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The new age of soil microbiome

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Head of the ENEA Laboratory
SSPT-BIOAG-SOQUAS

November 27, 2023
Hybrid course
c/o ENEA CR Casaccia
Sala Blu, Via Anguillarese 301, Roma





Microbiome: the new definition

Berg et al. *Microbiome* (2020) 8:103
<https://doi.org/10.1186/s40168-020-00875-0>

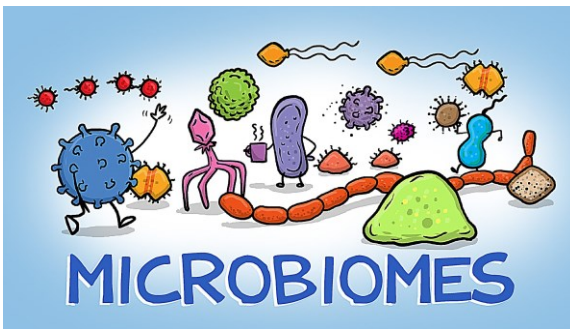
COMMENTARY

Open Access

Microbiome definition re-visited: old concepts and new challenges



Microbiome



Microbiome

Microbiota

Bacteria

Archaea

Fungi

Protists

Algae

+ "Theatre of activity"

Microbial structural elements

Proteins/
peptides

Lipids

Poly-
saccharides

Nucleic acids
structural DNA/RNA

mobile genetic elements
incl. viruses/phages relic DNA

Internal/external structural elements

Environmental
conditions

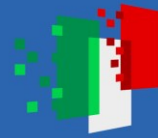
Microbial metabolites

Signalling
molecules

Toxins

(An)organic
molecules

Biome: a reasonably well defined habitat which has distinct bio-physio-chemical properties

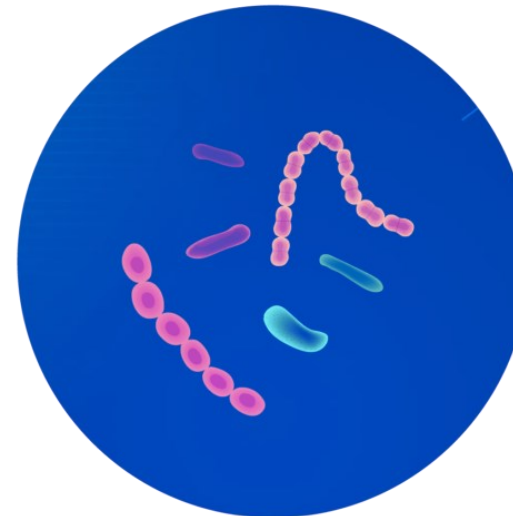


The microbiome vs. the microbiota

The **microbiome** is defined as a characteristic 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 encompasses their theatre of activity, which results in the formation of specific ecological niches. The microbiome, which forms a dynamic and interactive micro-ecosystem prone to change in time and scale, is integrated in macro-ecosystems including eukaryotic hosts, and here crucial for their functioning and health.

The **microbiota** consists of the assembly of microorganisms belonging to different kingdoms (Prokaryotes [Bacteria, Archaea], Eukaryotes [e.g. Protozoa, Fungi, Algae]), while "their theatre of activity" includes microbial structures, metabolites, mobile genetic elements (e.g. transposons, phages, viruses) as well as relic DNA embedded in the environmental conditions of the habitat.

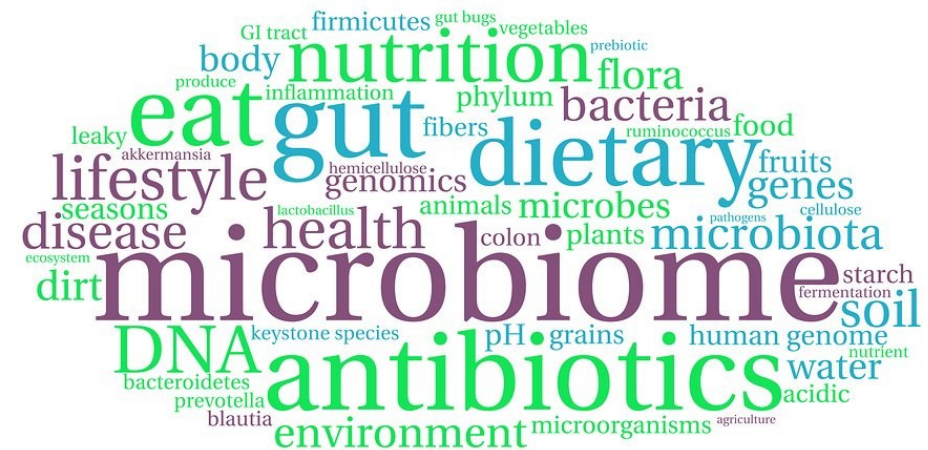
- MICROBIOME DEFINITION -



Microbiota



Microbiome

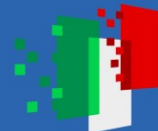




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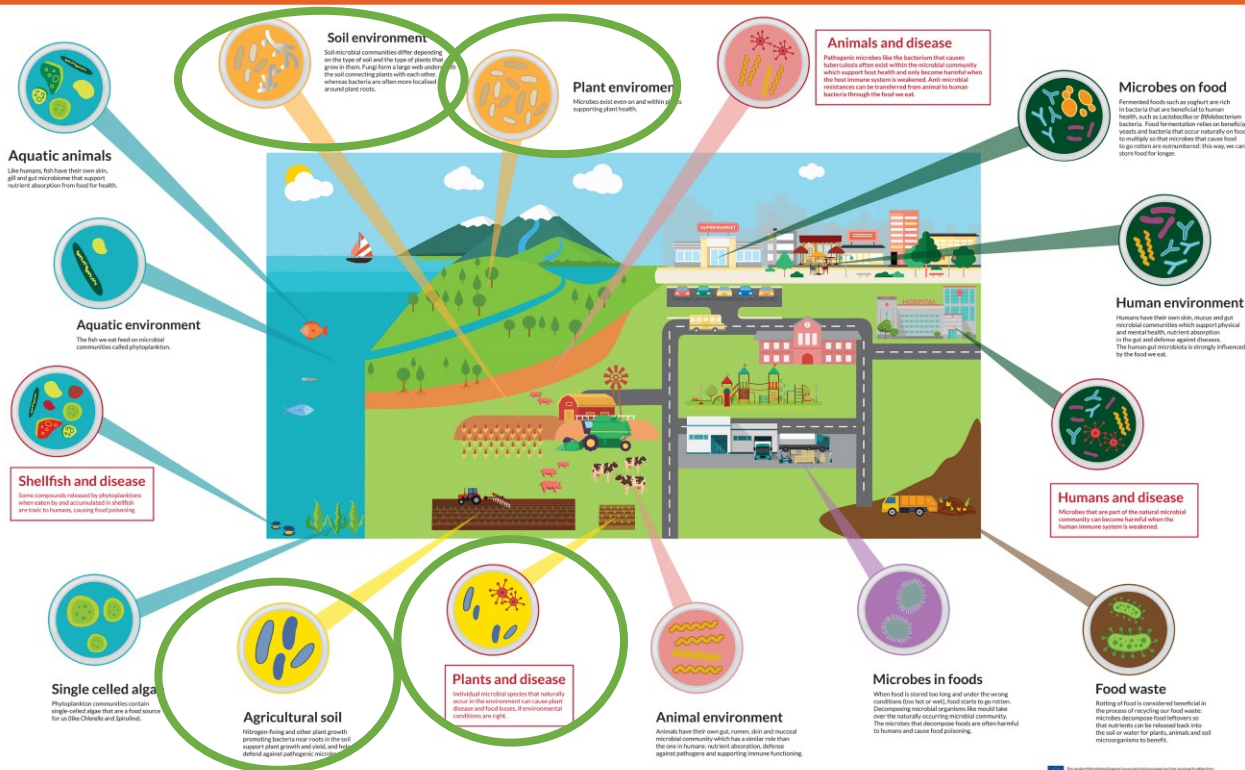


Soil and plant microbiome

Microbes are everywhere in the food system

Diverse microbial communities consisting of fungi, bacteria, protozoa and other micro-organisms occur in all parts of our food system and are essential in its functioning and health, for food security and climate change mitigation.

Individual microbes can be harmful to plant, animal and human health if environmental conditions are in their favour. These microbes are often a natural part of microbial communities in low numbers.



- Microbes, like bacteria, yeasts and other fungi, occur everywhere in the food system.
- Communities of microbes are essential in plant, animal, human and environmental health, with a consequent impact in terms of crop and livestock productivity, food quality and safety as well as for food waste decomposition and recycling processes.
- The composition of microbe communities differ across the food system, depending on the environmental conditions.



Soil microbes underpin food quality and security

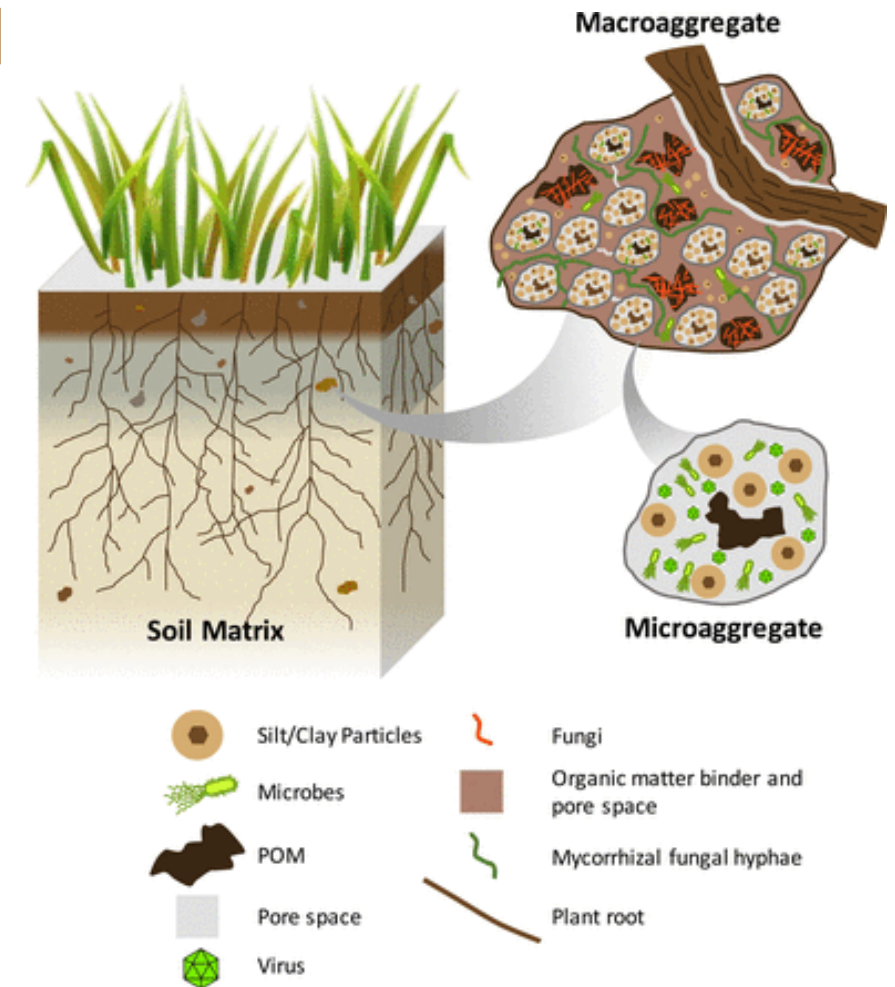


- Soil is a complex and dynamic biological system
- Soil is essential for the maintenance of biodiversity above and below ground
- Soil governs plant productivity of terrestrial ecosystems and it maintains biogeochemical cycles
- Soil is where food begins: 95% of all food comes from the soil
- Soil is fundamental to crop production
- Soil would be more resilient, and farmers would be less dependent on toxic pesticides and chemical fertilizers.



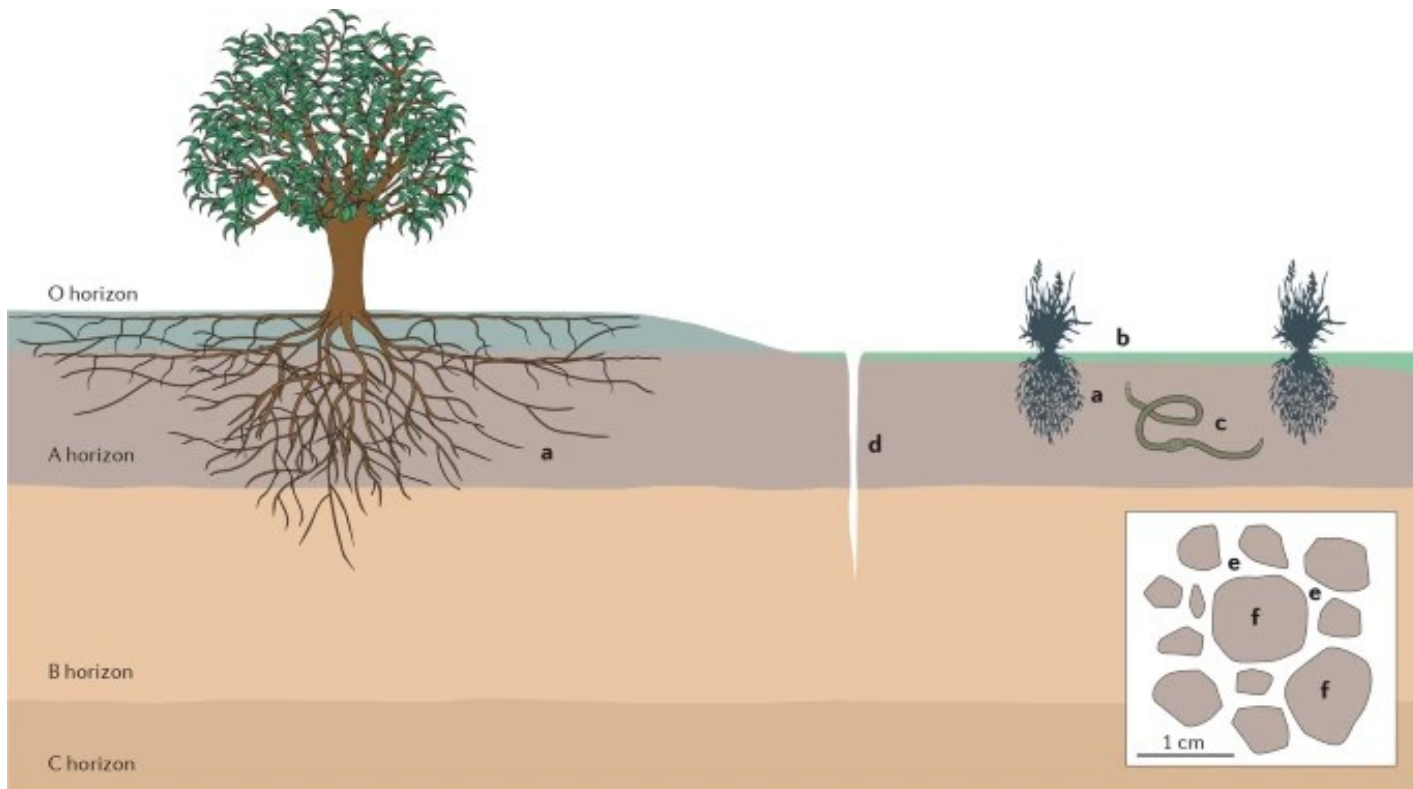
Simplified schematic of soil horizon and soil macro- and microaggregates

- Soils can generally be viewed as a complex three-dimensional structure consisting of packed aggregates and pore spaces
- Aggregates comprise clusters of mineral particles and organic carbon
- The architecture of a particular soil influences interactions between plants, microbes, and the soil matrix
- The porosity and connectivity of aggregates are influenced by the diversity of bacteria and fungi present during formation





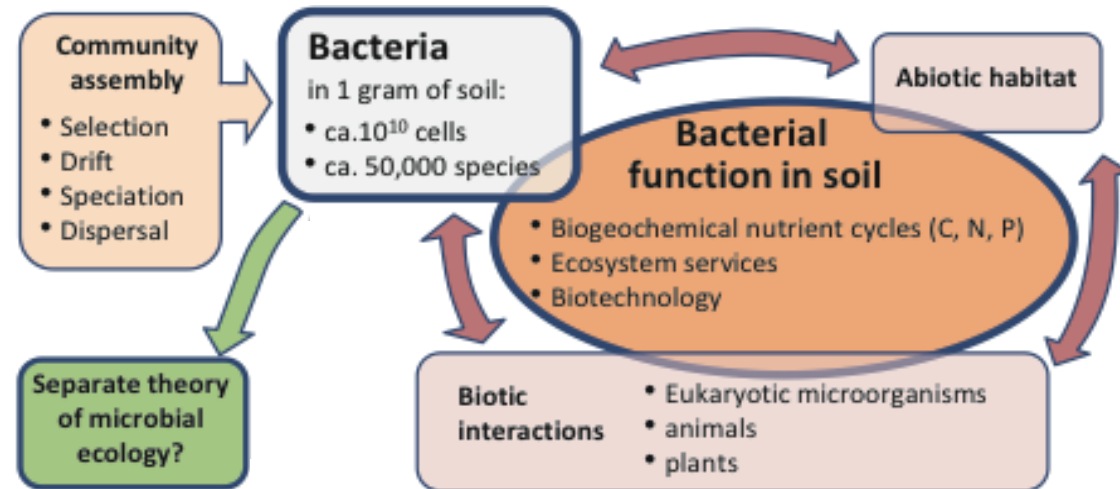
Embracing the unknown: disentangling the complexities



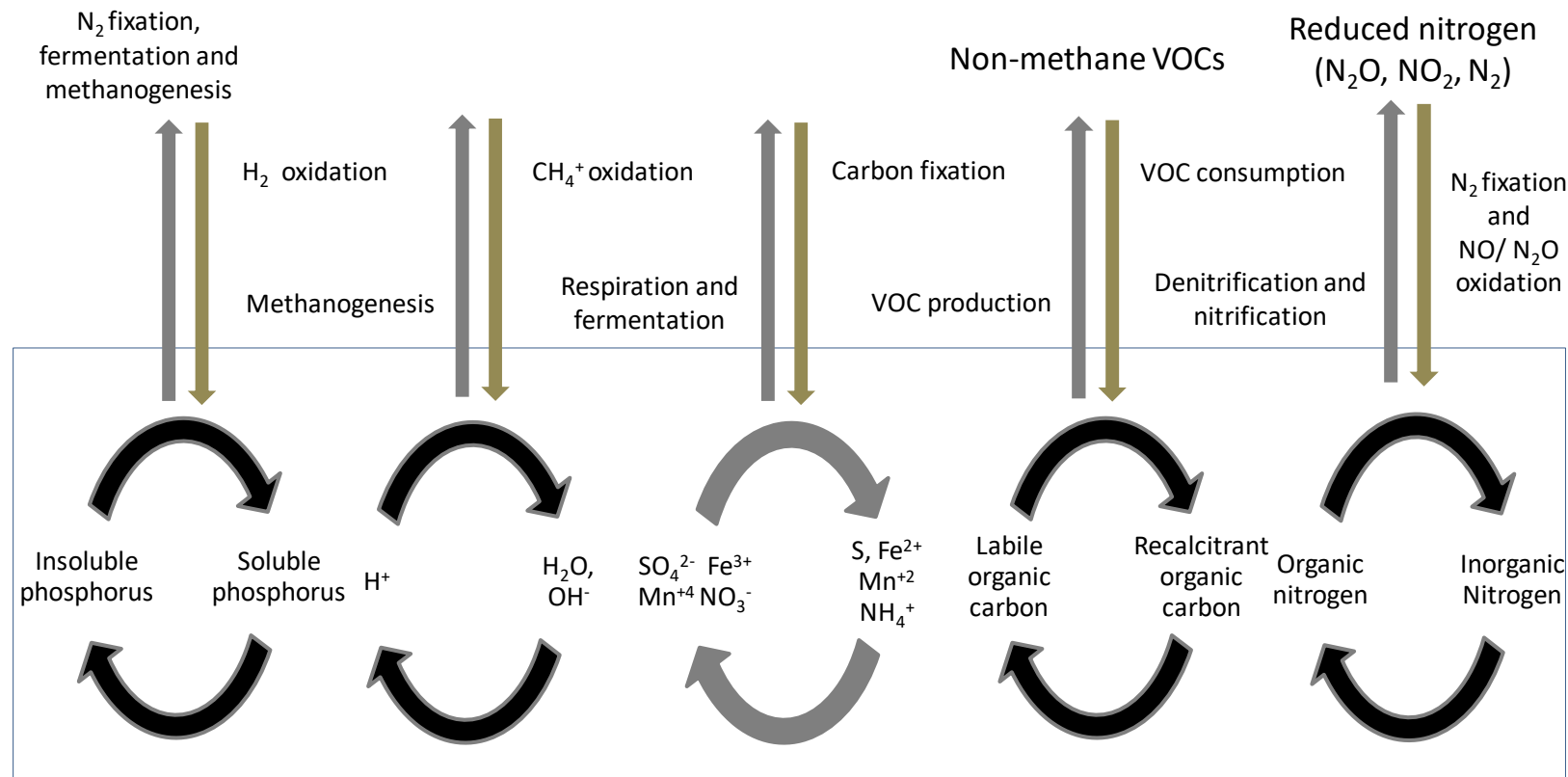
- Soil is not a single environment; instead, soil encompasses a broad range of different microbial habitats.
- These include the rhizosphere (soil in close proximity to plant roots; part a), surface layers that are exposed to light (part b; the photic zone), soil associated with earthworm burrows (the drilosphere; part c), and soil found in preferential water flow paths, including cracks in the soil (part d).
- One gram of healthy soil usually contains a microbiome comprising many millions of microbes, including archaea, bacteria and fungi.

The world beneath our feet

- The wealth of biodiversity below ground is vast and unappreciated: millions of microorganisms live and reproduce in a few grams of topsoil, an ecosystem essential for life on earth
- Soil microorganisms play an essential role in ecosystem functioning, decomposing organic matter, in determining the release of mineral nutrients, and in nutrient cycling
- Changes in soil microbial community may directly affect soil ecosystem function, particularly carbon and nitrogen cycling



Modified from Johannes Sikorski SOIL ORGANISMS 2015



Fierer, 2017. *Nature Microbiol.* doi:10.1038/nrmicro.2017.87

- The microbial biomass consists mostly of bacteria and fungi, which decompose crop residues and organic matter in soil. This process releases nutrients, such as nitrogen (N), sulfur (S) and, to a lesser extent, phosphate (P) into the soil that are available for plant uptake.

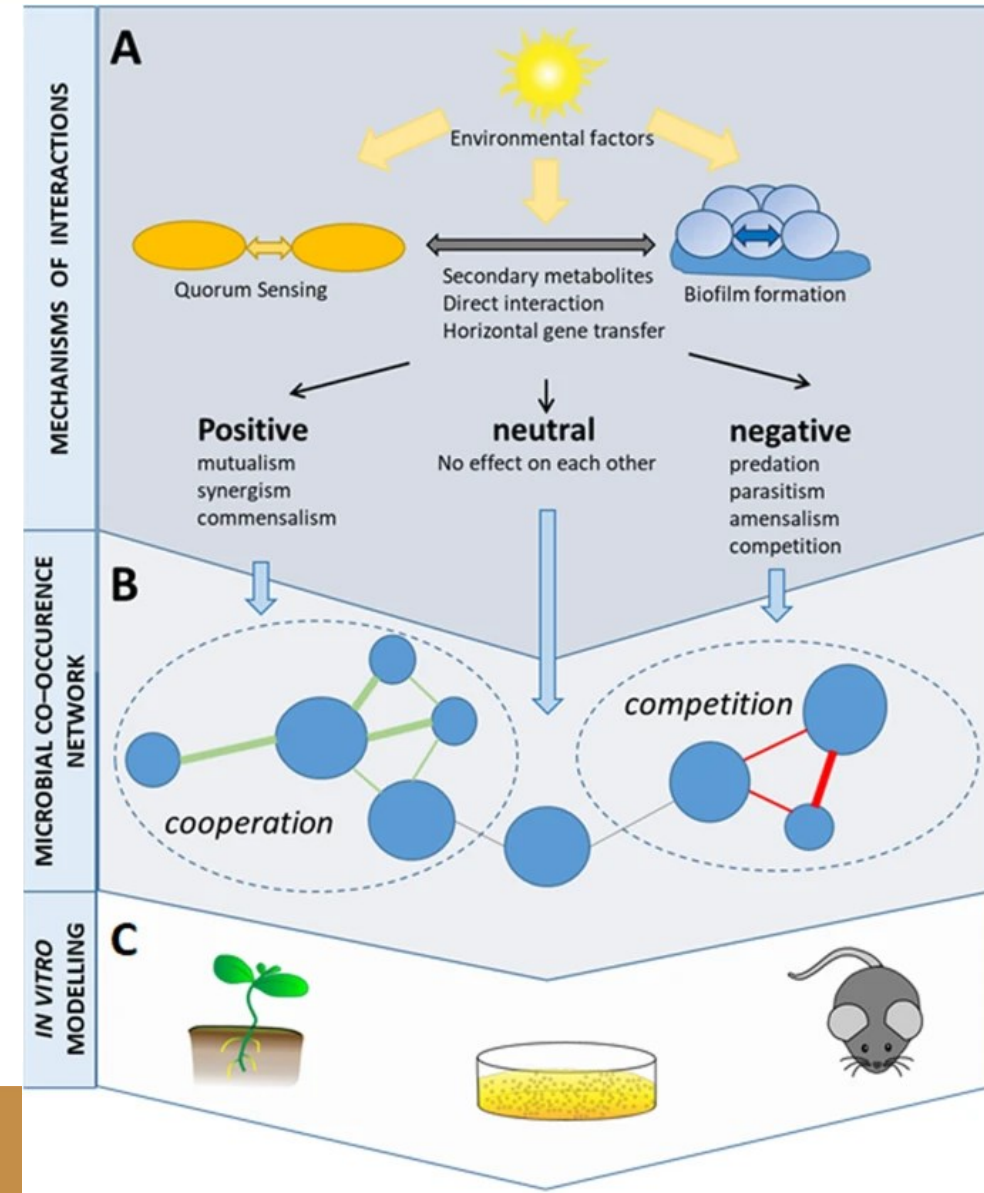
Microbial interaction

Microbial interactions visualized through microbial co-occurrence networks.

a Microbial interactions are influenced by environmental factors and are separated into positive, neutral, and negative interactions types.

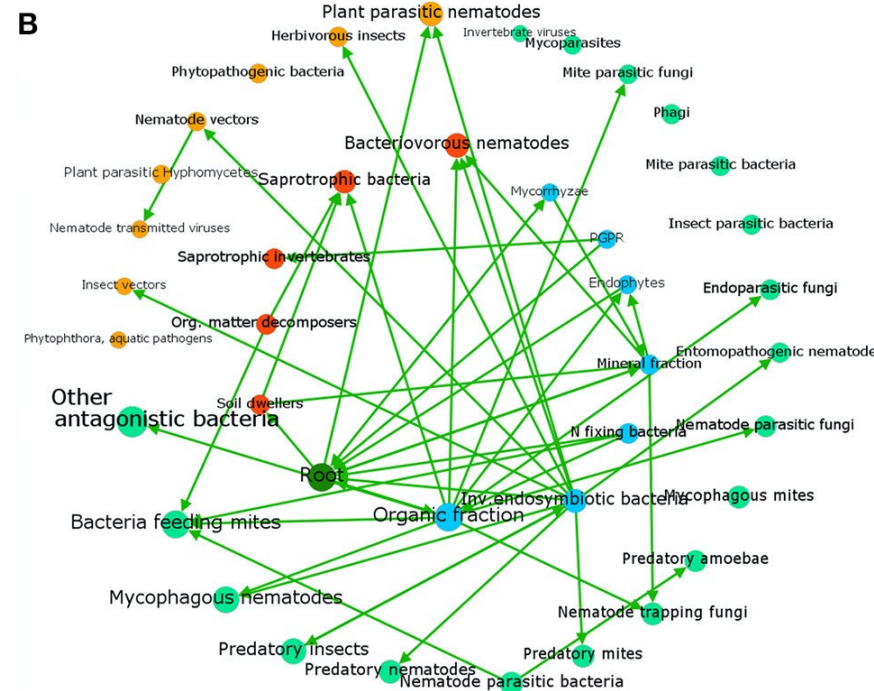
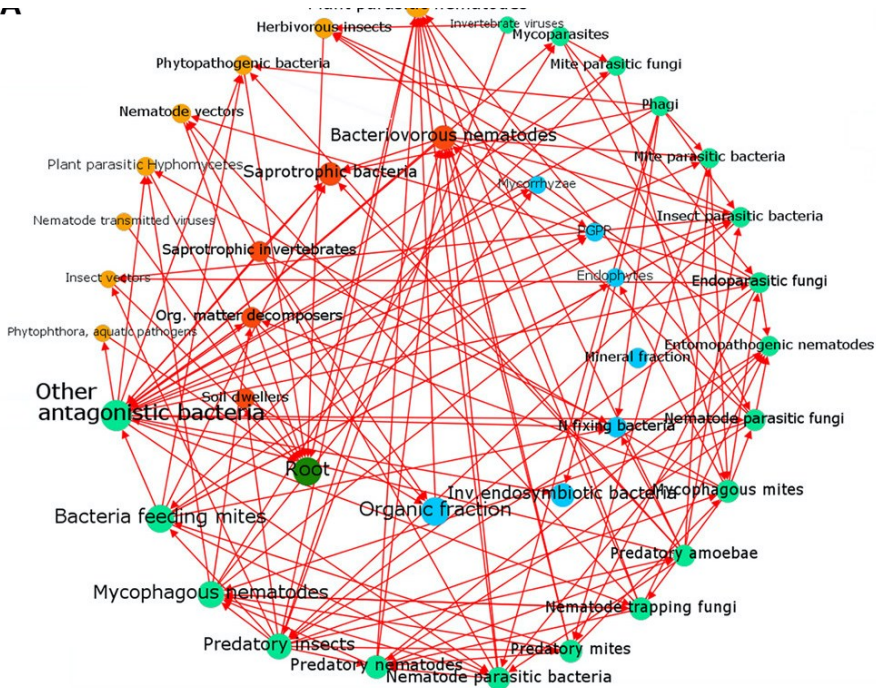
b Microbial co-occurrence and co-exclusion networks help visualizing microbial interactions. In such networks, nodes usually represent taxa of microorganisms, and edges represent statistically significant associations between nodes. Green edges usually stay for positive interactions, while red edges visualize negative interactions between the microorganisms.

c Testing of the hypotheses resulted from the network analyses in relevant model systems is required for a comprehensive study of microbial interactions





Soil microbes: networking



Belowground Microbiota and the Health of Tree Crops

Jesús Mercado-Blanco¹, Isabel Abrantes², Anna Barra Caracciolo³, Annamaria Bevivino^{4*}, Aurelio Ciancio⁵, Paola Greni³, Katarzyna Hryniewicz⁶, László Kredics⁷ and Diogo N. Proença⁸

Arrows show negative effects (A), such as predation, parasitism, pathogenicity or (B) positive links, such as growth promotion, symbiosis or alimentary provision.

A simplified food web describing main soil components and their relationships. The nodes are classified by roles as: primary root (dark green), beneficial soil components, organisms or promoters, including soil factors (blue), decomposers (brown), pathogens (orange) and biocontrol agents or antagonists (pale green).



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Healthy soil for a healthy life



- The healthiest soils are those with a diversity and abundance of life
- The use of beneficial microbes represents a promising tool that may respond to the challenges for modern agriculture

Bulk soil vs. rhizosphere

- In 1904, Lorenz Hiltner first coined the term "rhizosphere" to describe the plant-root interface
- The rhizosphere is the area around a plant root that is inhabited by a unique population of microorganisms influenced by the chemicals released from plant roots
- This complex plant-associated microbial community, also referred to as the second genome of the plant, is crucial for plant health.

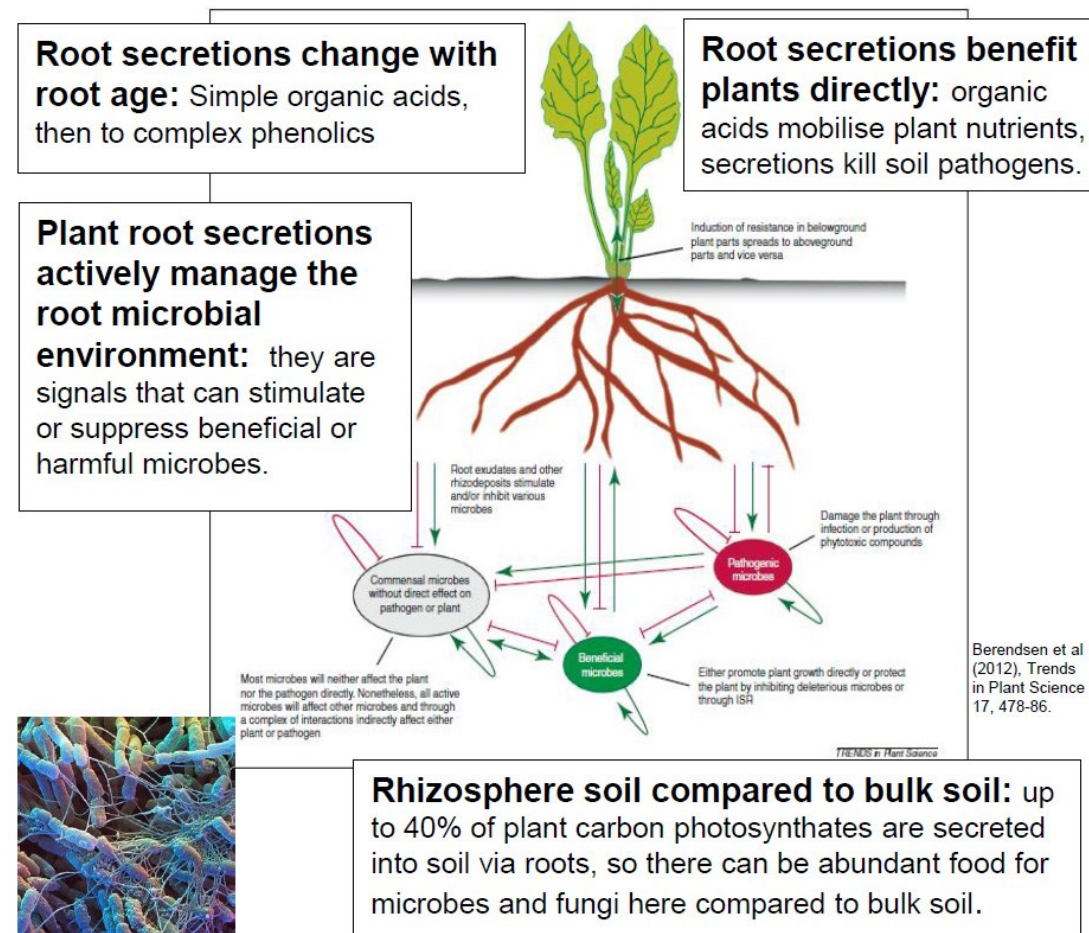


REVIEW ARTICLE

The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms

Rodrigo Mendes¹, Paolina Garbeva² & Jos M. Raaijmakers³

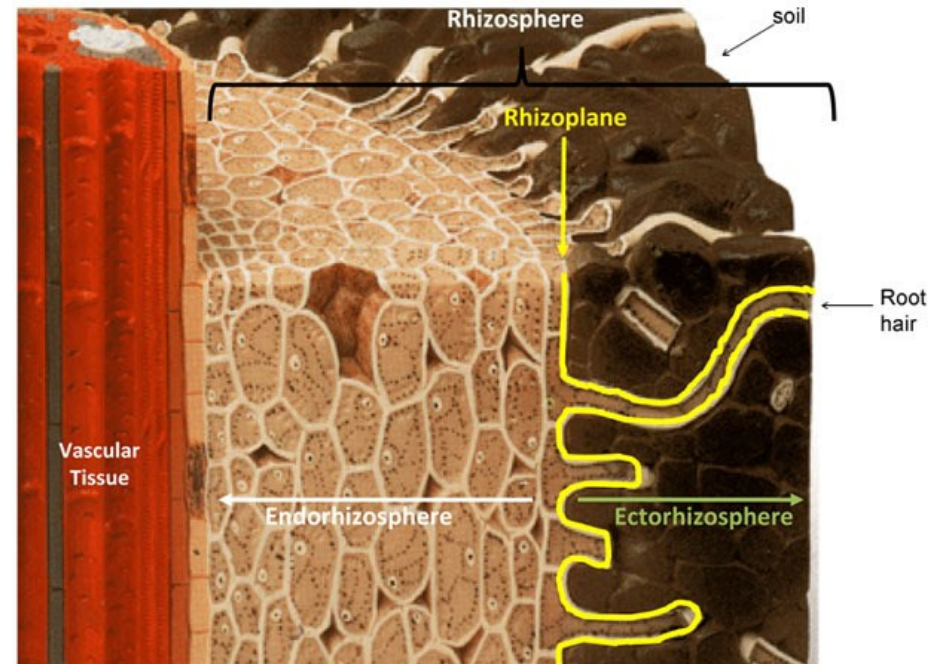
¹Laboratory of Environmental Microbiology, Embrapa Environment, Jaguariuna, Brazil; ²Netherlands Institute of Ecology, Royal Dutch Academy of Arts & Sciences (NIOO-KNAW), Wageningen, The Netherlands; and ³Laboratory of Phytopathology, Bacterial Ecology & Genomics, Wageningen University, Wageningen, The Netherlands





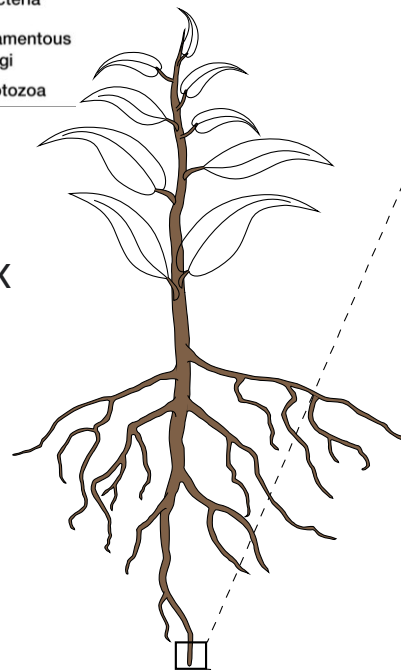
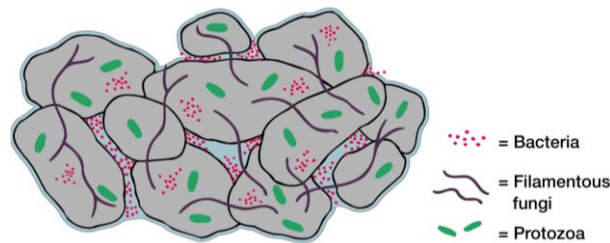
The rhizosphere

- The **rhizosphere** is a dynamic region governed by complex interactions between plants and the organisms that are in close association with the root.
- The rhizosphere includes **three zones** which are defined based on their relative proximity to the root
- **Endorhizosphere**: includes portions of the cortex and endodermis
- **Rhizoplane**: the medial zone directly adjacent to the root including the root epidermis and mucilage
- **Ectorhizosphere** which extends from the rhizoplane out into the bulk soil

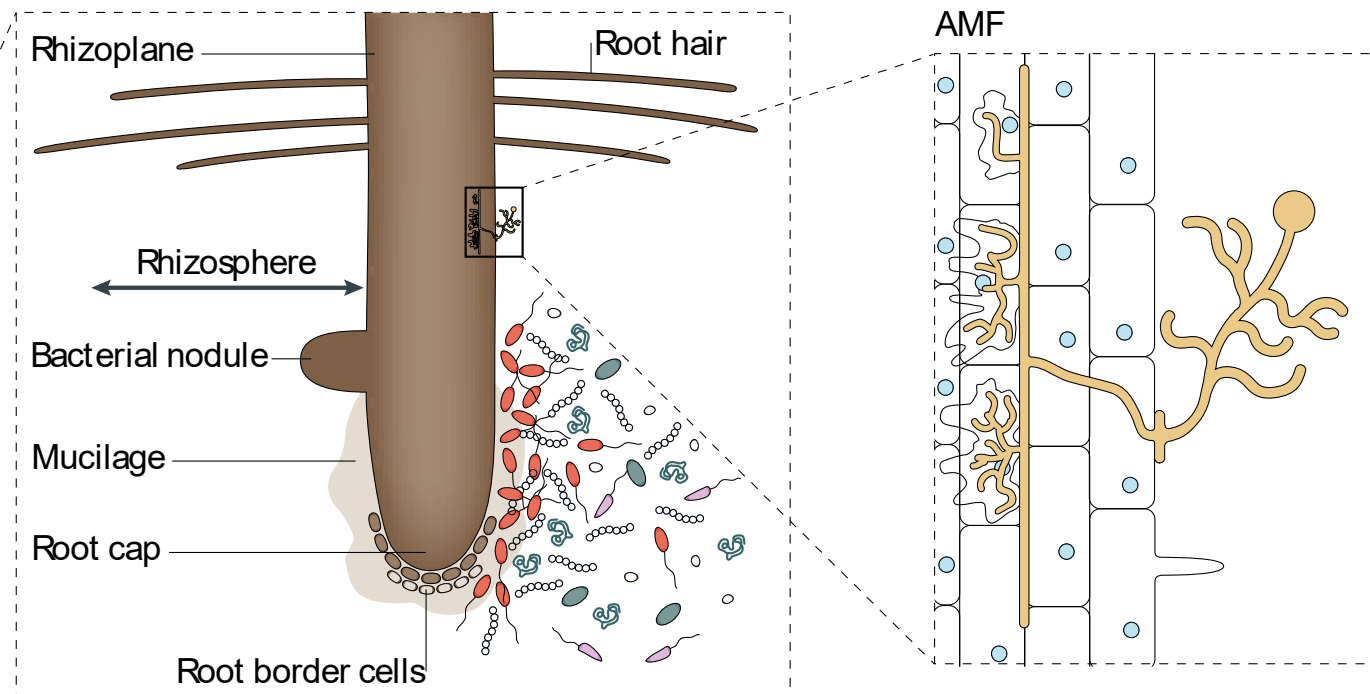




Bulk soil vs. rhizosphere



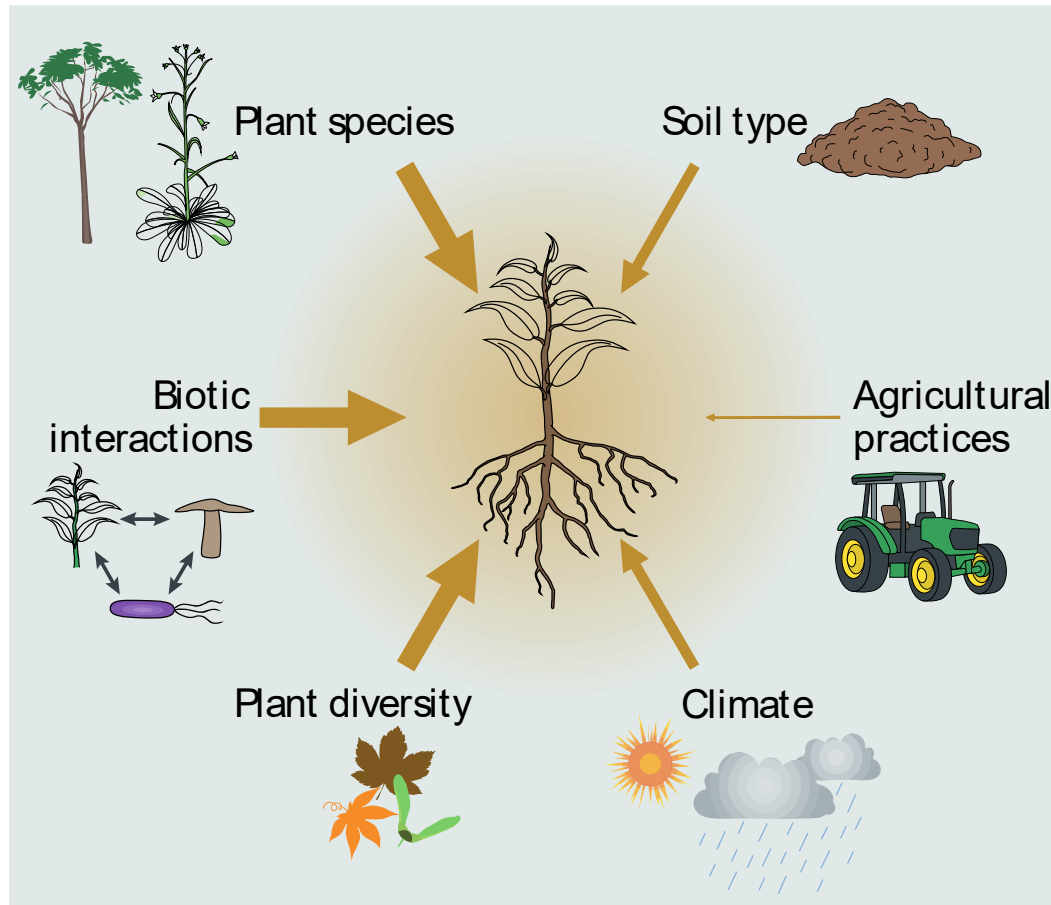
Microbes are recruited from the bulk soil to the rhizosphere by root exudates, which include primary metabolites mainly responsible for attraction and secondary metabolites mainly responsible for screening the recruited microbes.



- The bulk soil microbial community is the seed bank for the plant root-associated microbiota.
- Rhizosphere community has greater microbial biomass and activity compared with that in bulk soil.



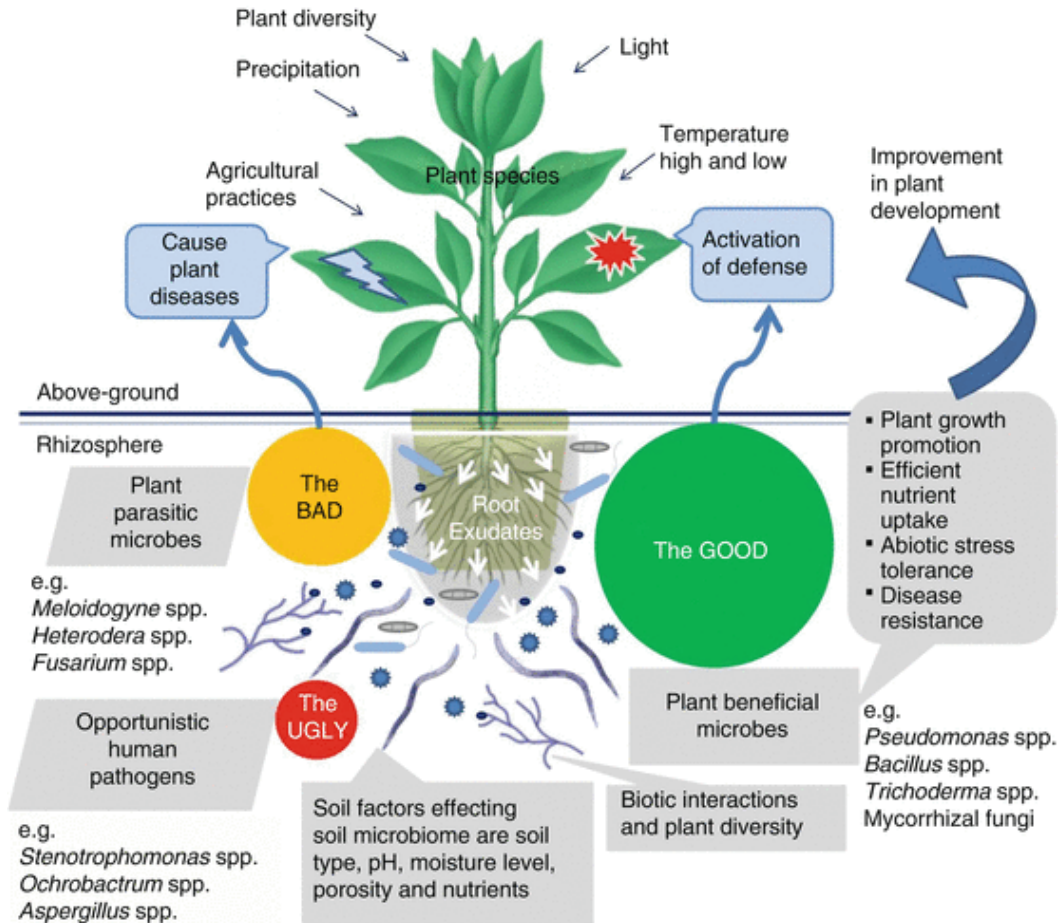
Factors driving the assembly of the rhizosphere microbiota



- The assembly of the rhizosphere microbiota is governed by the abiotic factors, soil properties, climate, and biotic factors of plant species, biotic interactions and agricultural management.



The rhizosphere microbiome: the function

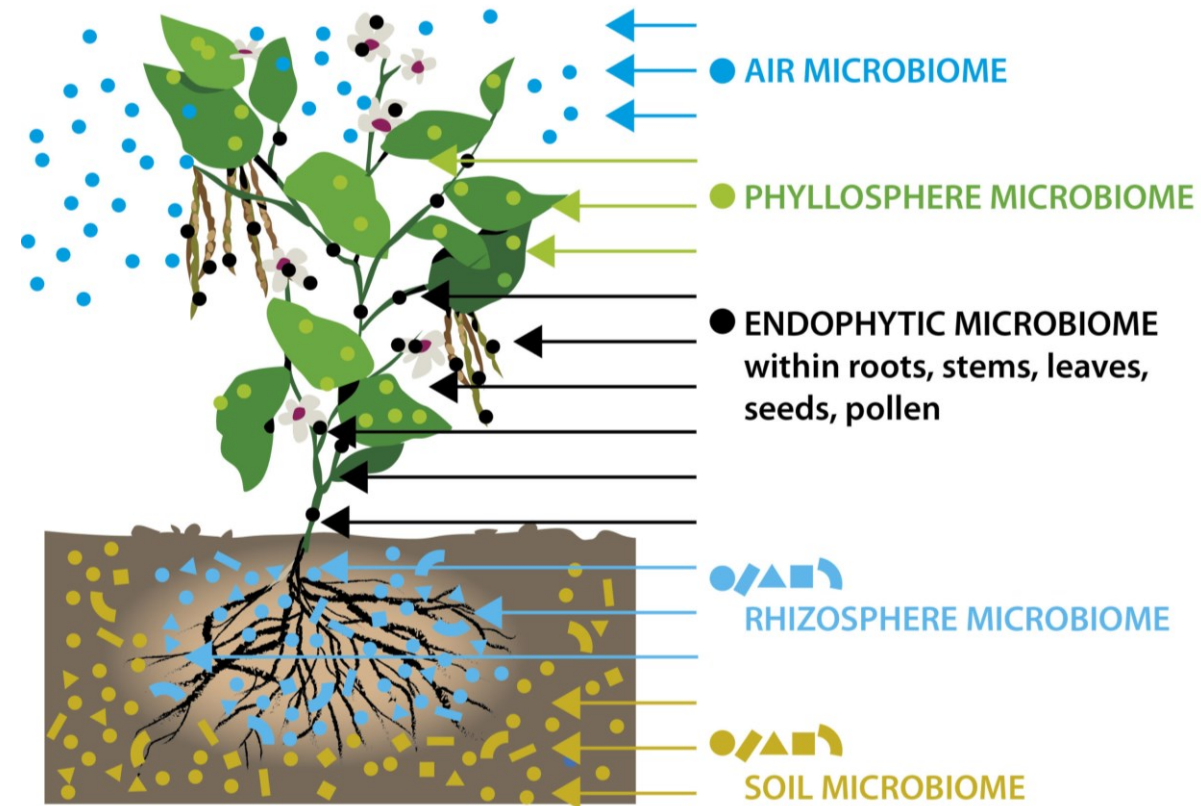


- Schematic overview of the functions and impact of plant beneficial ('the good'), plant pathogenic ('the bad'), and opportunistic human pathogens ('the ugly') on the host plant

Ali et al. (2017) The Good, the Bad, and the Ugly of Rhizosphere Microbiome. In: Kumar V., Kumar M., Sharma S., Prasad R. (eds) Probiotics and Plant Health. Springer, Singapore.

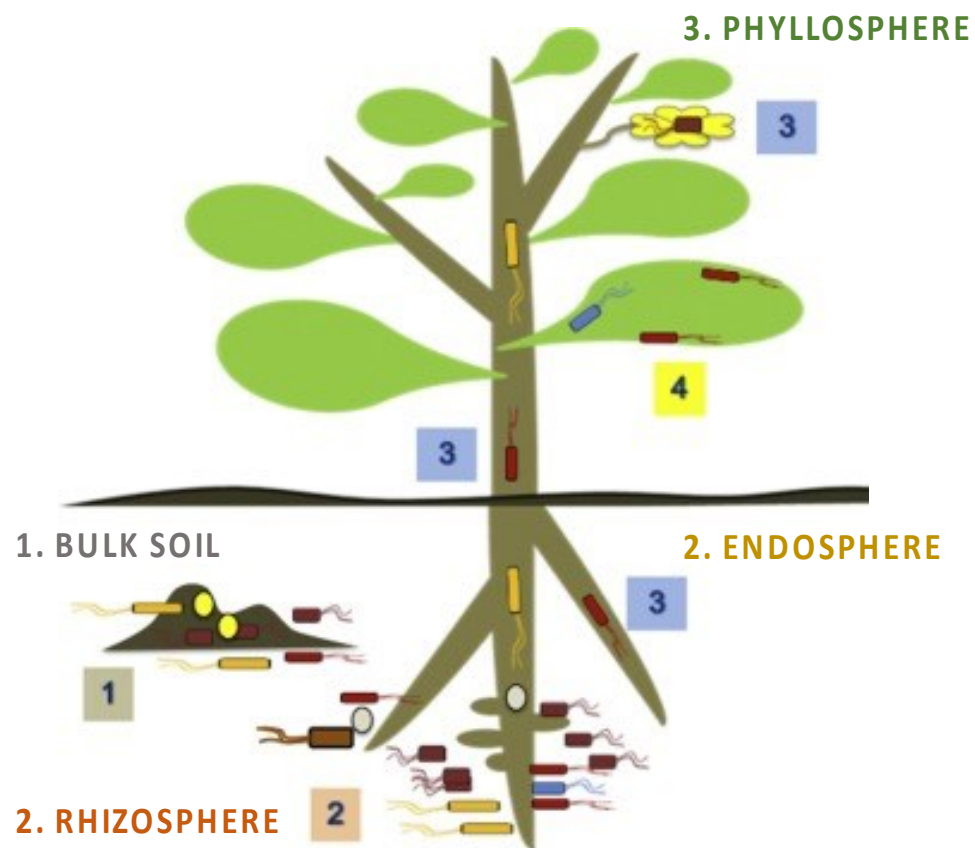


The 'Plant Microbiome'

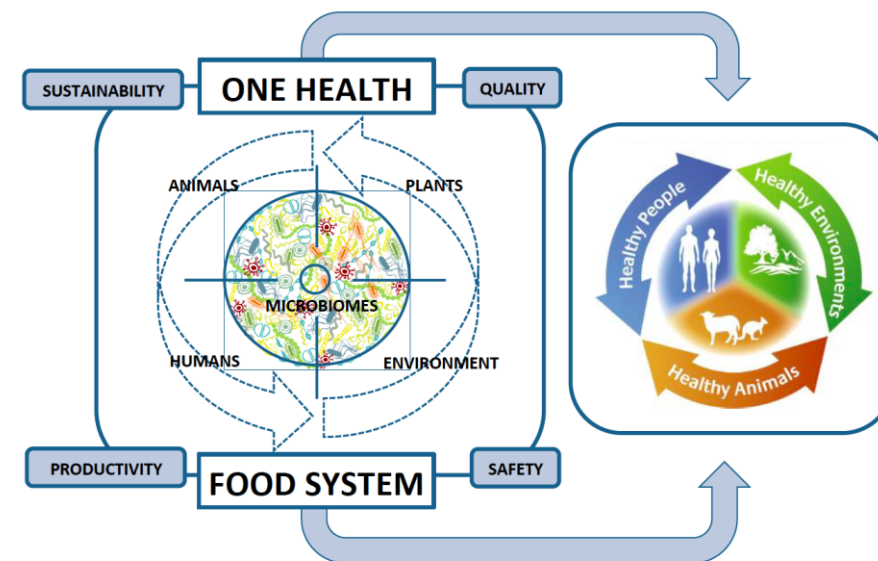


The 'Plant Microbiome' can be described as the sum total of the genomic contribution made by the diverse microbial communities that inhabit the surface and internal tissues of the plant parts.

The concept of «Holobiont»



- In the ecological perspective, the plant holobiont and not the plant as an individual, is now known to respond to the various biotic and abiotic perturbations in a given environment.
- Plant-associated microbiome is important for plant growth, health and stress resilience

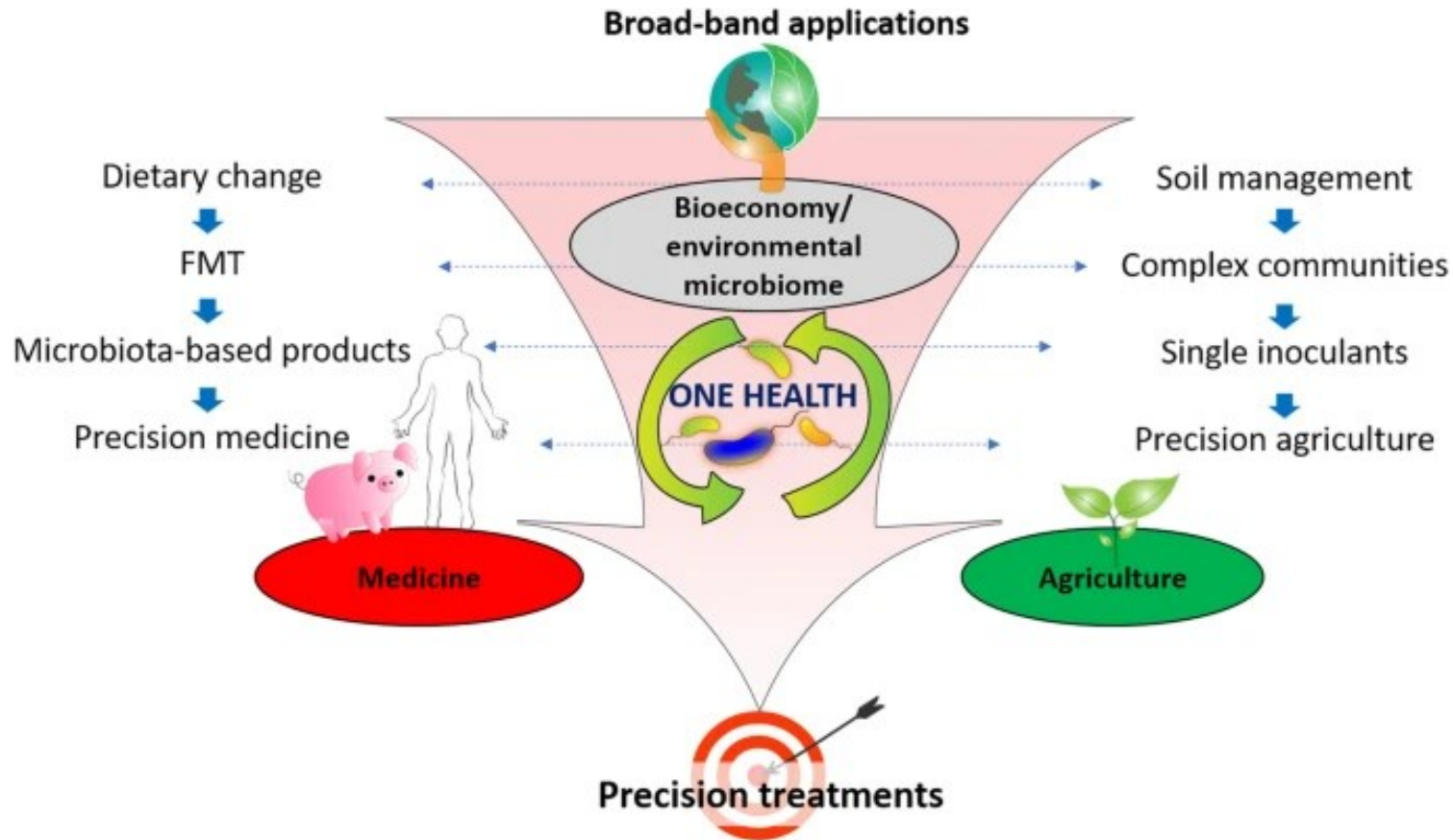


Based on: Gopal M and Gupta A (2016) *Front. Microbiol.* 7:1971. doi: 10.3389/fmicb.2016.01971

The One Health concept



Precision agriculture & personalised medicine



- The use of microbiome-based therapies represents a promising tool that may respond to the challenges for translational medicine

- The use of beneficial microbes represents a promising tool that may respond to the challenges for modern agriculture



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How can restore the sustainability of agricultural systems ?

Soil organisms are an integral component of ecosystems, but their activities receive little recognition in agricultural management strategies.

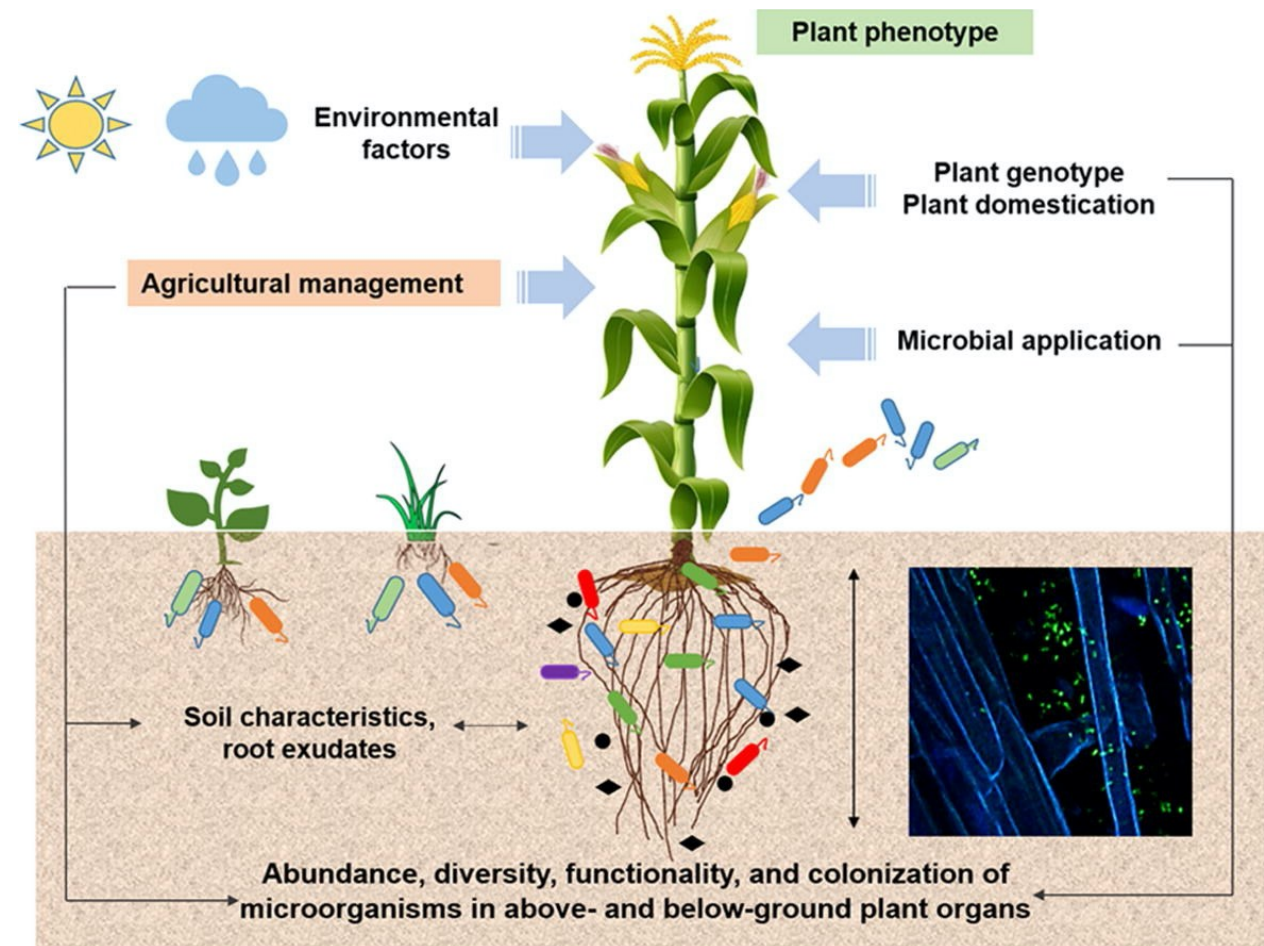
- Stimulating soil life and internally regulated ecosystem processes
- Enhance and maintain the soil biodiversity and function
- Inoculation with key microbiota members
- Targeted manipulations of soil organisms





Soil microbiome as a solution

- **Understanding soil-root microbiome diversity and function** to uncover novel microbes that can be used as biofertilisers and biopesticides
- **Promoting crop-microbe associations** through the development and optimisation of microbial inocula
- **Enhancing beneficial soil microbiome** diversity and function through optimising soil management methods



Compant *et al.*, 2019 *J Adv Res* 19:29-37



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Microbiome engineering

A targeted soil biological engineering approach to improving ecosystem functioning and services.

Microbial strains with verified function can be combined into simple synthetic microbiomes containing few to several dozen species and use to promote plant health and improve soil fertility

 **frontiers**
in Plant Science

OPINION
published: 04 December 2018
doi: 10.3389/fpls.2018.01801



Microbial Consortia: Promising Probiotics as Plant Biostimulants for Sustainable Agriculture

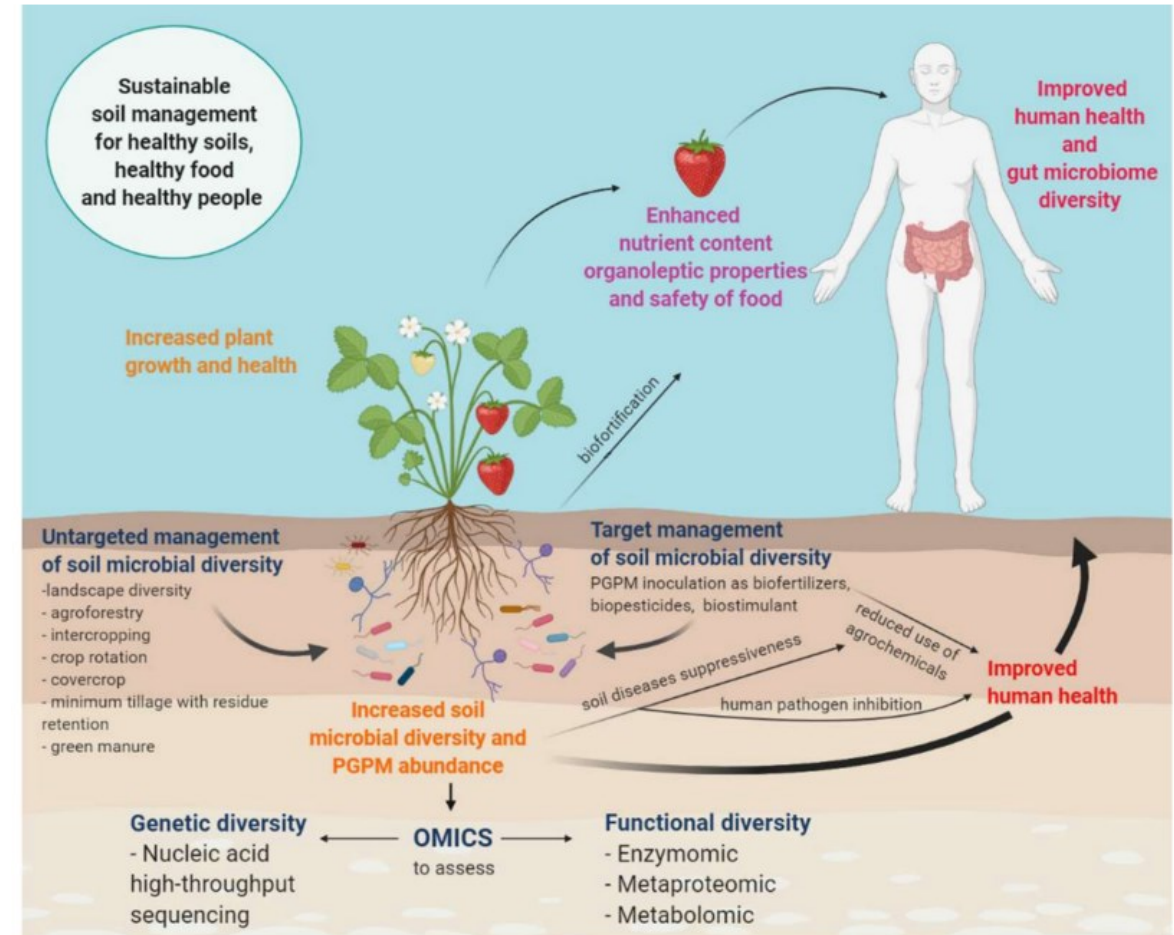
Sheridan L. Woo^{1,2,3*} and Olimpia Pepe^{3,4,5*}

¹ Department of Pharmacy, University of Naples Federico II, Naples, Italy, ² National Research Council, Institute for Sustainable Plant Protection, Portici, Italy, ³ Task Force on Microbiome Studies, University of Naples Federico II, Naples, Italy, ⁴ Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy, ⁵ CIRAM-Interdepartmental Center for Environmental Research, University of Naples Federico II, Naples, Italy



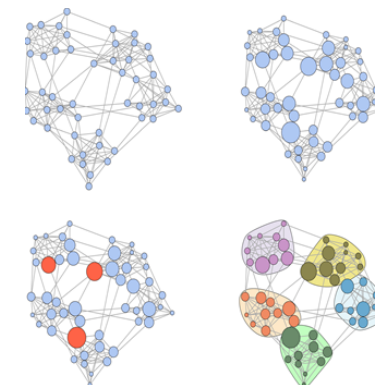
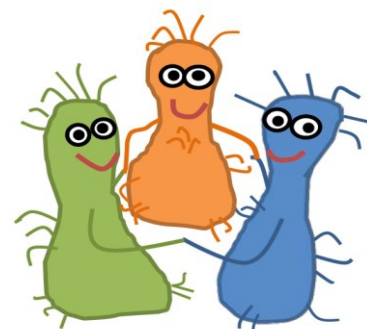
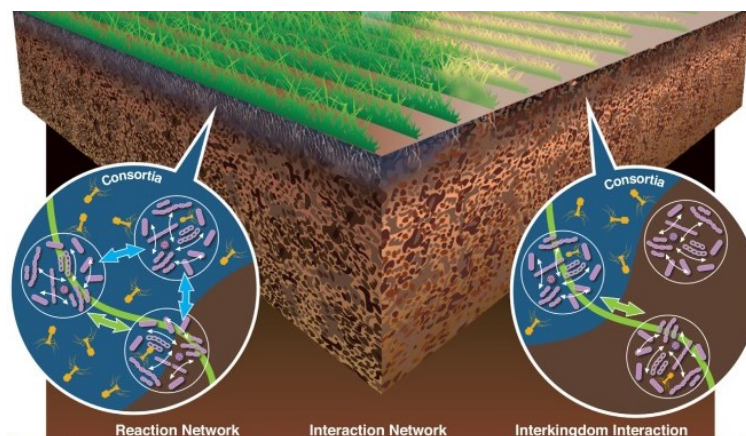
Targeted and untargeted management of soil microbial diversity

- The development of high-throughput technologies applied to the study of soil microbial functional diversity will help strengthen the link between soil well-being, food quality, food safety and human health.



Microbial consortia for sustainable crop systems

- Microbial consortia have a higher potential to increase plant growth-promoting (PGP) effects compared to single inoculants



Microbial consortia» indicates not only living together but also

- division of labor among members to increase community efficiency and productivity
- interactions between members at various scales of time and space (physical contact, chemical signaling and metabolic exchange)



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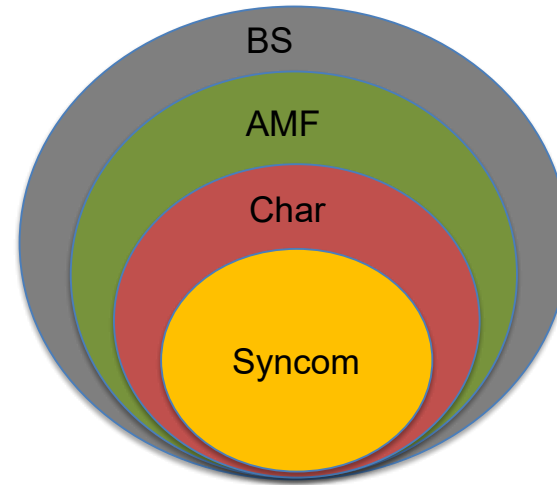
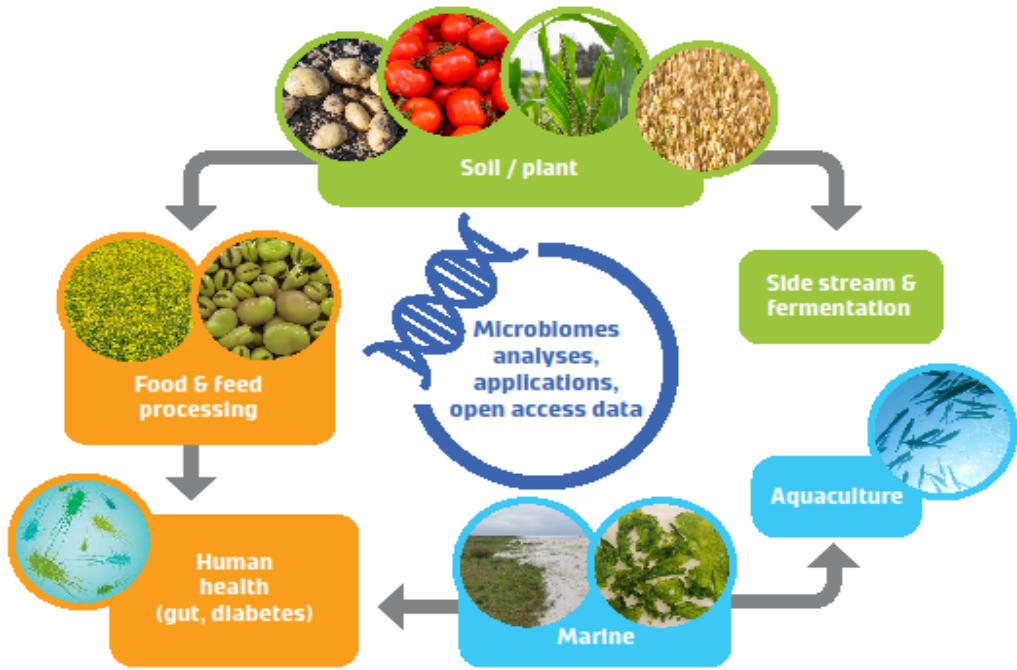
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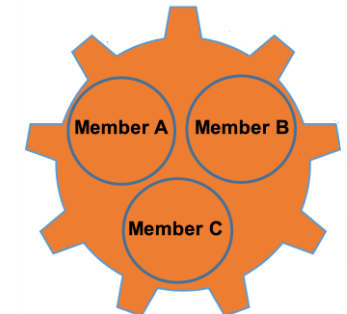
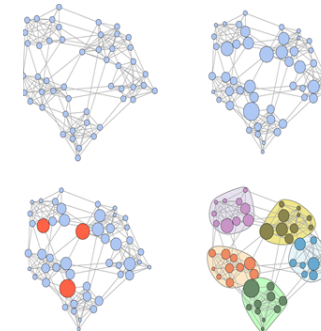
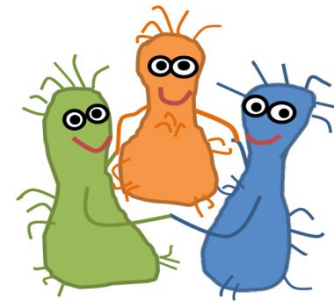
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Microbiome-based solutions in SIMBA project



Seaweed extracts, Plant-derived protein hydrolysate



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 818431 (SIMBA). This output reflects only the author's view and the European Union cannot be held responsible for any use that may be made of the information contained therein.

@SIMBAproject_EU

www.simbaproject.eu



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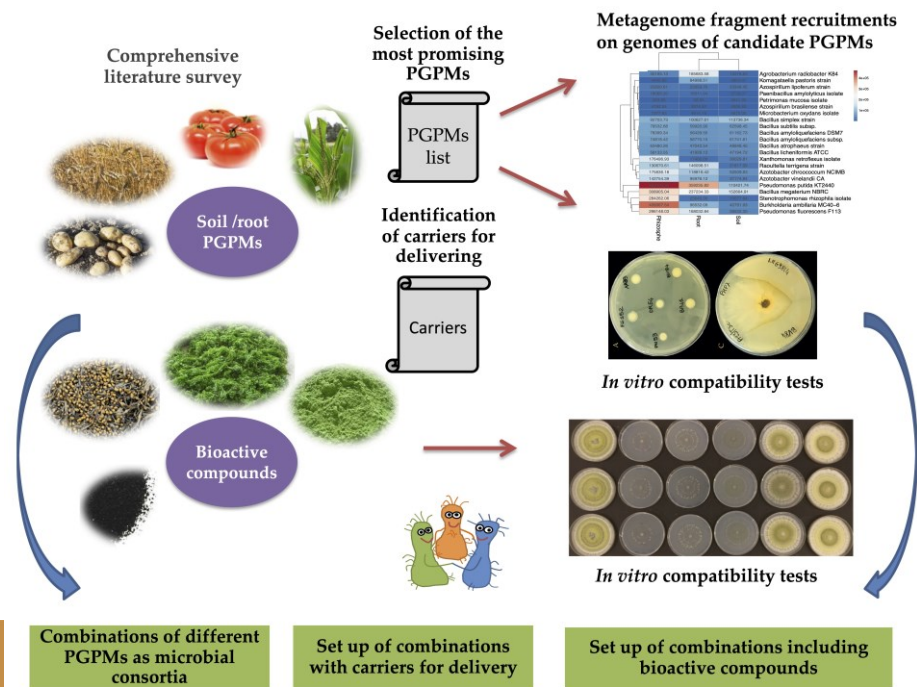
microorganisms



Article

Identification of Beneficial Microbial Consortia and Bioactive Compounds with Potential as Plant Biostimulants for a Sustainable Agriculture

Silvia Tabacchioni ^{1,†}, Stefania Passato ², Patrizia Ambrosino ², Liren Huang ³, Marina Caldara ⁴, Cristina Cantale ^{1,†}, Jonas Hett ⁵, Antonella Del Fiore ¹, Alessia Fiore ¹, Andreas Schlüter ³, Alexander Sczyrba ³, Elena Maestri ⁴, Nelson Marmiroli ⁴, Daniel Neuhoff ⁵, Joseph Nesme ⁶, Søren Johannes Sørensen ⁶, Giuseppe Aprea ¹, Chiara Nobili ¹, Ombretta Presenti ¹, Giusto Giovannetti ⁷, Caterina Giovannetti ⁷, Anne Pihlanto ⁸, Andrea Brunori ¹ and Annamaria Bevivino ^{1,*}



fermentation



Article

Designing a Waste-Based Culture Medium for the Production of Plant Growth Promoting Microorganisms Based on Cladodes Juice from *Opuntia ficus-indica* Pruning

Rosaria Alessandra Magarelli ¹, Mario Trupo ^{1,*}, Alfredo Ambrico ^{1,*}, Vincenzo Larocca ¹, Maria Martino ¹, Salvatore Palazzo ¹, Roberto Balducci ¹, Vesa Joutsjoki ², Anne Pihlanto ² and Annamaria Bevivino ³





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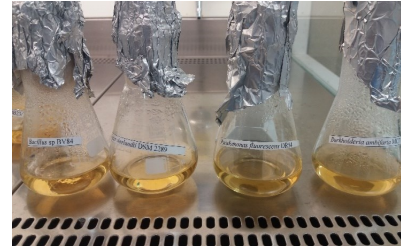
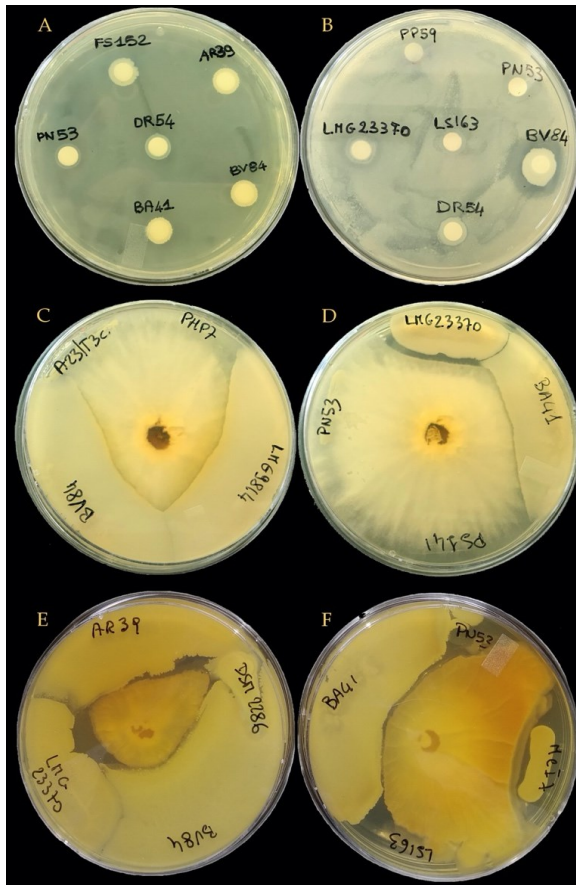
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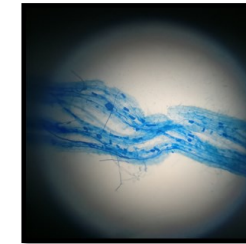
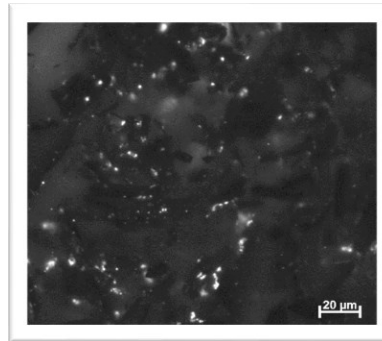
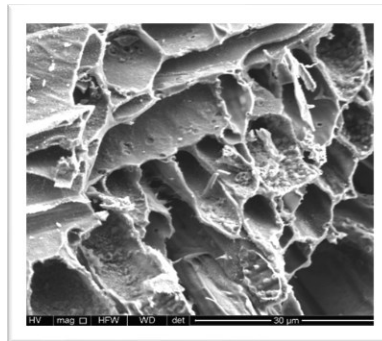
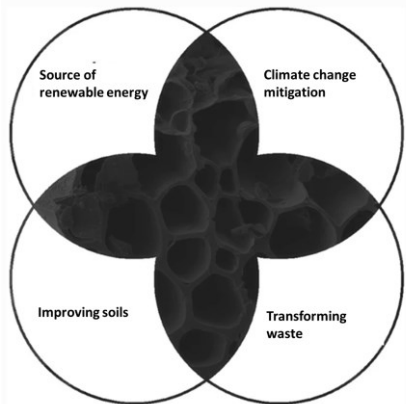
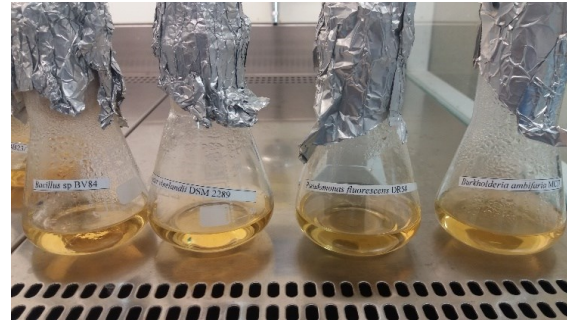
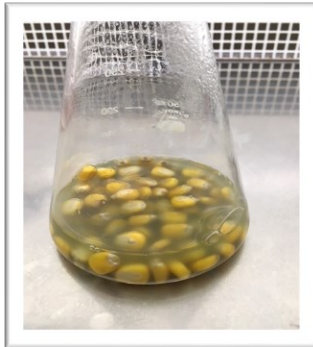
From PGPM selection to scaling-up microbial production





Delivery methods

Microbial consortia were applied *via* seed coating or delivered as a powder (with zeolite-based amendments), liquid suspension or incorporated in wood biochar, with or without arbuscular mycorrhizal fungi (AMF).





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From LAB to FIELD



Lab/Climate chamber



Picture: www.weiss-technik.com



Greenhouse



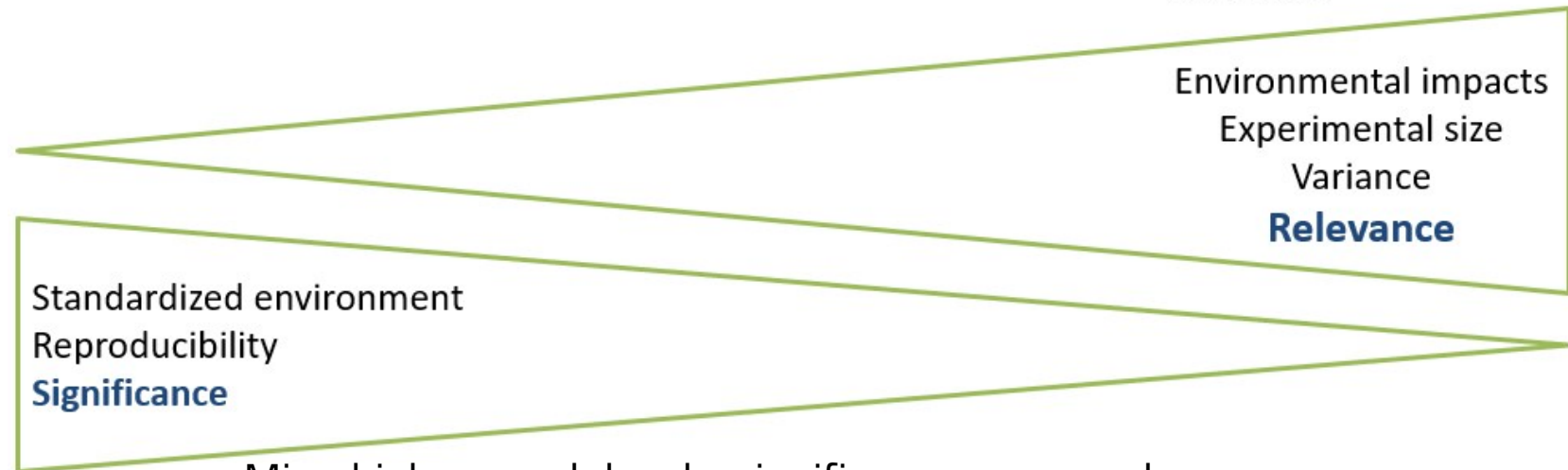
Picture : Jonas Hett



Field



Picture : Jonas Hett



Microbial research levels, significance versus relevance

From microbial production to application in greenhouse and field

European Journal of Agronomy 144 (2023) 126743



Article
Effects of Multi-Species Microbial Inoculants on Early Wheat Growth and Litterbag Microbial Activity



Article
A Metagenomic and Gene Expression Analysis in Wheat (*T. durum*) and Maize (*Z. mays*) Biofertilized with PGPM and Biochar

Sara Graziano ¹, Marina Caldara ², Mariolina Gullì ^{1,2}, Annamaria Bevivino ³, Elena Maestri ^{1,2} and Nelson Marmiroli ^{1,2,4,*}



Contents lists available at ScienceDirect

European Journal of Agronomy

journal homepage: www.elsevier.com/locate/eja



Impact of microbial consortia on organic maize in a temperate climate varies with environment but not with fertilization

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