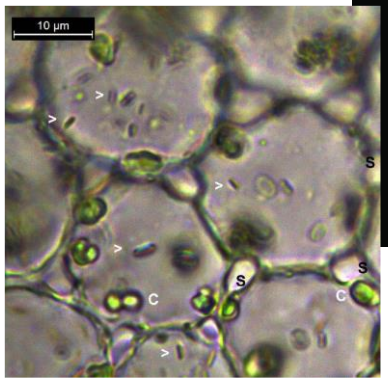
Chelius and Triplett (2000)

Intracellular endosymbionts

- Rhyzobium are legume plant endosymbiotic diazotrophs that inhabit plant cells and form symbiosomes.

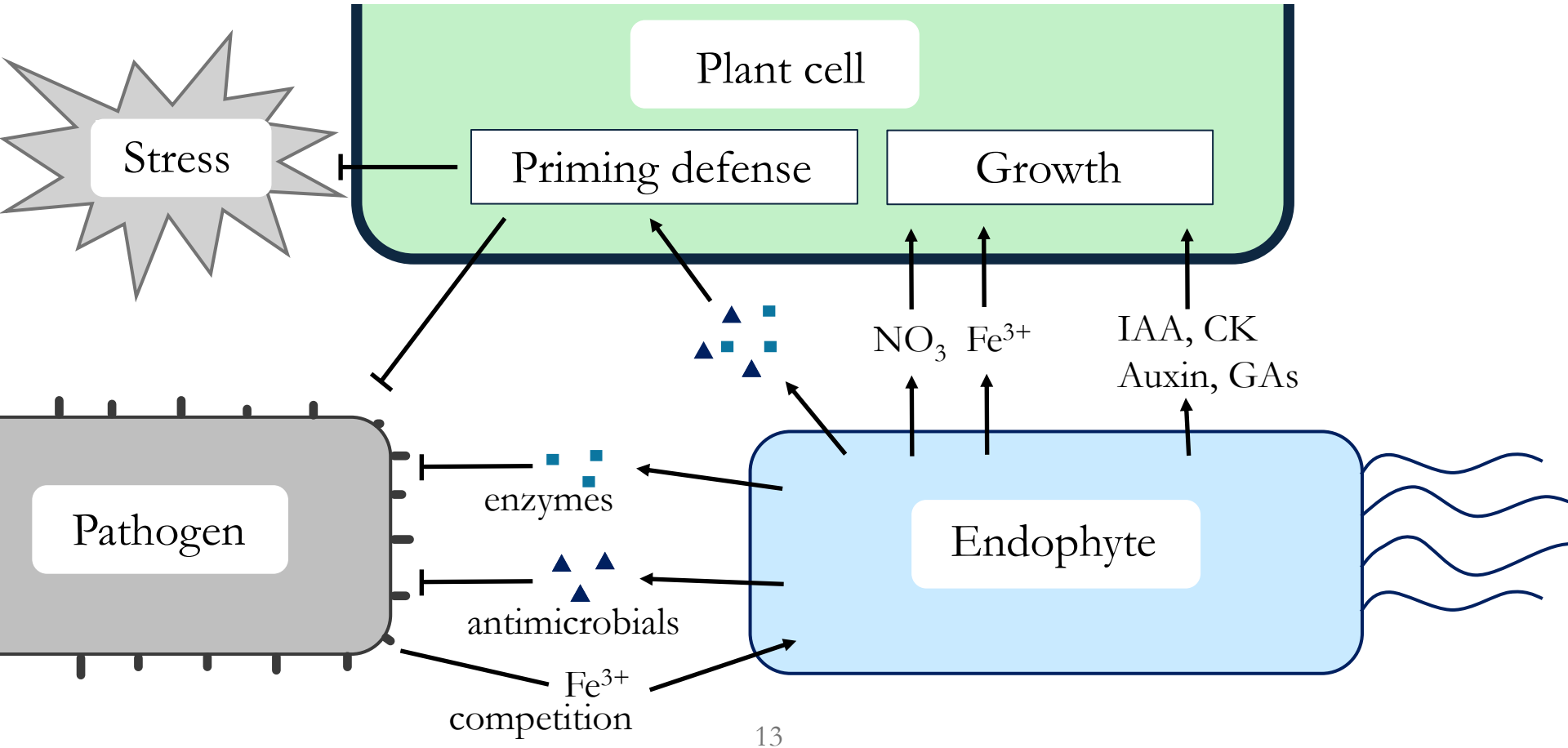
- A posibility of presence of endophytic bacteria living inside the cytoplasm of live plant cells (cytobacts) is proposed.



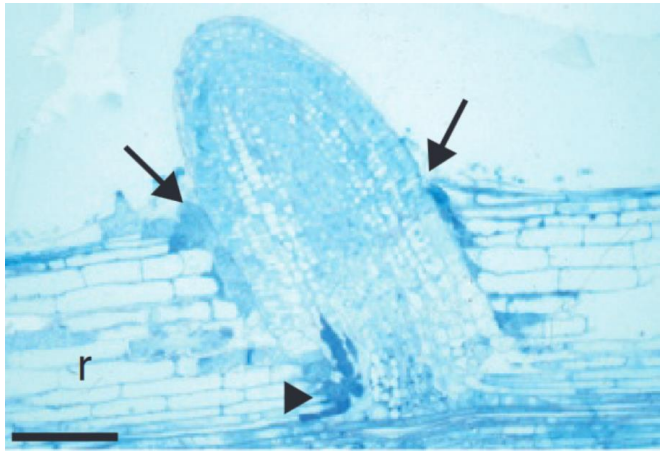


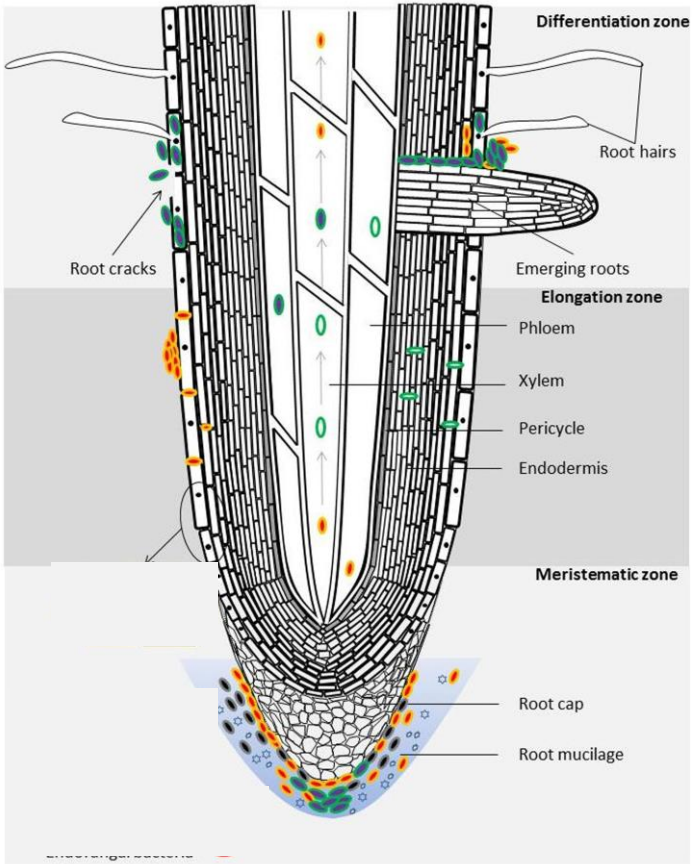
Marcela Mendoza-Suárez et al. (2020); Thomas et al. (2022)

Growth enhancing and barrier effect



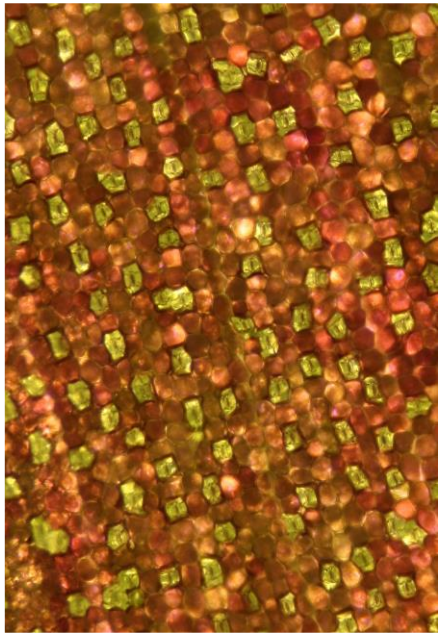
Horizontal transmission

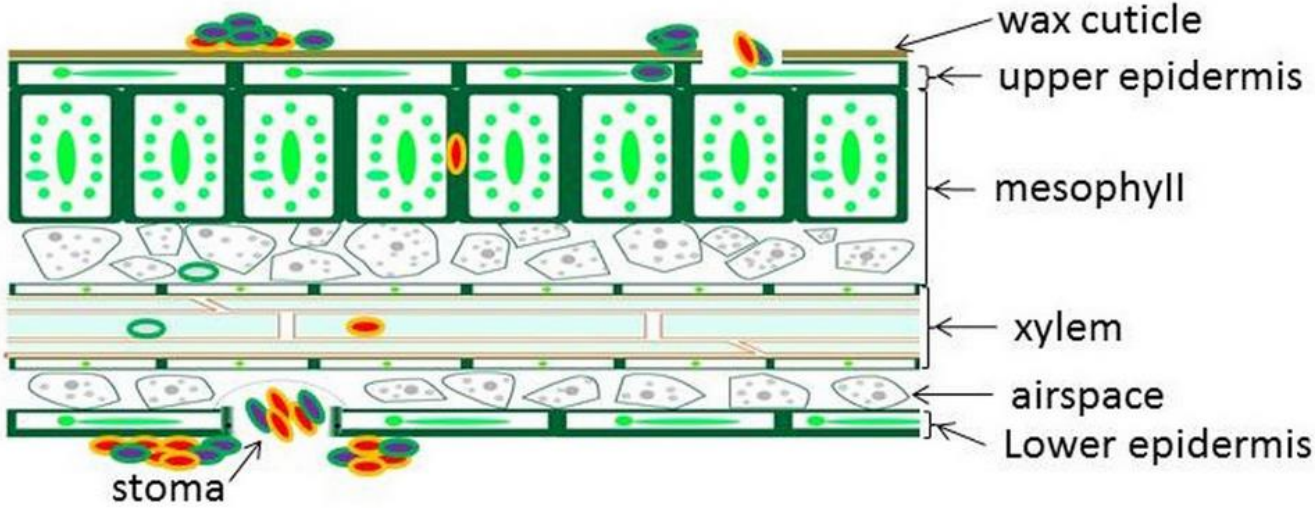




Turner et al. (2013)
Liu et al. (2017)

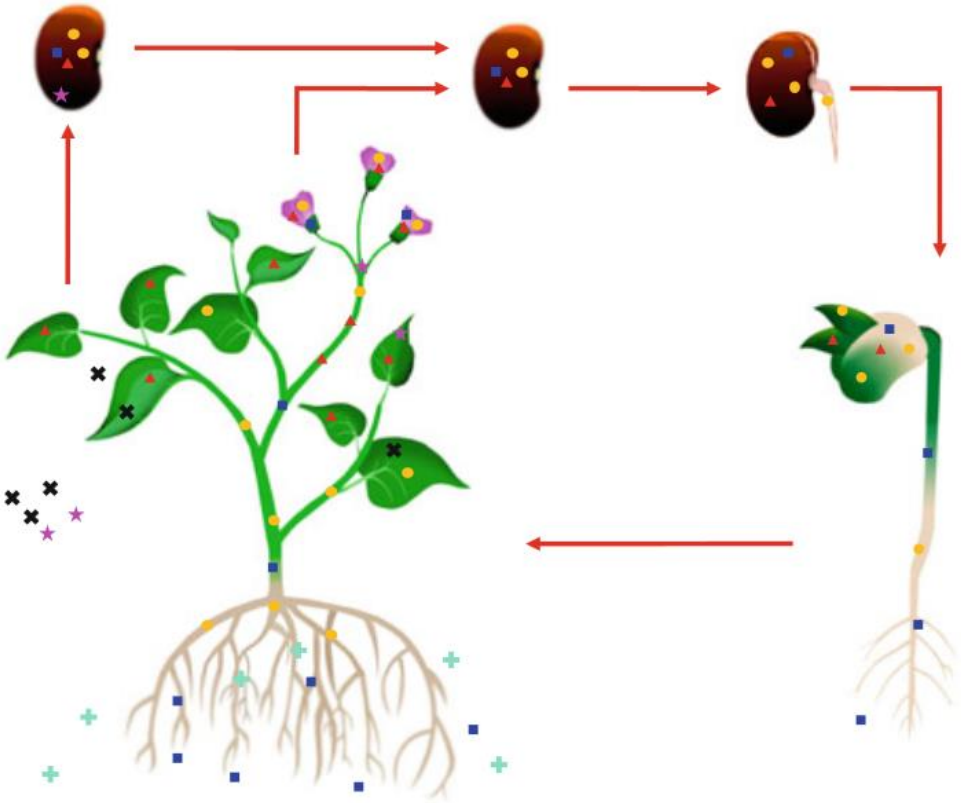
Horizontal transmission





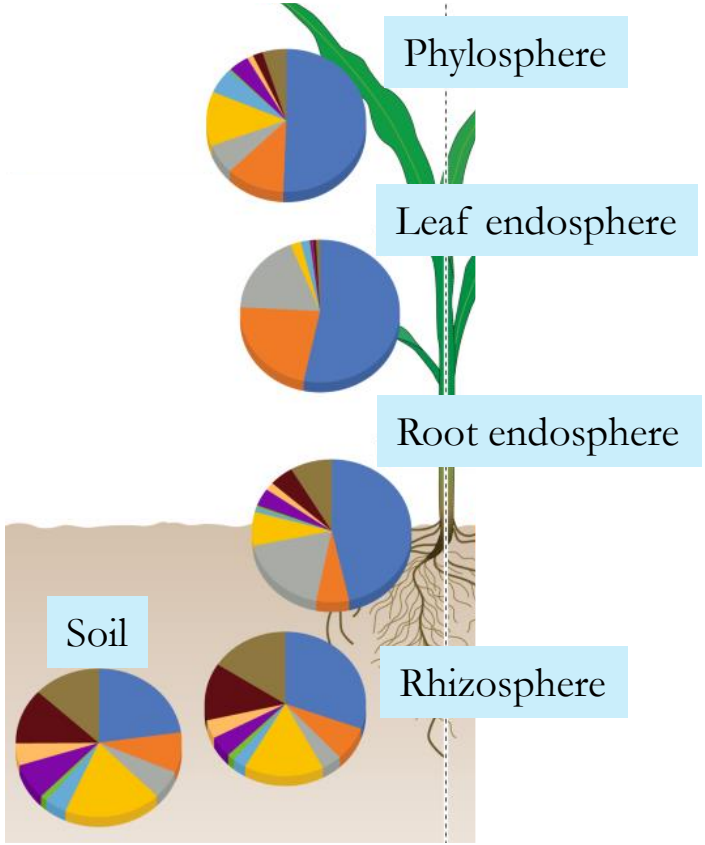
Liu et al. (2017);
Zephyris (Richard Wheeler)
https://en.wikipedia.org/wiki/File:LeafUndersideWithStomata.jpg

Vertical transmission



adapted from Li et al. (2019)

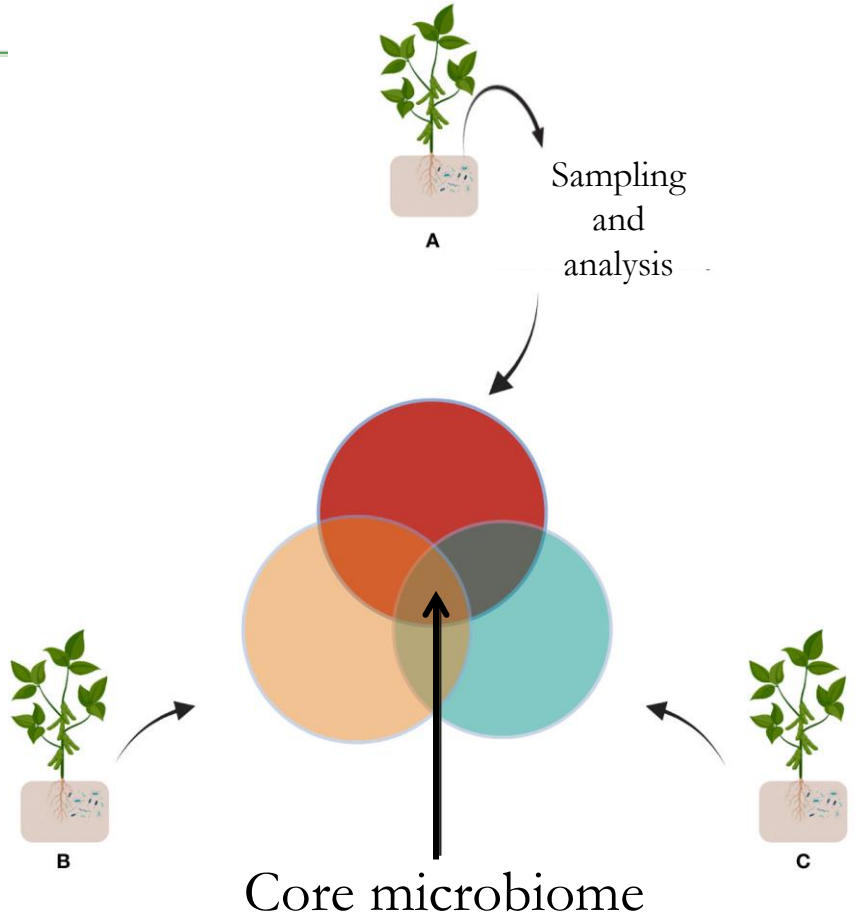
Selective recruitment



- *Proteobacteria*
- *Bacteroidetes*
- *Bacillota/Firmicutes*
- *Actinobacteria*
- *Cyanobacteria*
- *Plantomycetes*
- *Verrucomicrobia*
- *Gemmatimonades*
- *Acidobacteria*
- *Others*

adapted from Liu et al. (2017)

Core microbiome is a set of microbial taxa that are characteristic of a host or environment of interest



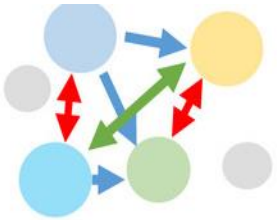
adapted from Shayanthan et al., (2022)

Core microbiome

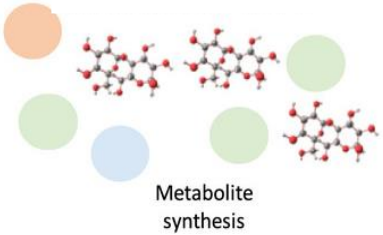
Common core

Temporal core

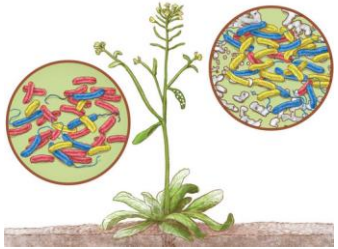
Ecological core



Functional core

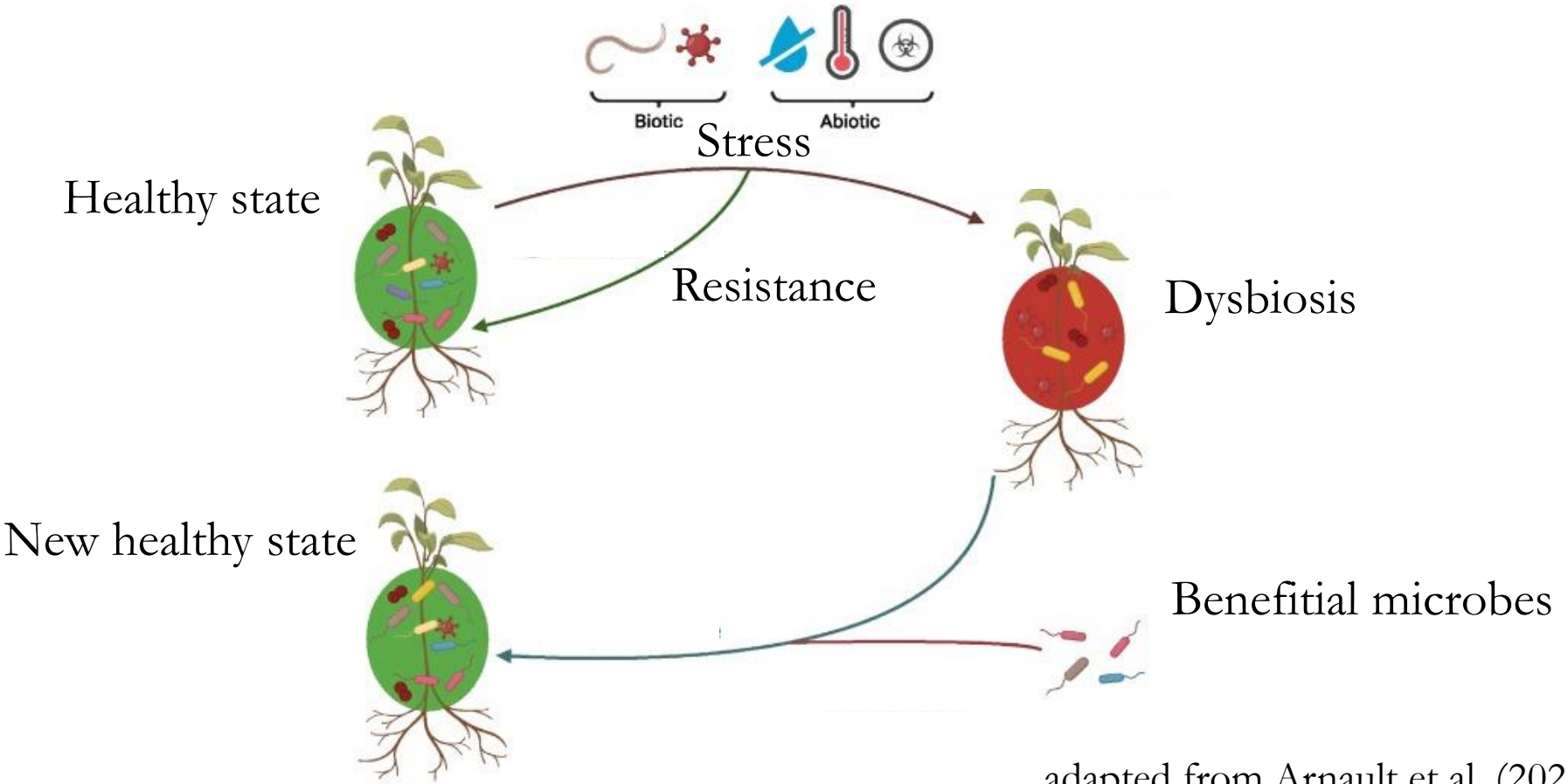


Host-adapted core



adapted from Risely (2020)

Dysbiosis



adapted from Arnault et al. (2022)

Seed-associated bacterial population

- Epiphytic:

from 10^4 CFU/g seed to 10^6 to 10^8 CFU/g seed

- Endophytic:

from 10 to 10^2 CFU/g seed to as high as 10^6 to 10^8 CFU/g seed

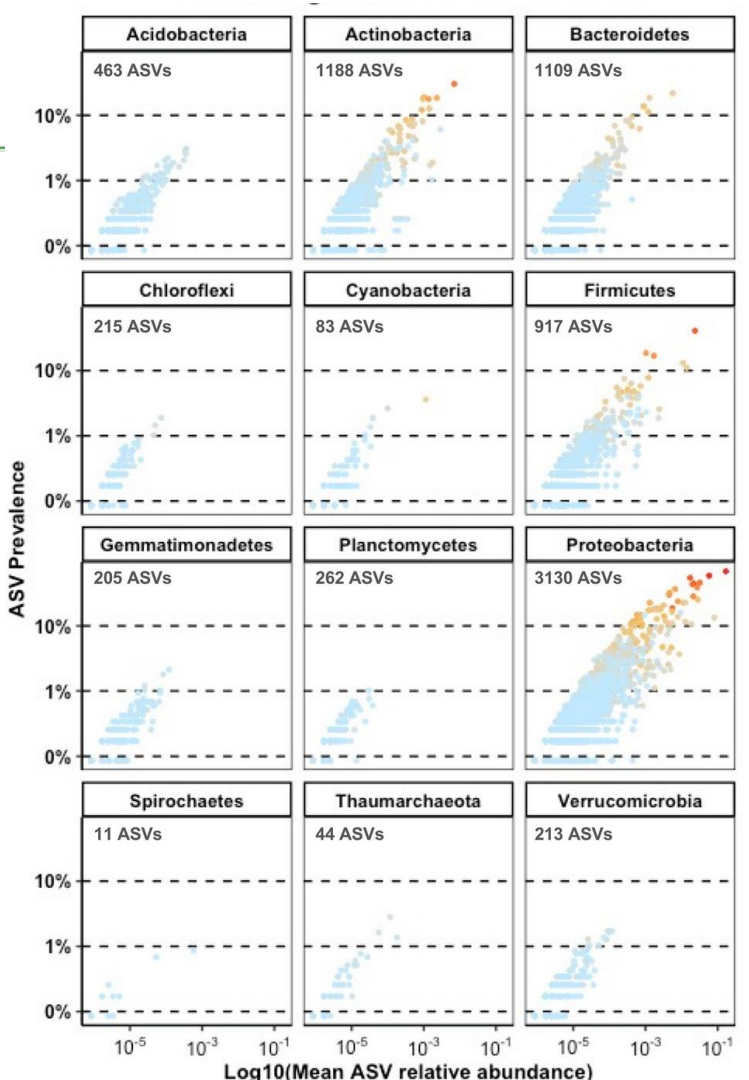
Seed microbiome

Meta-analysis study by M. Simonin et al. (2022):

- 63 seed microbiota studies
- 3190 seed samples
- 50 plant species
- 28 countries

Microbiome of seeds

- Across 2 archaeal and 41 bacterial phyla 12 phyla were dominant in terms of relative abundance (99.7% of reads)
- Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes are the most dominant in terms of abundance and diversity
- Archaea represent only 0.1% of reads



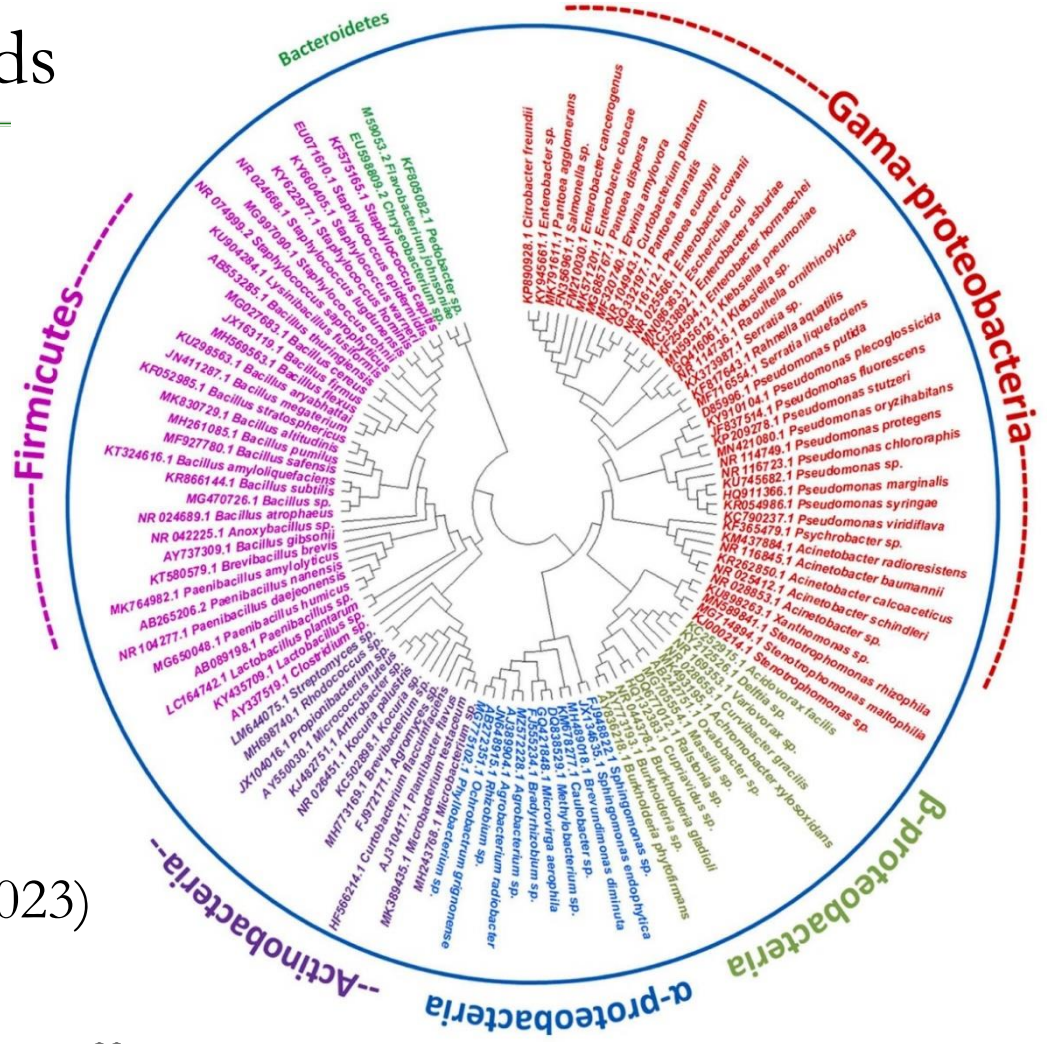
adapted from Simonin et al. (2022)

Microbiome of seeds

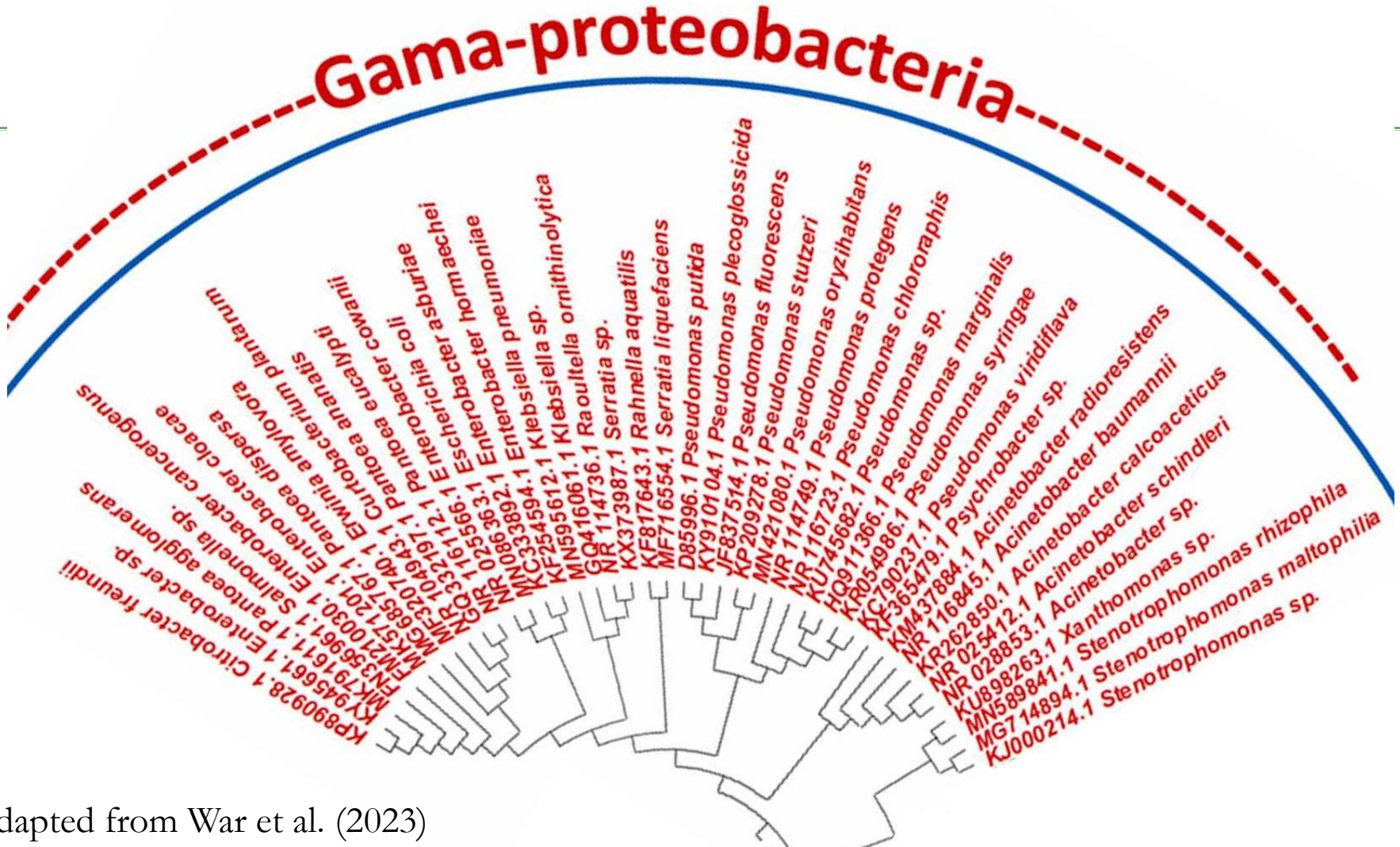
Proteobacteria (125 genera):
- γ-proteobacteria (55 genera)
- α-proteobacteria (12 genera)
- β-proteobacteria (12 genera)

Actinobacteria (49 genera)

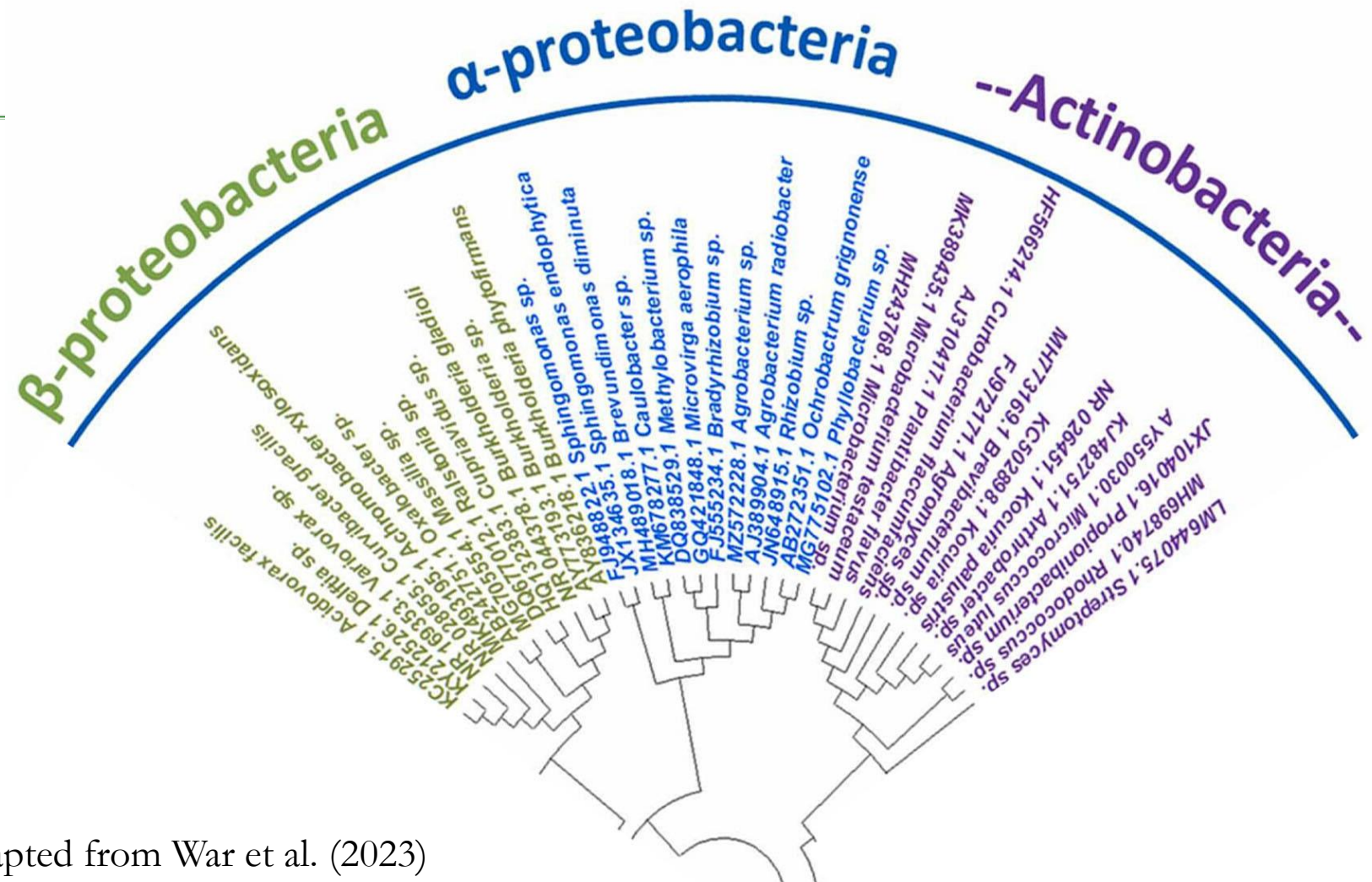
Firmicutes (36 genera)



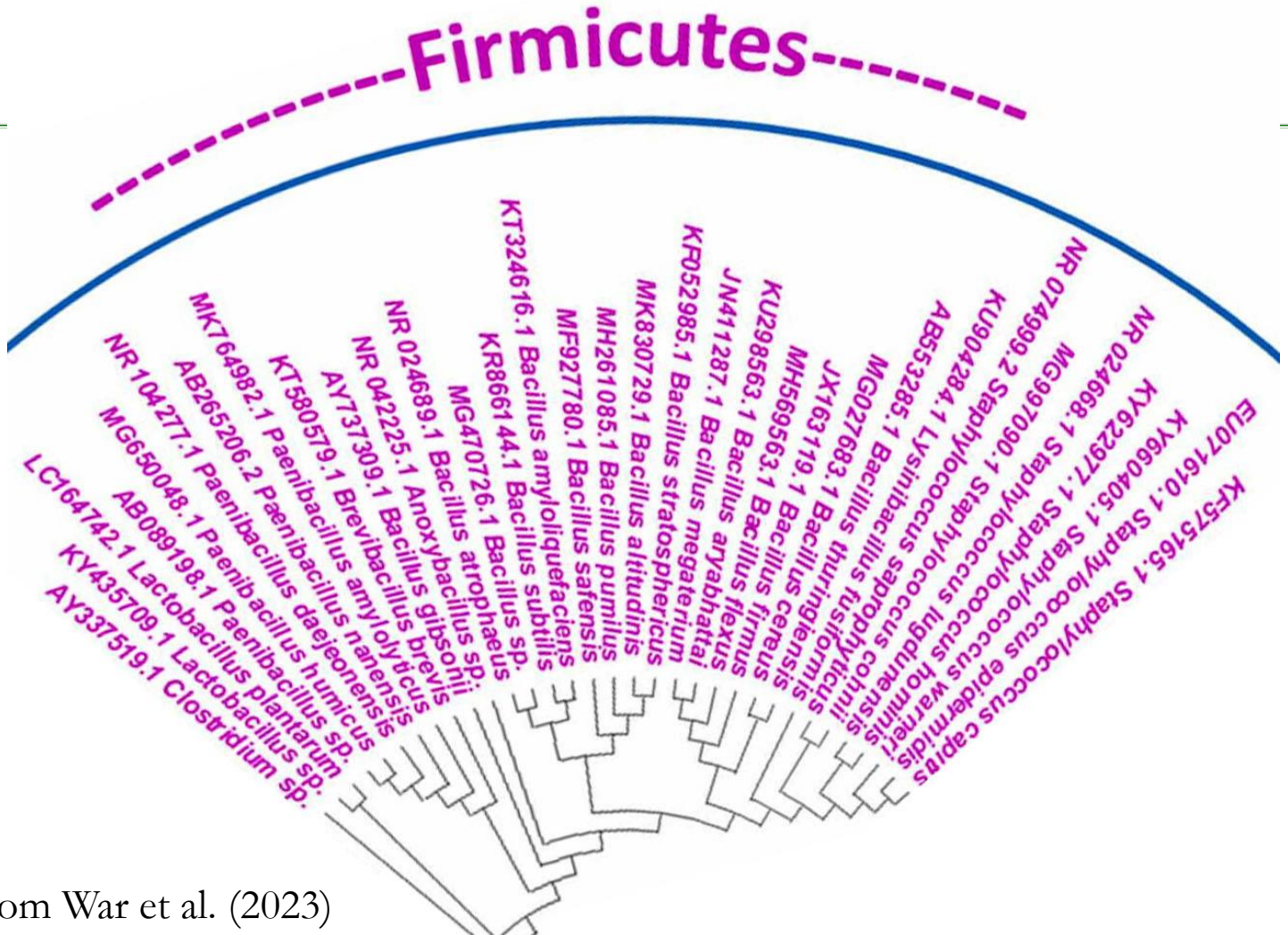
War et al. (2023)



adapted from War et al. (2023)



adapted from War et al. (2023)



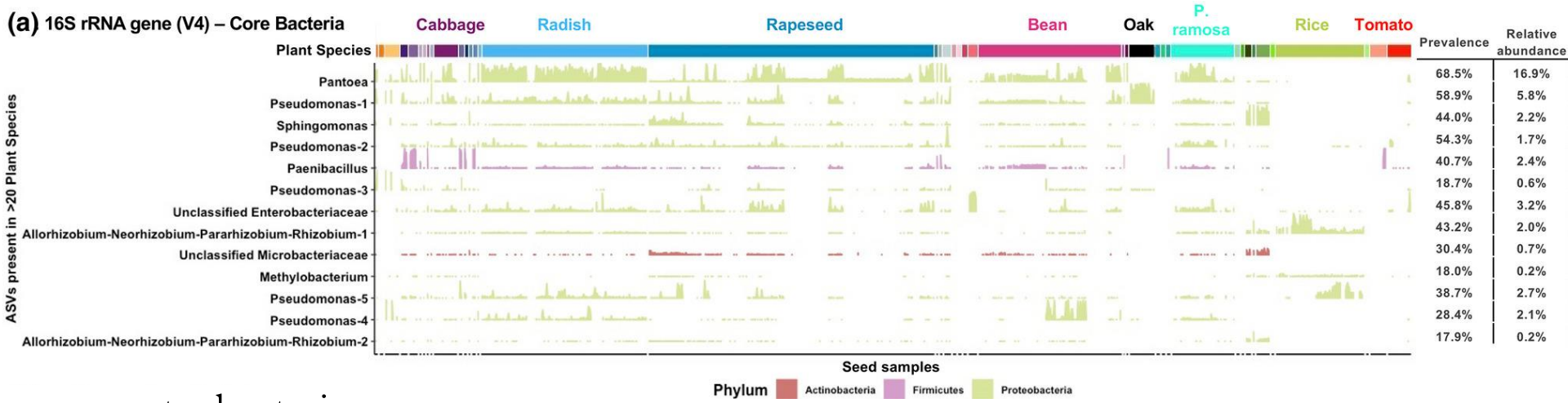
adapted from War et al. (2023)

Seed microbiome

Individual bacterial species vary for:

- plant species
- genotype
- stages of seed development
- geographical locations

Seed core microbiome



γ-proteobacteria:

Pantoea (16.9%; 68.5%)

Pseudomonas (0.6-5.8%; 18.7-58.9%)

α-proteobacteria:

Sphingomonas (2.2%; 44.0%)

Methylobacterium (0.2%; 18.0%)

Rhizobium (0.7%; 30.4%)

Firmicutes:

Paenibacillus (2.4%; 40.7%)

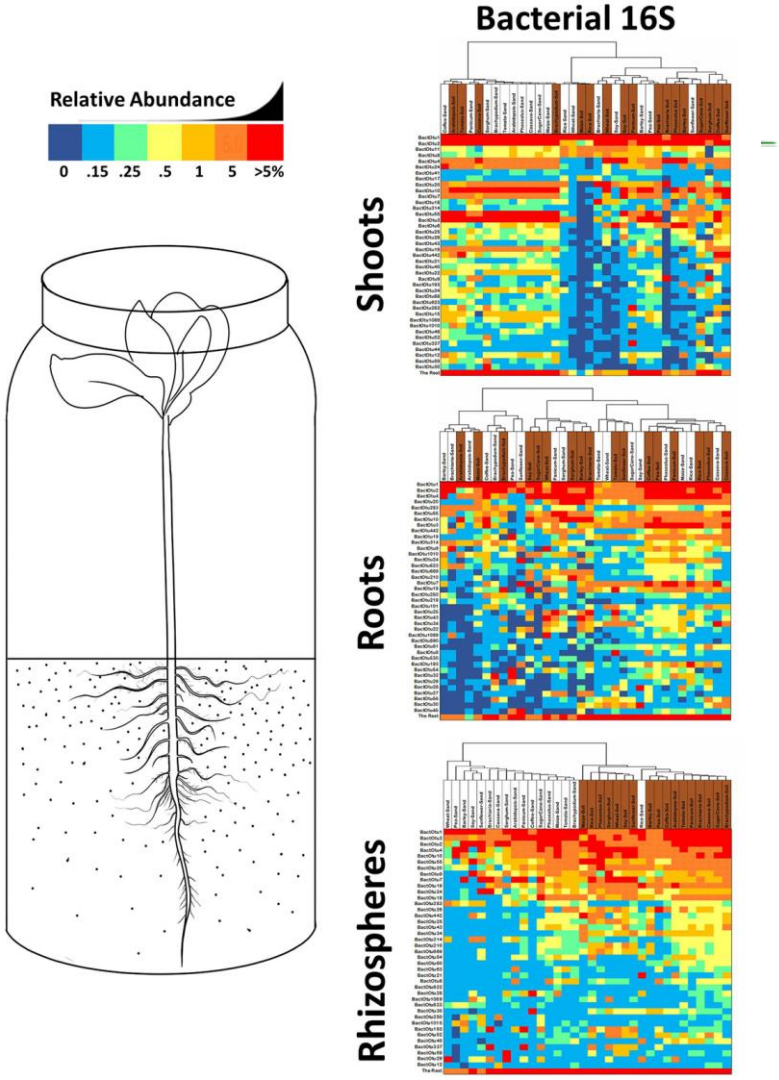
adapted from Simonin et al. (2022)

Seedling microbiome

Seed-associated and endophytic bacteria may provide a bulk of the species pool from which the seedling microbiome is recruited.

Johnston-Monje et al. (2021):

- for 17 plant species core seed-transmitted bacteria were also found in field soil-grown plants.
- soil served as a minor source of bacterial diversity to juvenile plants.



adapted from Johnston-Monje et al. (2021)

NTP effect on seed microbiota

- Microbial cell and spore inactivation
- Microbial growth and biochemical activity stimulation
- Plant growth promoting effect

Bacteria and spore inactivation

NTP-generate components that impair cell membrane and wall integrity and damage DNA and protein:

- ROS and RNS produced in air atmosphere
- UV irradiation

Bacteria and spore inactivation

Bacteria inactivation by NTP treatment was reported for seeds:

- chickpea [Mitra et al., 2014],
- alfalfa, onion, radish, cress [Butscher et al., 2016],
- cucumber, pepper [Štepánová et al., 2018],
- lentil [Waskow et al., 2018],
- rice [Khamsen et al., 2016],
- barley [Los et al., 2019]

Bacteria and spore inactivation

- prolongs shelf life of seeds
- beneficial for safety of seed-derived foods, such as sprouts
- reduce occurrence of seed-born bacterial diseases

Stimulation of bacterial growth

Depending on dose of the NTP treatment and composition of generated reactive species plasma treatment may enhance the vitality of bacteria.

- *Salmonella enteritidis* - increase in the abundance of proteins related to carbohydrate and nucleotide metabolism [Ritter et al., 2018].

- *Pseudomonas aeruginosa* - upregulation of bacterioferritin B protein to NTP-induced oxidative stress response [Yau et al., 2018].

- *E. coli* and *Deinococcus radiodurans* - activation of the oxidative stress response and DNA repair processes [Sharma et al., 2009] and [Roth et al., 2010].

No data for bacteria on NTP-treated seeds

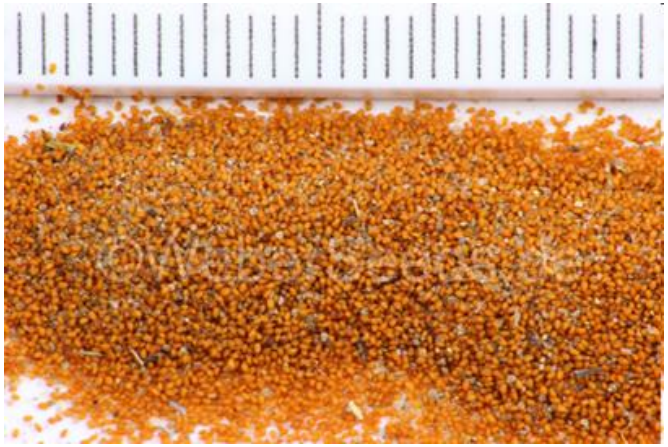
Effect of NTP on plant-associated microbiota

- NTP-mediated inactivation or activation of seed-associated microbiota could lead to a long-term effect on plant development, resistance to pathogens and productivity.

- NTP seed treament could induce changes in plant and, especially, in root physiology that could result in altered interaction with the soil microbiome and colonization by rhizosphere and endophytic bacteria.

NTP treatment of seeds

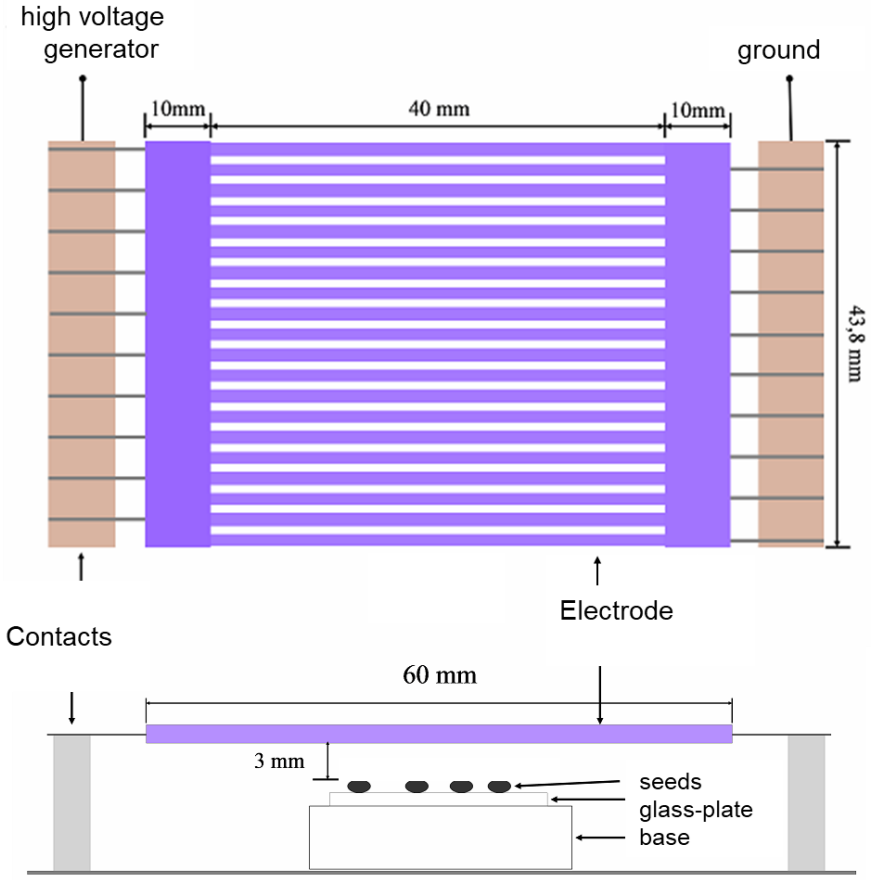
Species:	Common sunflower (*Helianthus annuus*)	Thale cress (*Arabidopsis thaliana*)
Family:	Asteraceae	Brassicaceae



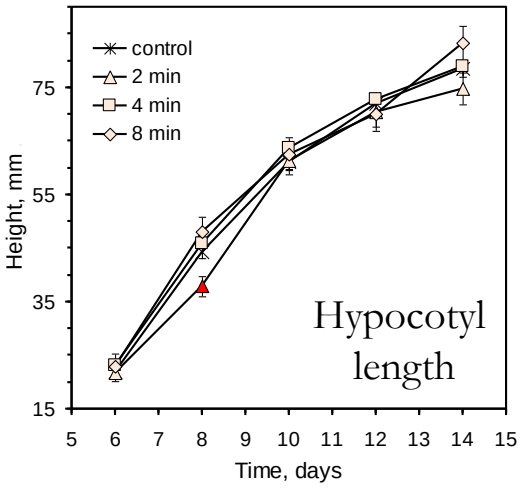
DBD plasma source

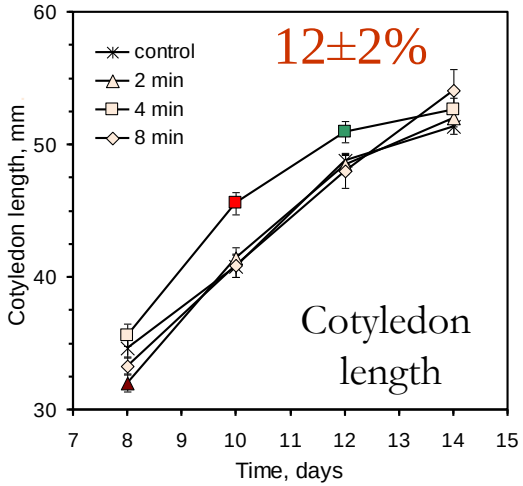
Koga et al., (2015)

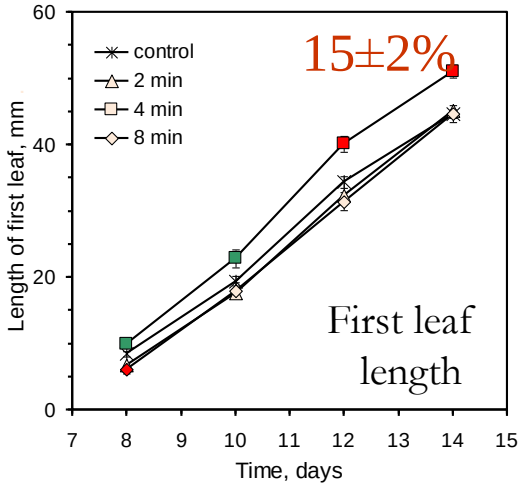
discharge voltage: 7.0 kV,
frequency: 14.4 kHz
power: 4.64 W
distance: approx. 3 mm.
relative humidity: 40%–60%.
irradiation duration: 2 min
intervals: 1 min
seed surface temperature: <45 °C



Sunflower seedlings (2 weeks)

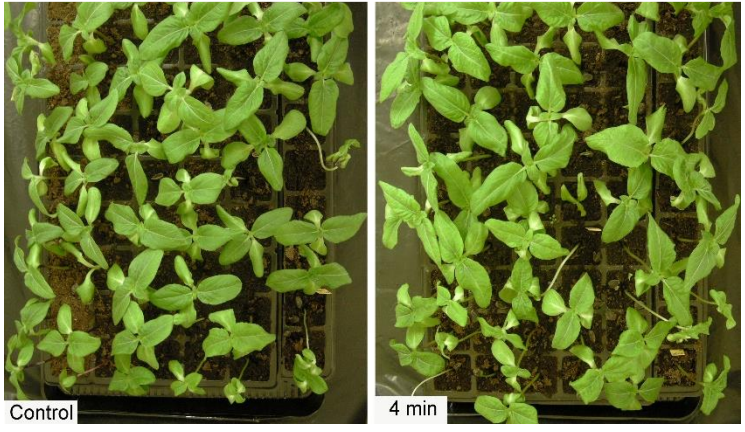






red, $p<0.01$
green, $p<0.05$

Roots of the seedlings germinated from the 4 min treated seeds were 8.7% ± 2.7% longer compared to control



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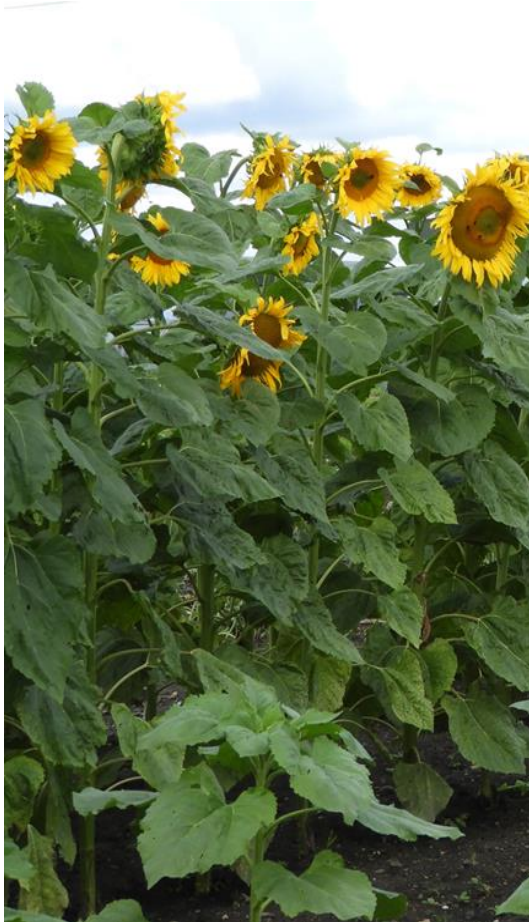
Sunflower plants (2 months)

Parameter	Experimental group		
	Control	4 min (CP4)	8 min (CP8)
Plant height, cm	221.8±3.9 (n=38)	218.0±3.3 (n=37)	228.7±6.7 (n=18)
Area of capitulum, cm^2	747.7±26.4 (n=33)	**842.5±30.4** * (n=37)	754.8±34.3(n=18)
		13±4%	
Relative leave length, % #	– (n=284)	**108.1 ±1.8** * (n=164)	100.4±2.1 (n=142)
Relative leave width, % #	– (n=282)	**109.9±2.5** * (n=164)	98.0±2.4 (n=142)

cumulative value of relative dimensions compared to control was estimated separately for five true leaves



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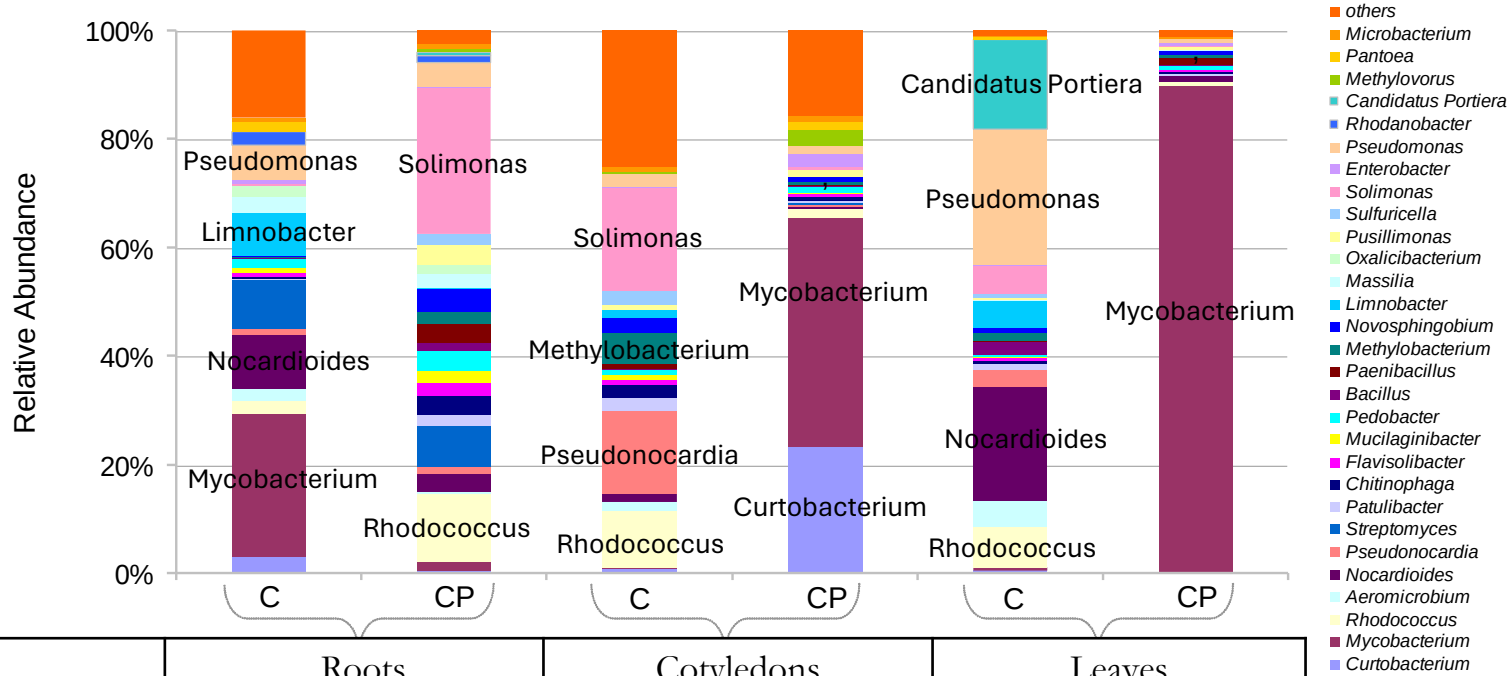
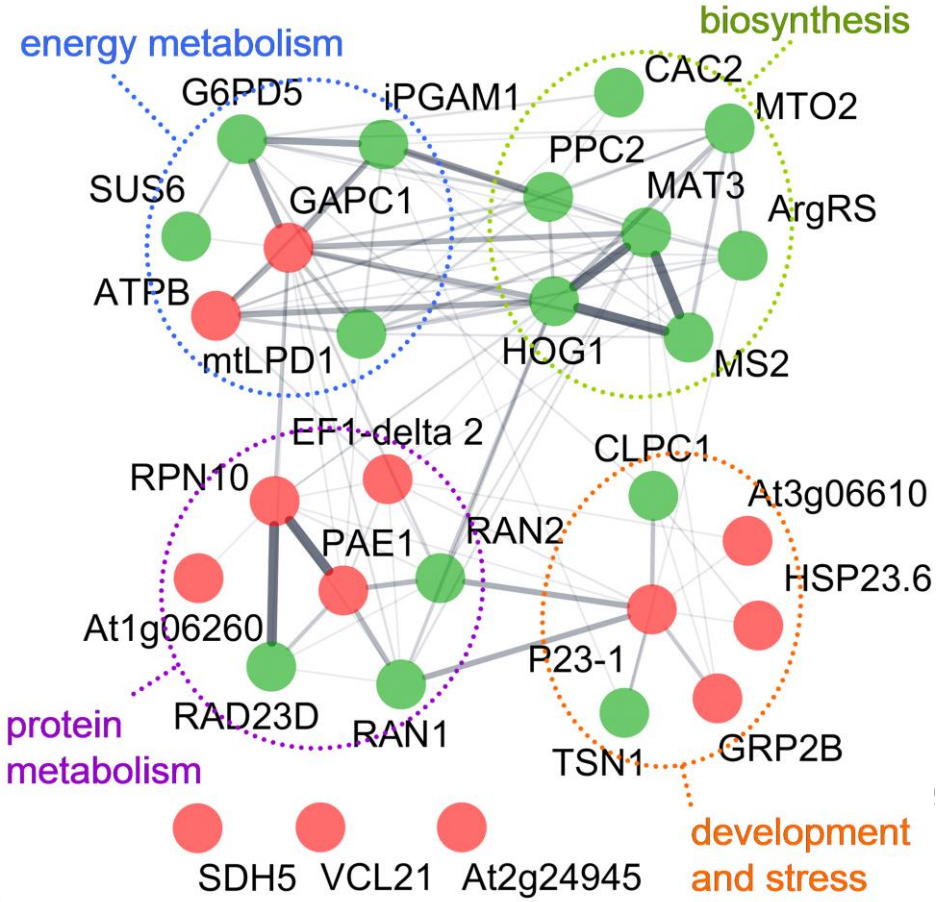
Sunflower seedling microbiome



Parameter	Roots		Cotyledons		Leaves	
	Control	CP4	Control	CP4	Control	CP4
Simpson index (S)	0.897	0.905	0.885	0.643	0.893	0.193
Shannon index (H')	4.402	4.428	3.975	2.295	4.244	0.955
Chao1 estimator/ OTUs	113	97	65	41	114	58

Sunflower seedling proteome

Sample	Differentially expressed spots (CP 4 min)
Roots	67
Cotyledons	2
Leaves	3



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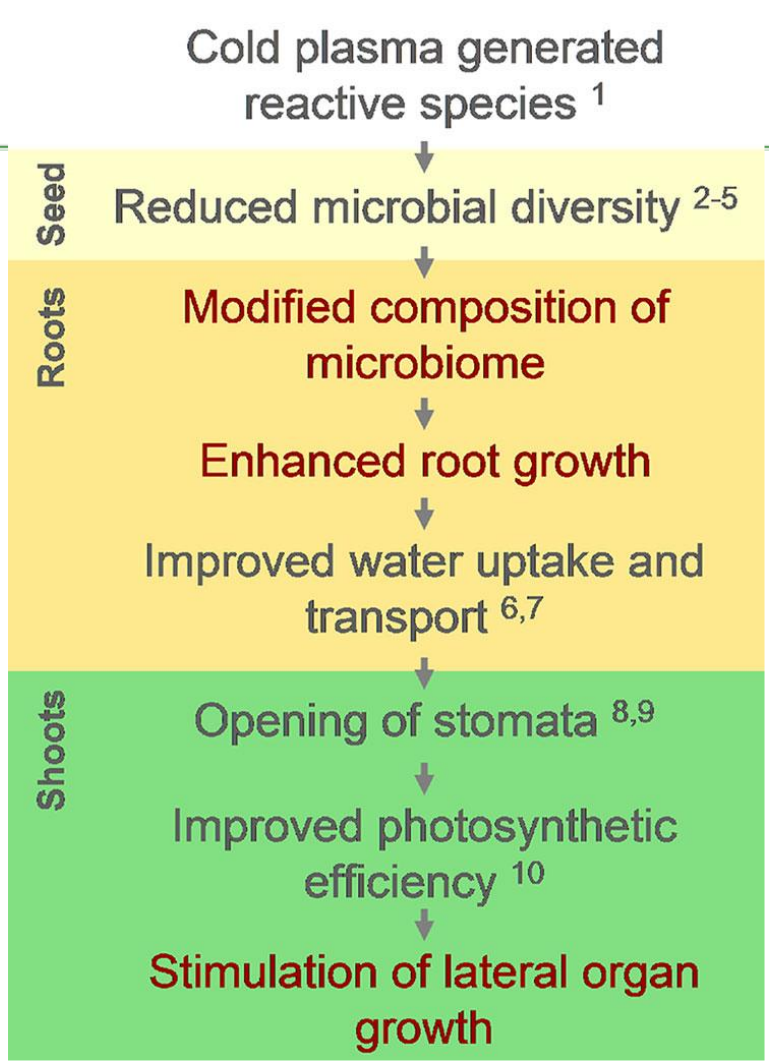
Seedling response to NTP

- NTP treatment have a long-term effect on microbial composition in the rhizosphere and/or endosphere which could be linked to stimulation of root elongation.

- Growth stimulation of the roots is likely the basis for enhanced lateral organ growth due to an increase in water uptake and/or direct root signaling



1 (Whitehead, 2016); 2 (Patil et al., 2016); 3 Han et al., 2016);
4 (Hong et al., 2009); 5 (Reineke et al., 2015);
6 (Bogre et al., 2008); 7 (Chitwood and Sinha, 2016);
8 (Pinheiro and Chaves, 2010); 9 (Hepworth et al., 2016);
10 (Zukiene et al., 2019).

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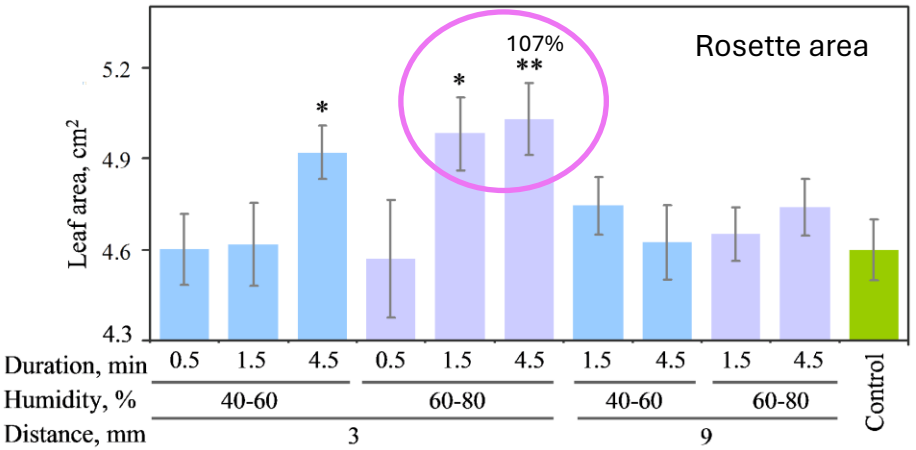
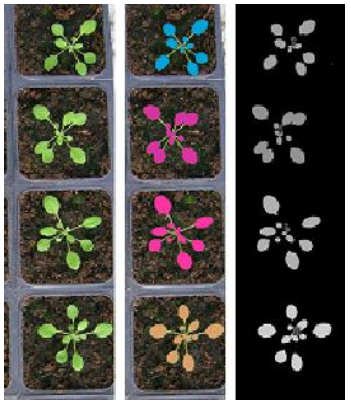
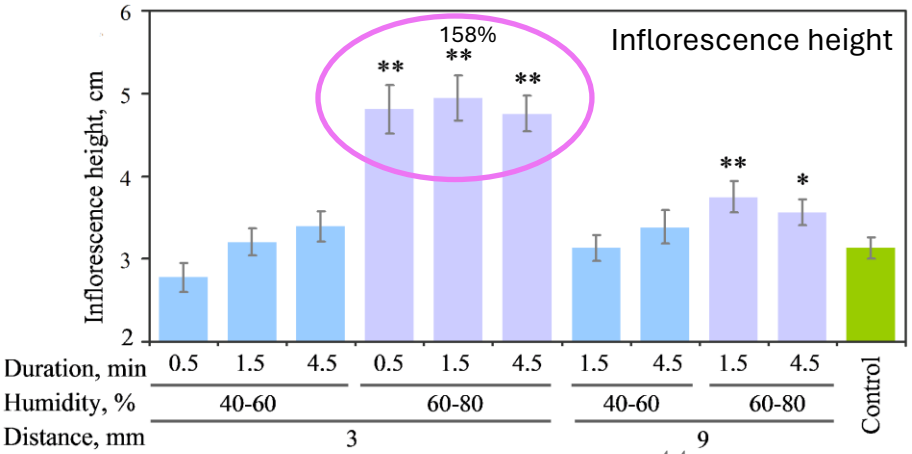
Arabidopsis plants







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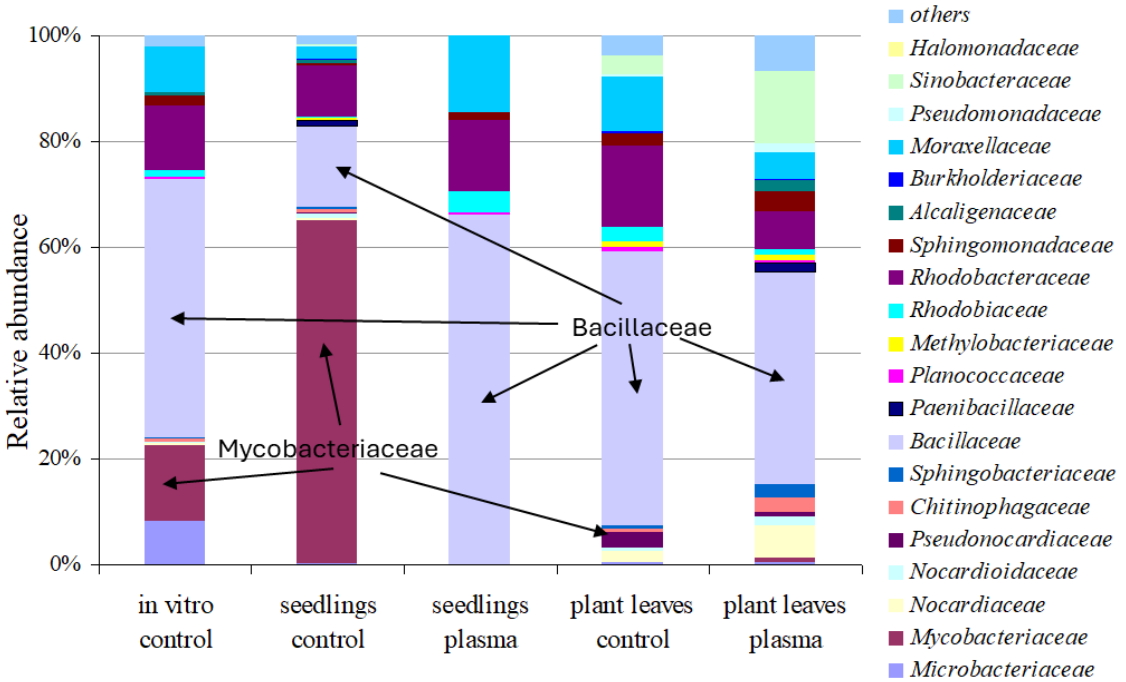
Arabidopsis microbiome



Parameter	In vitro	Seedlings		Leaves	
	Control	Control	CP3	Control	CP3
Simpson index (S)	0,795	0,630	0,769	0,806	0,868
Shannon index (H')	2,859	2,307	2,490	3,057	3,745
Chao1 estimator / OTUs	41	53	14	70	77

Summary

- NTP treatment of seeds stimulated growth of roots and lateral organs (leaves and inflorescences) in sunflower and Arabidopsis in a treatment conditions-dependent manner.

- NTP reduces bacterial diversity in seeds and has a long-term effect on composition of the plant-associated microbiome that is likely involved in regulation of the NTP-stimulated plant growth.

Future research perspective

It remains to be answered whether NTP-induced changes in plant-associated microbiome occur due to:
1) a direct effect of plasma on microorganisms residing on seed surface or inside the seeds,
2) or is a consequence of NTP-induced changes in plant physiology that result in altered interaction with the soil microbiota and colonization by endophytic bacteria.

Future research perspective

As experimental evidence suggests NTP-induced changes in seed and plant microbial diversity, a long-term impact of the new NTP-based seed decontamination and plant growth-enhancing technologies on diversity of plant and soil microbiota should be addressed.

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