

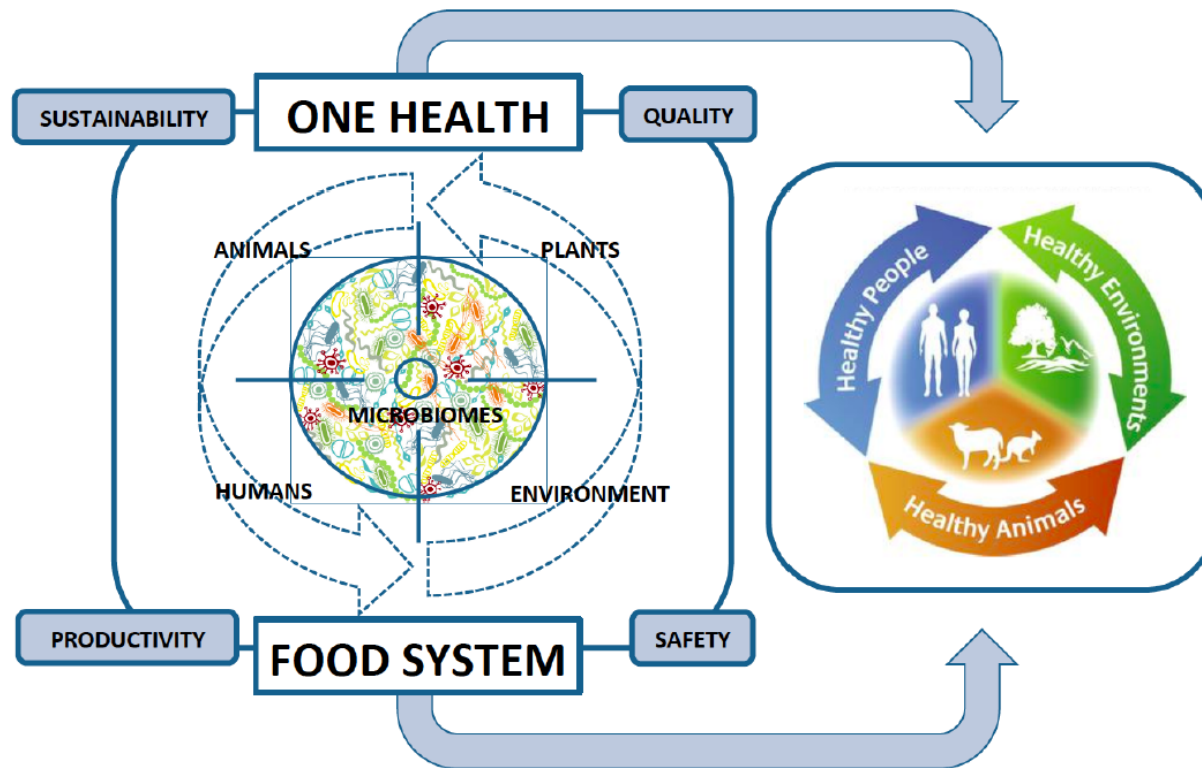
Microbioma: La produzione primaria

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Sala Monumentale Presidenza del Consiglio, Roma,
Italy 12th June 2019

The CNNBBSV standpoint - primary production



- Soil and sediments
- Crop primary production
- Livestock primary production
- Marine primary productions

(CNBBSV 2019)

Main challenges in crop production

- Increased productivity
- Sustainable production
- Waste recovery and by-products exploitation
- Climate changes
- Demographic development
- How the system management has to improve/change?



(Mitter et al. 2016. *Microb. Biotechnol.* 9:635)

Soil microbiome

Soil often contains **>1,000** kg of microbial biomass carbon per hectare

Distinct soil environments are only **micrometres** to millimetres apart

Soil is not a single environment, it encompasses a broad range of **different microbial habitats**

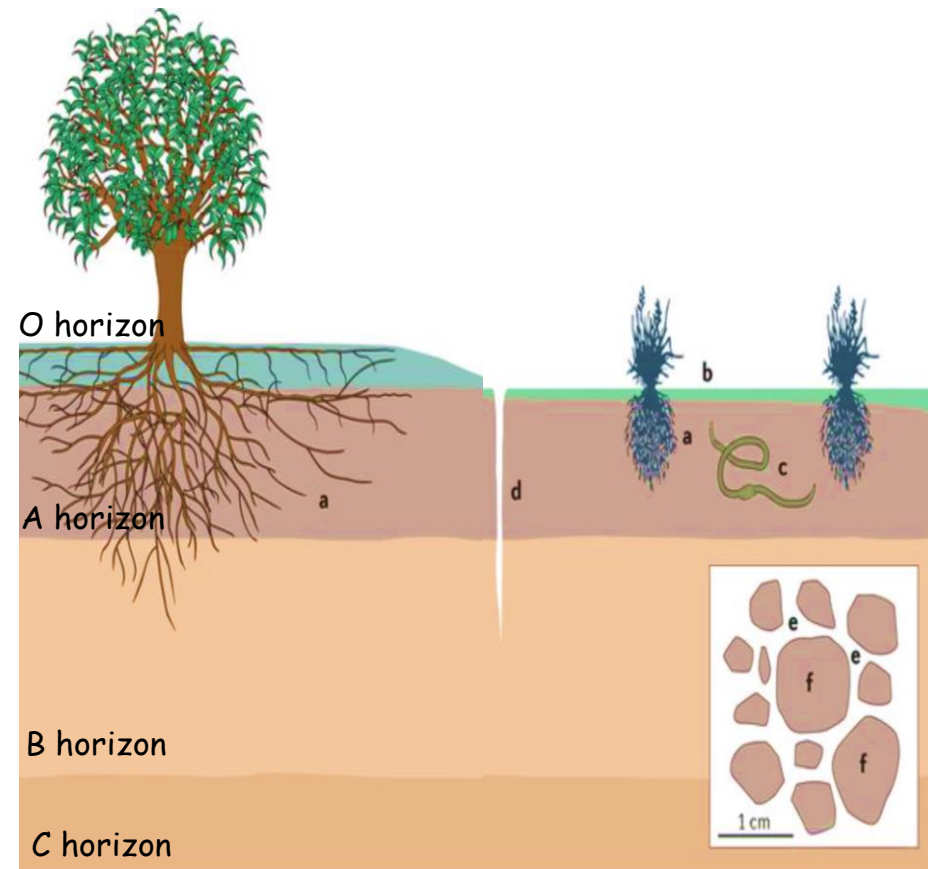
Climate, organisms, relief, parent material and time



Soil properties (e.g., pH, organic carbon, salinity, texture and available nitrogen)

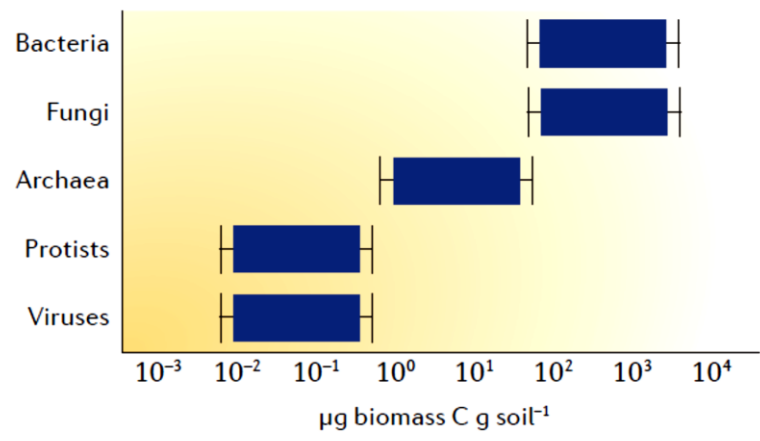
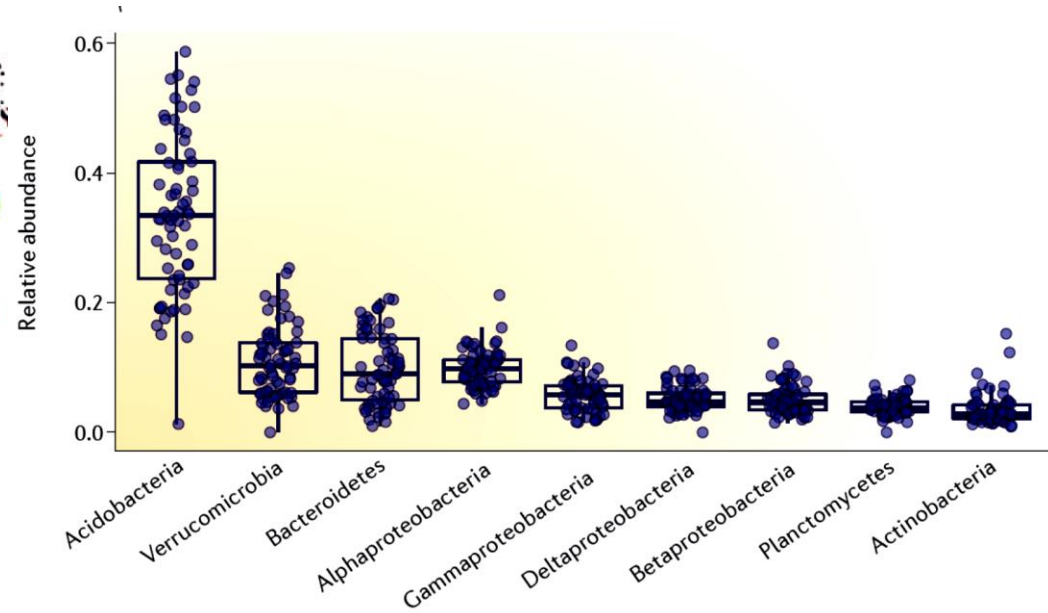
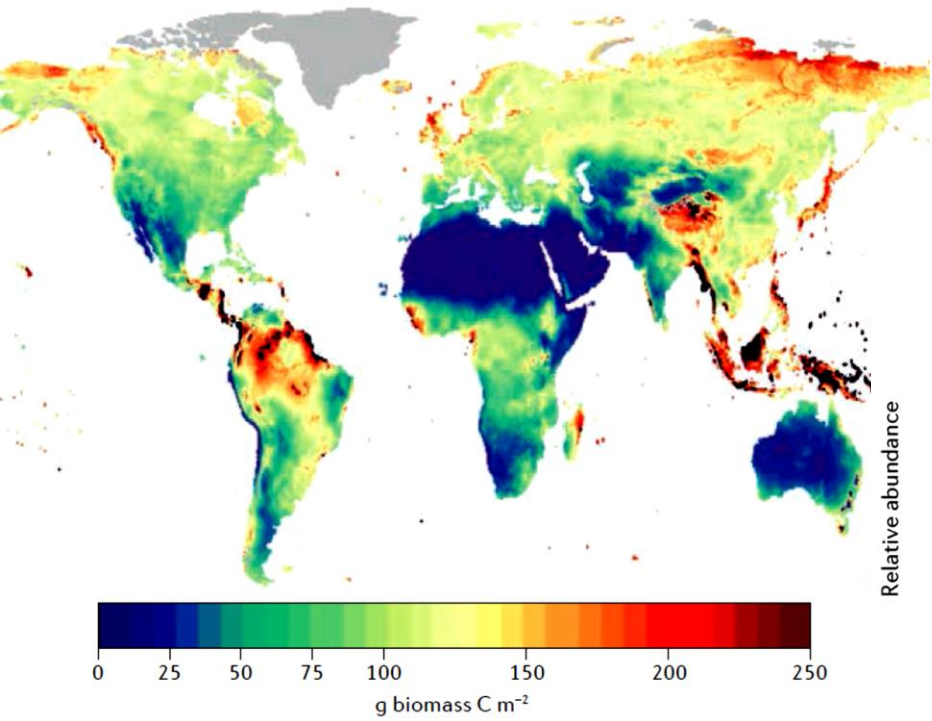


Soil microbiome with crucial roles in nutrient cycling, fertility and carbon sequestration



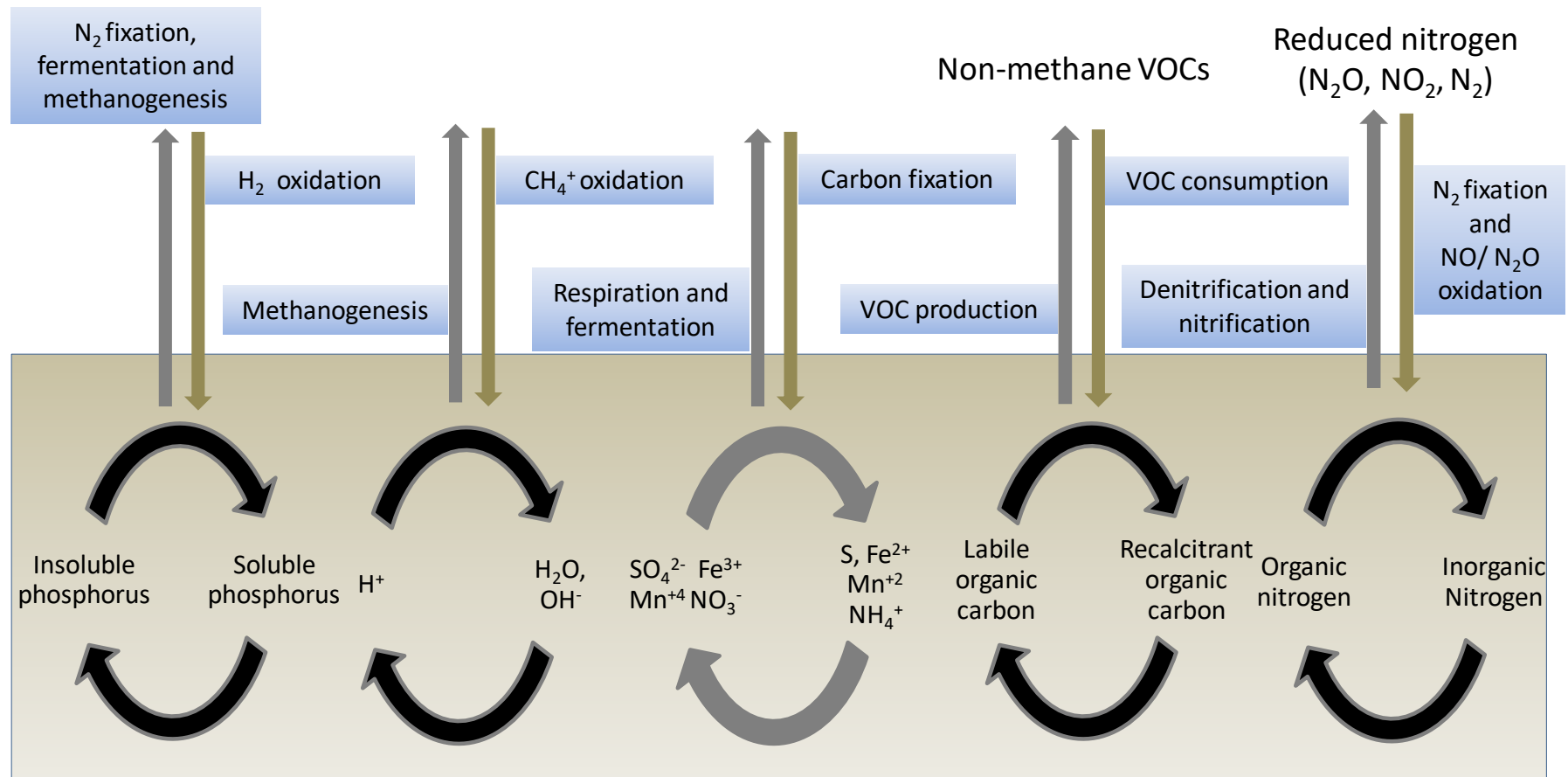
(Fierer, 2017. *Nature Microbiol.* doi:10.1038/nrmicro.2017.87)

Global microbial biomass found in soil



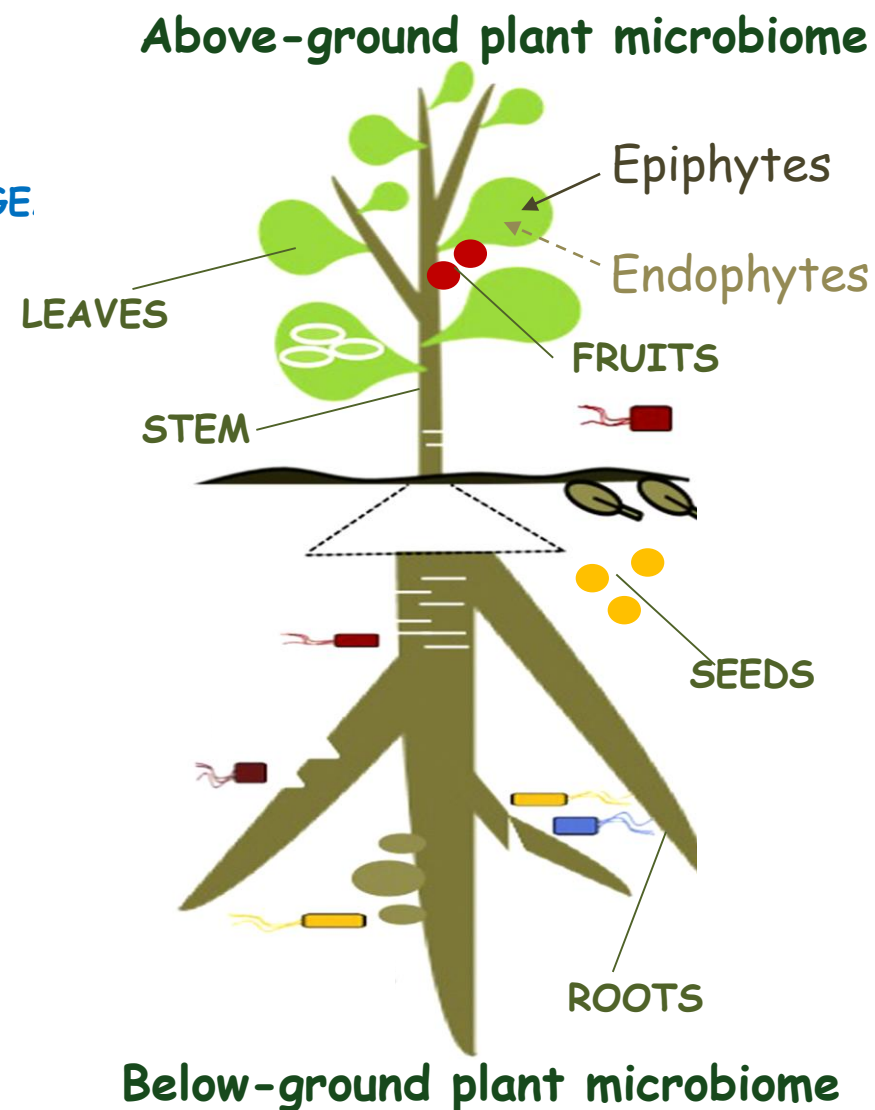
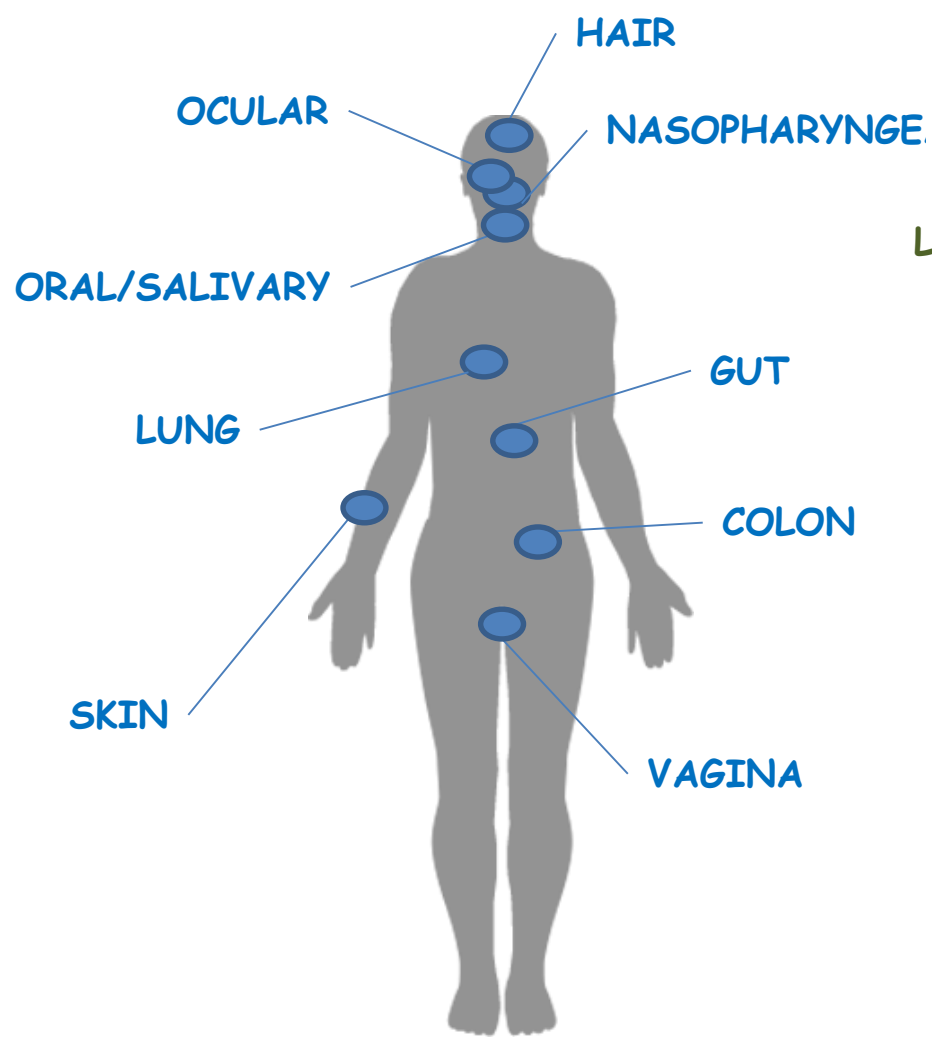
(Fierer, 2017. *Nature Microbiol.* doi:10.1038/nrmicro.2017.87)

Soil biogeochemical processes modulated by microbiome



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

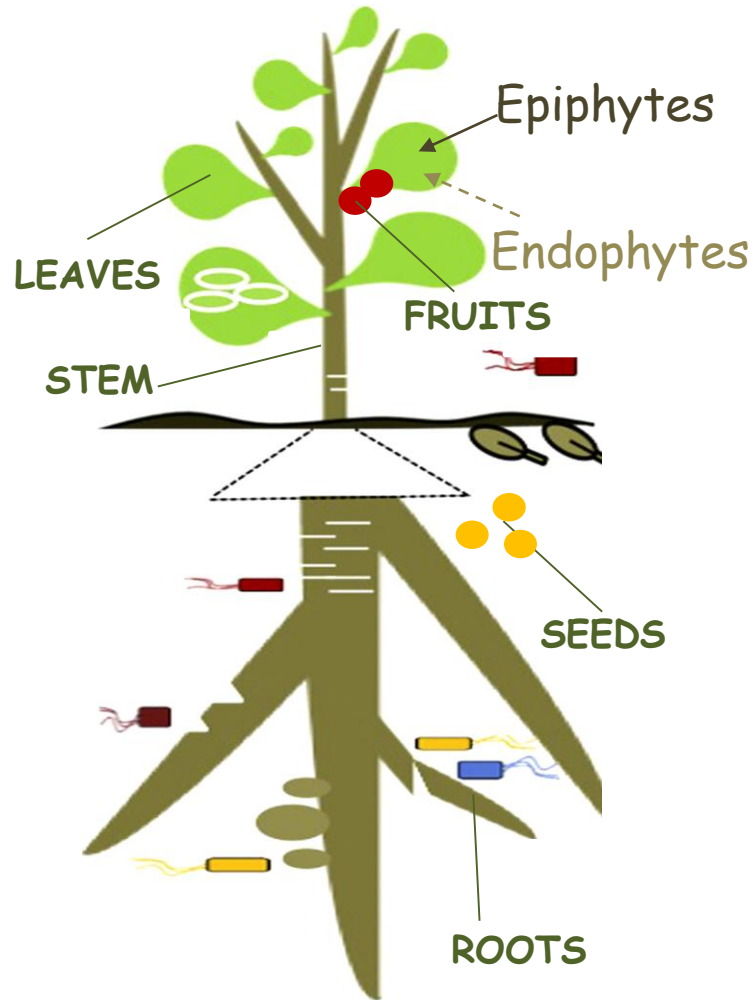
Plant as an holobiont, a meta-organism



(Orozco-Mosqueda et al., 2018. *Microbiol. Res.* 1016:25)

The plant holobiont:

Above-ground plant microbiome

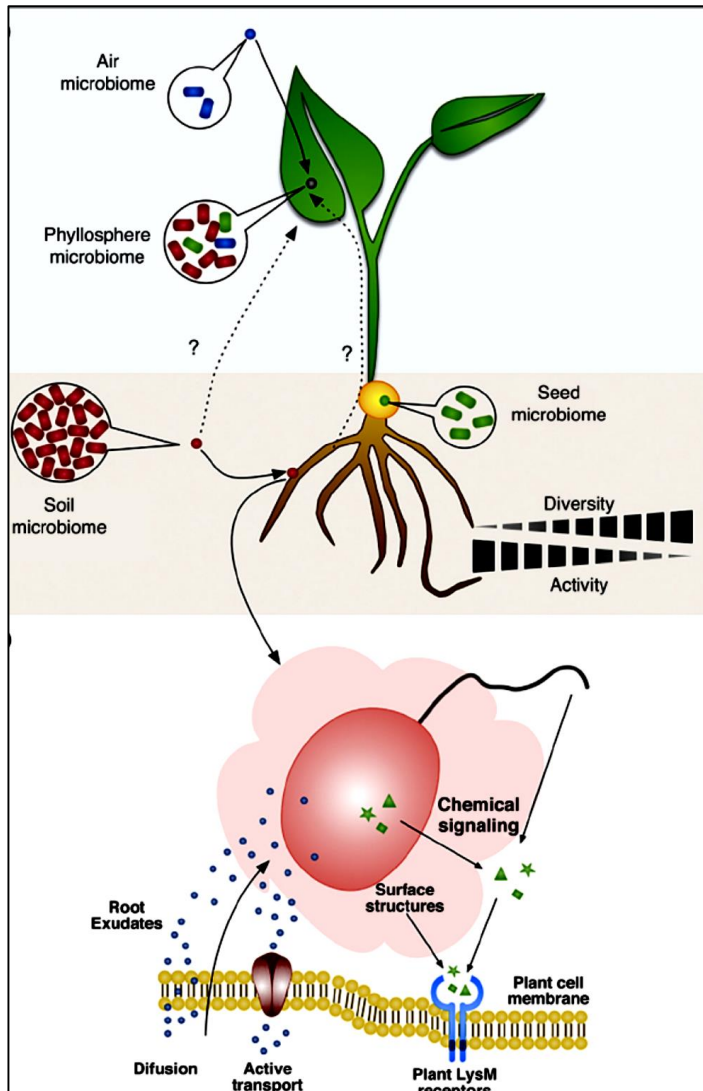


Below-ground plant microbiome

(Hassani et al. 2018. *Microbiome* 6:58)

- Plants evolved with a plethora of bacteria, fungi, archaea, protozoa and virus. Testamentary mycorrhizal fungi date back 700 million years ago
- *Arabidopsis thaliana*, and *Hordeum vulgare*, *Zea mays*, *Oryza sativa*, *Glycine max* and *Triticum aestivum*
- The plant bacterial microbiome is dominated by Proteobacteria, Actinobacteria, and Bacteroidetes
- Diverse and well-balanced microbiome at the plant-soil interface is vital in crop production

Where does the plant microbiome come from?

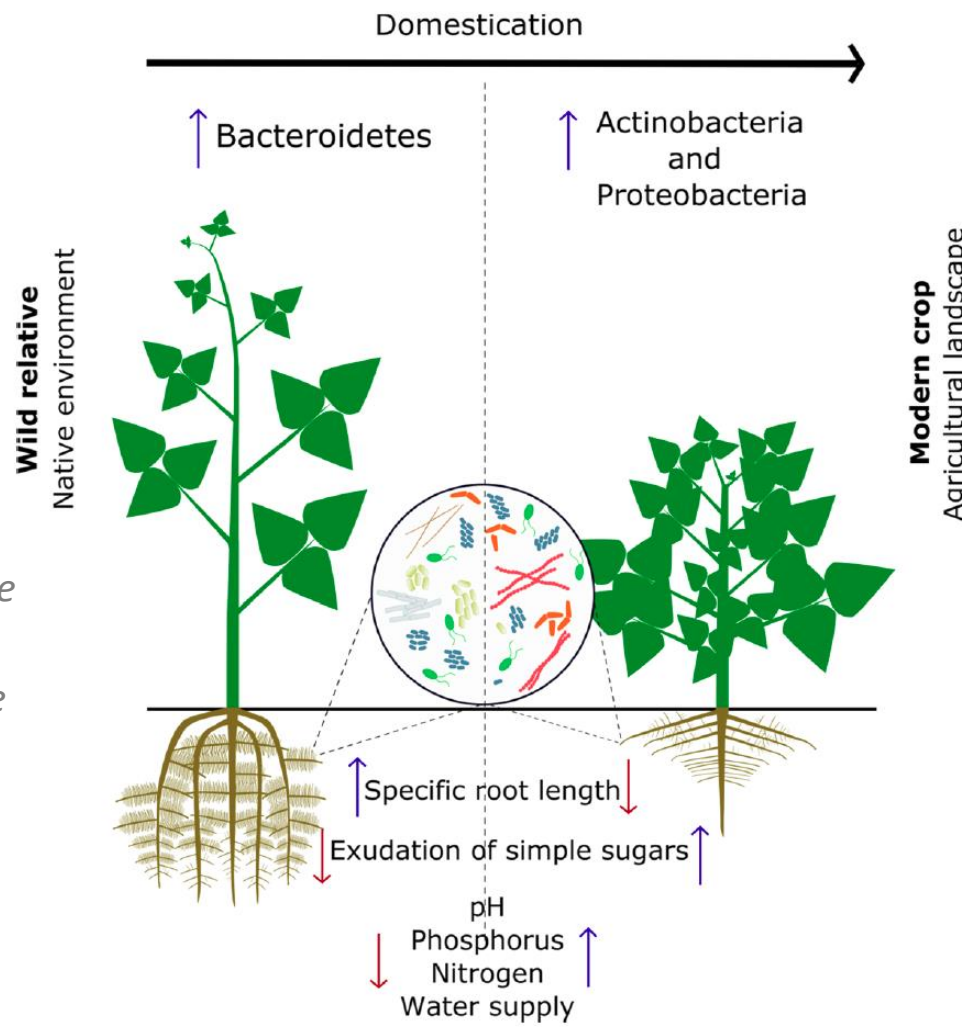
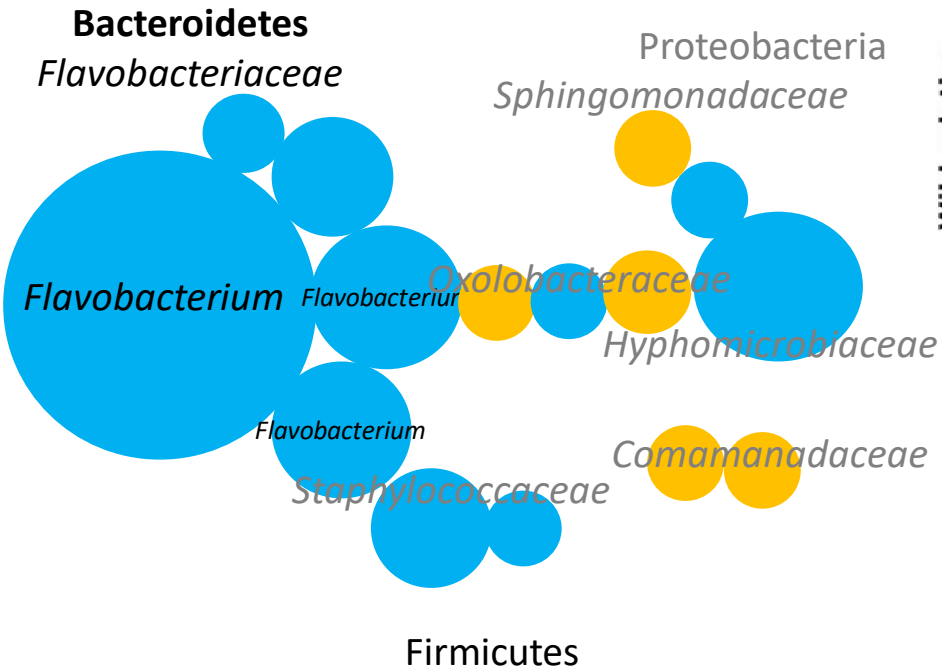


- Surrounding environment: soil (main reservoir) and air (minute contribution)
- Bulk soil (not influenced by the plant root), rhizosphere (soil influenced by the root), rhizoplane and the endosphere (microbiota inside the root)
- Part of it could also be inherited from the seed
- **Plants fine-tune their microbiomes**

(Sanchez-Canizares et al. 2017. *Current Opinion Microbiol.* 38:188)

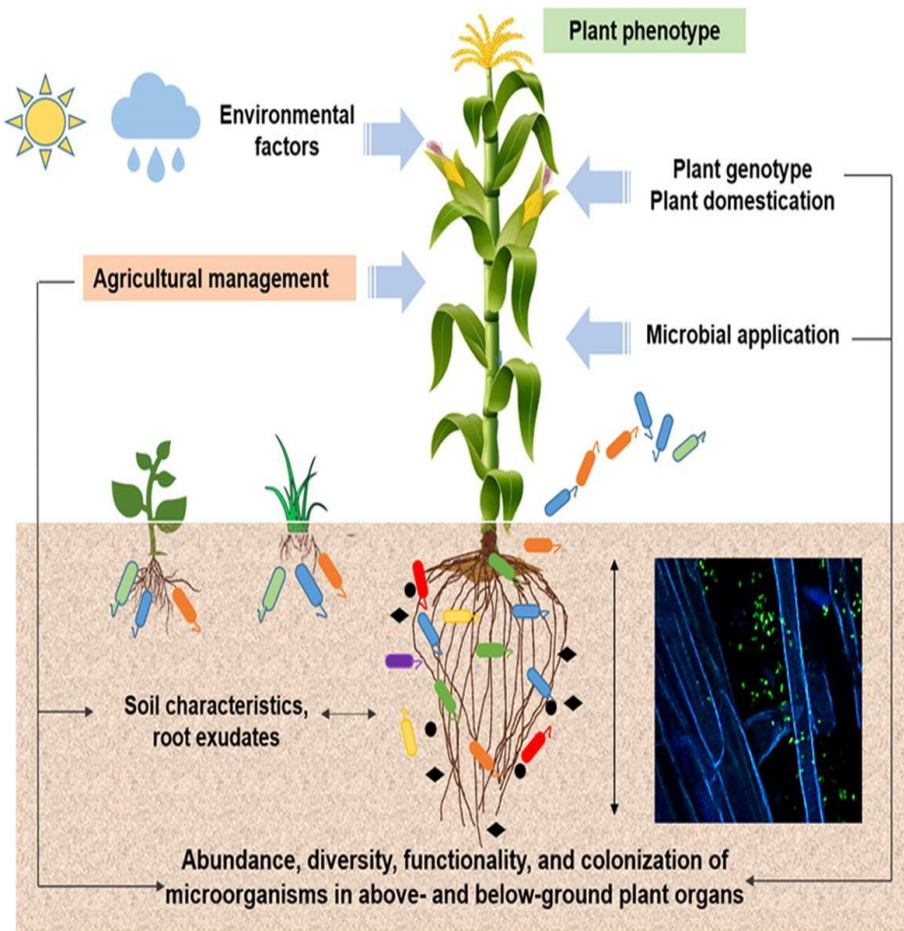
Plant domestication

Hordeum vulgare ssp. *spontaneum* vs
Hordeum vulgare ssp. *vulgare*



(Pérez-Jaramillo et al., 2018. *Microbiome* 6:143)

Biotic and abiotic drivers affecting the plant microbiome

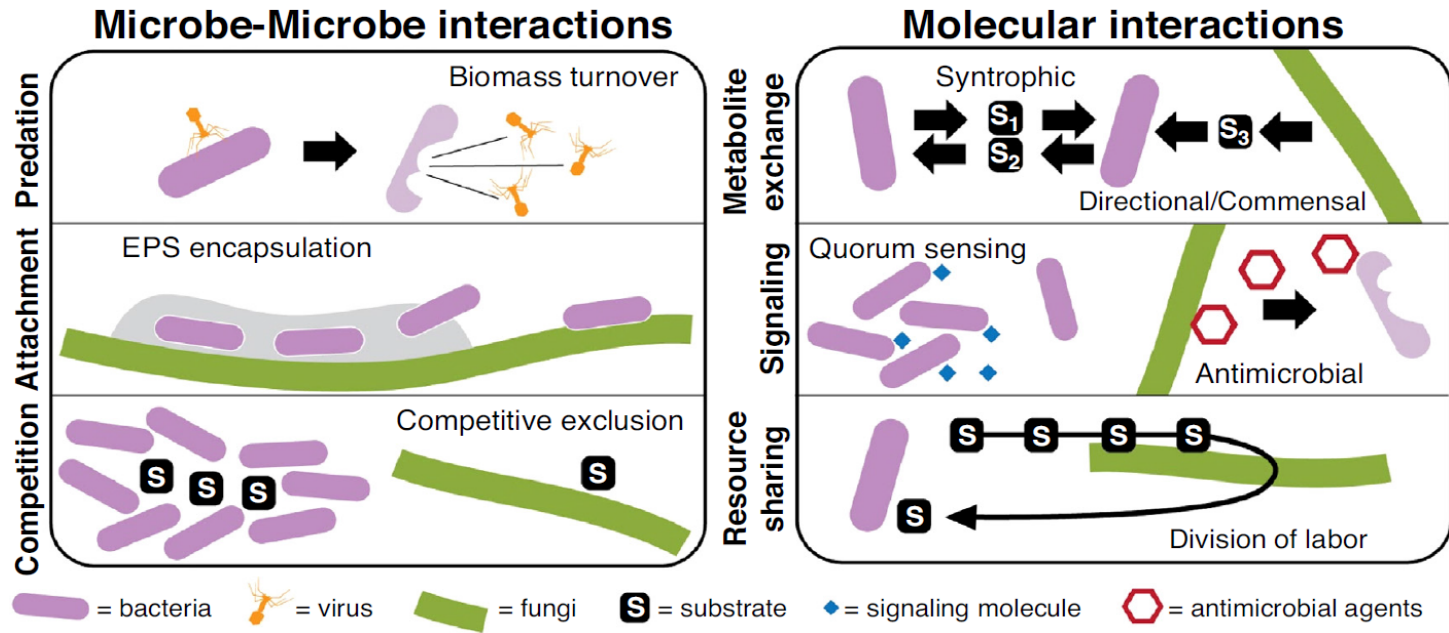


- Root exudates (e.g., organic acids, amino acids, phenolics, plant growth regulators ... rhizosphere effect)
- Soil type and factors (e.g., pH, salinity, structure, moisture, organic matter)
- Environmental factors (e.g., climate, agricultural practices, pathogen presence)
- Plant compartments and species (e.g., genetics, host innate immune system, age, development stage, fitness, signaling)

(Compant *et al.*, 2019. *J. Adv. Res.* 8:51)

Microbial and molecular interactions at the plant microbiome level

(Jansson and Hofmockel, 2018. *Current Opinion Microbiol.* 43:162)



Core plant microbiome: tightly associated with a certain plant genotype

Satellite plant microbiome: occurring in low abundance or in a reduced number of sites

Meta-community: collection of populations connected by genetics, metabolic networking and organismal flow (holobiont - super-organism)

Functions of plant microbiome

- Plant growth (e.g., phytohormones - auxin, cytokinin and gibberellin)
- Cycling nutrient (e.g., phosphate and iron solubilisation, and nitrogen fixation)
- Promotion of the establishment of mycorrhizal associations
- Increased nutrient uptake and stress tolerance (e.g., indole and acetic acid)
- Adaptive advantage and biocontrol activities (e.g., lytic enzymes, pathogen-inhibiting volatile compounds and siderophores)
- Modulating plant hormones level, and priming plant immune system and systemic resistance
- Decrease of the level of stress hormone ethylene (e.g., 1-aminocyclopropane-1-carboxylate -ACC- deaminase)
- Sustainability (reduced chemical inputs, reduce emissions of greenhouse gases)
- Driving food processing

(Compant *et al.*, 2019. *J. Adv. Res.* 8:51)

Fermented foods

Forbes

Fermented Foods Are Up 149% - As Long As They're Unfamiliar

Forbes, Feb 6, 2019

Fermented foods are one of the top 10 food trends in 2016 (Riley, 2015), continuing the trend over the last few years.



Traditional

Novel



The sourdough definition (pasta madre, lievito madre, impasto acido)

Art. 5. (Prodotti utilizzabili per la lievitazione nella panificazione) - punto 5: È definito «pasta madre» o «lievito naturale» l'impasto ottenuto con farina e acqua, sottoposto a una lunga fermentazione naturale acidificante utilizzando la tecnica dei rinfreschi successivi al fine di consentire la lievitazione dell'impasto. **La fermentazione deve avvenire esclusivamente a opera di microrganismi endogeni della farina o di origine ambientale. ...**

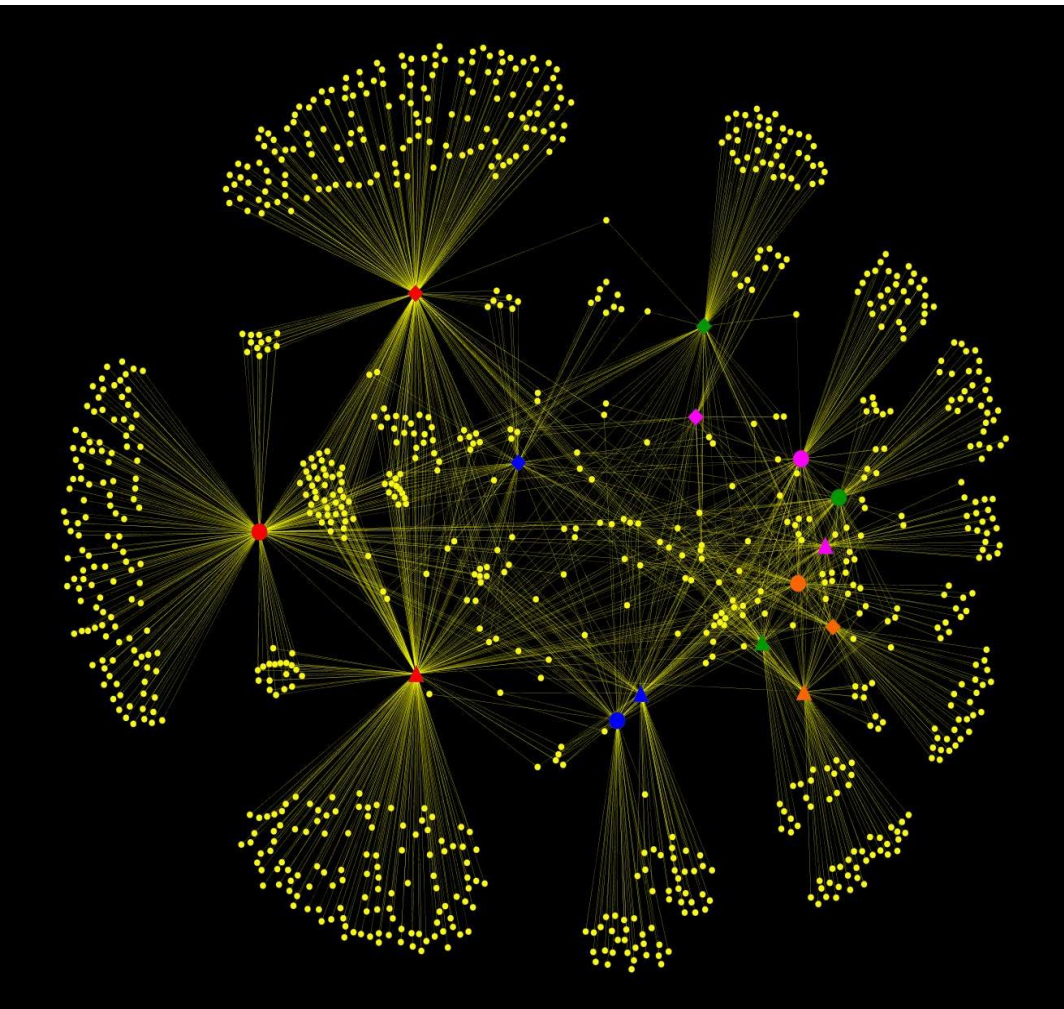
The sourdough preparation

Jan Vermeer 1658

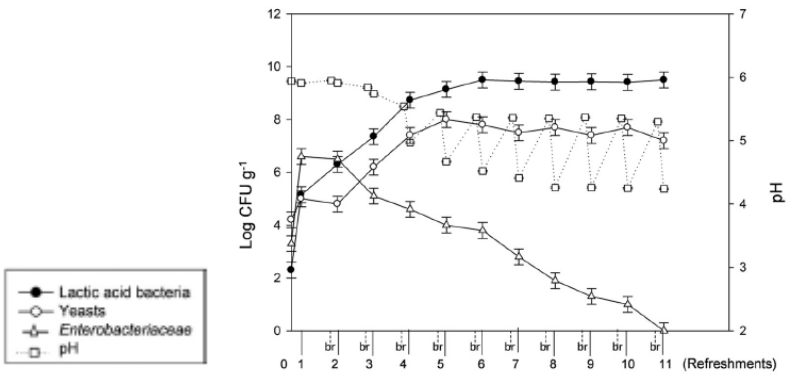
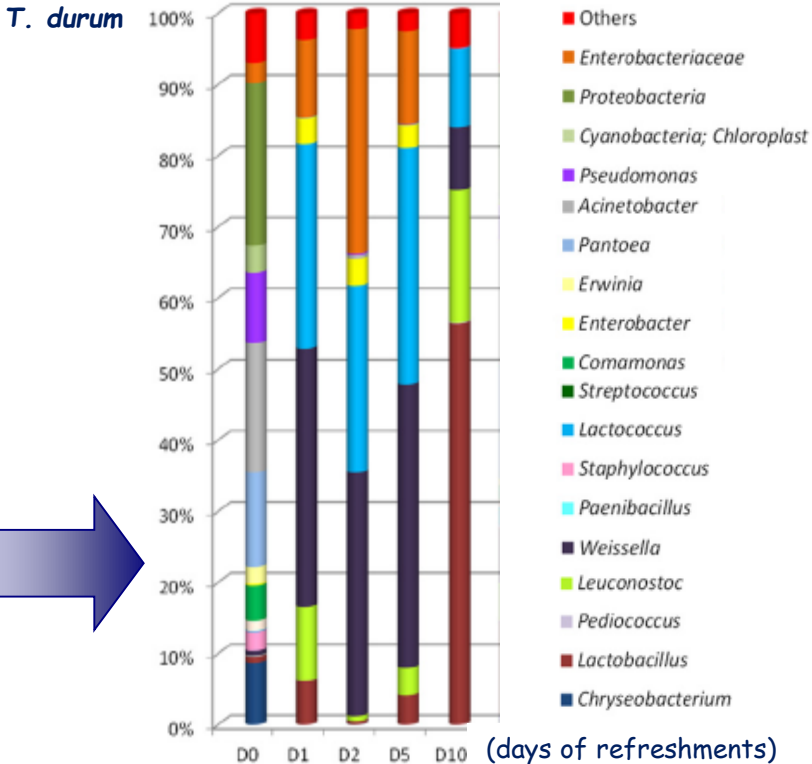


Microbial dynamics during sourdough preparation

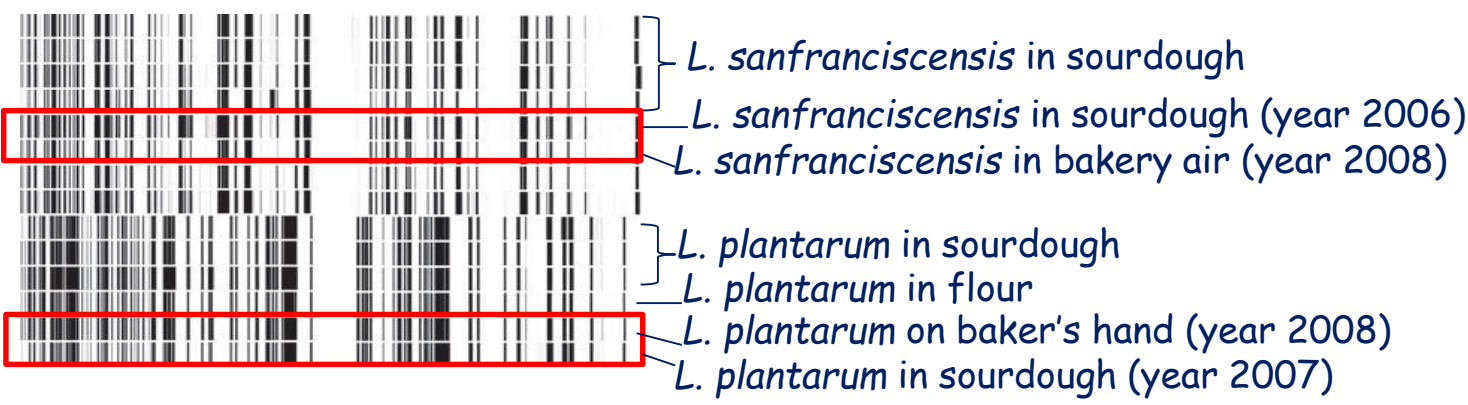
(Ercolini et al., 2013. Appl. Environ. Microbiol., 79:7827)



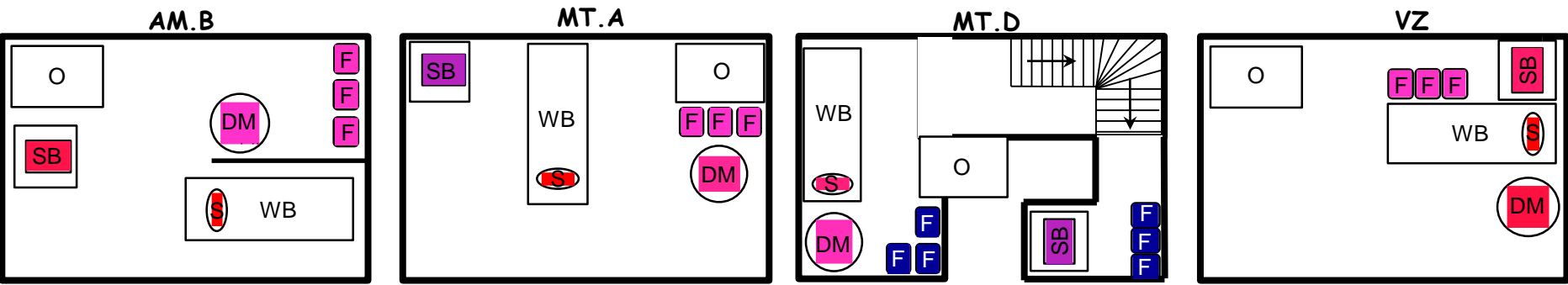
0 1 2 5 10 0 1 2 5 10 0 1 2 5 10 days of refreshments
Rye Triticum durum T. aestivum



The house microbiota



(Scheirlinck et al., 2009. *J Appl Microbiol*, 106:1081-1092)



SB, storage box; DM, dough mixer; F, flour; S, sourdough

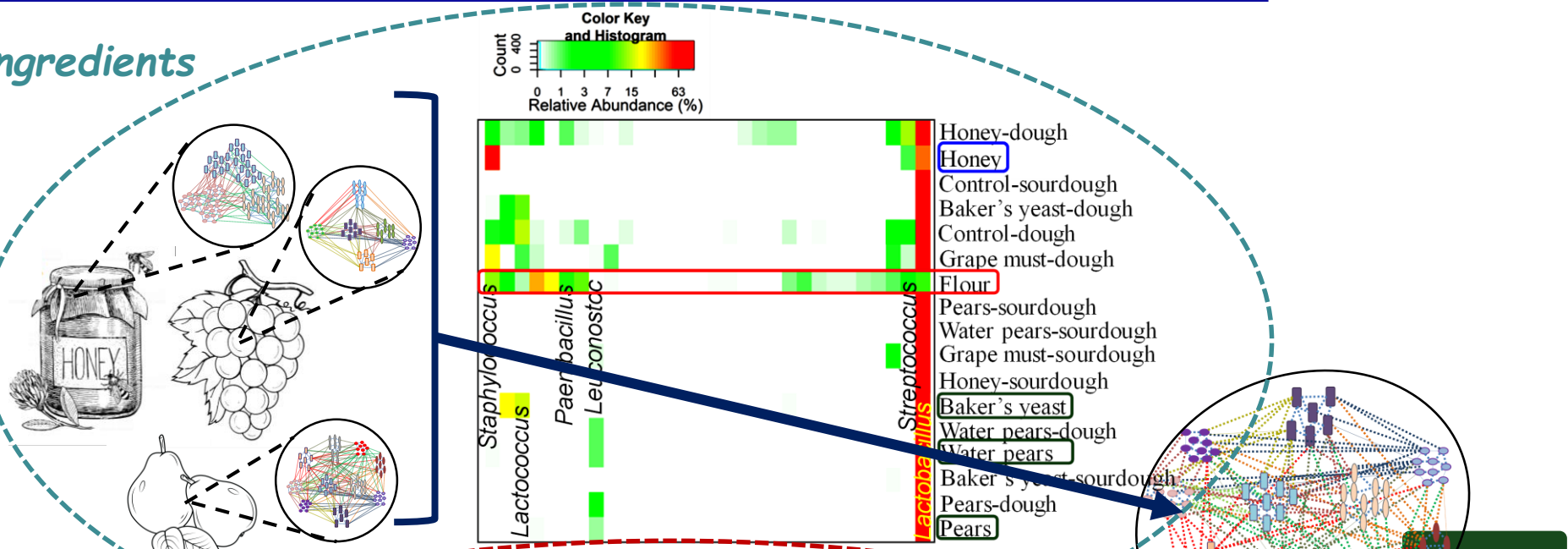


(Minervini et al., 2015. *Food Microbiol*. 52:66-76)

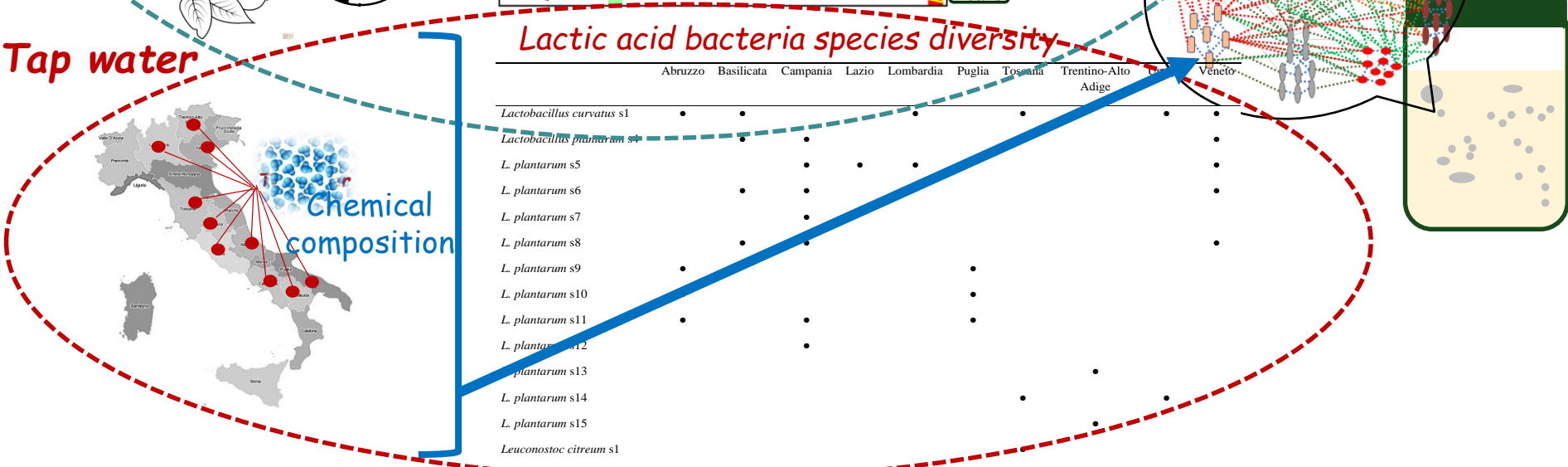
Ingredients and tap water

(Minervini et al., 2016. Food Microbiol. 60:112; Minervini et al., 2019. Sci. Reports 9:250)

Ingredients



Tap water



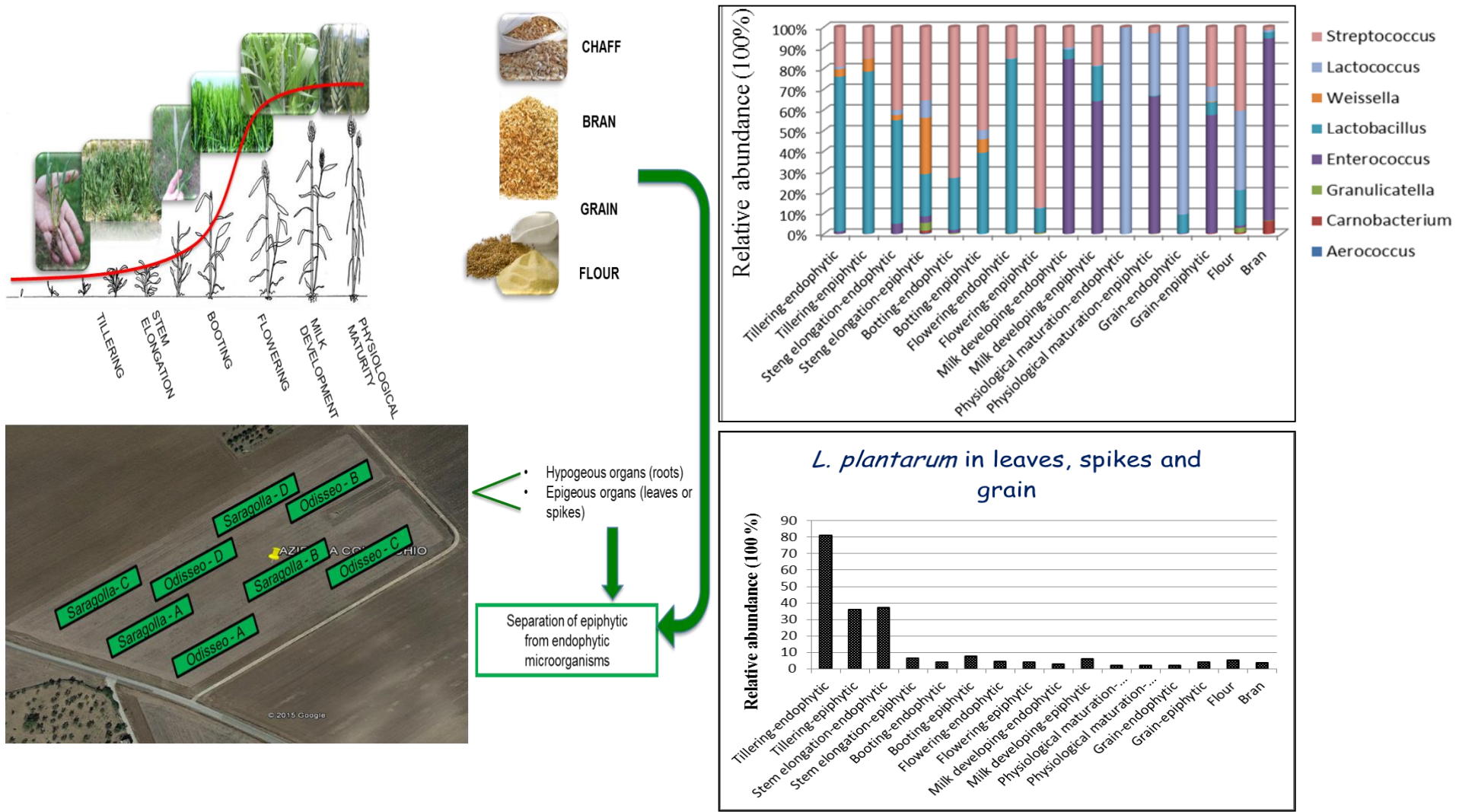
Flour harbors lactic acid bacteria



(Alfonzo *et al.*, 2013. *Food Microbiol*, 36:343-354; Coda *et al.*, 2010. *J Appl Microbiol*, 108:925-935)

Lactic acid bacteria as endophyte components of the durum wheat plant

(Minervini et al., 2015. *Appl. Environ. Microbiol.* 81:6736)



Robustness of endophyte components

Durum wheat dough singly
inoculated with
Lactobacillus
sanfranciscensis A4 and...

Wheat endophytic strains:

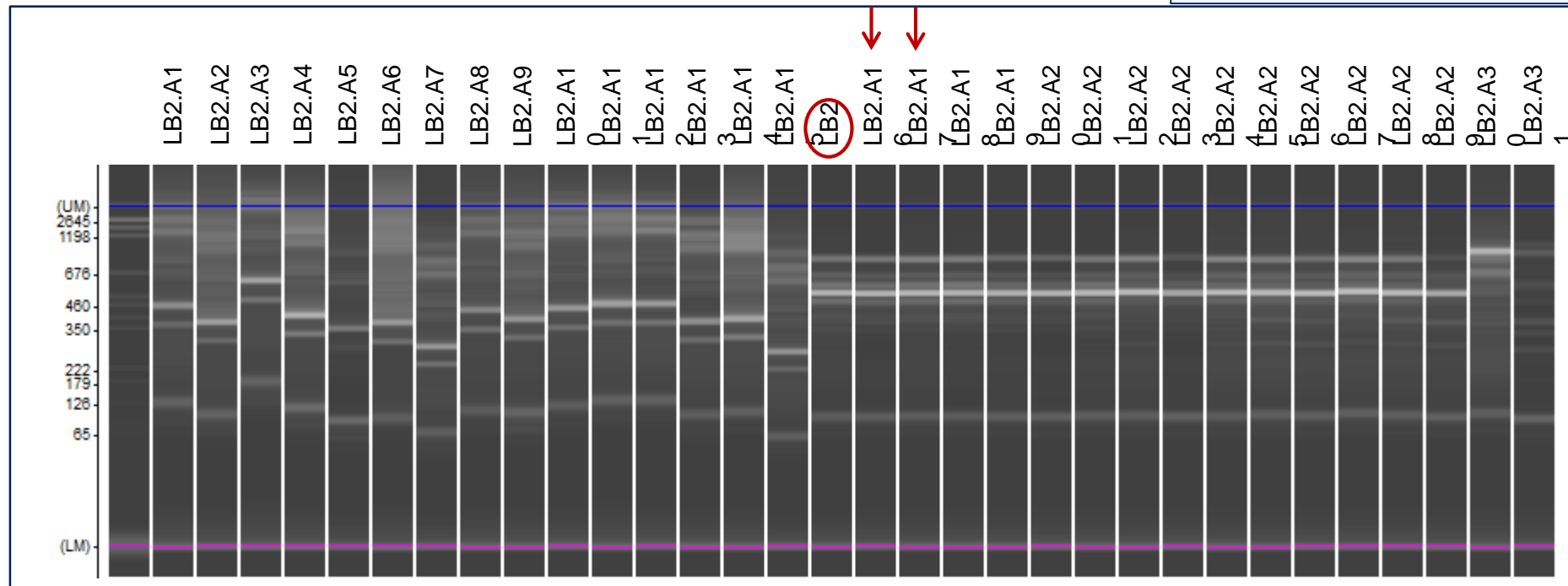
1. *Lactobacillus plantarum* LA1
2. *L. plantarum* LB2
3. *L. plantarum* OLB3
4. *L. plantarum* OLD1
5. *L. plantarum* OLB4
6. *L. plantarum* OLC4
7. *Lactobacillus rossiae* OLC1
8. *Enterococcus faecalis* LA2

Nine doughs (including the control, with no microbial inoculation)

Propagation



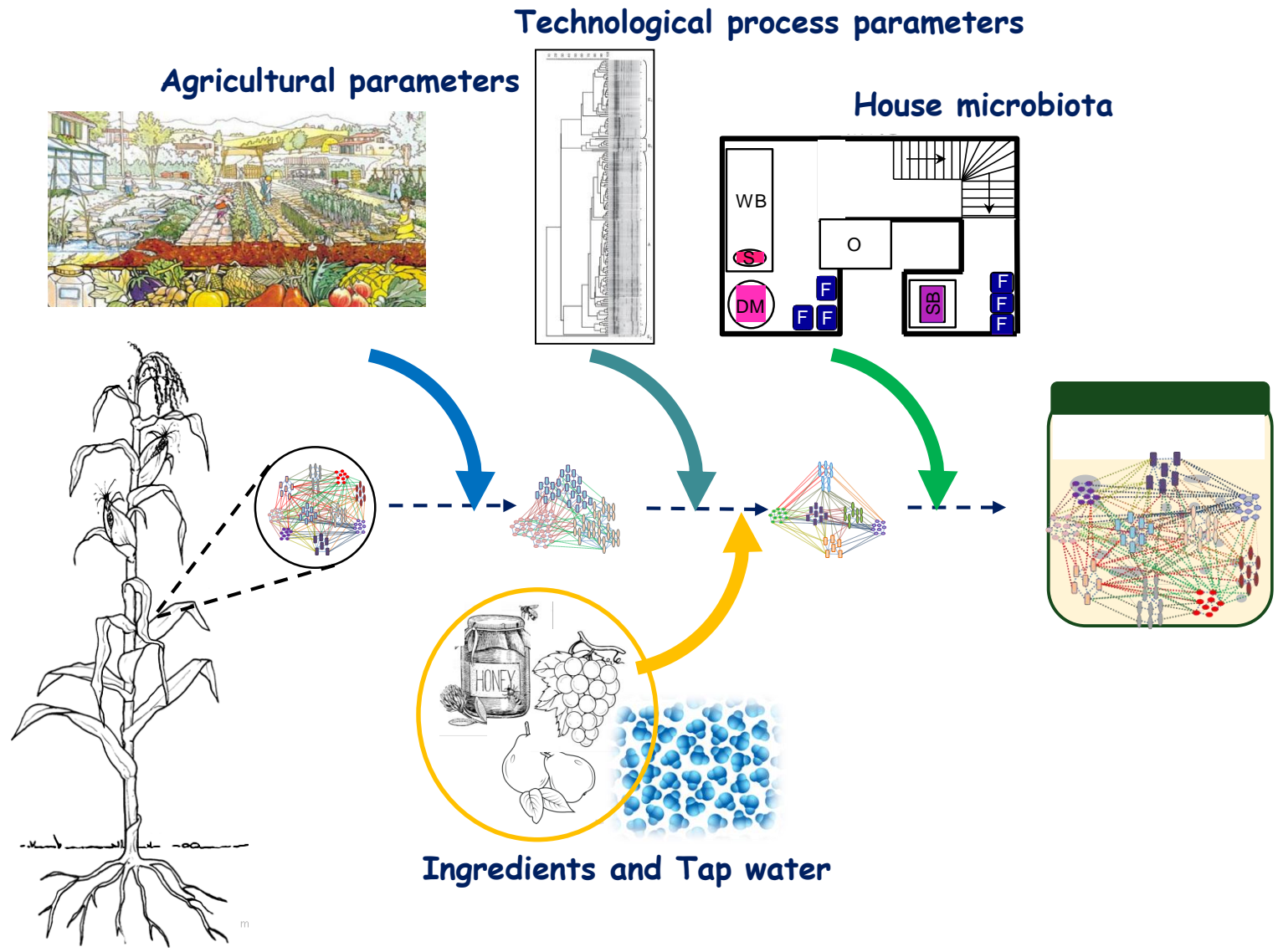
De novo sourdoughs



- ✓ Parallel propagation (one week) at artisan bakery and laboratory level
- ✓ *L. plantarum* LB2 dominated all the sourdoughs under all the different conditions of propagation

(Minervini *et al.*, 2018. *Food Microbiol.* 70:162)

From wheat plant to sourdough: the microbiome fil rouge



(Gobbetti et al., 2019. Crit. Reviews Food Sci Nutr. In press)

The sourdough microbiome is extremely diverse

L. acetotolerance, *L. acidifarinae*, *L. acidophilus*
L. alimentarius, *L. amylovorus*, *L. amylolyticus*
L. arizonensis, *L. brevis*, *L. buchneri*, *L. casei*,
L. cellobiosus

L. collinoides, *L. crispatus*,
L. crustorum, *L. curvatus*
L. delbrueckii, *L. farciminis*,
L. fermentum
L. fructivorans, *L. frumenti*,
L. gasseri, *L. gallinarum*,
L. graminis, *L. guizhovensis*

L. hammesii, *L. helveticus*, *L. hilgardi*,
L. homoiochii, *L. kimchi*, *L. kunkeei*
L. johnsonii, *L. mindensis*
L. mucosae, *L. nagelii*, *L. namurensis*

L. coryniformis, *L. colehominis*,
L. nantensis, *L. nodensis*, *L. oris*
L. parabrevis, *L. parabuchneri*,
L. paracasei, *L. paralimentarius*,
L. pentosus, *L. perolens*
L. plantarum, *L. pontis*, *L. reuteri*

L. rhamnosus, *L. rossiae*,
L. saivarius, *L. sakei*,
L. sanfranciscensis,
L. siliginis, *L. secaliphilus*,
L. spicheri, *L. panis*
L. vaginalis, *L. zaea*,
L. zymae

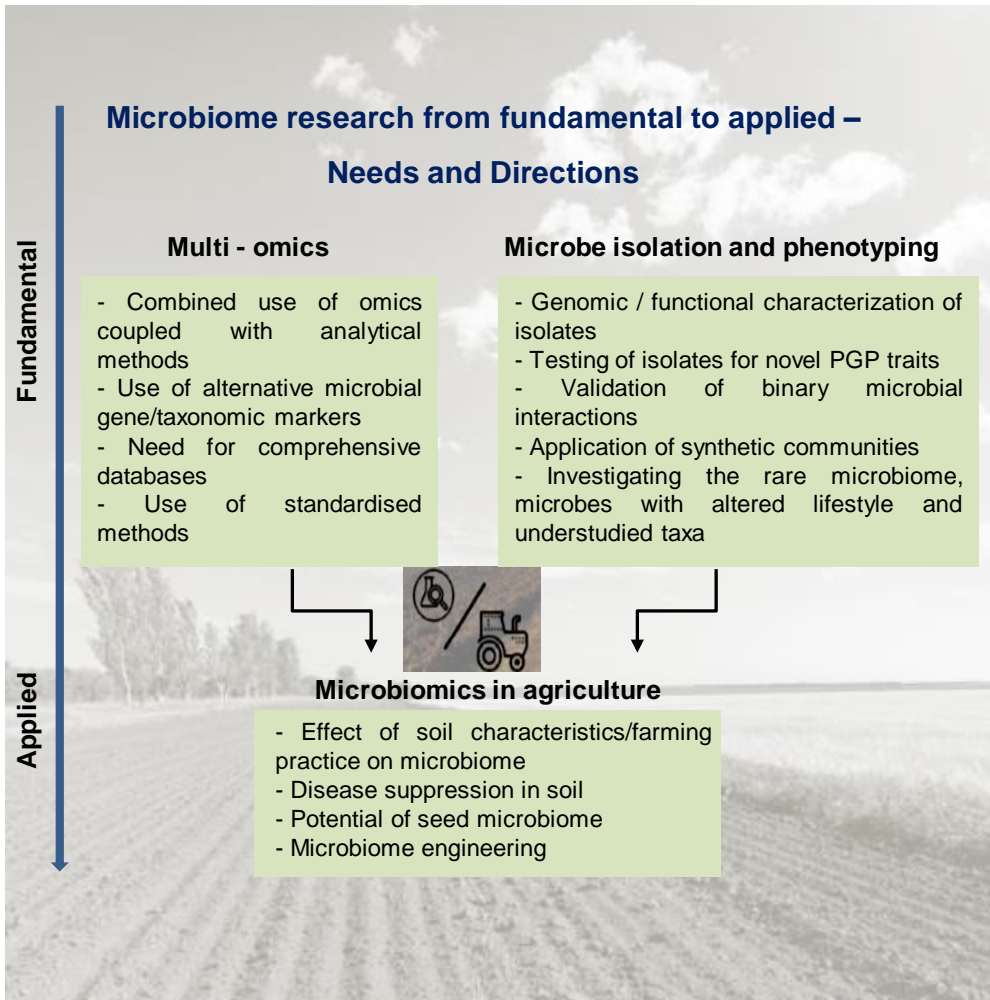


(De Vuyst et al., 2014; Minervini et al., 2012; Kashiuvagi et al., 2009; Vancanneyt et al., 2005; Meroth et al., 2003)

The first sourdough “microbiome library” (Saint-Vith, Belgium) in the world



Modulation of plant microbiota



- ✓ Inoculation of single strain
- ✓ Application of microbial consortia
- ✓ Plant selection
- ✓ Mode of delivery
- ✓ Microbiome engineering
- ✓ **Meta-community approach**

(Compant *et al.*, 2019. *J. Adv. Res.* 8:51)

Micro4food Lab: running EU projects

1. FUNBREW - Biotransformation of brewers' spent grain: increased functionality for novel food applications (European Union ERANET/SUSFOOD)
2. SMART Protein - Alternative proteins for food and feed (Horizon 2020, LC-SFS-17-2019)
3. Knowledge Platform on Food, Diet, Intestinal Microbiomics and Human Health (JPI, ERA-HDHL INTIMIC)
4. Knowledge Hub on Food and Nutrition Security (JPI, ERA-HDHL)