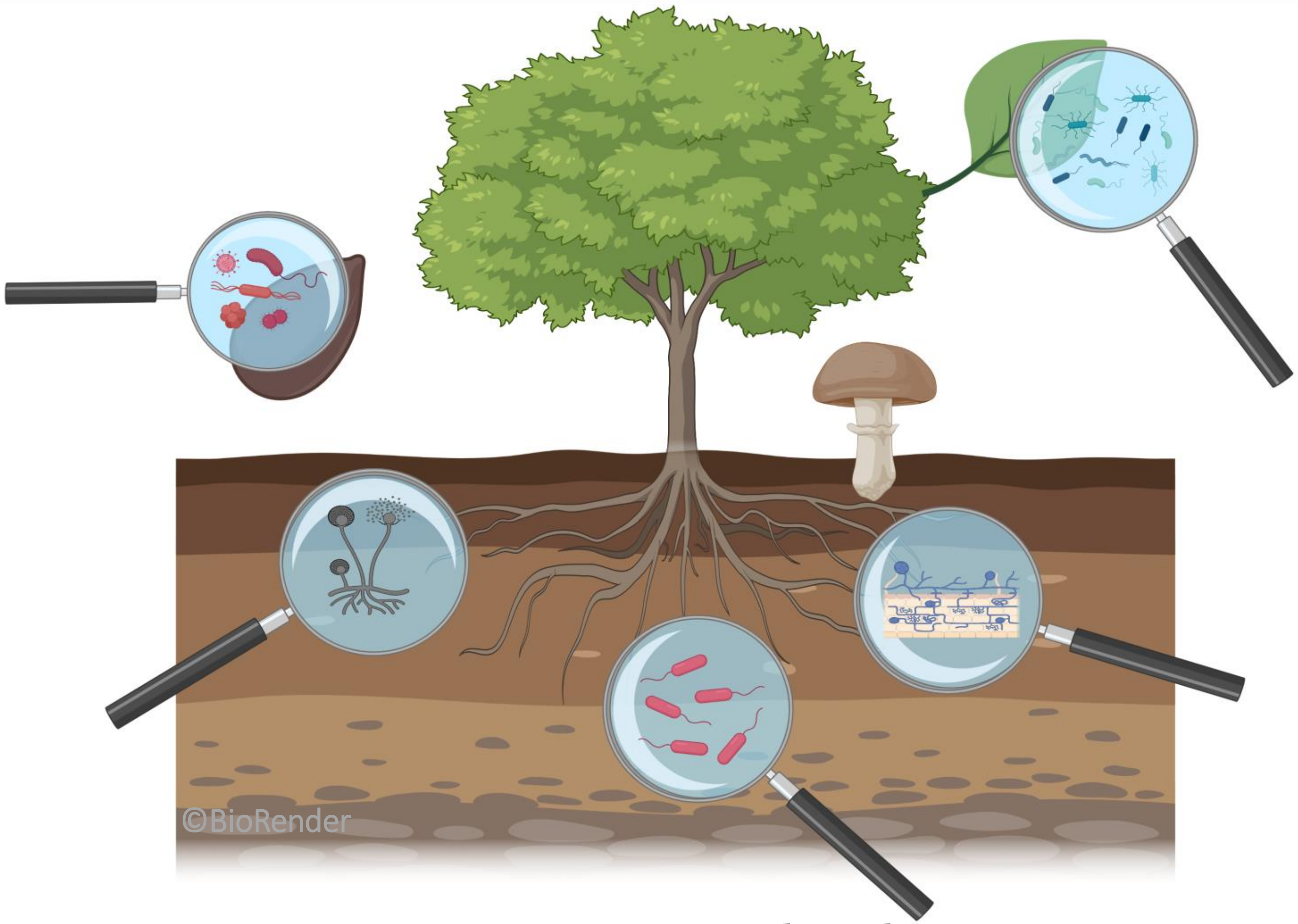
The Biology of Plant-Microbial Interactions



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CA19110
Plasma applications
for smart and
sustainable agriculture

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Biotechnical Faculty

Biotechnical faculty, UL

- **University of Ljubljana**
  - Oldest & largest University in Slovenia (1919)
  - **23 faculties + 3 academies**
  - **~ 40.000 students**
- **Biotechnical Faculty UL:**
  - One of the largest members
  - **~ 3.000 students; ~ 670 employees**
  - 13 study programmes on BSc
  - 14 study programmes on MSc
  - 1 study programme on PhD (18 scientific fields)
  - **8 departments**


↑ ANIMAL SCIENCE
(Rodica, Domžale)
BIOLOGY,
MICROBIOLOGY
AGRONOMY,
BIOTECHNOLOGY
LANDSCAPE
ARCHITECTURE
FOOD SCIENCE
DEANS OFFICE
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Arah Piano
Rastlinjak sredozemskih
rastlin in sočnic...
Fakulteta za kemijo in
kemijsko tehnologijo
Biotehniška fakulteta,
Oddelek za gozdarstvo
Najem, izposoja
potniškega in...
Didas Ljubljana, d.o.o
Programerske storitve
in svetovanje, Jakob...
Otroška igralnica
Malina doo
HGI, software
& IT solutions
Slaščičarna Galerija Grad
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Biotechnical faculty, UL

UNIVERSITY OF LJUBLJANA
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- **Chair for Botany and Plant Physiology, Dept. of Biology**
  - Staff:
    - 3 Professors (lecturers), 6 Teaching assistants
    - 5 Laboratory associates/technicians
    - 1 Researcher, 2 PhD Students
    - 2-3 MSc & BSc students on "student work"
  - Research:
    - Cellular changes during plant development (microscopy)
    - Elemental composition of plant tissues (XRF, PIXE)
    - Invasive & indigenous flora (taxonomy)
    - Plant-microbe interactions (mycorrhiza, seed endophytes)
  - Teaching:
    - Botany, Plant Systematics, Plant Physiology, Plant Cell Biology, Plant-microbe Interactions…

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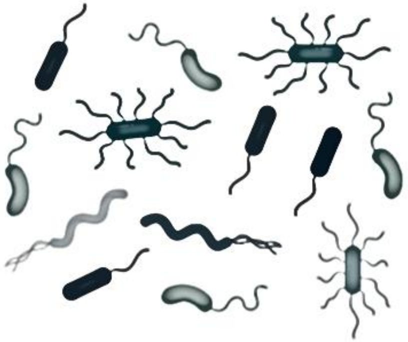

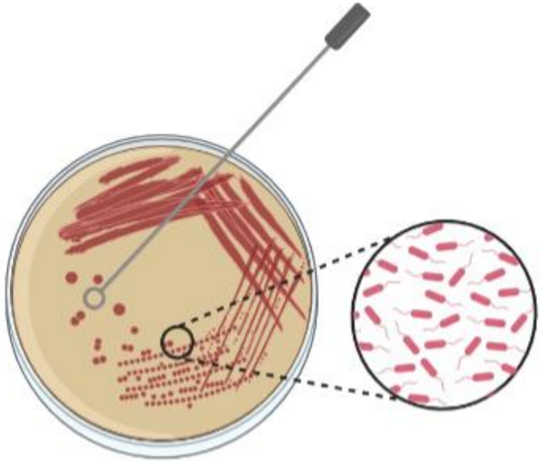

I. The Evolution of Life on Earth

II. Microbial Diversity

III. Interactions of Plants and Microorganisms

i. Plant as a Holobiont

ii. Symbiotic MO (mycorrhizal fungi)

iii. Endophytic continuum

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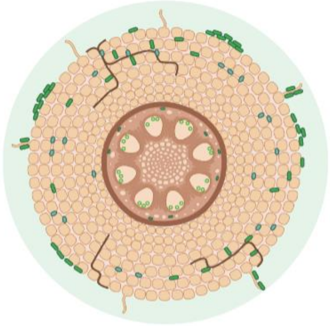
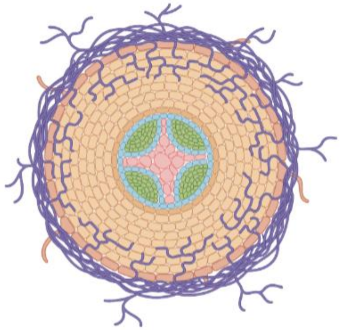
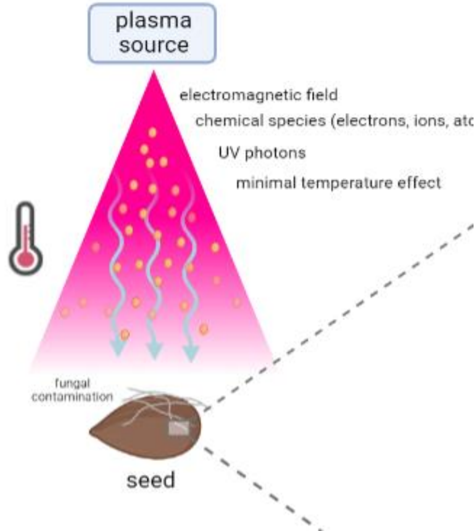


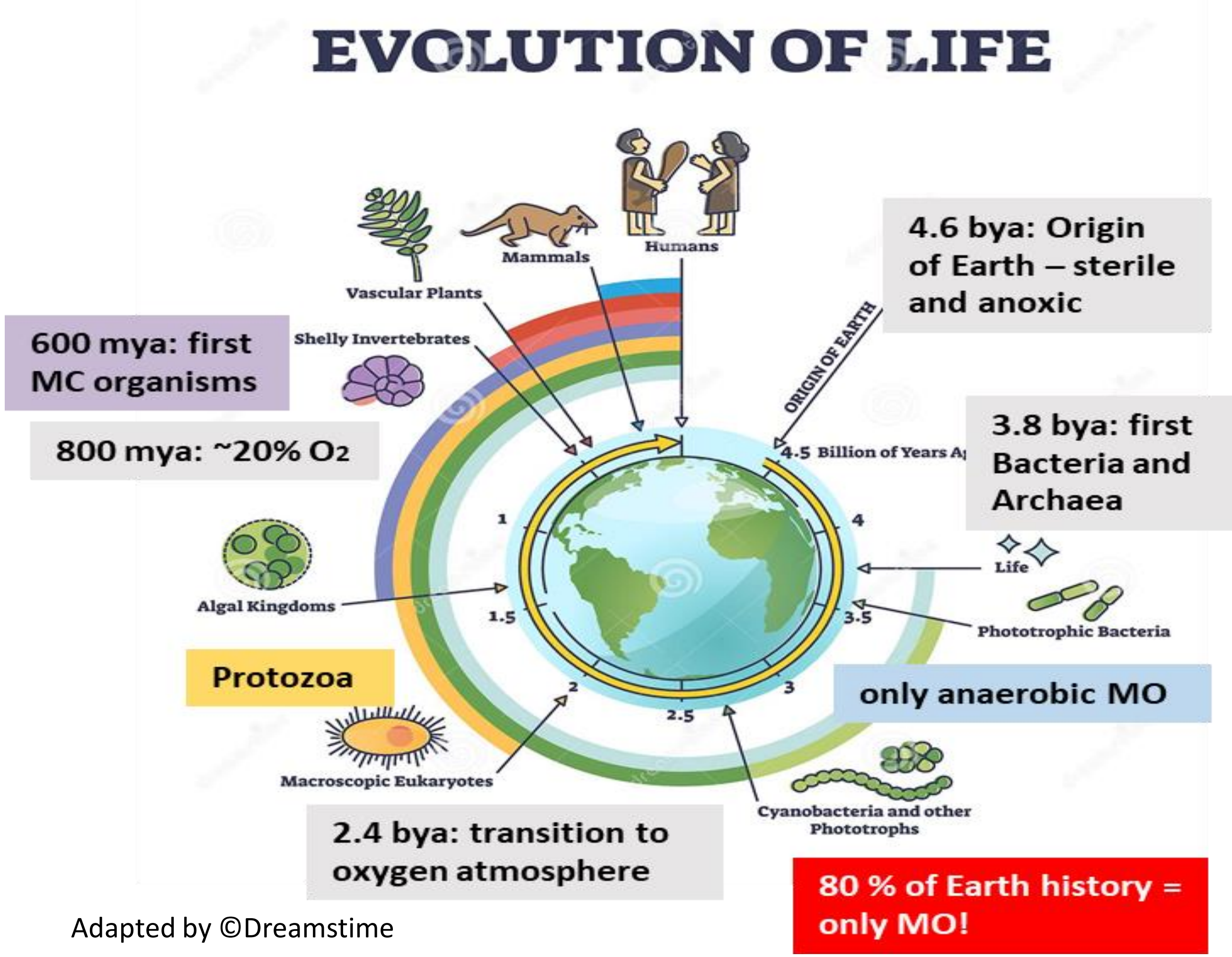




I. The Evolution of Life on Earth

- **How old is life?**
  - Earth: 4,6 bya
  - Life on Earth: > 3,5 bya

- **Evolution**
- **Diversity – biodiversity**
  - Ecosystems
  - Organisms
  - Genes



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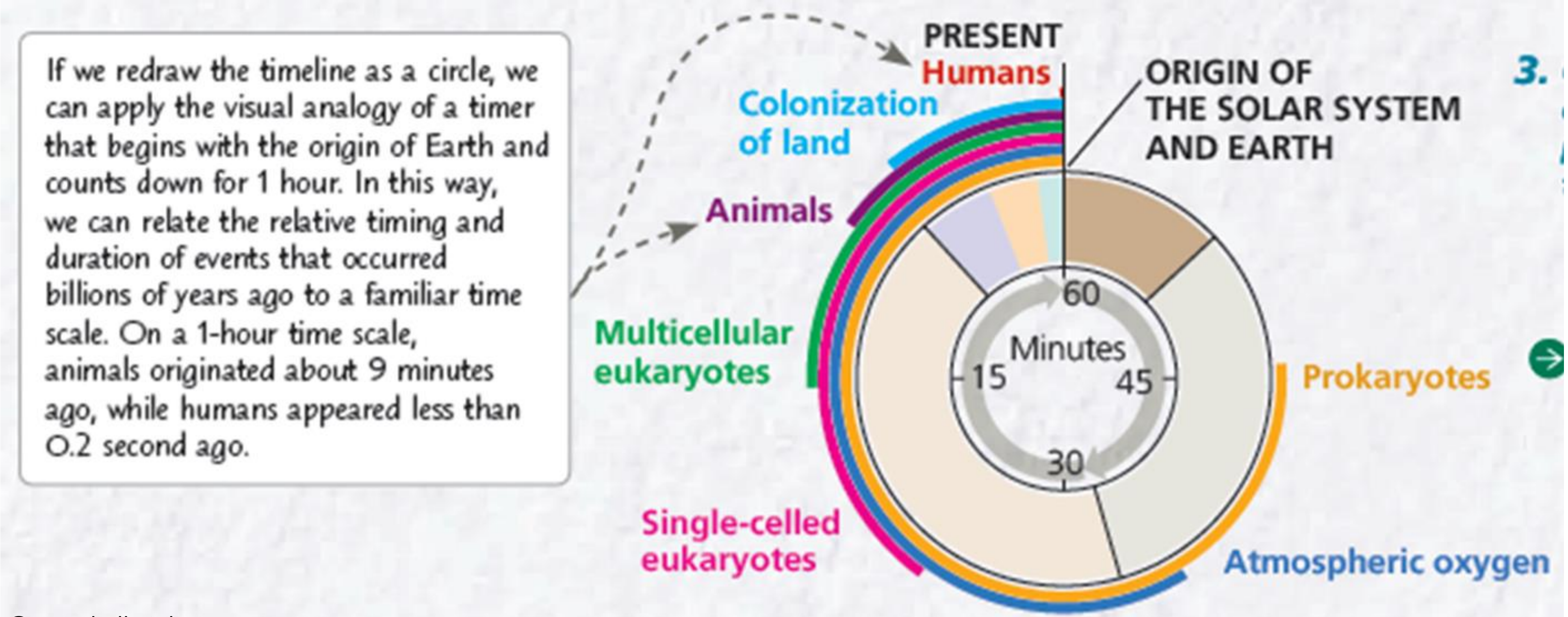
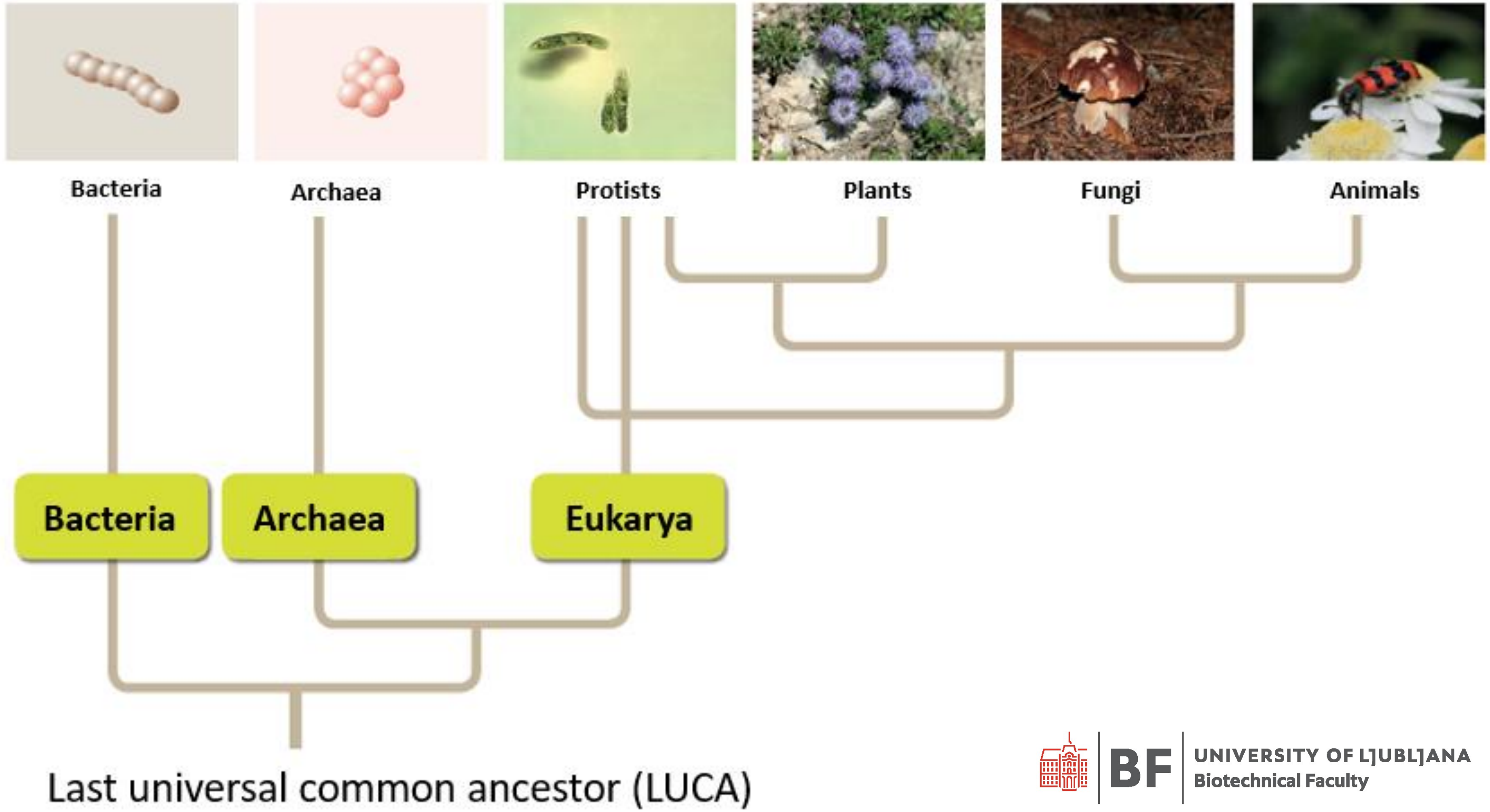
I. The Evolution of Life on Earth

- *If Planet Earth was 1 hour old…*



I. The Evolution of Life on Earth

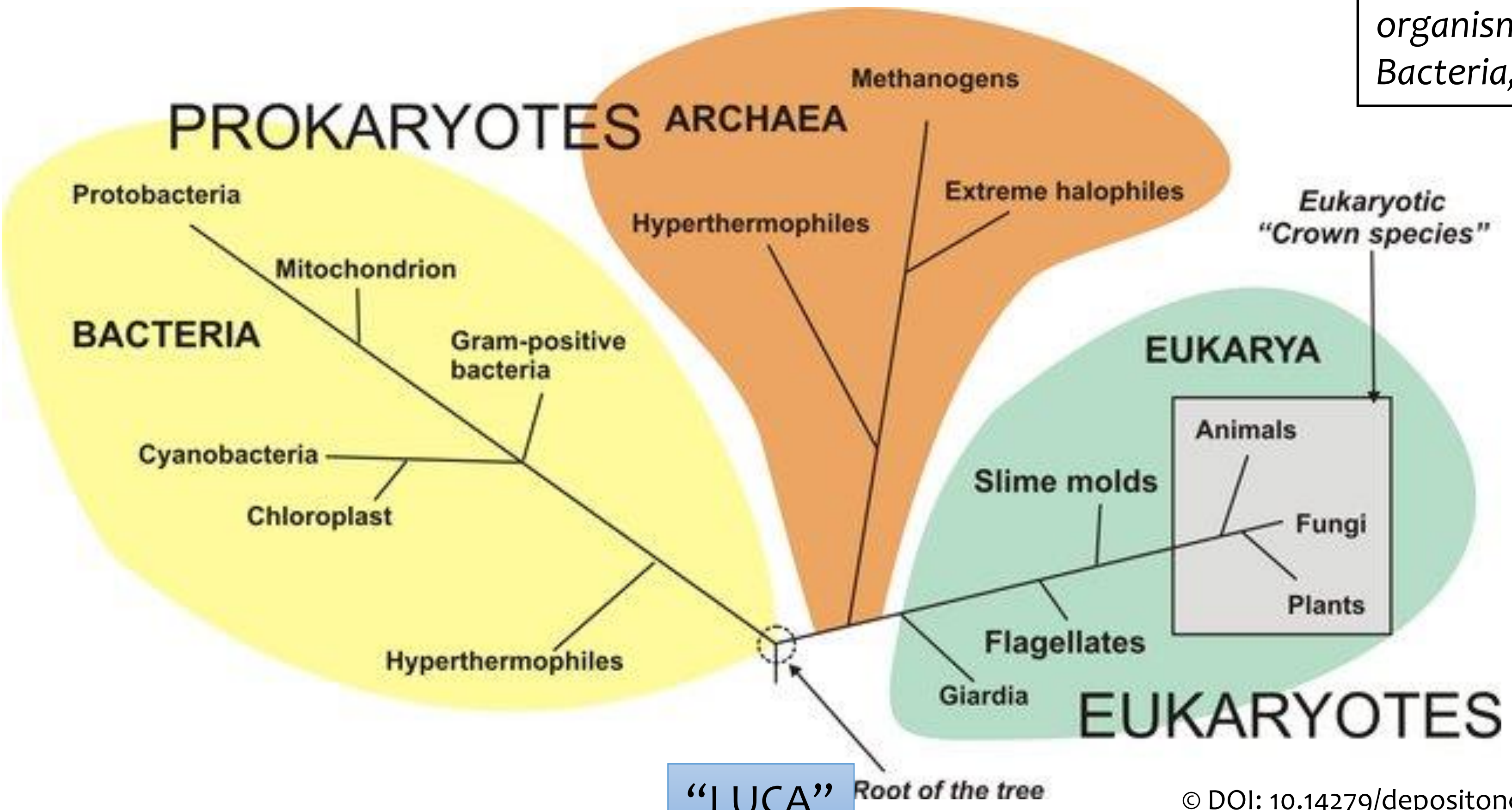
- **Diversity of Life**



The Evolution of Life on Earth

- **All 3 domains of living organisms**

The modern phylogenetic tree of life based on the analysis of rRNA gene sequences as proposed by **Carl Woese, 1990** (*Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya*)



"LUCA"

Highlights that much of the history of life has been single-celled organism!

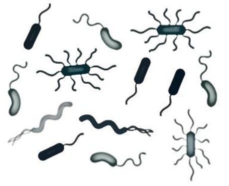
© DOI: 10.14279/depositonce-2755

I. The Evolution of Life on Earth

- **Microorganisms (MO)** = far the **most abundant and diverse** living organisms
  - 2 x 10 ^30 cells
- inhabited the planet Earth for **more than 3.5 billion years** (long before the first P & A appeared!)
- They have **evolved and adapted to nearly every type of environment**, even *the most extreme ones*.
  - **land** and in **water** (+ice); **soils** (+volcanos) and **air** (+clouds); **on** and **inside** other living organisms.
- the most dominant form of life, **a majority of biomass** on our planet → ***essential for sustaining life on Earth***!
- MO activities play critical roles in **biogeochemical cycles:**
  - N circulation & fixation (+ C, O, P, S… cycle)
  - disintegration of organic matter (decomposition).

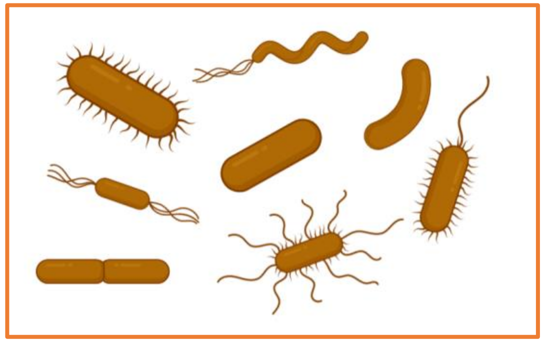
▲ **Figure 27.18 Extreme thermophiles.** Orange and yellow colonies of thermophilic prokaryotes grow in the hot water of Yellowstone National Park's Grand Prismatic Spring.

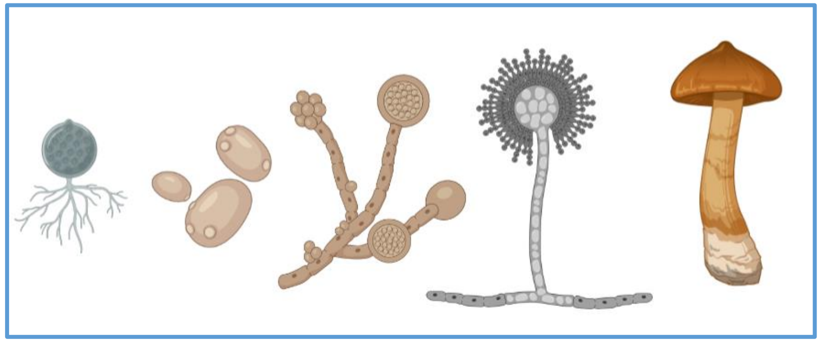
II. The Microbial Diversity

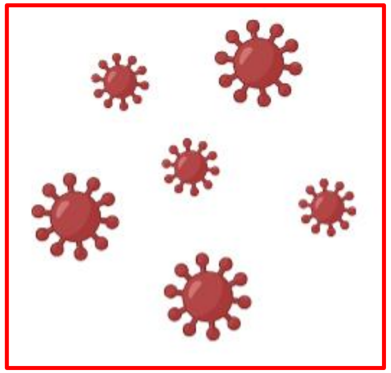


- **Microbiology** = science of microorganisms (MO)
- MO = *microscopic organisms, mostly single-celled, some can form complex multicellular structures.*
- **Prokaryotic** and **eukaryotic** **organisms** from all 3 domains:
  - **Bacteria**
  - **Archaea**
  - **Eucarya**
    - **Protists**
    - **Fungi**
  - Viruses*

"The microbial world is strange and fierce. It is teeming with life, ancient, diverse, and constantly changing. MO are Earth's life support system, and from our first breath they influence nearly every moment of our lives." (Brock Biology of MO, 2021)







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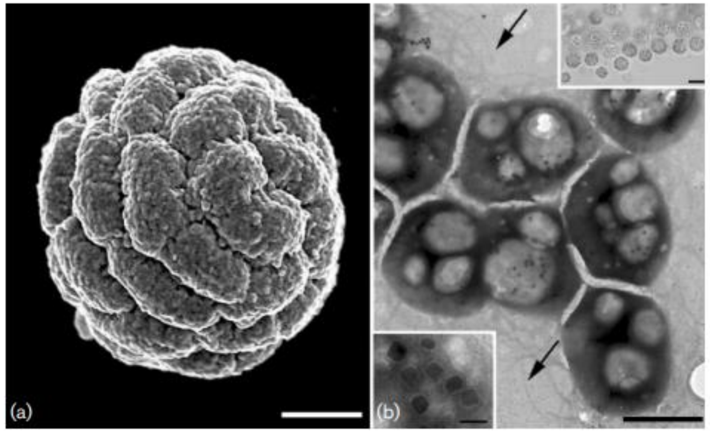
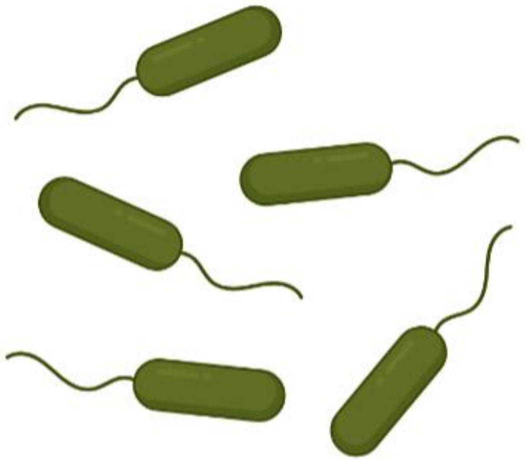
II. The Microbial Diversity

- **Bacteria**
  - **prokaryotic**, undifferentiated, **single cells** (0.5 – 10 µm)
    - *multicellular (e.g. *Magnetoglobus* sp.)
  - 30 phyla that can be grown in lab. cultures
  - More than 90% of cultivated bacteria belong to only 4 phyla: ***Actinobacteria*, *Firmicutes*, *Proteobacteria*,** and ***Bacteroidetes*.**
  - eDNA seq → evidence for the existence of 80+ bacterial phyla!

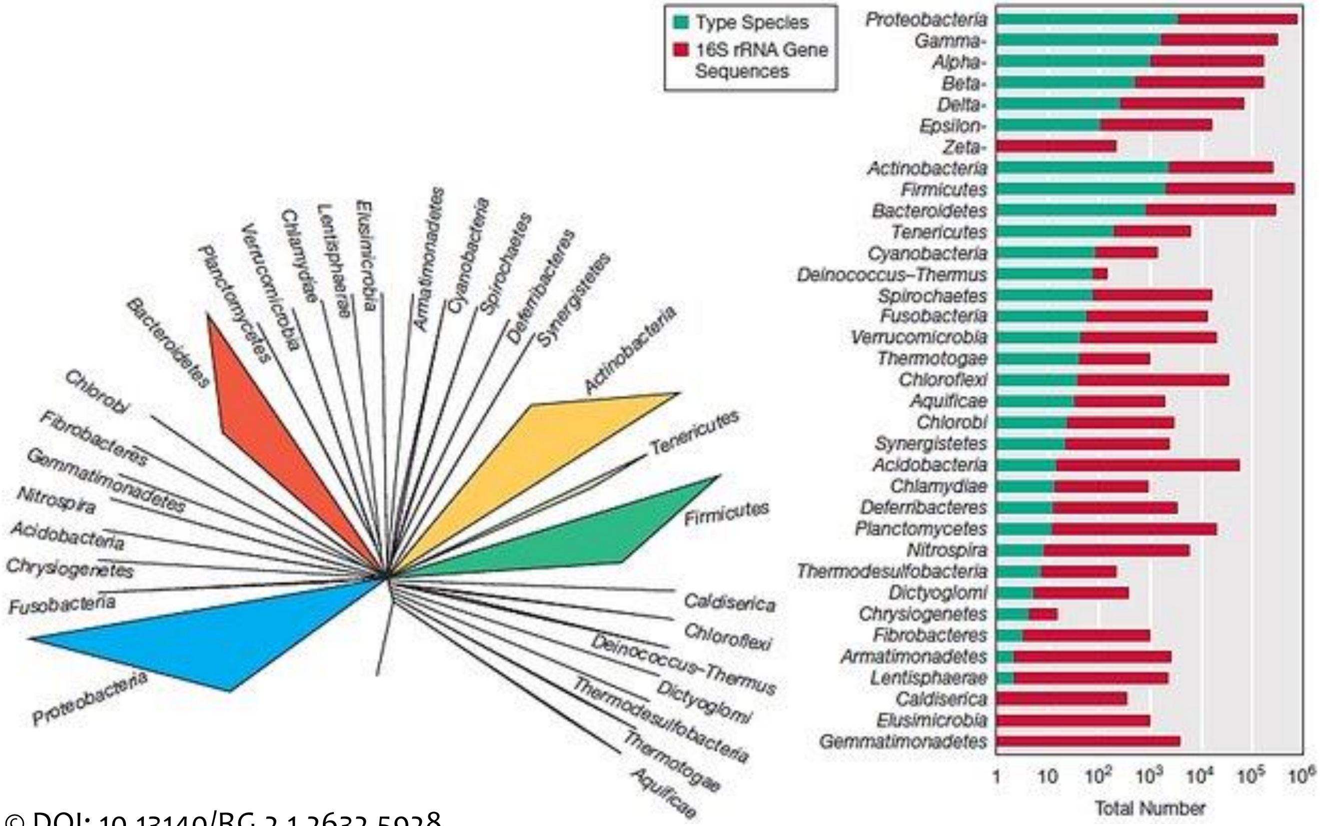


© DOI: 10.1099/ijs.0.64857-0



II. The Microbial Diversity

- **Bacteria**
  - **Major phyla** (based on 16S ribosomal RNA gene sequence comparisons)



*Note: The area of each wedge is roughly proportional to the number of described cultivated species in group.

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II. The Microbial Diversity

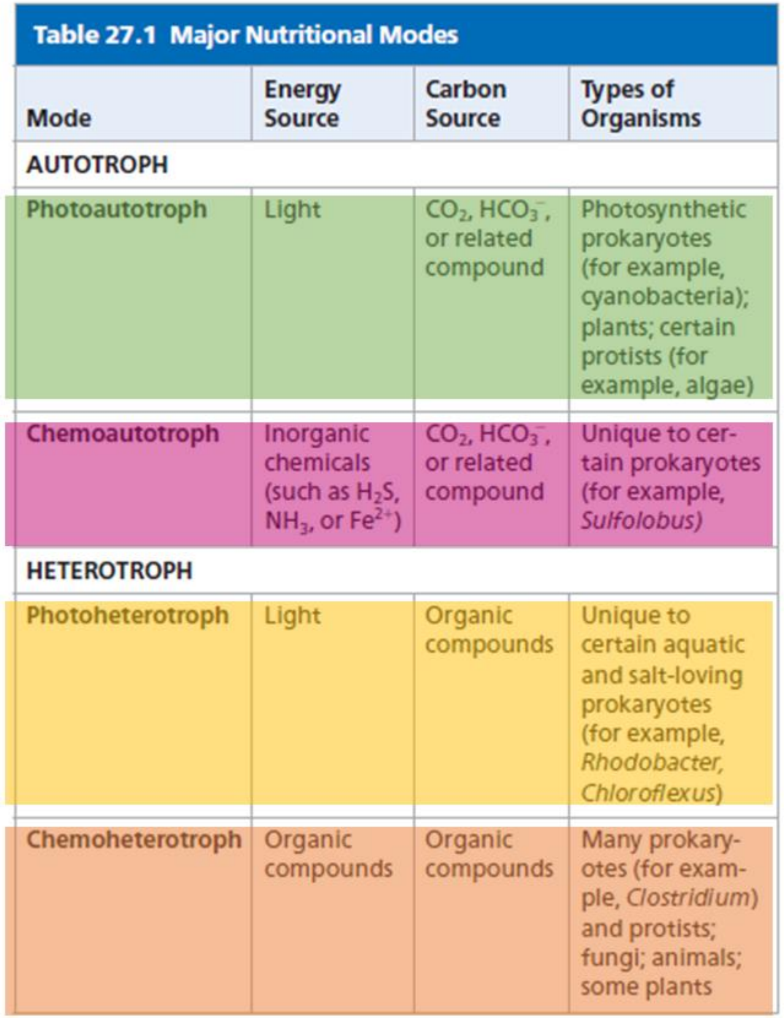
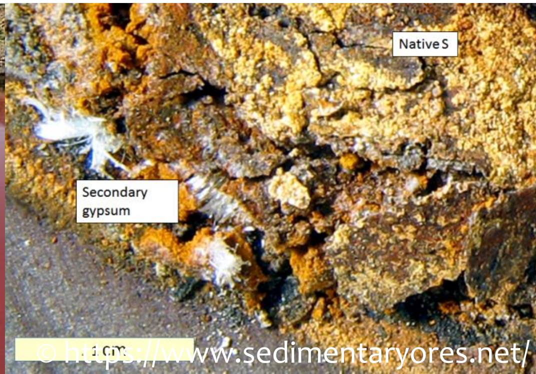


Table 27.1 Major Nutritional Modes			
Mode	Energy Source	Carbon Source	Types of Organisms
AUTOTROPH			
Photoautotroph	Light	CO_2, HCO_3^-, or related compound	Photosynthetic prokaryotes (for example, cyanobacteria); plants; certain protists (for example, algae)
Chemoautotroph	Inorganic chemicals (such as H_2S, NH_3, or Fe^{2+})	CO_2, HCO_3^-, or related compound	Unique to certain prokaryotes (for example, *Sulfolobus*)
HETEROTROPH			
Photoheterotroph	Light	Organic compounds	Unique to certain aquatic and salt-loving prokaryotes (for example, *Rhodobacter*, *Chloroflexus*)
Chemoheterotroph	Organic compounds	Organic compounds	Many prokaryotes (for example, *Clostridium*) and protists; fungi; animals; some plants

- **Bacteria – metabolic diversity:**
  - **Cyanobacteria**: phototrophic (oxygenic photosynthesis), *"blue-green algae"*
  - **Purple Sulfur Bacteria**: anoxygenic phototrophs (H_2S): lakes, marine sediments and "sulfur springs"
  - **Nitrogen Fixing Bacteria**: fix gaseous N_2 into NH_3, symbiotic relationships
  - **Nitrifiers**: ammonia oxidizers ($NH_3 \rightarrow NO_2$), nitrite oxidizers ($NO_2 \rightarrow NO_3$),
  - **Denitrifiers**: anaerobic respiration (NO_3, $NO_2 \rightarrow NO$, N_2O, N_2)
  - **Sulfate-Reducing Bacteria**: Sulfur metabolism probably fueled the earliest forms of life on our planet; anaerobes (anoxic, marine sediments).
  - **Sulfur-Reducing Bacteria**: respiratory reduction of S to conserve energy; more diverse - obligate anaerobes, facultatively aerobic).
  - **Sulfur-Oxidizing Bacteria**: dissimilative chemolithotrophs.
  - **Dissimilative Iron-Reducers**: anoxic freshwater, marine sediments
  - **Dissimilative Iron-Oxidizers**: acidophilic, neutrophilic, anaerobic
  - … *and a lot more.* ☺



II. The Microbial Diversity

- **Bacteria: Worlds toughest**

  - ***Deinococcus radiodurans***
    - ***Extremely radiation & desiccation resistant***
    - Red or pink (carotenoids),
    - Resistant to **ultraviolet (UV)** radiation or even **gamma rays**
      - survives 15,000 grays (Gy) of ionizing radiation!
      - Most vertabrates (human) killed by exposure to less than 10 Gy!
      - Most bacterias can survive up to 200 Gy
    - **highly efficient in repairing damaged DNA,** even from small chromosome fragments (enzyme RecA + more RecA-independent DNA-repairing enzyme systems)
    - Cells in pairs or tetrads: unique toroidal (coiled, rings) structure of DNA → fusion when repairing damages.

© DOI: 10.1038/nrmicro2073

II. The Microbial Diversity

• … to….

• Resista…

…g damages.

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Biotechnol. Prog. 2003, 19, 776–783

Impact of Low-Temperature Plasmas on *Deinococcus radiodurans* and Biomolecules

Rakesh Mogul,[†] Alexander A. Bol'shakov,[‡,§] Suzanne L. Chan,[†] Ramsey M. Stevens,[⊥] Bishun N. Khare,[||] M. Meyyappan,[#] and Jonathan D. Trent[†,*]

Astrobiology Technology Branch, Plasma Research Group, Center for Nanotechnology, NASA A… Center, Moffett Field, California 94035, Eloret Corporation, Sunnyvale, California 94… Mountain View, California 94043

J Ind Microbiol Biotechnol (2012) 39:1367–1375
DOI 10.1007/s10295-012-1137-6

ENVIRONMENTAL MICROBIOLOGY

Contact-free cold atmospheric plasma treatment of *Deinococcus radiodurans*

Tim Maisch · Tetsuji Shimizu · Anindita Mitra · Julia Heinlin · Sigrid Karrer · Yang-Fang Li · Gregor Morfill · Julia L. Zimmermann

Planetary and Space Science 90 (2014) 60–71

Contents lists available at ScienceDirect

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Cold atmospheric plasma – A new technology for spacecraft component decontamination

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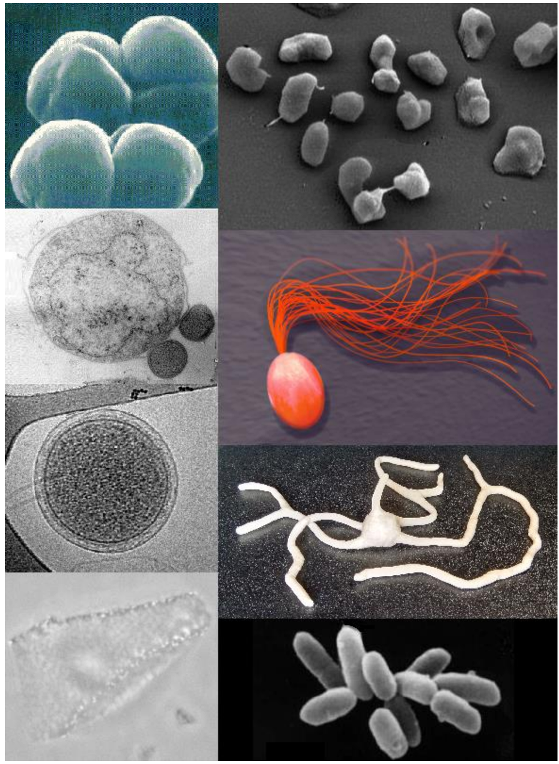
II. The Microbial Diversity

- <u>**Archaea**</u> *Once classified as bacterias (Archaebacteria)*
  - *metabolic pathways + gene expression more similar to eukaryotes.*
  - **associated with extreme environments**: hot, salty, acidic sites.
    - define chemical and physical limits of life.
  - **methane-producing** Archaea (methanogens):
    - Wetlands
    - Guts of animals (including humans) → big impact on the global greenhouse gas emissions!
  - Important in C- and N-cycle;
  - No disease-causing (pathogenic or parasitic) species!
  - 5 described phyla: ***Crenarchaeota, Euryarchaeota***, *Korarchaeota, Nanoarchaeota* and *Thaumarchaeota*.
    - Most can not be isolated (grown) in laboratory (only seq).



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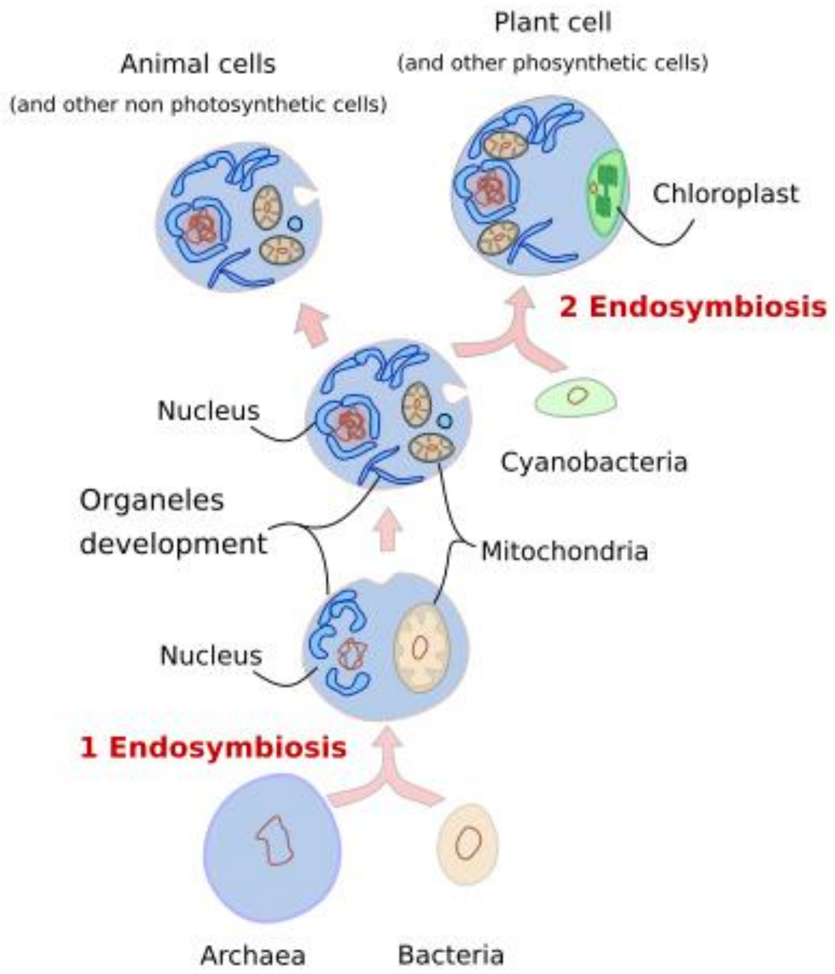
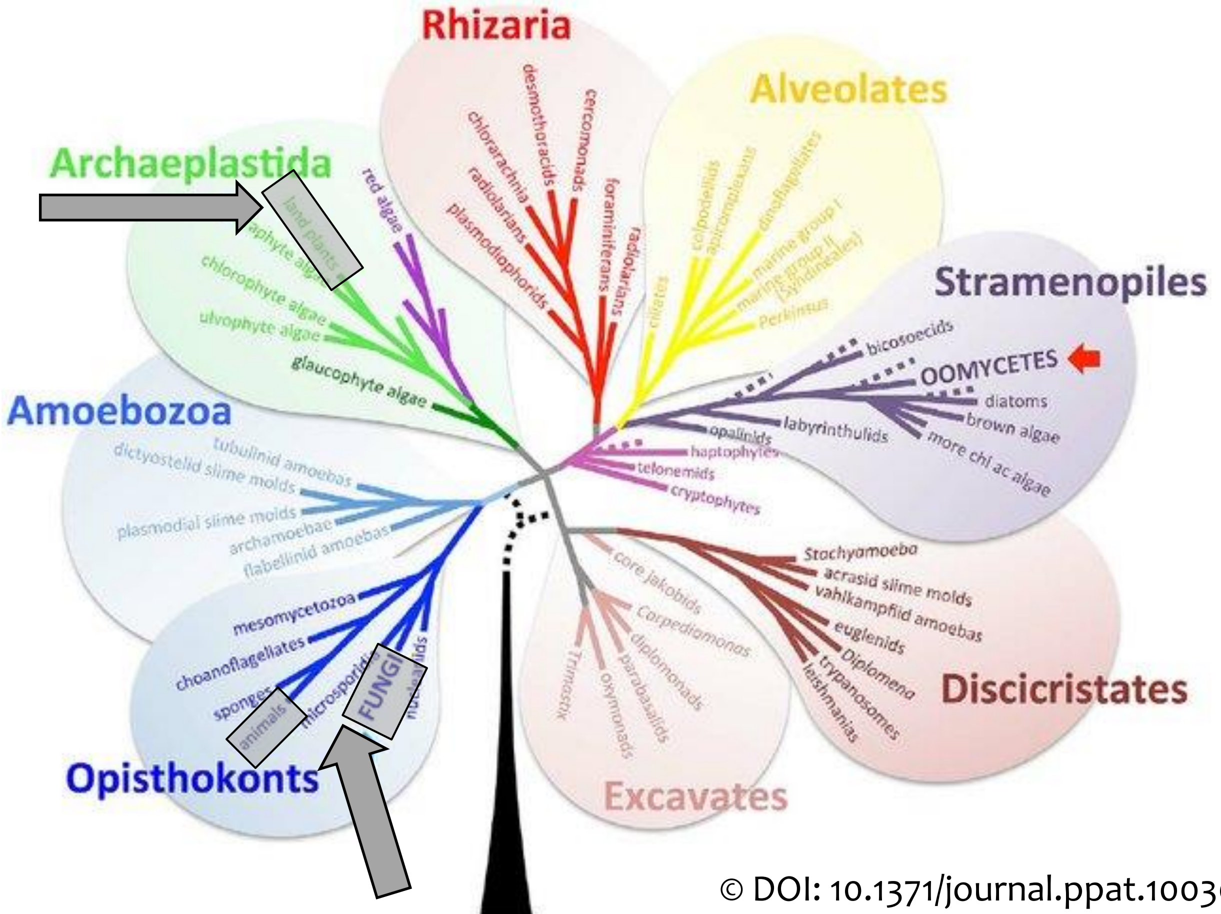
II. The Microbial Diversity

- **<u>Eukarya: Protists</u> (+*Algae*)**
  - Microbial eukaryotes appeared ~ 2 bya
  - *"any eukaryotic microorganism that is not a plant, animal, or fungus"*
  - Complex cell structure, true organelles → allowed development of multicellularity!
  - **Endosymbiosis theory:**
    - ***primary and secondary endosymbiosis***



II. The Microbial Diversity

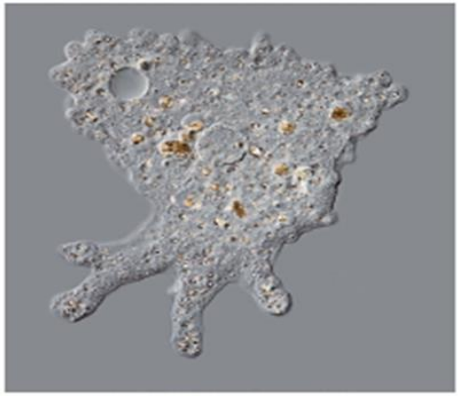
- **Eukarya: Protists (+*Algae*) vs. "higher" Eukarya**



© DOI: 10.1371/journal.ppat.1003028

II. The Microbial Diversity

- **Eukarya: Protists (+*Algae*)**
  - **Very variable in size** (even smaller than bacteria), **shape, and physiology**
    - *Ostreococcus* sp. (picoplanktonic green alge) ~ 0.8µm
    - Amoeba-like *Xenophyophores* sp. ~ 10 cm
    - Slime-molds ~ 30 cm



Slika 1.5: *Amoeba proteus*:

Unicellular
500 – 1000 µm

Slika 1.6: *Rotifera sp.*:

Multicellular
100 – 5000 µm

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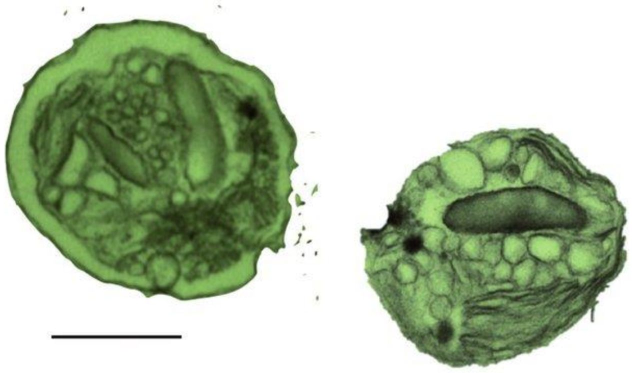

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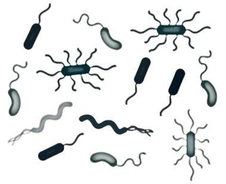
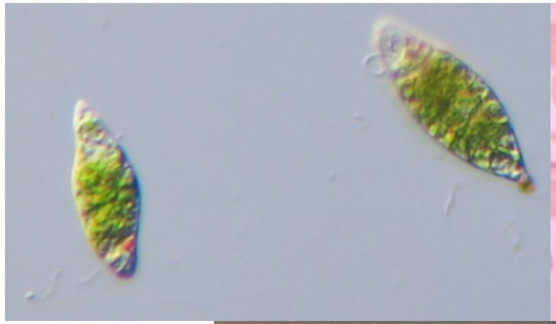
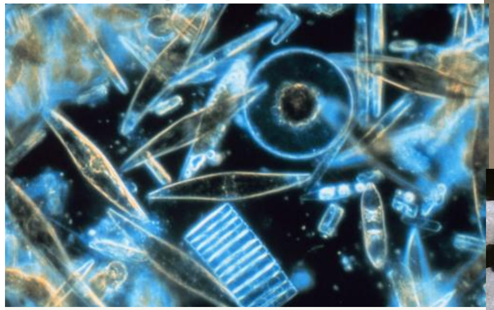
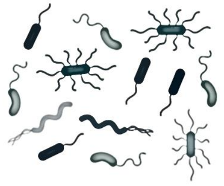


II. The Microbial Diversity

- **Eukarya: Protists (+*Algae*)**
  - **Euglenida, Kinetoplastida** (*Trypanosoma*)**…**
  - **Alveolata: Ciliates** (*Paramecium*)**, Dinoflagellates…**
  - **Diatomeas**
  - **Oomycetes** (*"water molds", once Fungi*)
  - **Golden, Brown Algae**
  - **Foraminifera, Radiolaria**
  - **Amoebozoa** (*including slime molds*)

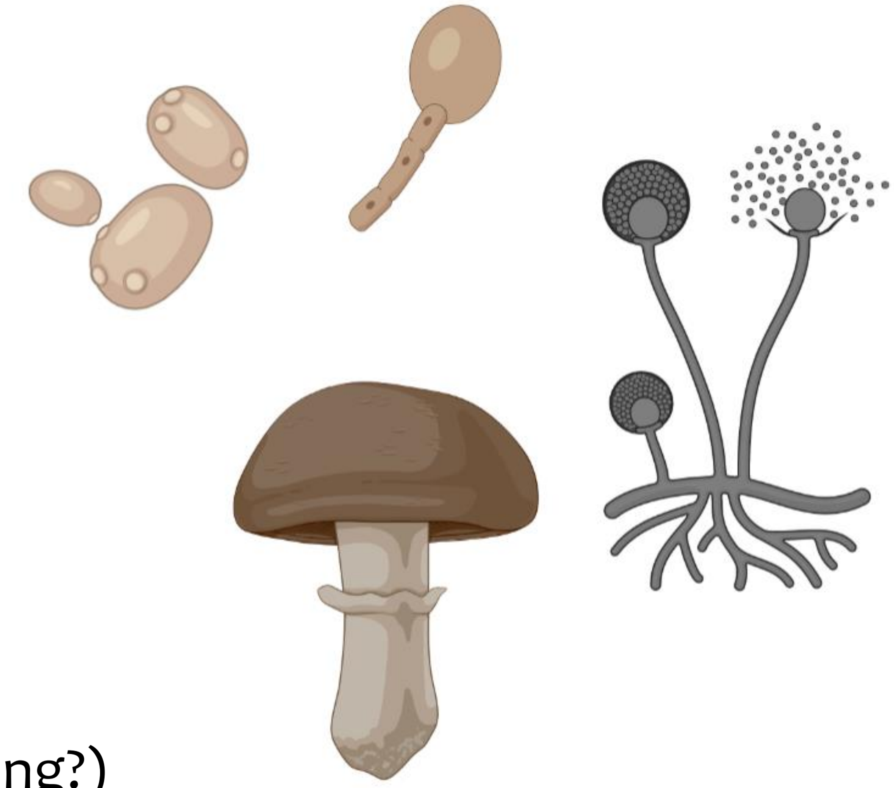






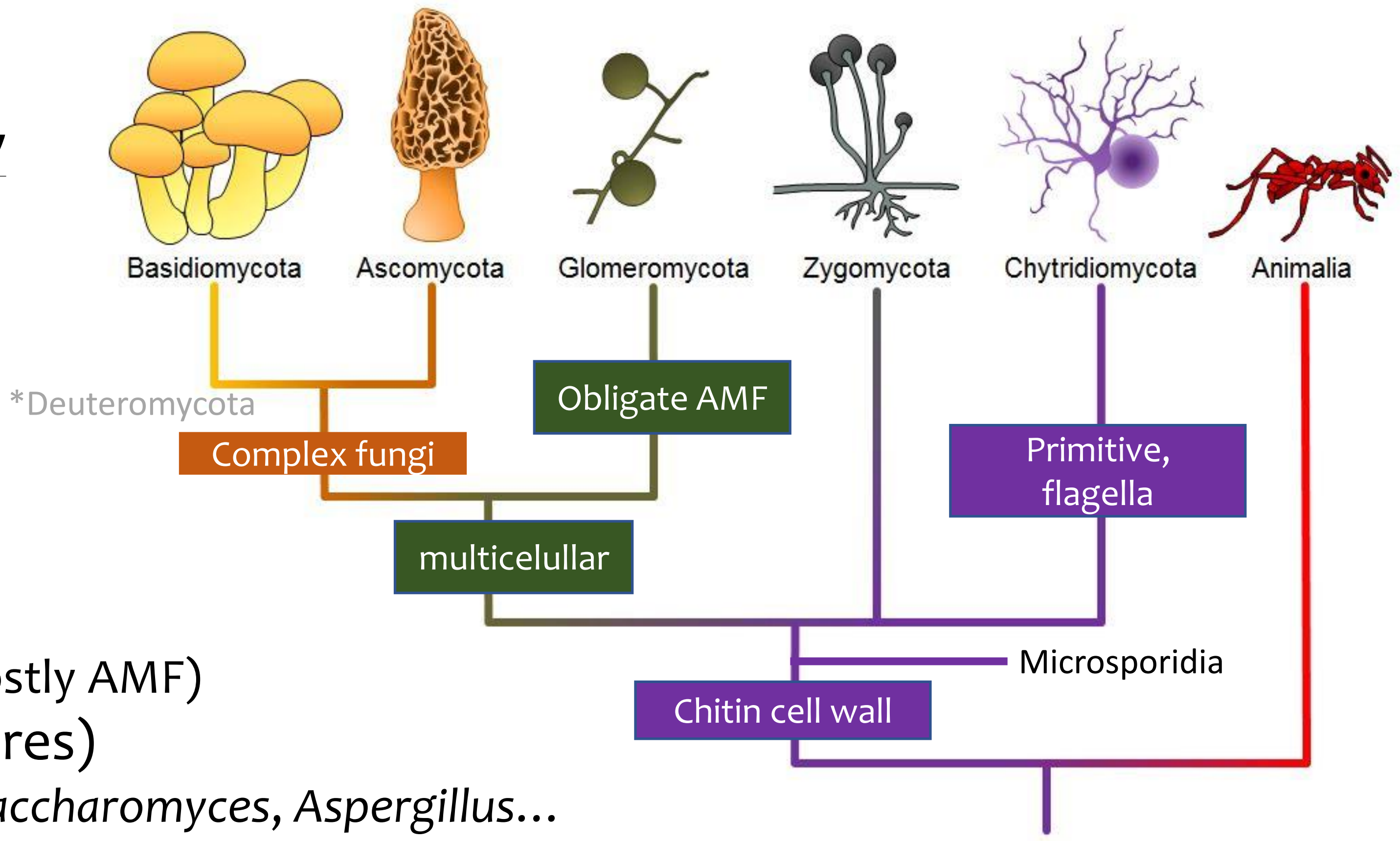
II. The Microbial Diversity

- **Eukarya: Fungi**
  - **Non-phototrophic** eukaryotic microorganisms
  - **saprotrophic and/or symbiotic** → decomposition
  - **Single cells** / **hyphae** (mycelia)
    - rigid **cell walls** (*chitin*)
  - Spores
    - Conidia (asexual spores)
    - sexual spores: asco-, basidio-, zygo-, zoospores
  - **Yeasts, molds and mushrooms**
  - ~ 100,000 described species (approx. 1,5 million existing?)
    - soil/dead plant material
    - pathogens (mostly plants, some also animals/humans)
    - Symbiotic relationships (plants – mineral acquisition)
  - Importance for humans: food, fermentation, antibiotics…



II. The Microbial Diversity

- **Eukarya: Fungi - Diversity**
  - **Microsporidia**
    - Unicellular parasites of animals
  - **Chytridiomycota**
    - Flagellated zoospores
  - **Zygomycota** (zygospores)
    - *Rhizopus*, *Mucor*
  - **Glomeromycota**
    - Obligate endomycorrhizae (mostly AMF)
  - **Ascomycota** (ascus → ascospores)
    - Largest, most diverse group: *Saccharomyces*, *Aspergillus*…
  - **Basidiomycota** (basidium → basidiospores)
    - 30.000+, mushrooms: *Agaricus*, *Amanita*, *Cryptococcus*…
  - *Once *Deuteromycota ("Fungi imperfecti")*



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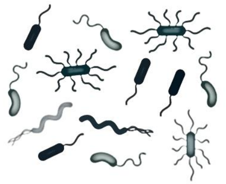
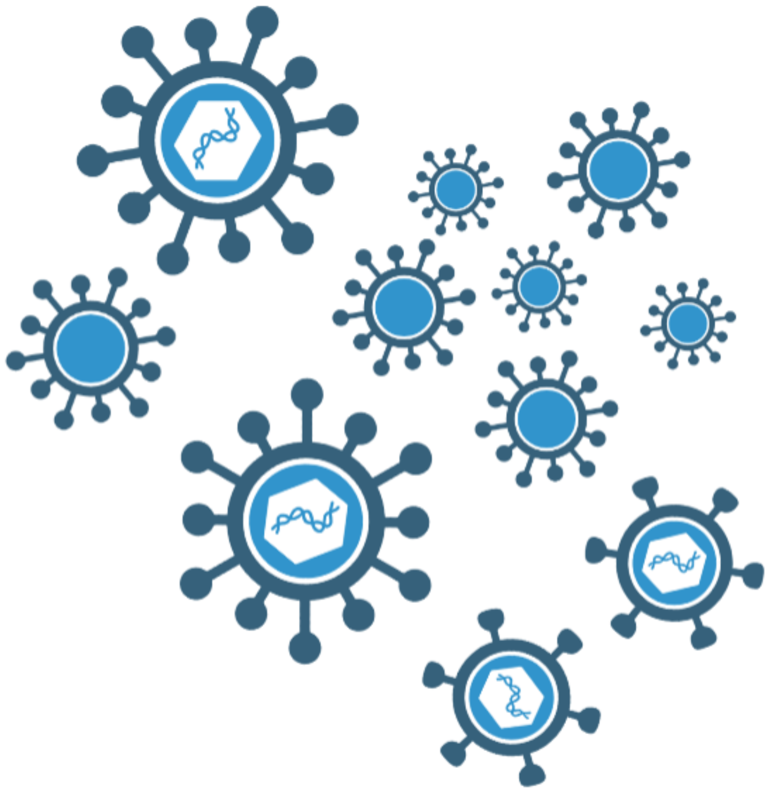


II. The Microbial Diversity

- **Viruses**
  - Are they alive or not ?!
  - **Can only replicate in a host cell!** (are "parasites")
  - Are not cells! (no membrane, cytoplasm, ribosomes)
  - *Do not have their own metabolic processes*
  - Small genome: DNA or RNA, ss or ds
  - Infect B, A and E!
  - Classification is based on:
    - structure,
    - genome composition,
    - host specificity.



III. Interactions of Plants and MO

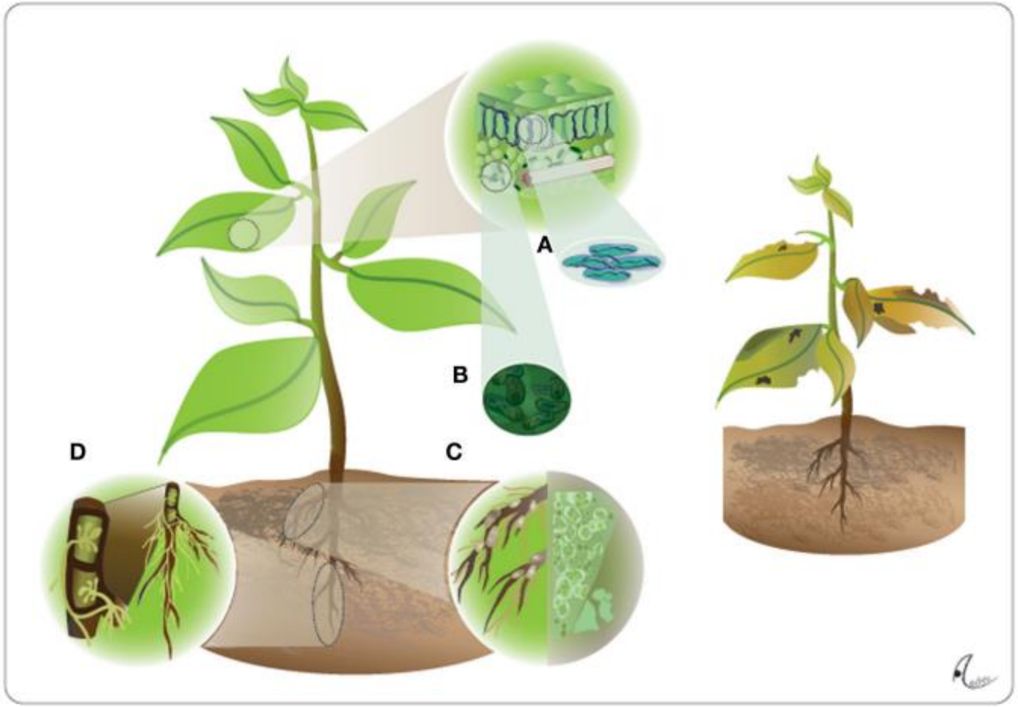
- **<u>Plants and MO in nature</u>**
  - Plant–microbe interactions are ubiquitous
  - Plants are full of MO!
  - ***Plant phenotype = product of plants and MO gene expression!***
  - Mutualistic, neutral or pathogens

  - *Mycorrhizal fungi, N-fixing bacteria (Rhizobia in nodules) and PGB bacteria (B, P, R).*

frontiers in
PLANT SCIENCE

The microbe-free plant: fact or artifact?

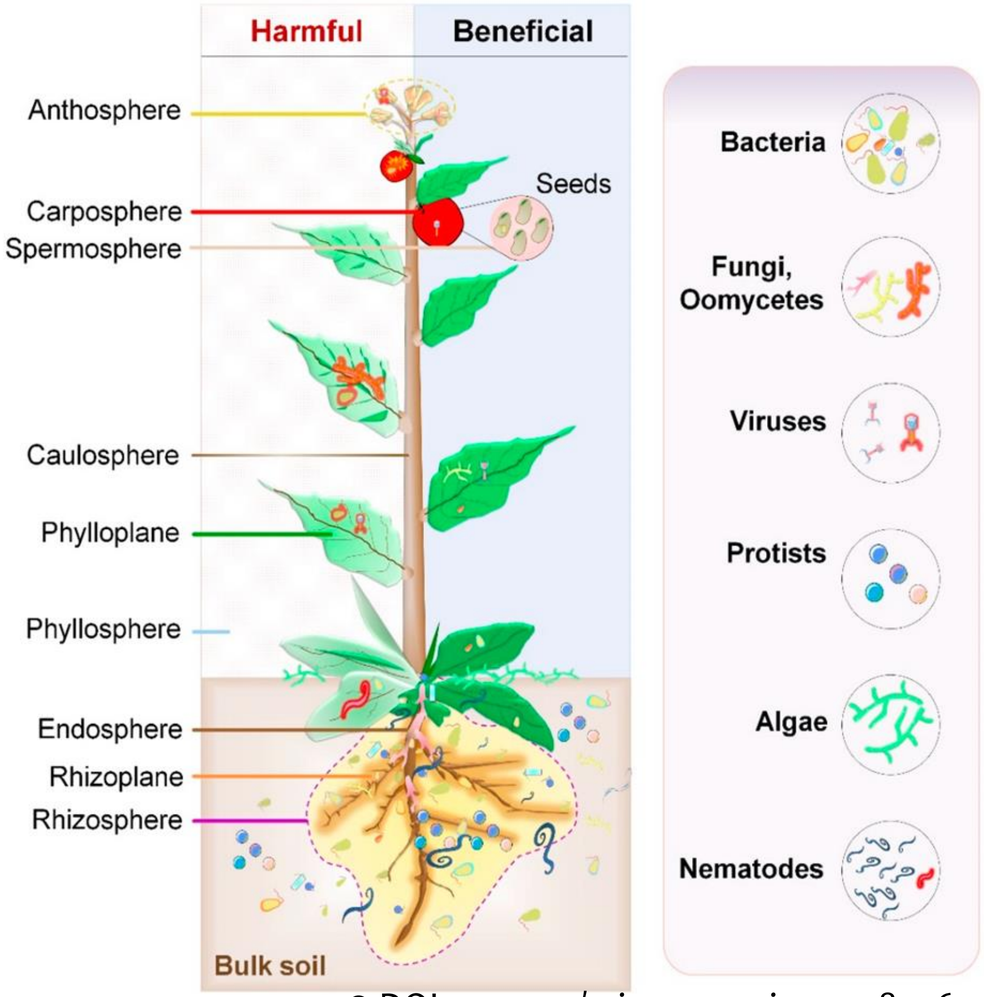
Laila P. Partida-Martínez and Martin Heil *
Departamento de Ingeniería Genética, Centro de Investigación y de Estudios Avanzados – Irapuato, Irapuato, México

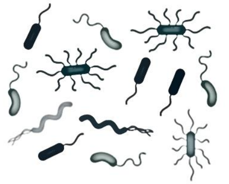


© DOI: 10.3389/fpls.2011.00100

III. Interactions of Plants and MO

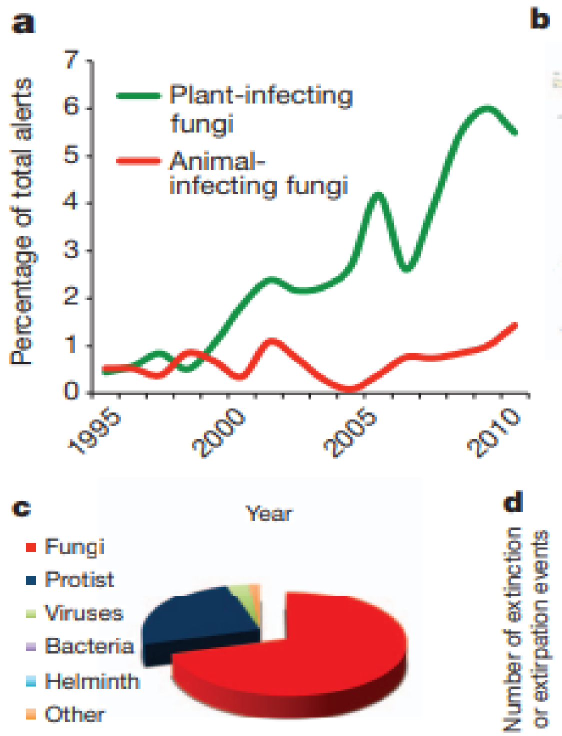
- **<u>Plant as a HOLOBIONT</u>**
  - *"the host plant together with all its associated microbiome"*
  - Contribute to the whole performance.
  - Credits: **Lynn Margulis (1991)** ?
    - *Symbiosis as a Source of Evolutionary Innovation*
  - Introduced by **Adolf Meyer-Abich (1943)!**
    - *"The theory of holobiosis says that all higher and more complex organisms have developed through biontic processes, that is, parabioses, antibiosis, symbioses, and finally holobioses between simpler and lower forms of organisms."*

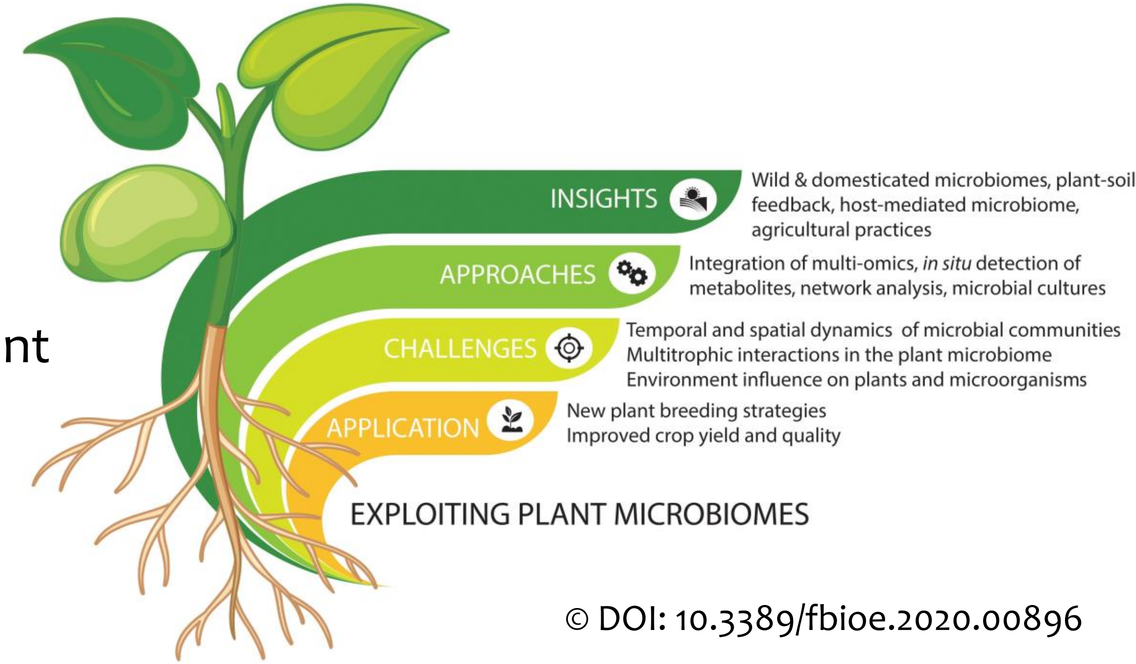




III. Interactions of Plants and MO

- **Plants and MO**
  - **Epiphytic** or **endophytic**
    - **Beneficial, neutral** or **harmful**
  - Important role in:
    - Plant growth & development
    - Physiological state
    - Immunity against biotic & abiotic stress





© DOI: 10.3389/fbioe.2020.00896

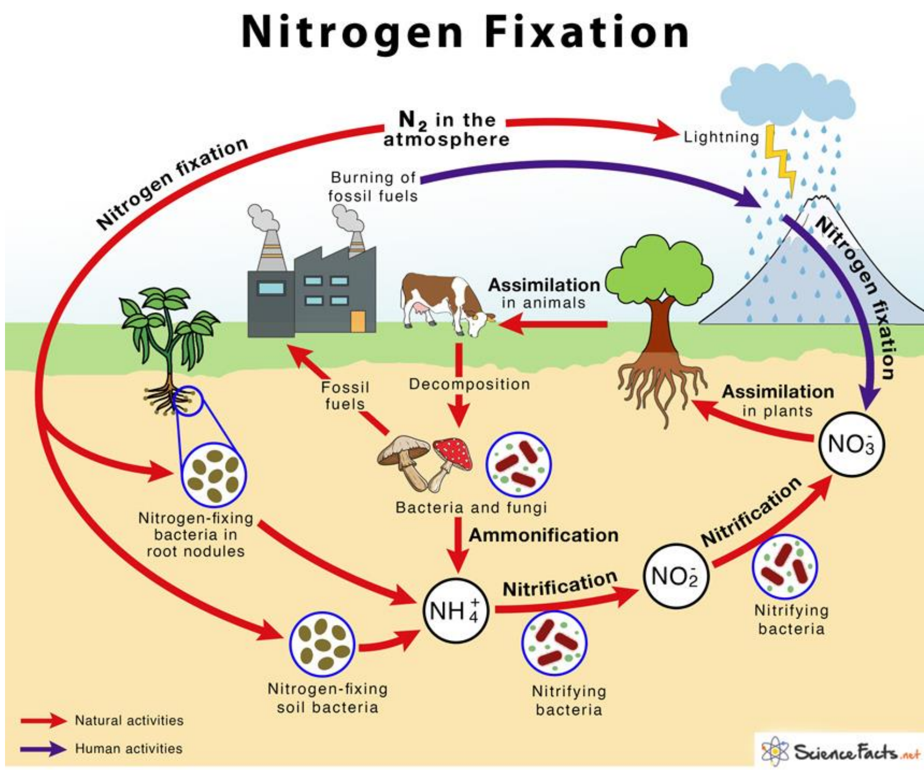
In the context of climate changes?

© DOI: 10.1038/nature10947

III. Interactions of Plants and MO

- **Symbiosis with N-fixing Bacteria**
  - ***N deficiency = most limiting for plant growth*** (N in proteins, NA…)
  - Plants: only nitrate (NO3-) and ammonia (NH4+)
  - **Bacteria & Nitrogen cycle**
    - Nitrification (NH3 $\xrightarrow{ox}$ NO2-; NO2- $\xrightarrow{ox}$ NO3-) Nitrifying bacteria
    - Nitrogen fixation (N2 $\xrightarrow{red}$ NH3) Nitrogen-fixing bacteria
    - Denitrification (NO3- $\xrightarrow{red}$ NO2- $\xrightarrow{red}$ N2) Denitrifying bacteria
    - Protein → amino acids → NH3 → NH4+

      MO decomposition   Ammonifying bacteria



III. Interactions of Plants and MO

- **Symbiosis with N-fixing Bacteria**
  - **Root nodules:**
    - *Rhizobium* + legumes (*Fabaceae*: beans, peas, chickpea, soy, lentil, peanuts, alfalfa, clover…)
    - *Bradyrhizobium, Mesorhizobium, Proteobacteria…*
  - **Corraloid roots:**
    - *Cycadaceae: Cycas revoluta*
      - *Living fossils 200+ mya*
    - Cyanobacterias:
      - ***Nostoc***, *Calothrix, Scytonema and Richelia*

© DOI: 10.3390/genes10120991

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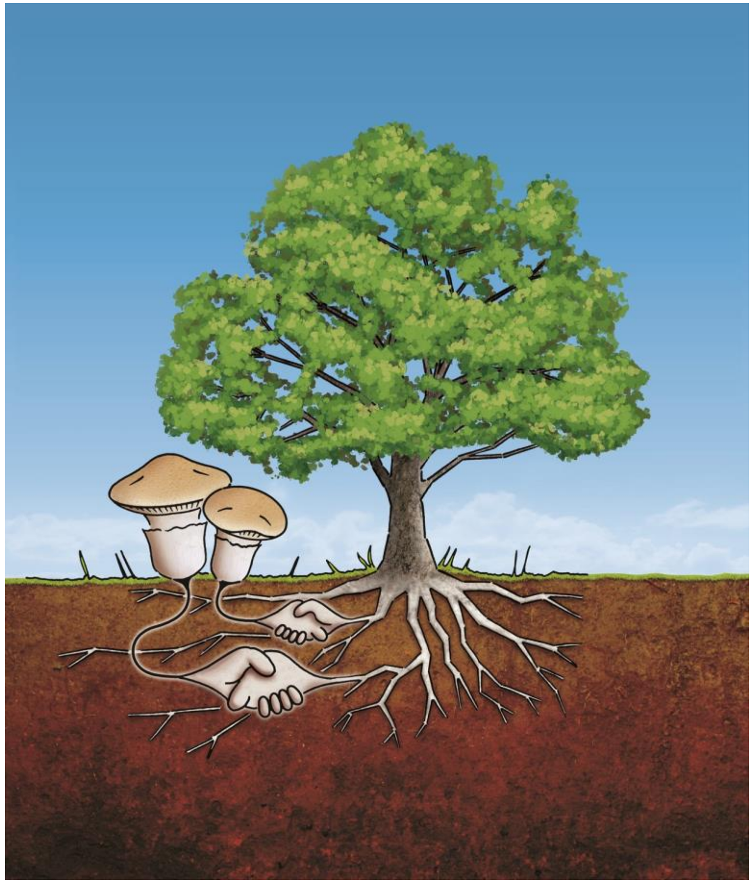
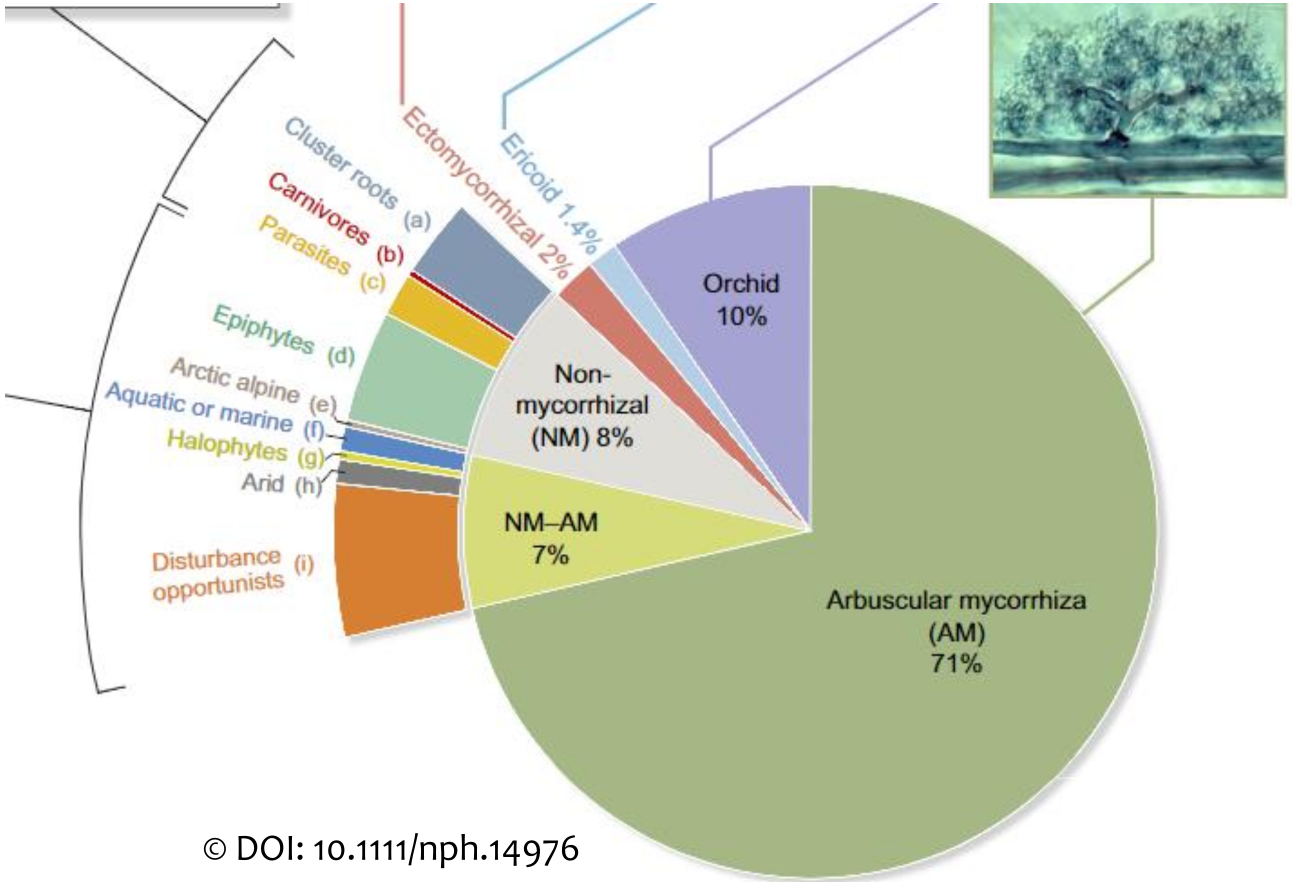
III. Interactions of Plants and MO

- <u>**Mycorrhiza**</u>
  - Symbiotic relationship between plant & fungi
  - *"fungus roots"* (gr. múkēs - fungus; ríza - root)
  - Mostly **mutualistic** (+, +)
  - **Primary for nutrient transport**
    - Fungal hyphae → ↑ water, mineral nutrition
    - Plants → sugars to fungi
  - **More than 90 % of all land plants** (+ ferns & mosses)
  - ↑ growth & development of plants, tolerance to abiotic (drought) and biotic stress (pathogens, parasites).
  - MF ↑ soil quality, plant biodiversity, key role in establishing & sustaining ecosystems!



III. Interactions of Plants and MO

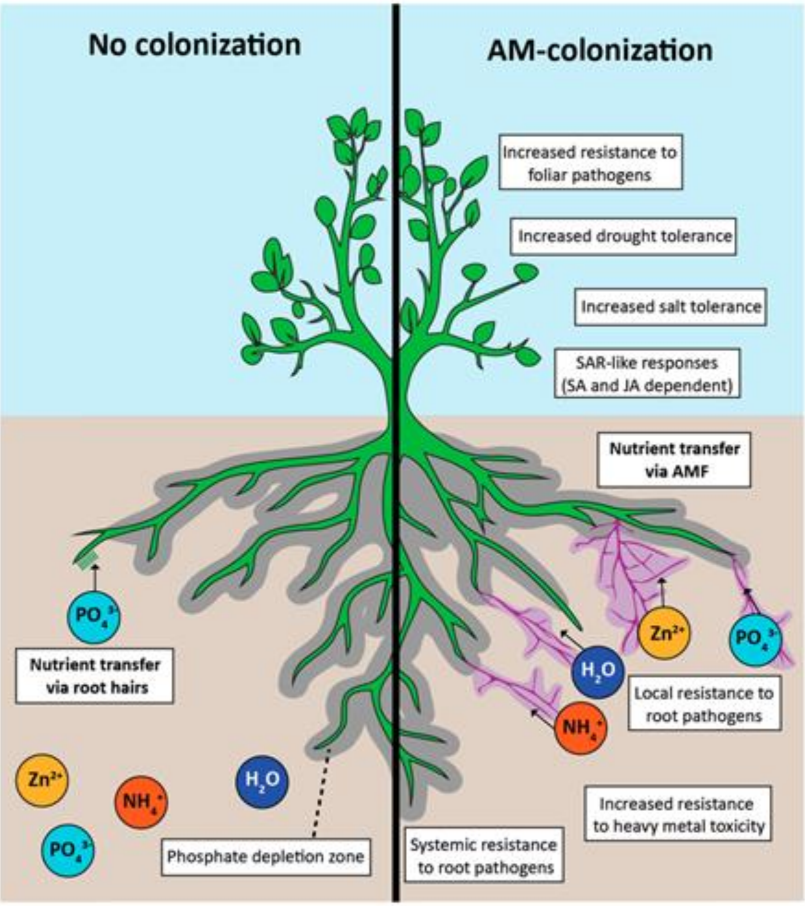
- **Mycorrhiza in plants**



© DOI: 10.1111/nph.14976

III. Interactions of Plants and MO

- **Arbuscular mycorrhiza**
  - **AMF key role in evolution of plants** (land colonisation) → first fossils more than 400 mya!
  - *The oldest & most widespread symbiosis on Earth*
  - Living fossils?
  - *Glomeromycota* (150+ species, nonspecific)
    - Obligate symbionts
  - Mainly herbaceous plants **(>80% of all plants)**
  - Typical structures:
    - **Arbuscules** = the most intimate symbiotic connection of 2 membranes
    - **Vesicles** = storage structures (lipids)
    - **Spores** = hardy structures + reproductive propagules
  - **Primary role = supply of P** (a very immobile, hard-to-access, often depleted in the soil)



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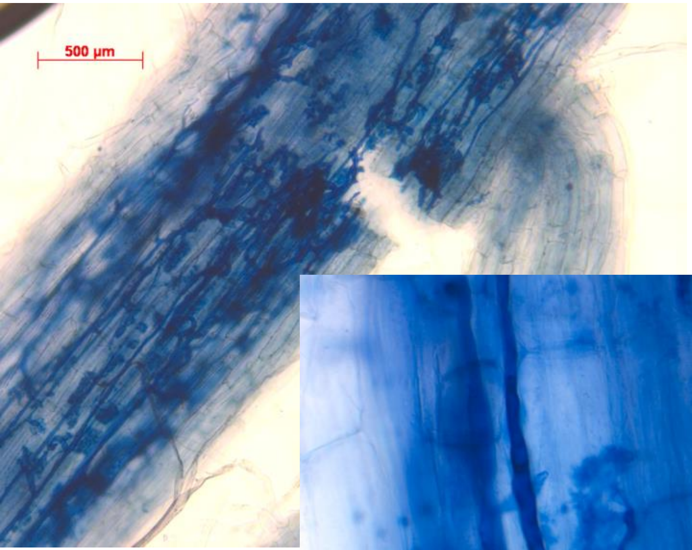
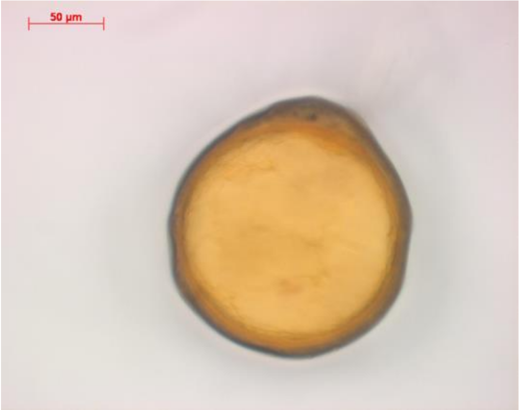
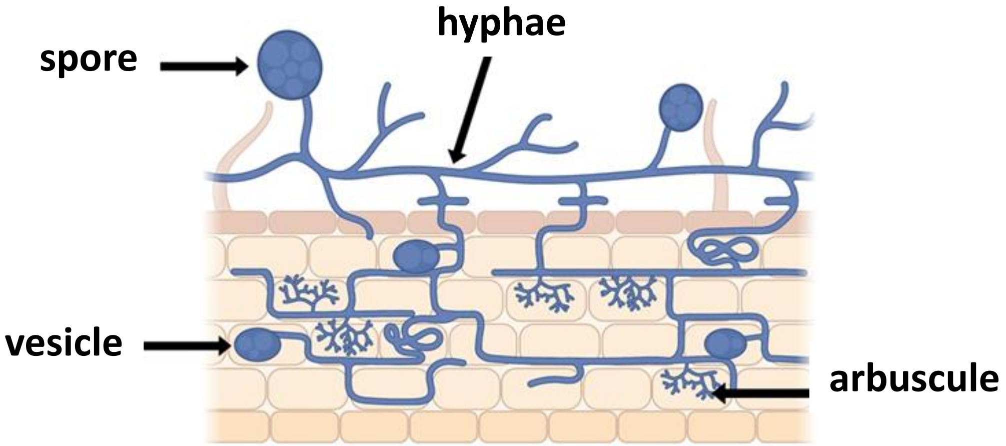
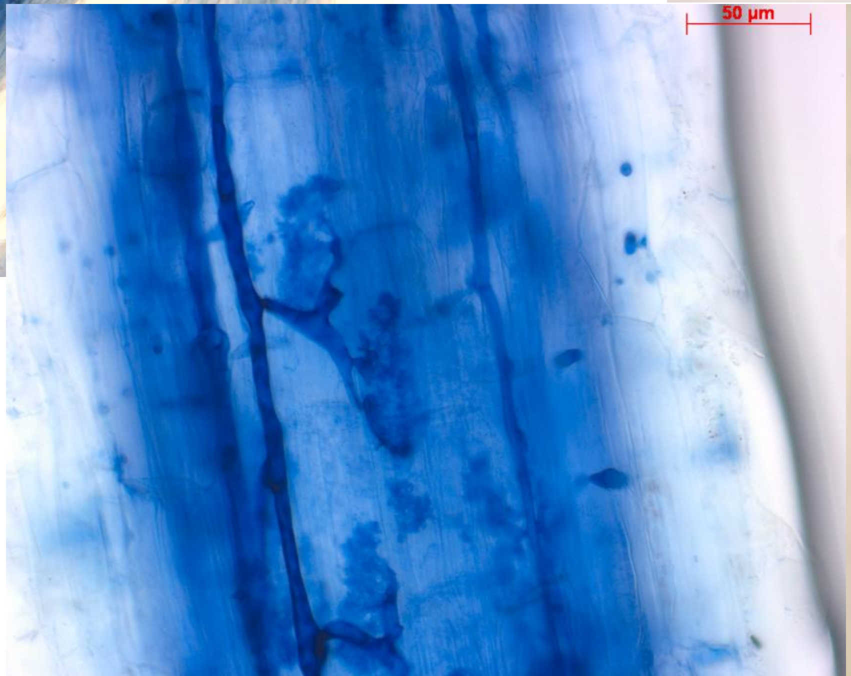
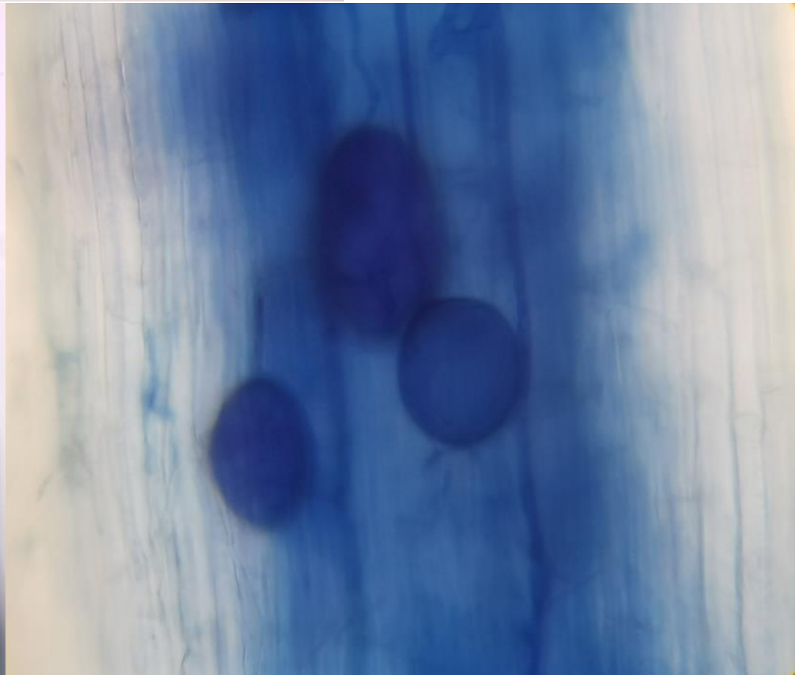
- <u>**Structures of AMF**</u>





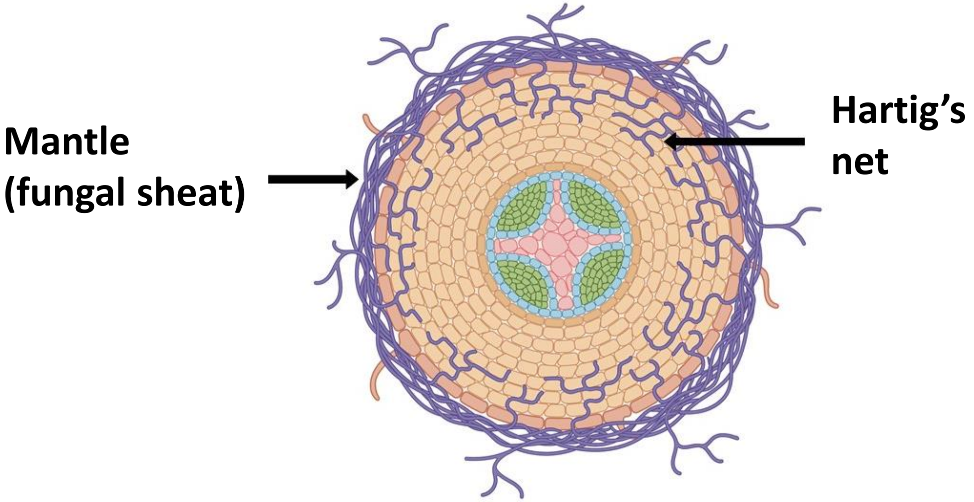




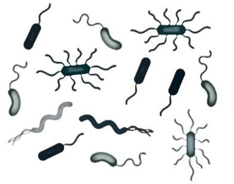


III. Interactions of Plants and MO

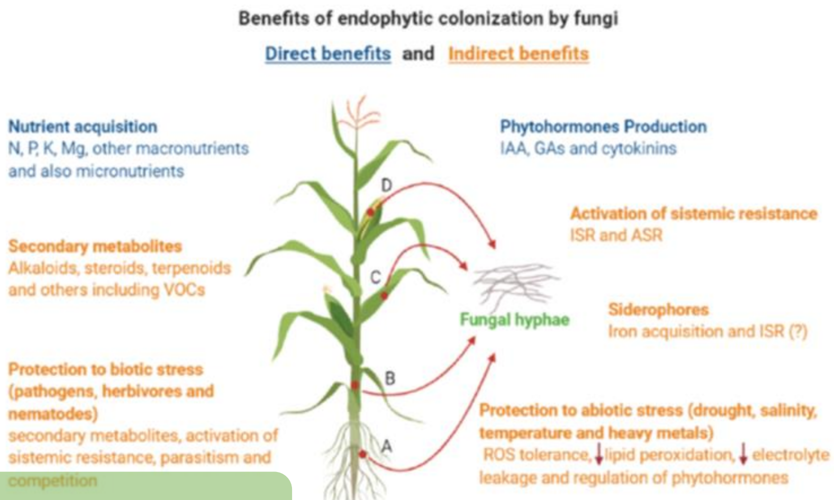
- <u>**Ectomycorrhiza**</u>
  - 6000+ Ascomycetes & Basidiomycetes
  - ***Facultative symbionts, very diverse***
  - Mostly woody species (10%)
  - Mantle, Hartig's net
  - **Primarily for better N acquisition**
  - Large underground network of hyphas
  - Fruiting bodies of EMF = **mushrooms**



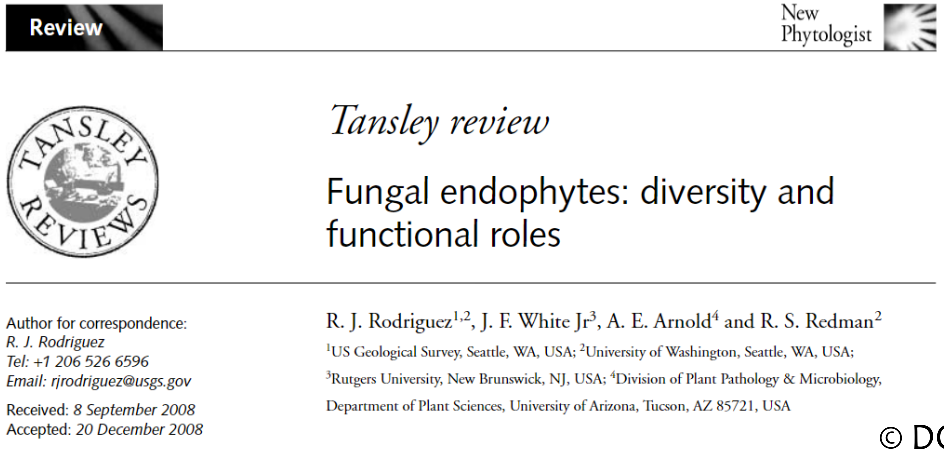
III. Interactions of Plants and MO



- **Plants and Fungal Endophytes**
  - Definition:
    - **De Bary (1886):** *“each organism living inside plant” (gr. éndon=inside, phyton=plant)*
    - ***Petrini (1991):*** *“fungi that colonise inside of plant tissues without causing visible damage (symptomes of disease) in host plants.”*
  - **All plants in natural ecosystems are colonised with fungal endophytes:**



© DOI: 10.1111/1462-2920.15392



Review

New Phytologist

Tansley review

Fungal endophytes: diversity and functional roles

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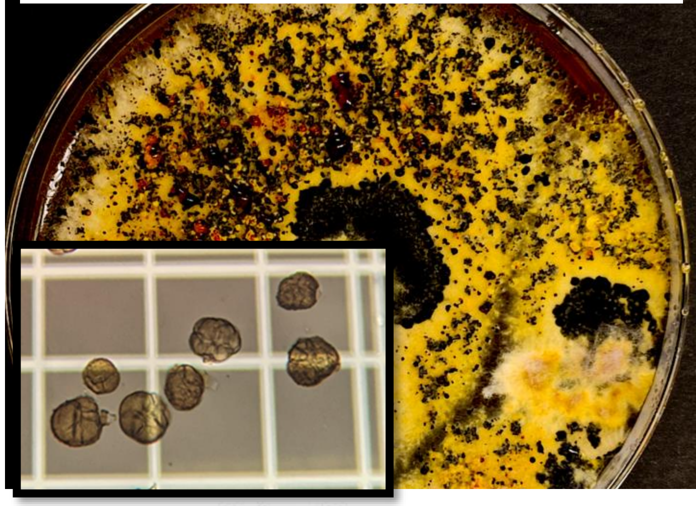
- Vertical (usually mutualists) and horizontal transmission (opportunists)
- Highly diverse → profound impacts on natural plant communities!
- Ecological significance → poorly characterized (100 yr+ research)

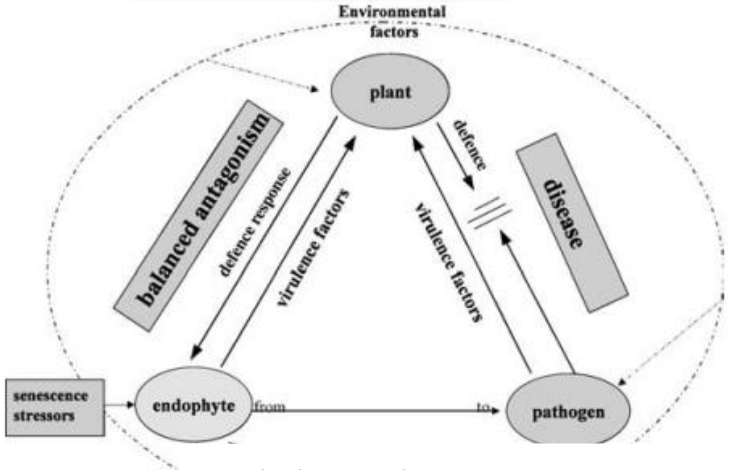
© DOI: 10.1111/j.1469-8137.2009.02773.x

III. Interactions of Plants and MO

- <u>**The concept of Endophytic continuum**</u>
  - *Schultz & Boyle, 2005*
  - **Developmental** and **evolutionary!**
  - No neutral interactions, rather a **balanced antagonism**
    - *Always at least a degree of virulence*
    - ***Host plant defence**: limits development disease*
  - **Endophytes ↔ *phenotypic plasticity* ↔ pathogens**
    - Local/extensive colonisation, virulence, pathogenity, latency, saprophytism
    - Motor of evolution!
    - saprophyte ↔ endophyte ↔ mutualist (MF)

Epicoccum nigrum (grown in culture + spores)

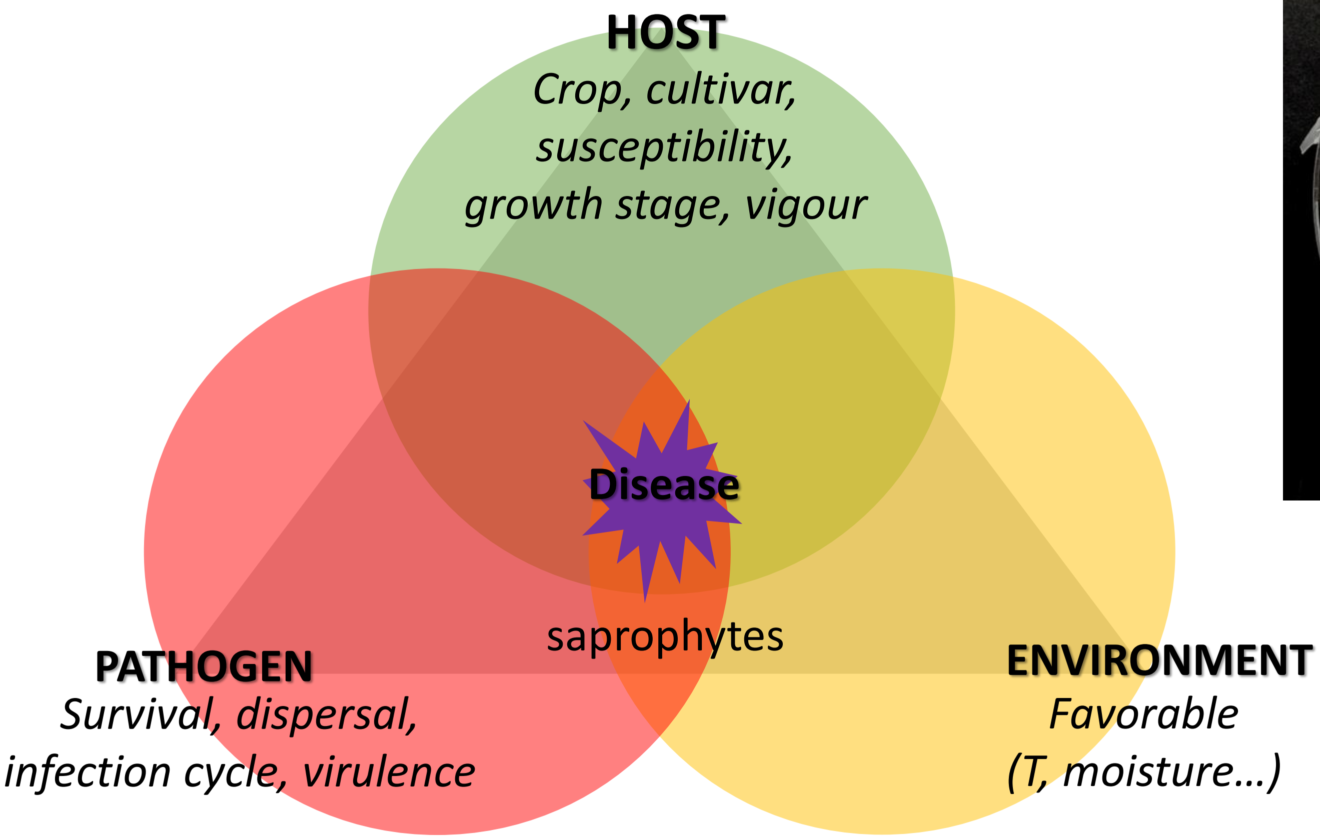


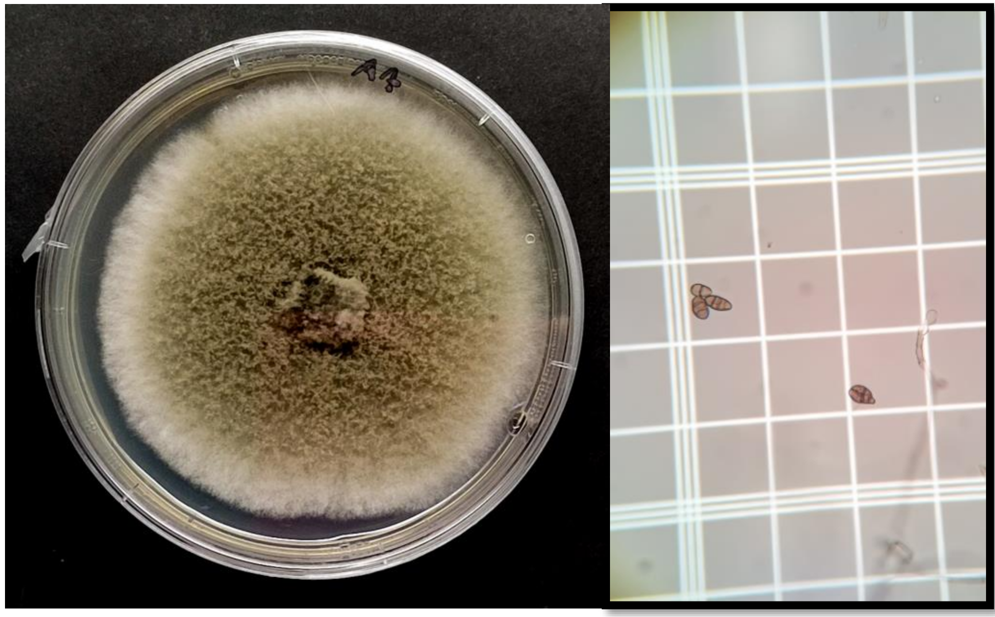


© DOI: 10.1017/S095375620500273X

III. Interactions of Plants and MO

- **Pathogens? The Disease Triangle**





Alternaria alternata isolated from buckwheat grains grown in pure culture (left) + spores (conidia) (right).

III. Interactions of Plants and MO

- **The Disease Cycle**

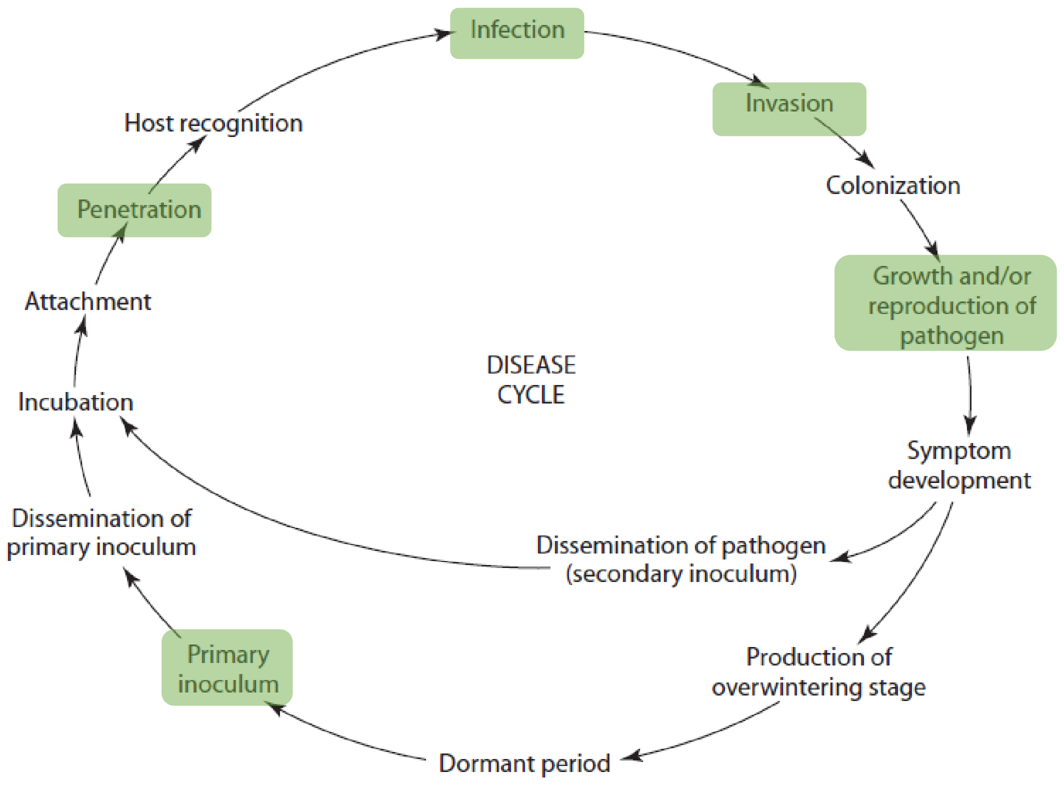


FIGURE 2-2 Stages in development of a disease cycle.

- **Disease cycle – pathogen life cycle**

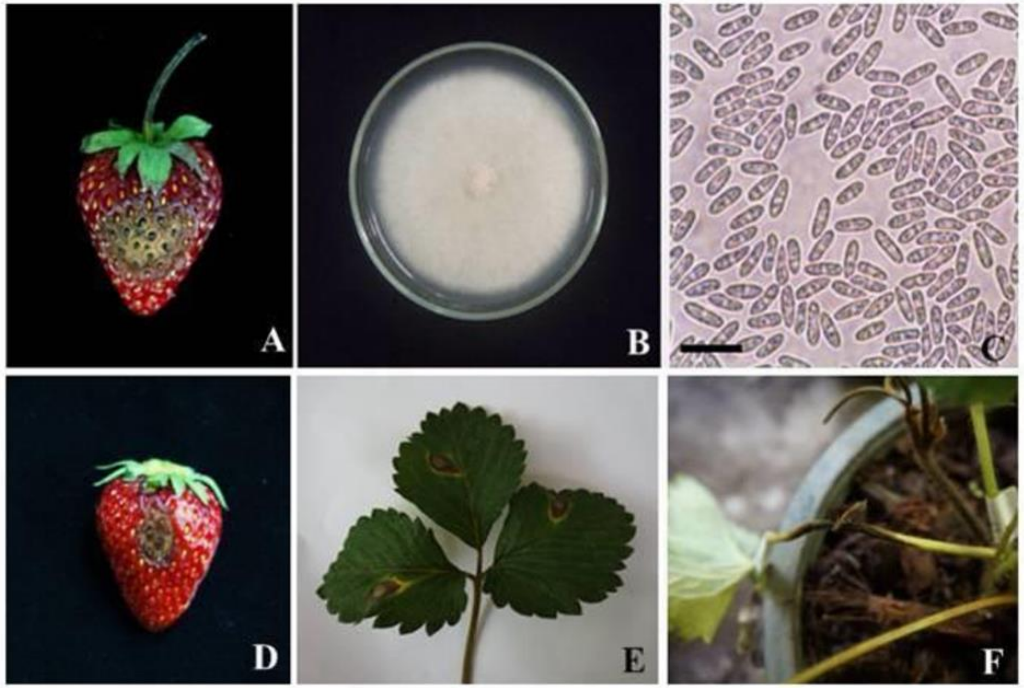
Appearance, development & perpetuation

1. Inoculation →
2. penetration →
3. establishment of infection →
4. colonization (invasion) →
5. growth & reproduction of the pathogen →
6. dissemination of the pathogen (or survival of the pathogen in the absence of the host: overwintering / oversummering)

III. Interactions of Plants and MO

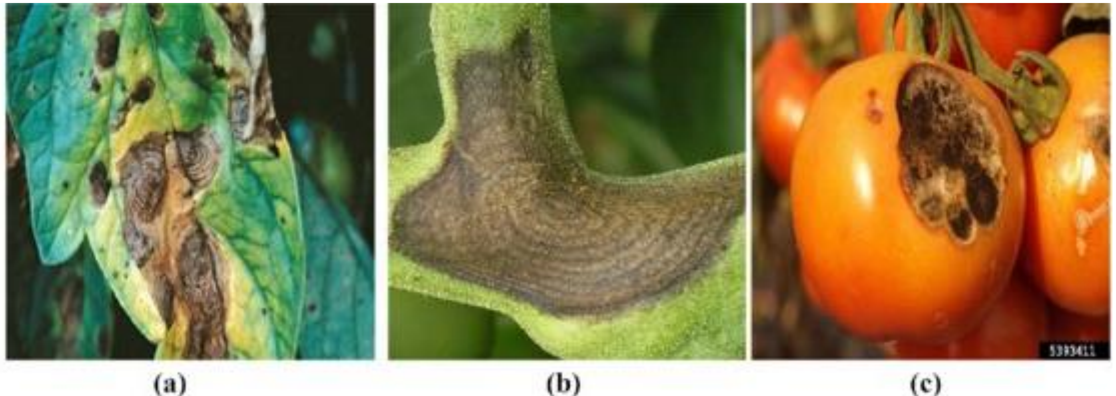
- **Fungal Diseases**

Botrytis cinerea **(grey mold on grapes)**
© https://blog.pestprophet.com/



Colletotrichum acutatum **(antrachnose on strawberry)** © Kumvinit & Akarapisan (2016). Journal of Agricultural Technology 12(4):693-706.

Fusarium oxysporum **(Fusarium wilt of banana - Panama disease)** © Wikipedia



Alternaria solani **(early blight disease of tomato)**
© Karthika, Varghese & Shanavas (2020). 3 Biotech 10(7)w2

III. Interactions of Plants and MO

• Fungal Diseases of Cereal Crops

- Ergot (*Claviceps purpurea*): rye, millet, wheat (barley, oat)
  - Ergot alkaloids → **ergotism**
- Fusarium head blight (*F. graminearum, F. culmorum*): small grains (wheat, barley, oat, rye, corn etc.)
  - Trichothecenes (Deoxynivalenol, T-2, HT-2), zearalenone…
- Powdery mildew (*Blumeria* sp.): corn, wheat, barley
- Cereal (leaf & stem) rust (*Puccinia* sp.): wheat, oat, barley
  - **~10% of world cereal crop loses**
- Corn smut / Loose smut (*Ustilago* sp.): corn, cereals

Ergot ***on rye*** © www.aokin.de/

FHB ***in wheat*** © www.apsnet.org/

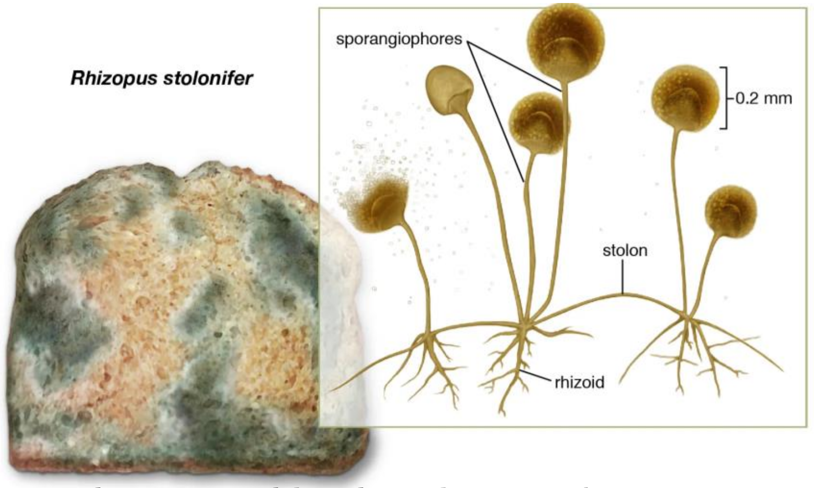
Powdery mildew ***in wheat***
© www.agric.wa.gov.au/

Corn smut
© www.macleans.ca/

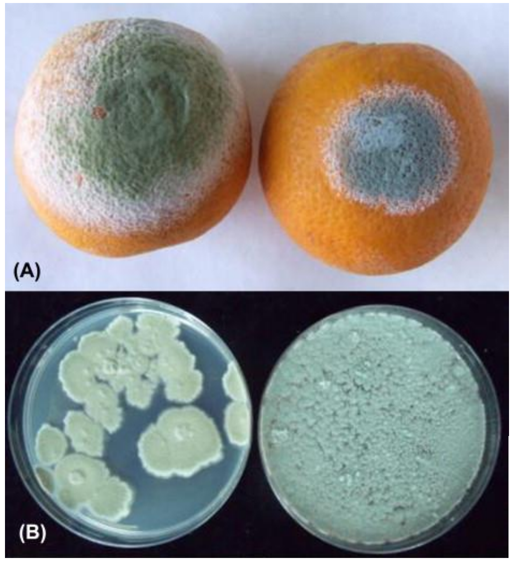
Stem rust ***in wheat***
© www.ars.usda.gov/

III. Interactions of Plants and MO

- **Postharvest Fungal Diseases**
  - after harvesting, grading, and packing, during transport to market, during storage
  - ***Destroy up to 30% of total yield***
  - **Mycotoxins!** (especially grains)
    - acute mycotoxicosis → severe illness → (death)
  - Ascomycota: ***Aspergillus***, ***Penicillium*** (grains)
    - Aflatoxins, ochratoxins
    - Low WA (10-12% moisture), *A. flavus*
    - On field or during storage
  - Zygomycota: ***Mucor***, ***Rhizopus***
  - Basidiomycota: ***Rhizoctonia***, ***Sclerotium*** (fleshy fruit & vegetables)



***Rhizopus* mold on bread** © www.britannica.com



Green (P. digitatum) and blue mold (P. italicum) on citruses DOI: 10.1016/B978-0-12-411552-1.00002-8

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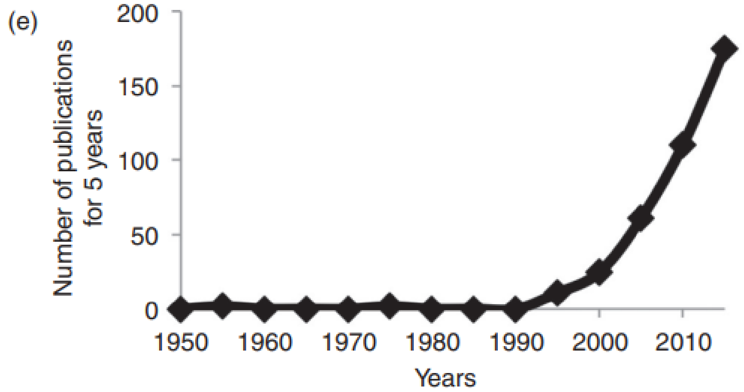
III. Interactions of Plants and MO

- **Dark Septate Endophytes**
  - *A continuum between mutualism and parasitism*
  - Ascomycetes
  - **dark melanised hyphae + microsclerotia**
  - Ubiquitous: *high tolerance to abiotic stress*
    - *Extreme environments, trace element-contaminated and other stressful soils*
    - **Important function for host survival!**
      - PGP by improving nutrition
      - producing secondary metabolites (phytohormones, VOC)
      - protecting against phytopathogens



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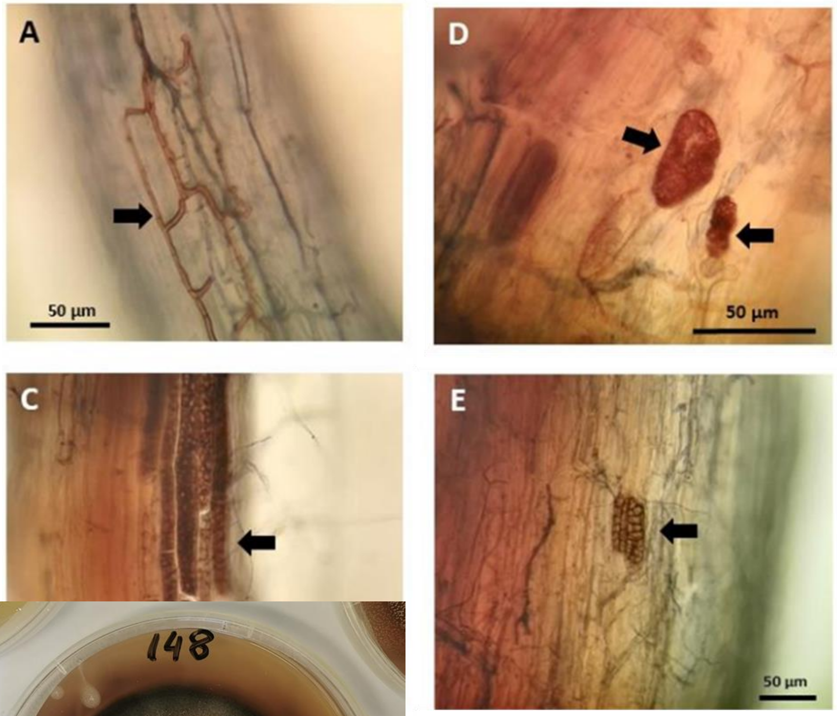
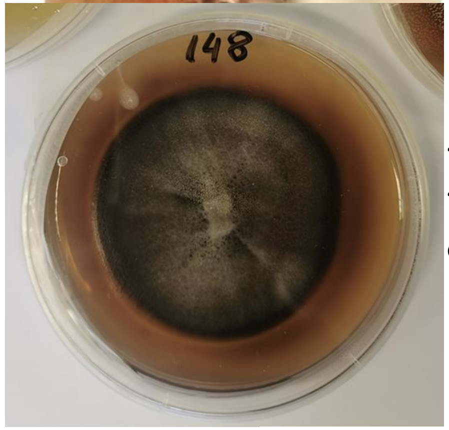




*DSE colonization in roots
* *Cadophora* sp. in pure culture (© J. Mravlje)

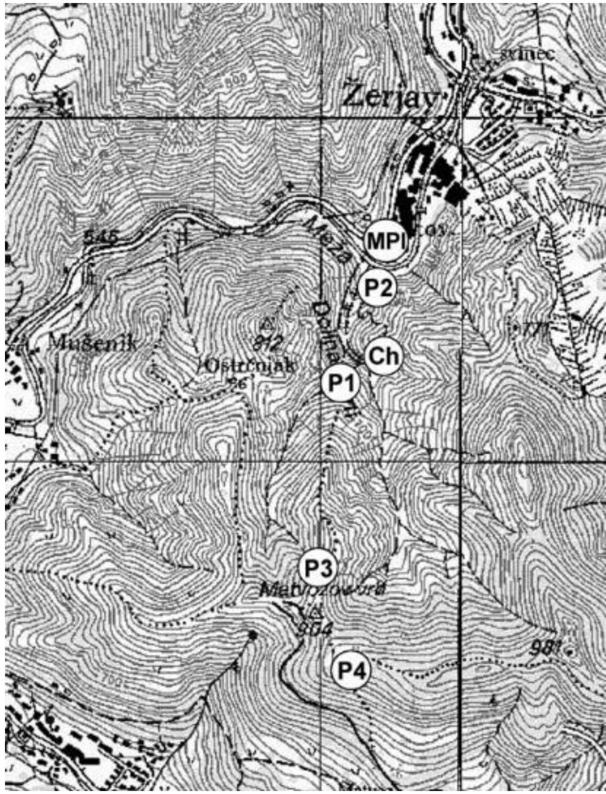
III. Interactions of Plants and MO

- **DSE in metal polluted area in SLO**
  - *Case study: almost 30 years of research at a heavily metal polluted site in N Slovenia (Žerjav, former lead smelter facility).*

PRILOGA 1

MEJNE IN KRITIČNE IMISIJSKE VREDNOSTI SNOVI V TLEH

Nevarna snov	Limit value (mg/kg suhih tal)	warning value (mg/kg suhih tal)	critical value (mg/kg suhih tal)
1. Kovine ekstrahirane z zlatotopko (razen Cr^{6+})			
kadmij in njegove spojine, izražene kot Cd	1	2	12
baker in njegove spojine, izražene kot Cu	60	100	300
nikelj in njegove spojine, izražene kot Ni	50	70	210
svinec in njegove spojine, izražene kot Pb	85	100	530
cink in njegove spojine, izražene kot Zn	200	300	720



© Regvar et. al. (2006) Env. Poll. 144: 976-984.

Table 1
Soil properties, botanical composition of vegetation, Raunkiaerian life forms of plants and Jaccard's coefficients of the studied plots

Soil properties	Distance to smelter	P1 50 m	P2 250 m	P3 500 m	P4 800 m
pH_{KCl}		7.1	6.8	7.0	6.0
Organic matter (mg/kg)		3.2	11.1	12.6	32.2
Cd (mg/kg; mean ± SD)		90.2 ± 1.2	148 ± 3.0	49 ± 0.4	55 ± 0.6
Pb (mg/kg; mean ± SD		51400 ± 206	24500 ± 73.5	7500 ± 15	5 600 ± 11.2
Zn (mg/kg; mean ± SD)		2340 ± 91.6	2690 ± 59.18	928 ± 19.5	868 ± 23.4

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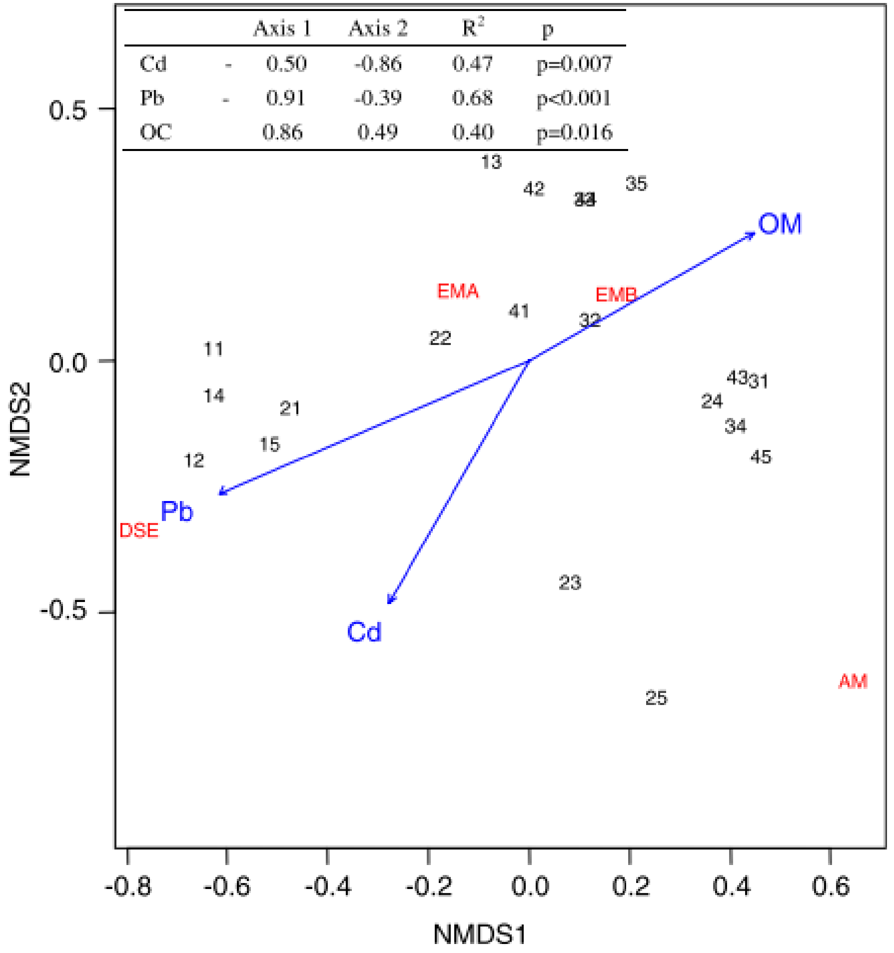
III. Interactions of Plants and MO

- **DSE in metal polluted area in SLO**
  - DSE colonisation ↑ with ↑ levels of pollution and ↓ levels of organic matter → ***suggesting a potential functional role of DSE for wilows*** (*Salix caprea*) growing at metal enriched site.



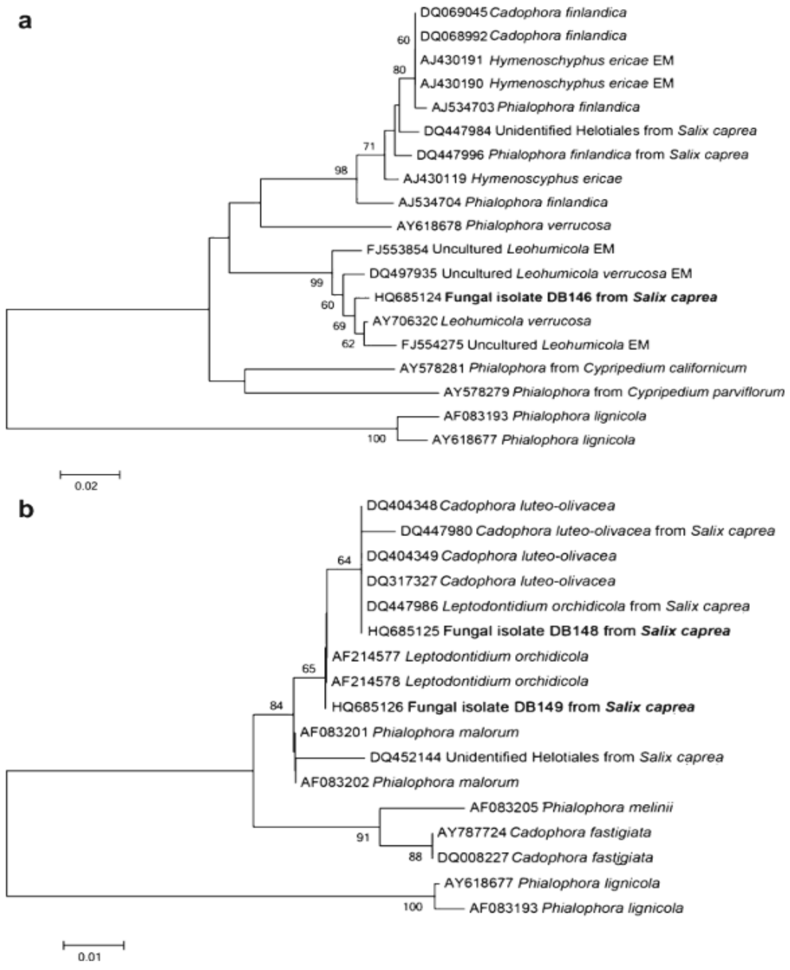
	P1	P2	P3	P4
Root fungal colonisation				
AM (%)	0±0	3.0±1.6	1±0.5	0.03±0.01
DSE (%)	20.4±7.0a	4.5±1.6b	0.1±0.01b	0.7±0.7b
EM (%)	45.3±1.9a	36.2±0.4b	46.3±0.7a	50.4±4.0a

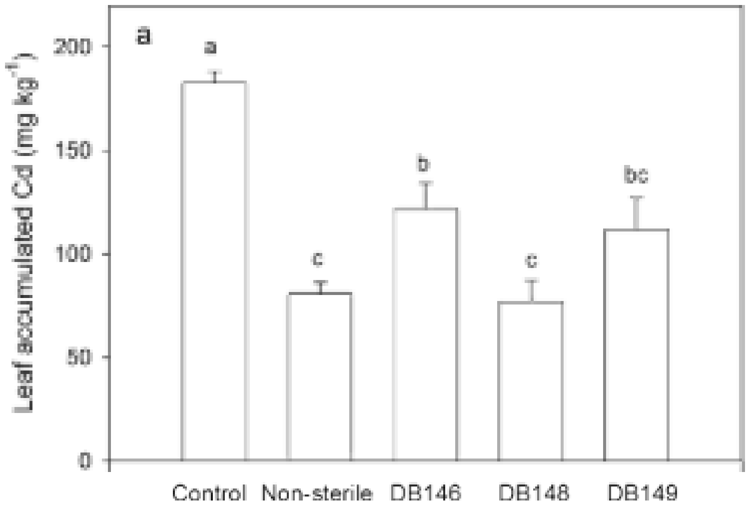
© Likar & Regvar (2009) Sci. Tot. Env. 407: 6179-6187

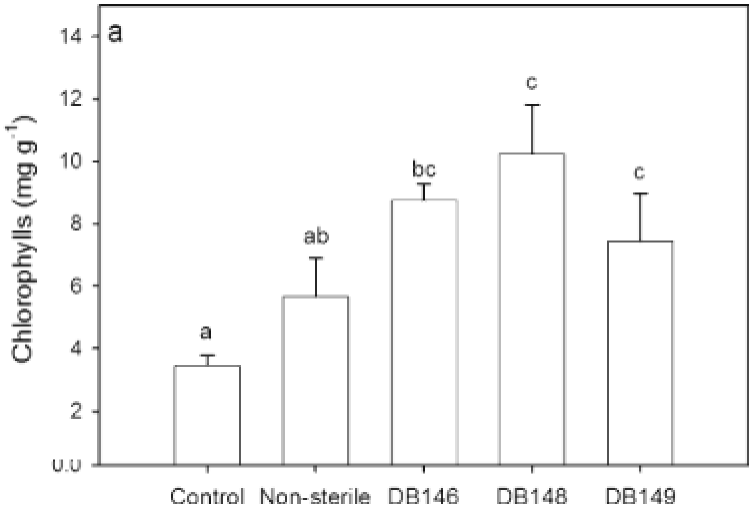
© Regvar et. al. (2010) Plant Soil 330:345–356.

III. Interactions of Plants and MO

- **DSE & their role in plant protection**
  - Fungal isolates: *Phialophora/Cadophora complex*
  - DSE-inoculated willows had ↓ leaf Cd content also ↓ Zn concentrations (isolates DB146 and DB148).
  - DSE isolates ↑ the chlorophyll levels, some also TR.
  - **DSE reduced the metal uptake by the willows → beneficial role of DSE in metal-enriched soils!**







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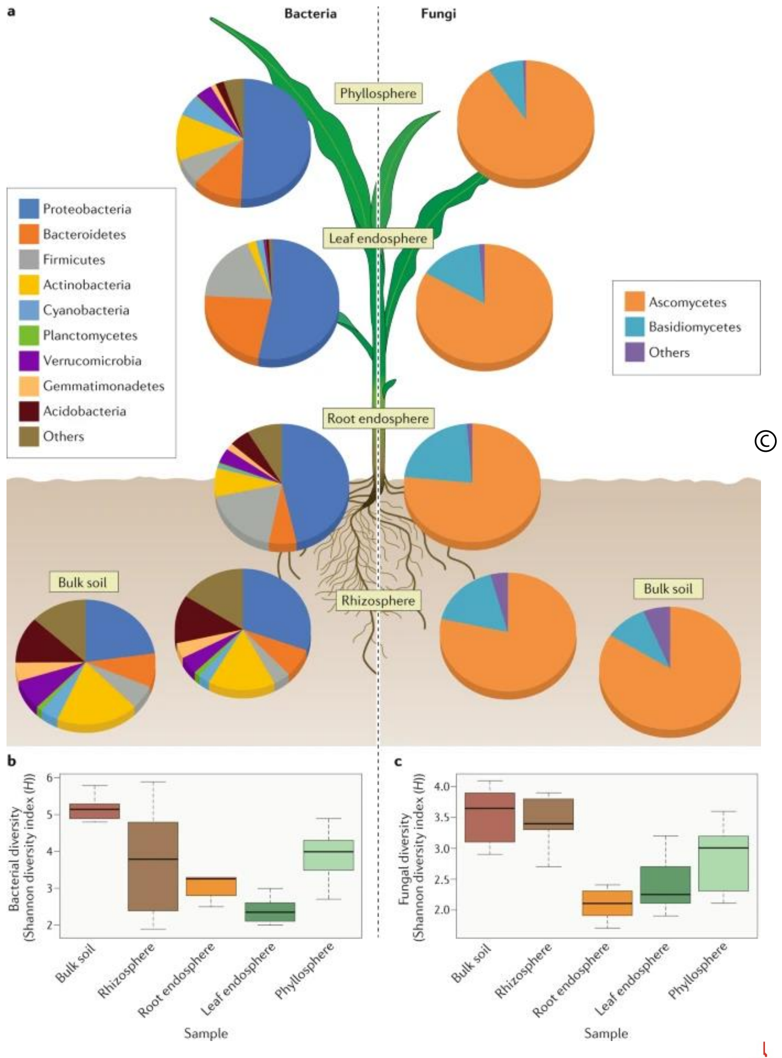
III. Interactions of Plants and MO

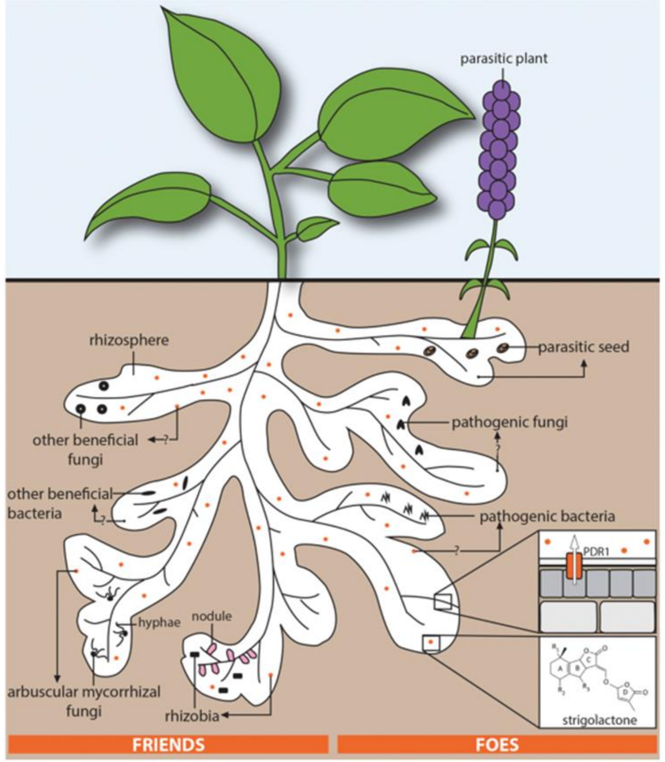
- **Plant microbiome**
  - Aboveground microbiome = more variable: open nature and rapidly fluctuating environment.
    - Transfer by aerosols, soil, pollen, insects, herbivores and/or migration via other plant tissues.
  - ***Smaller overlap between the fungal community of aboveground plant tissues and soil than bacteria →*** other sources are important reservoirs for the aboveground fungal community!



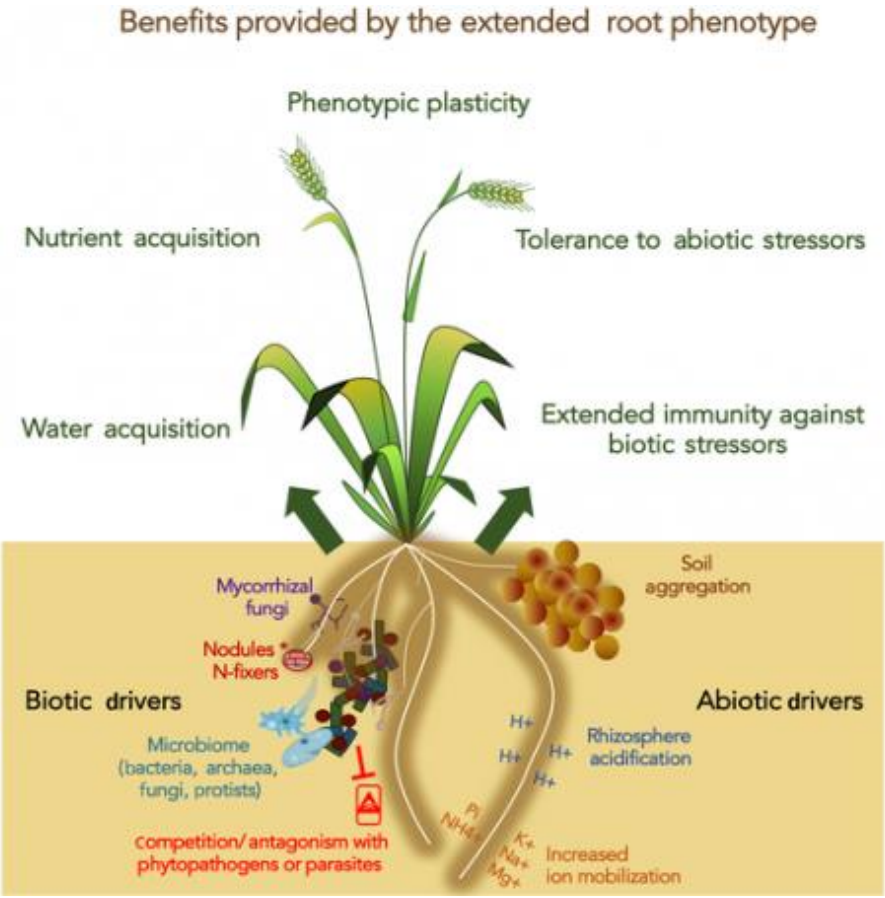
© DOI: 10.1038/s41579-020-0412-1

III. Interactions of Plants and MO

- **MO communities in rhizosphere**
  - *Rhizosphere = soil around root system, where interactions with MO occur (Hiltner, 1904).*
  - MO & plants:
    - **Friends**
    - **Foes**
  - strigolactones



© DOI:10.1094/MPMI-02-17-0051-CR



© DOI: 10.1111/tpj.14781

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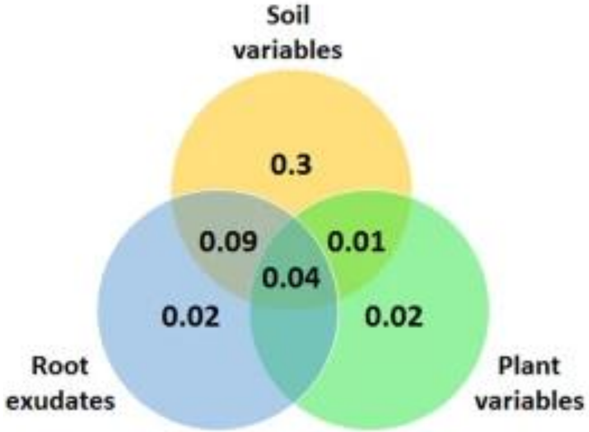
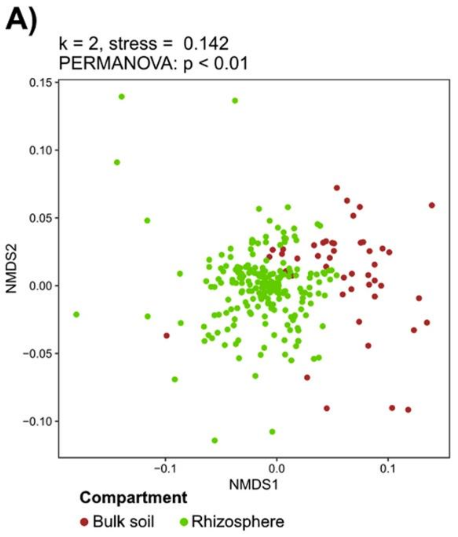
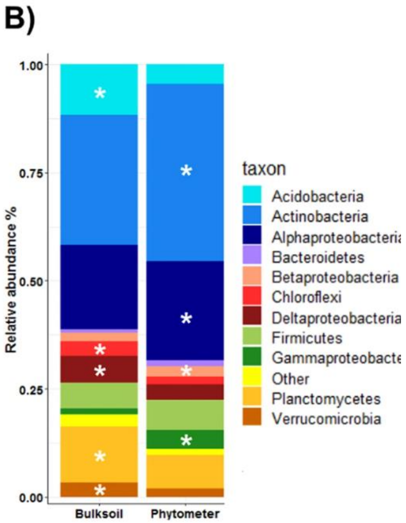
III. Interactions of Plants and MO

- **Bacterial communities in rhizosphere**
  - **Soil properties** (soil texture & type, water content, pH, salinity, OC…) **dictate bacterial structure!**
    - Minor role of:
      - plant communities
      - root exudates







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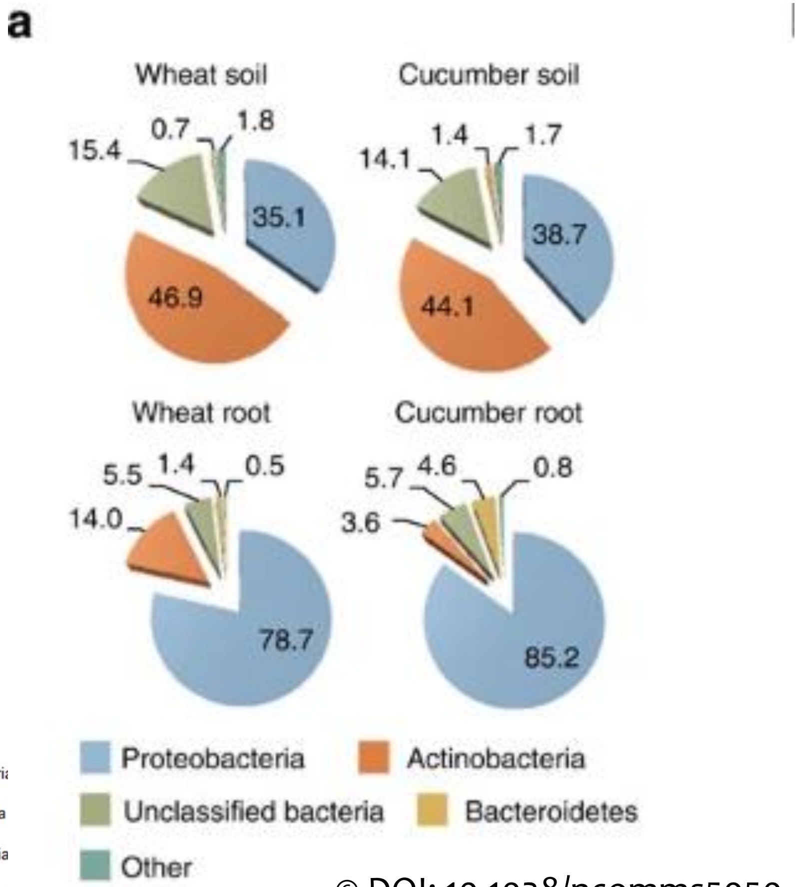

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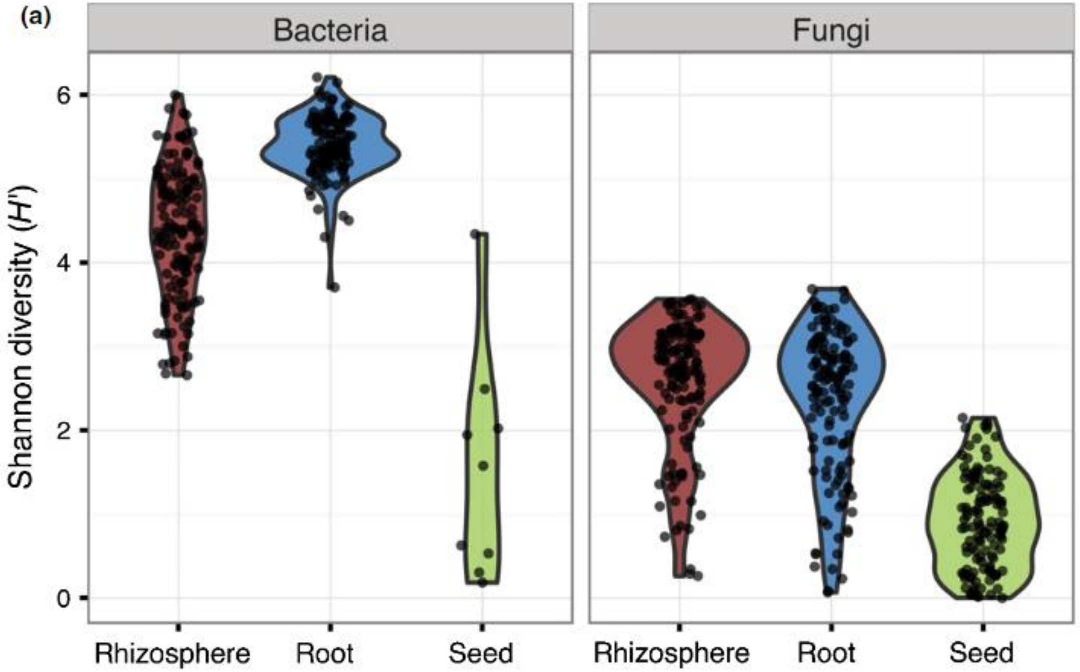
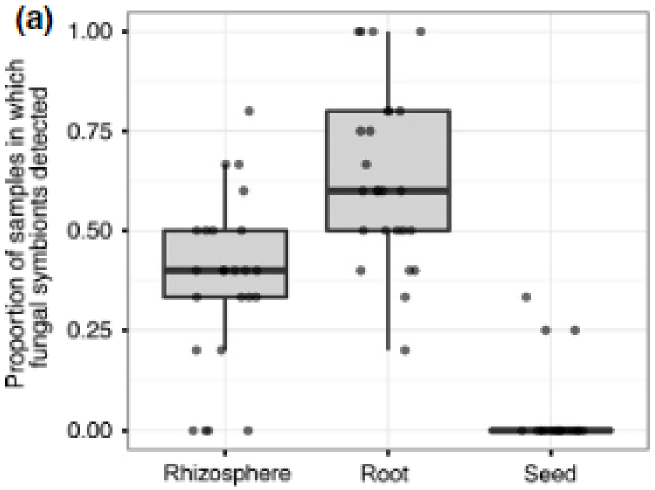
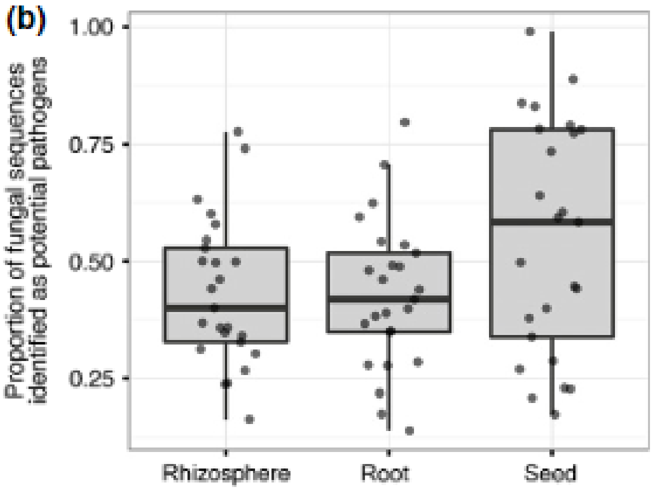
III. Interactions of Plants and MO

- **Bacterial & fungal communities**
  - Vertical transmission? Effect of soil?
    - **Bacterial communities** in rhizosphere and roots are similar, more diverse than in seeds!
    - **Fungal communities**: possible vertical transmission… interesting S/P ratio.







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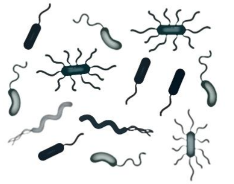
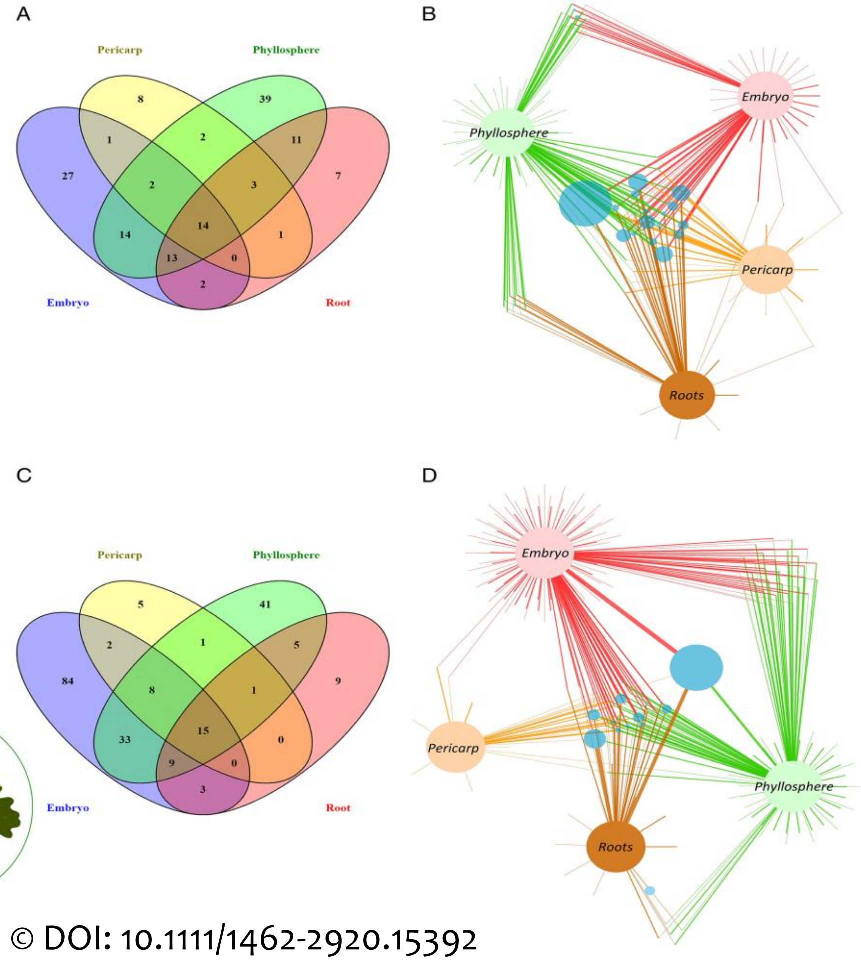


- **<u>Seed microbiome:</u>** *Inoculum for next generations? Vertical transmission?*
  - **seed microbiome = diverse and non-randomly distributed**
  - **large part of the seed microbiome is transmitted** to the phyllosphere and roots of the developing seedling!
  - **phyllosphere = diverse, strongly resembled the composition in the embryo!**
  - *roots and pericarp each had a less diverse and distinct microbial community.*



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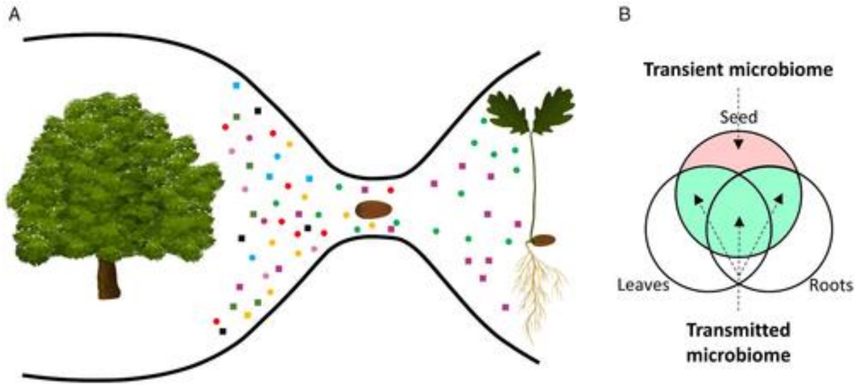

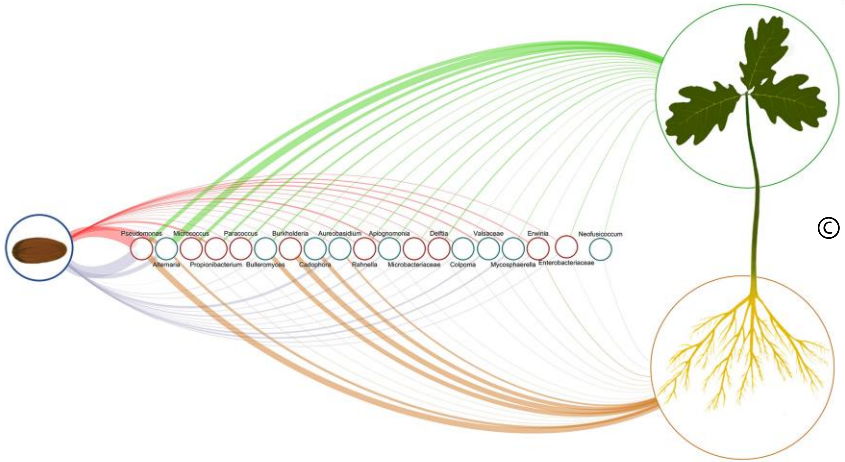

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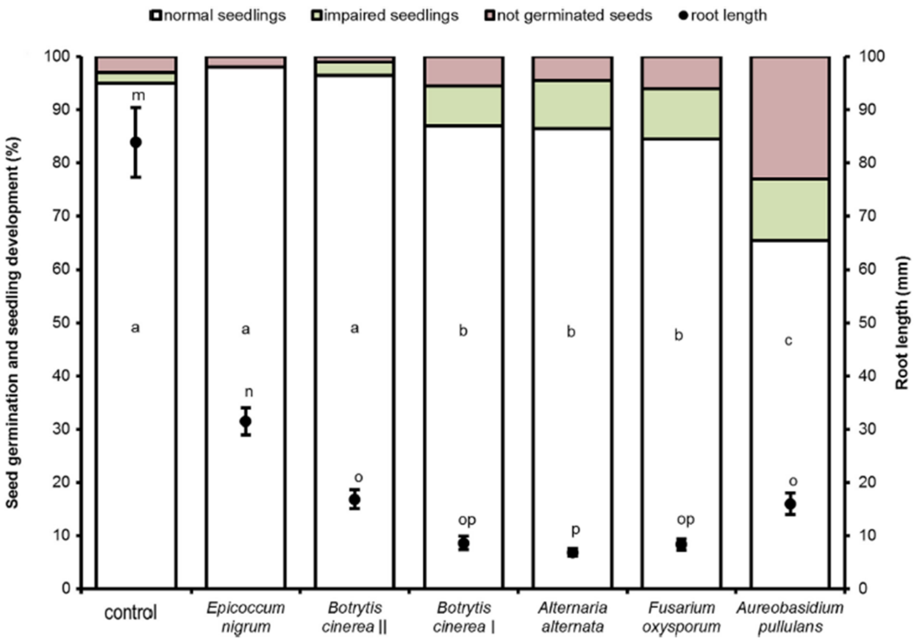
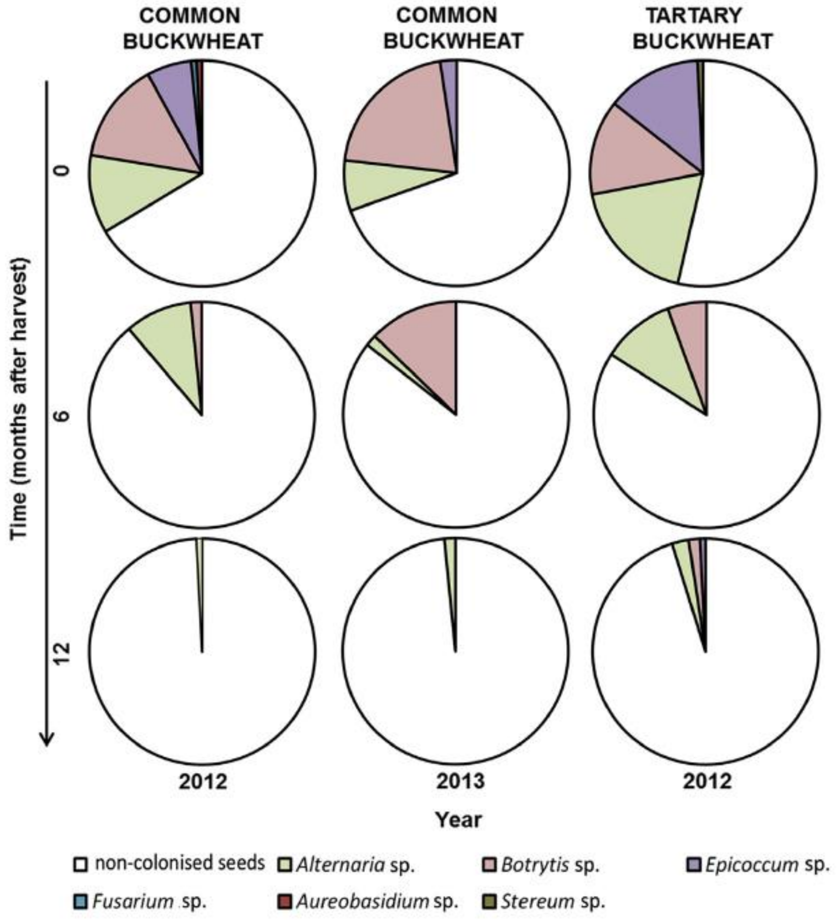
III. Interactions of Plants and MO

- **<u>Seed microbiome of Buckwheat grains</u>**
  - ***Fungal frequencies & diversity ↓ during storage!***
  - Interspecies interactions determine incidence of fungi? direct antagonism & VOC



- Different effect of fungi on germination and seedling growth.



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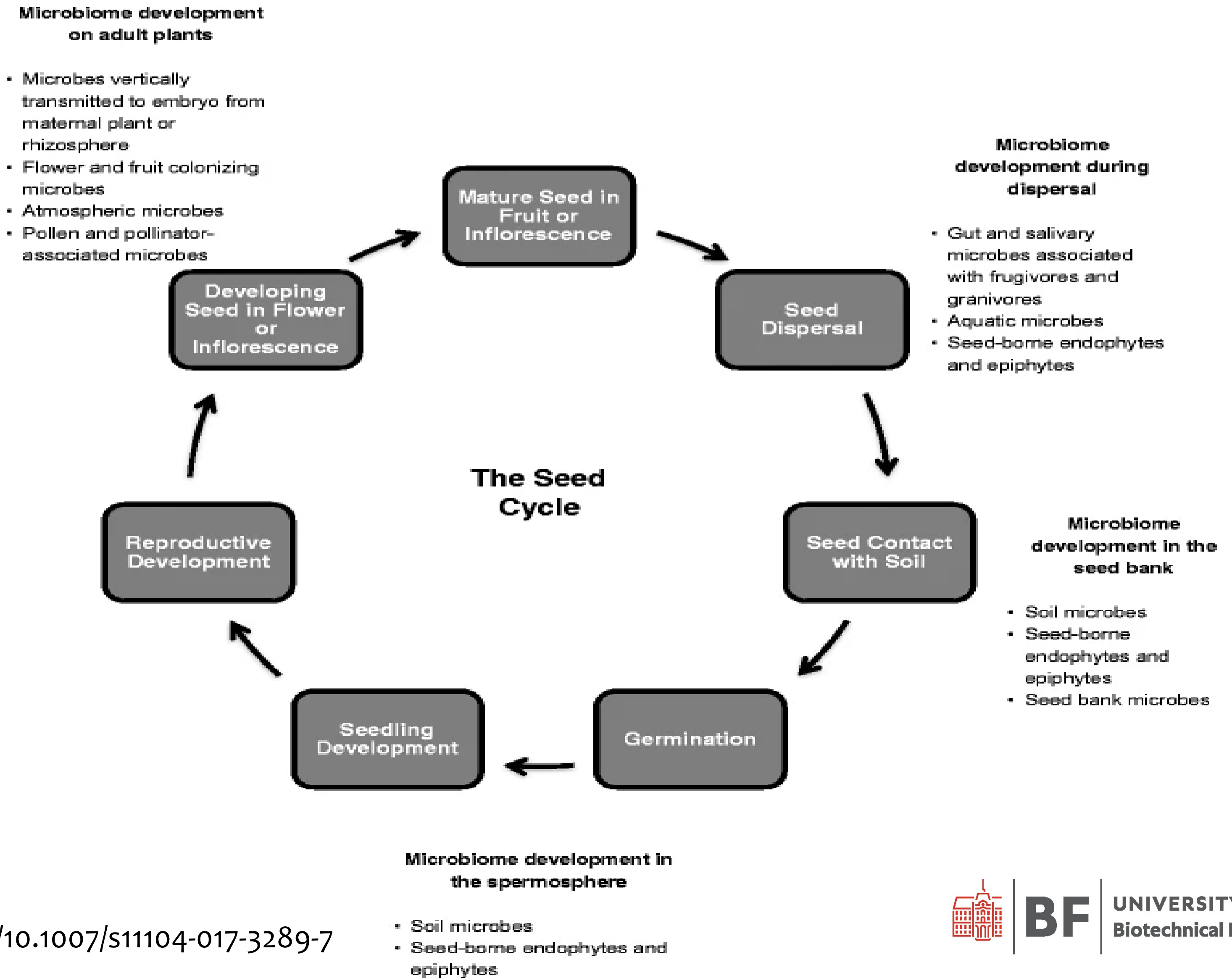
- **Seed microbiome**
  - *Influenced by many factors throughout its life-cyle!*
  - *V & H transmission!*



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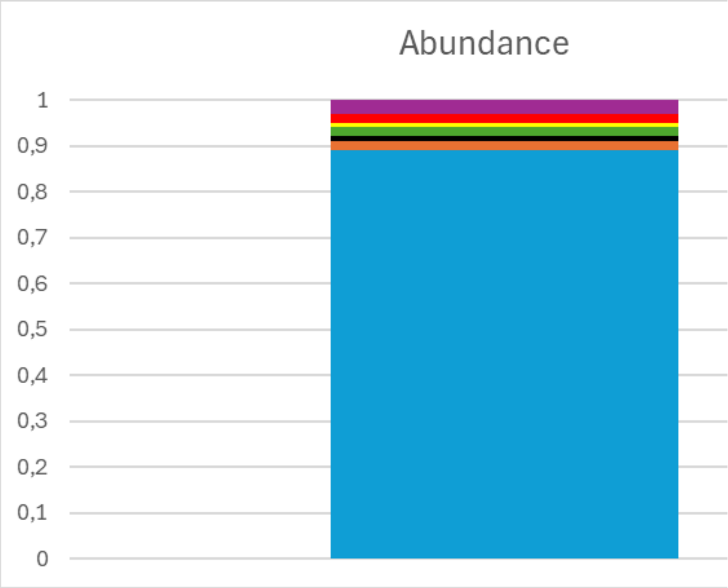
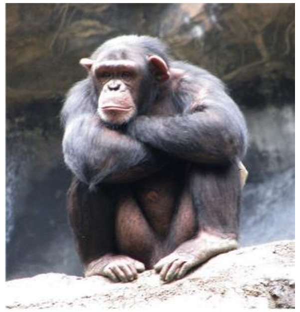
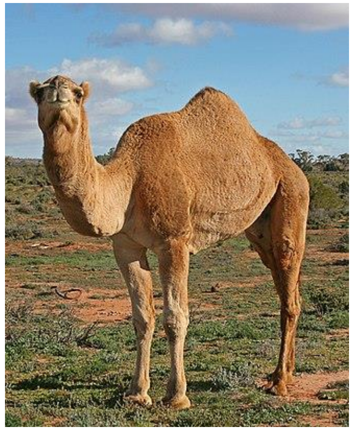
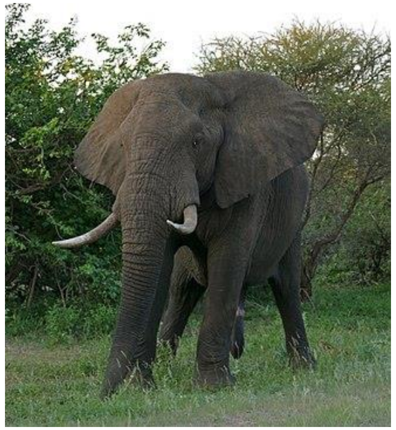
III. Interactions of Plants and MO

- **The beauty & the nightmare of NGS**
  - Mammals ~ 1300 genera

Panthera	3
Ursus	2
Pan	1
Camelus	2
Loxodonta	1
Mus	2
Mammalia	89
	100



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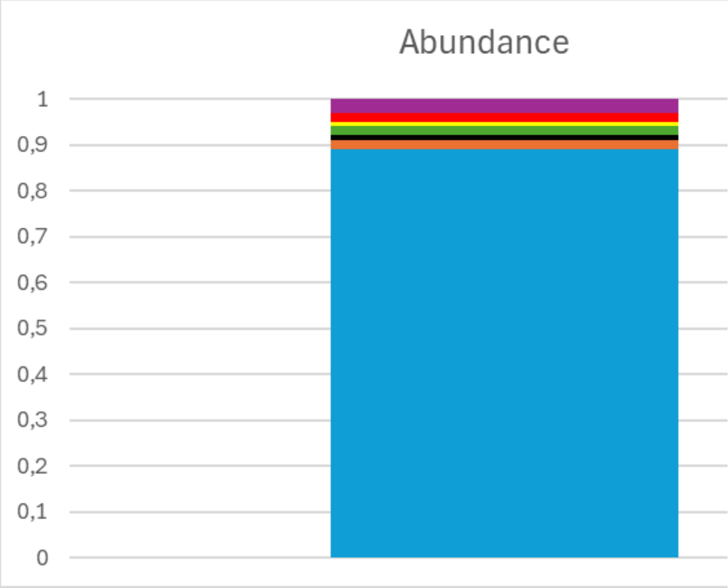

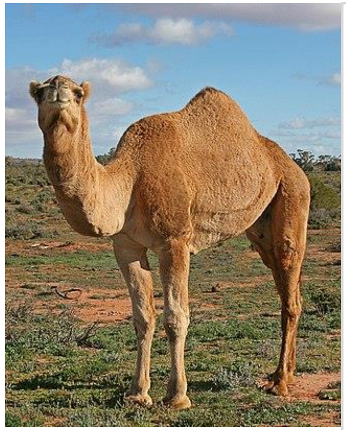

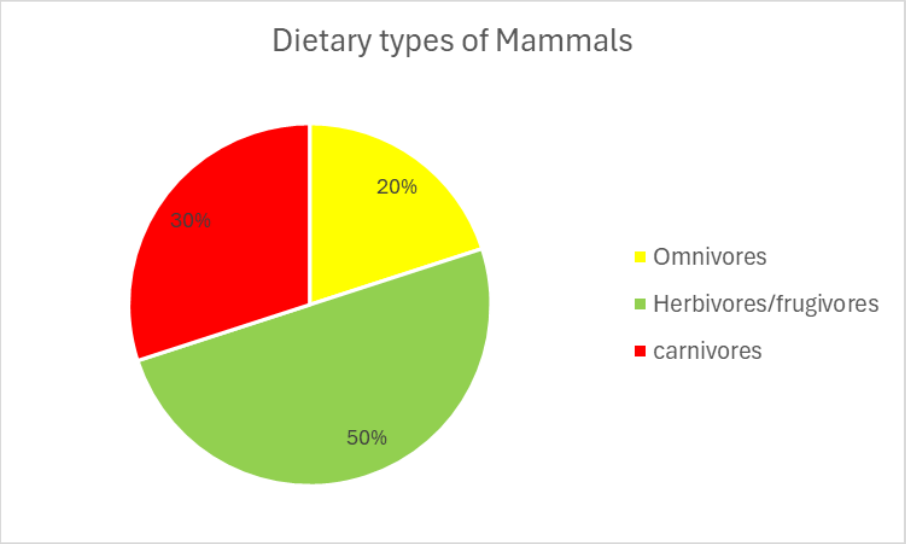

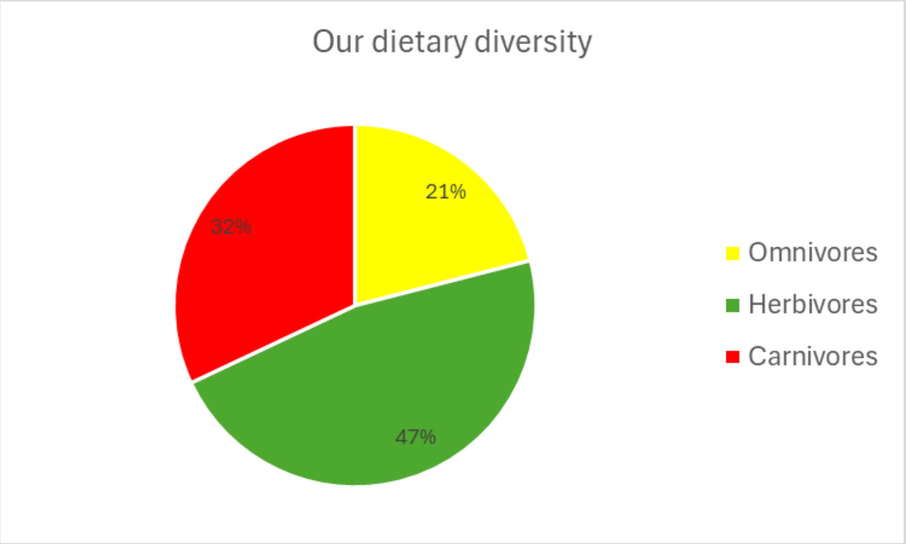

III. Interactions of Plants and MO

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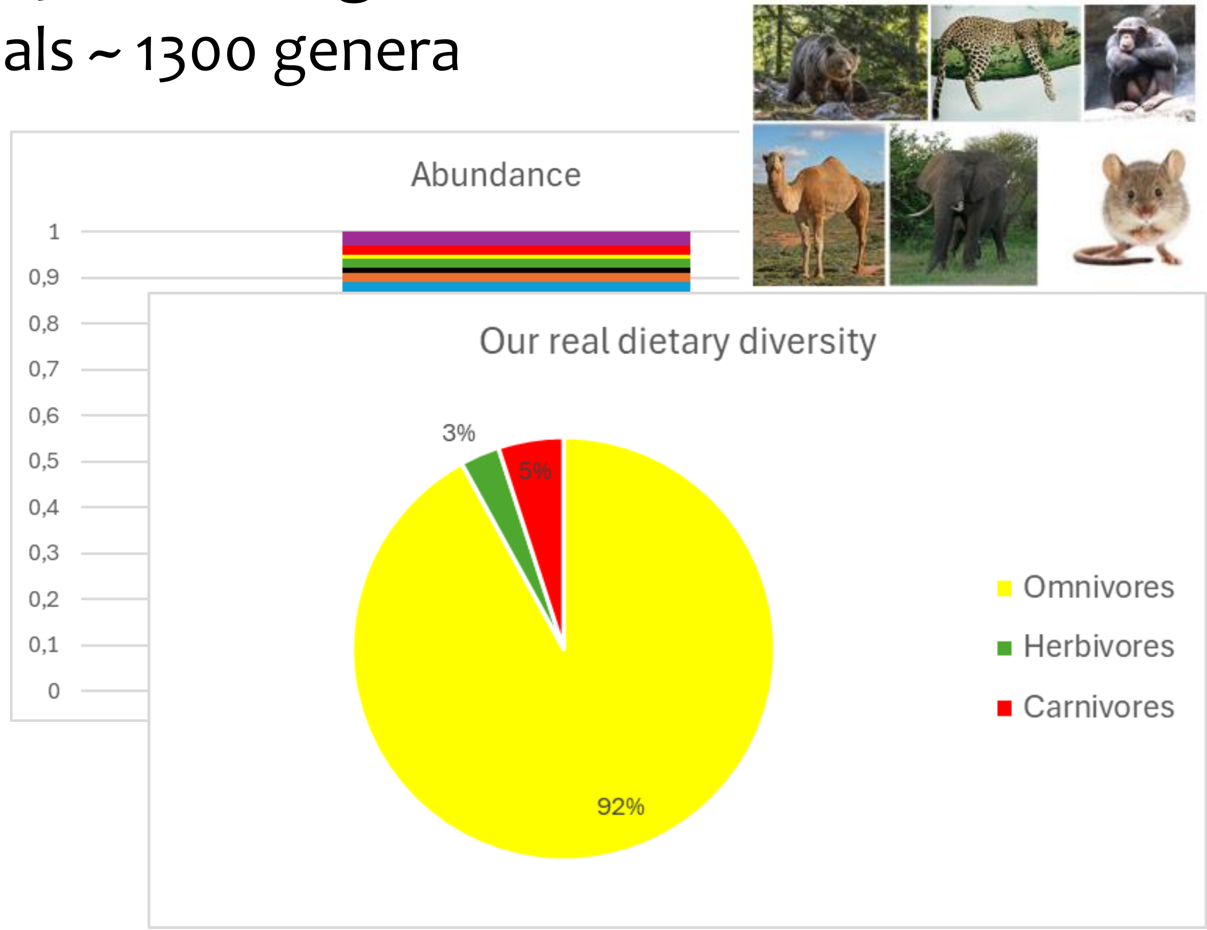
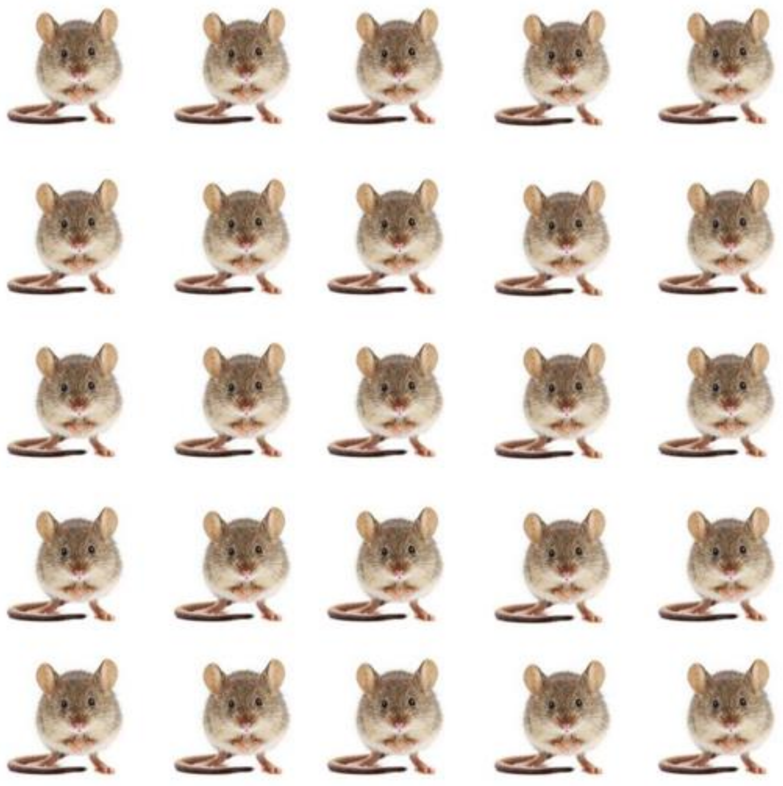
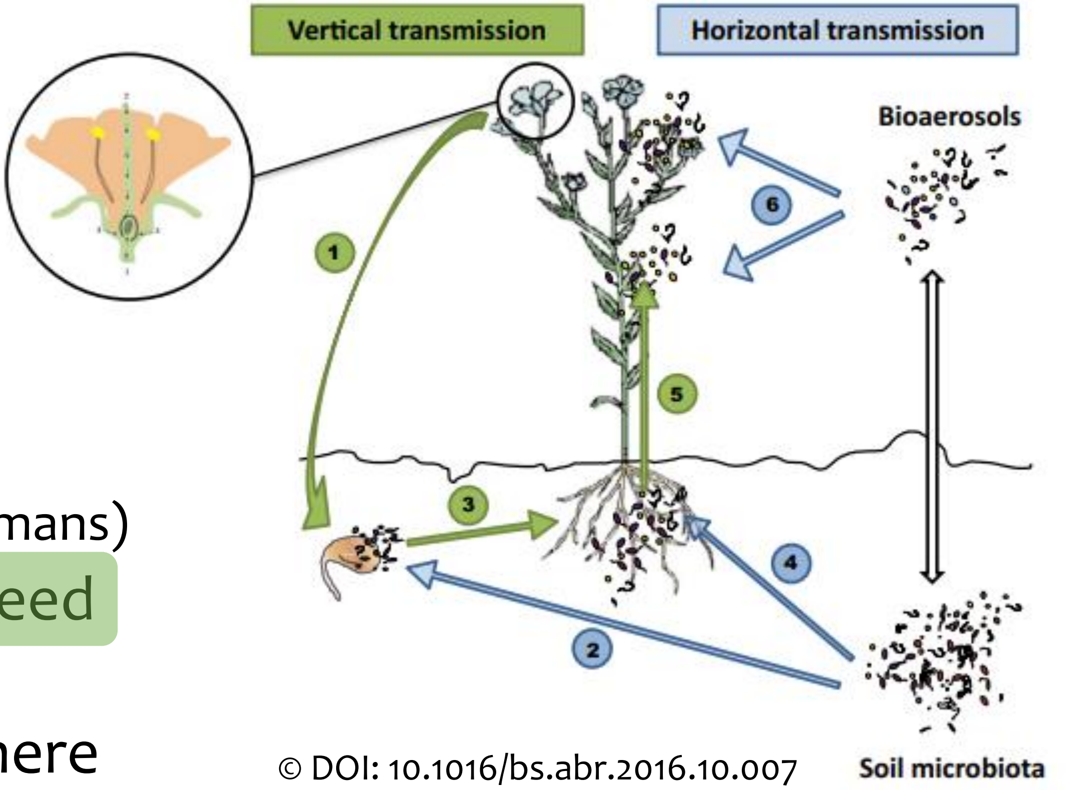




III. Interactions of Plants and MO

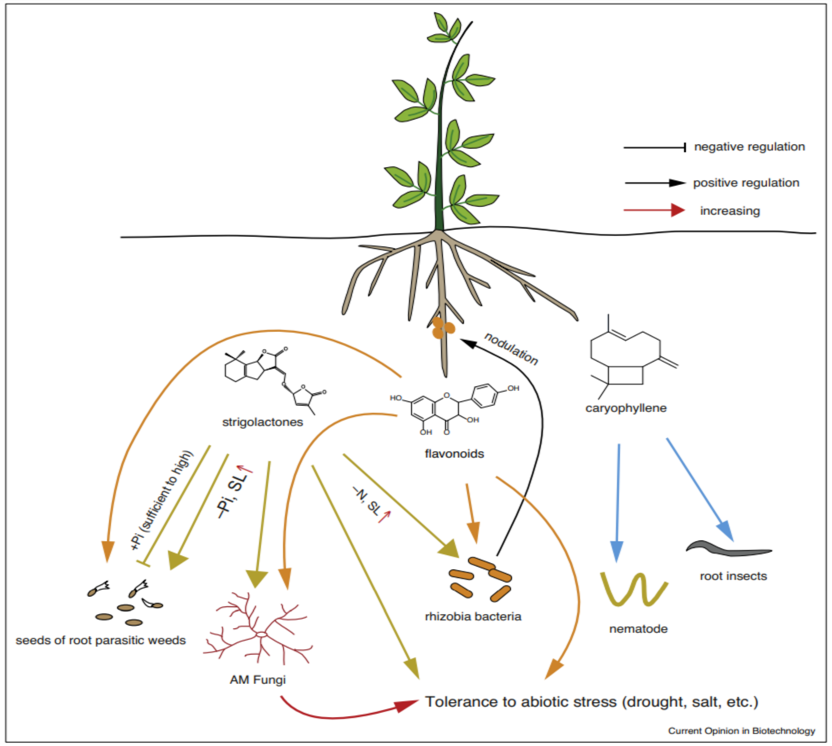
- **Transmission of MO in Plants**
  - MO are recruited:
    - **Horizontally:** from the environment
      - *Soil*
      - *Atmosphere*
      - *Among plants*
      - ***Other vectors*** (pollinators, herbivores, humans)
    - **Vertically:** from mother plant via the seed
      - **Via plant tissues**
  - spermosphere, rhizosphere and phyllosphere



© DOI: 10.1016/bs.abr.2016.10.007

III. Interactions of Plants and MO

- **<u>"MO engineering"</u>**
  - Manipulating with MO communities
  - ***Adapting plant microbiome*** (↑ beneficial, ↓ pathogens)



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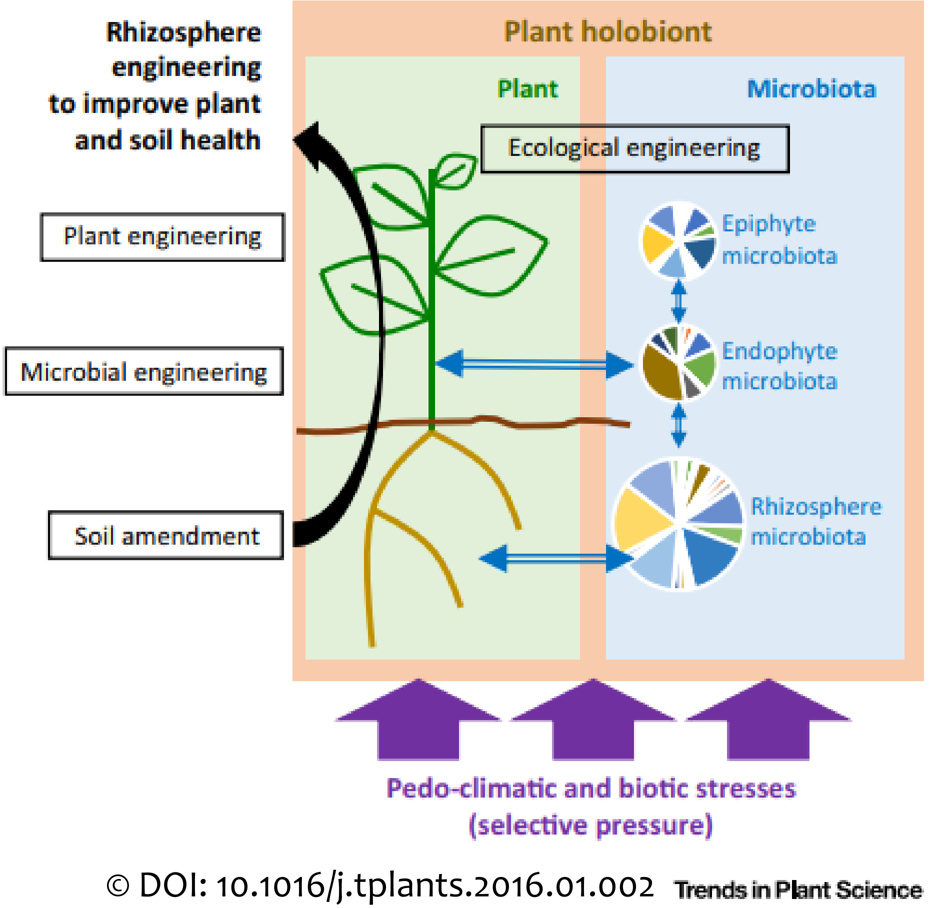
Engineering plant resistance for improving stress tolerance (biotic & abiotic)!



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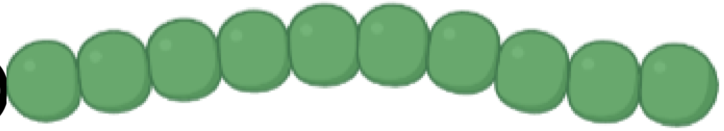
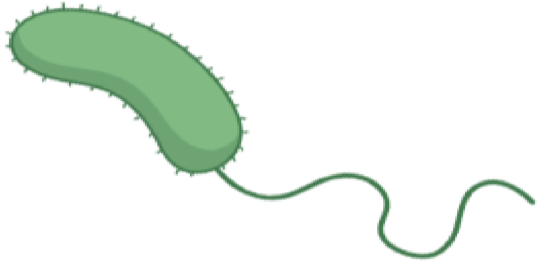
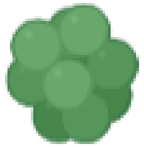
The End ☺





"(Ladies and) Gentlemen, ***it is the microbes who will have the last word!***" (Louis Pasteur)



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Literature & Sources

- Brock Biology of Microorganisms, 16th ed. (2021) Pearson Education.
- Campbell Biology, 12th ed. (2021) Pearson Education.
- Plant Pathology, 5th ed. (2005) Elsevier Academic Press.
- All the Articles cited (DOI in text).
- Personal archive (J. Mravlje & colleagues)