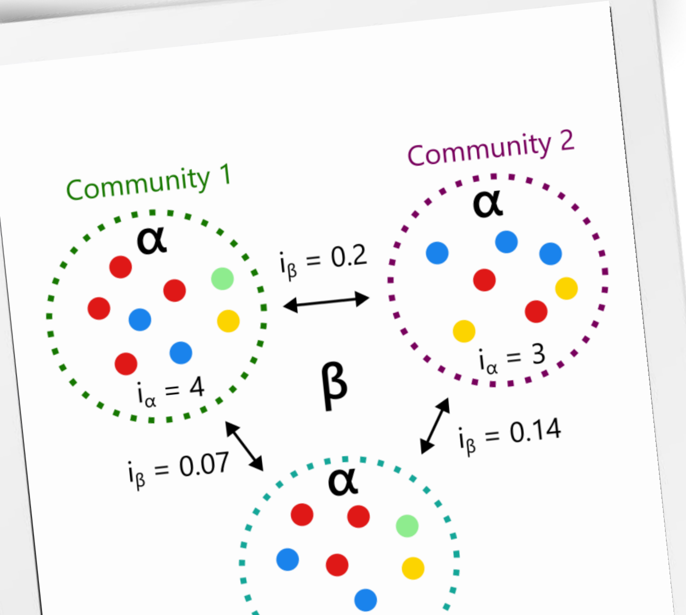


LiveSeeding

Training in organic breeding

Module 7: **Seed microbiota**

Unit 7.1: **Studying the seed microbiota**

Author: **Gaspard de Tournemire - ITAB**

Co-funded by the European Union

Funded by the European Union, the Swiss State Secretariat for Education, Research and Innovation (SERI) and UK Research and Innovation (UKRI).

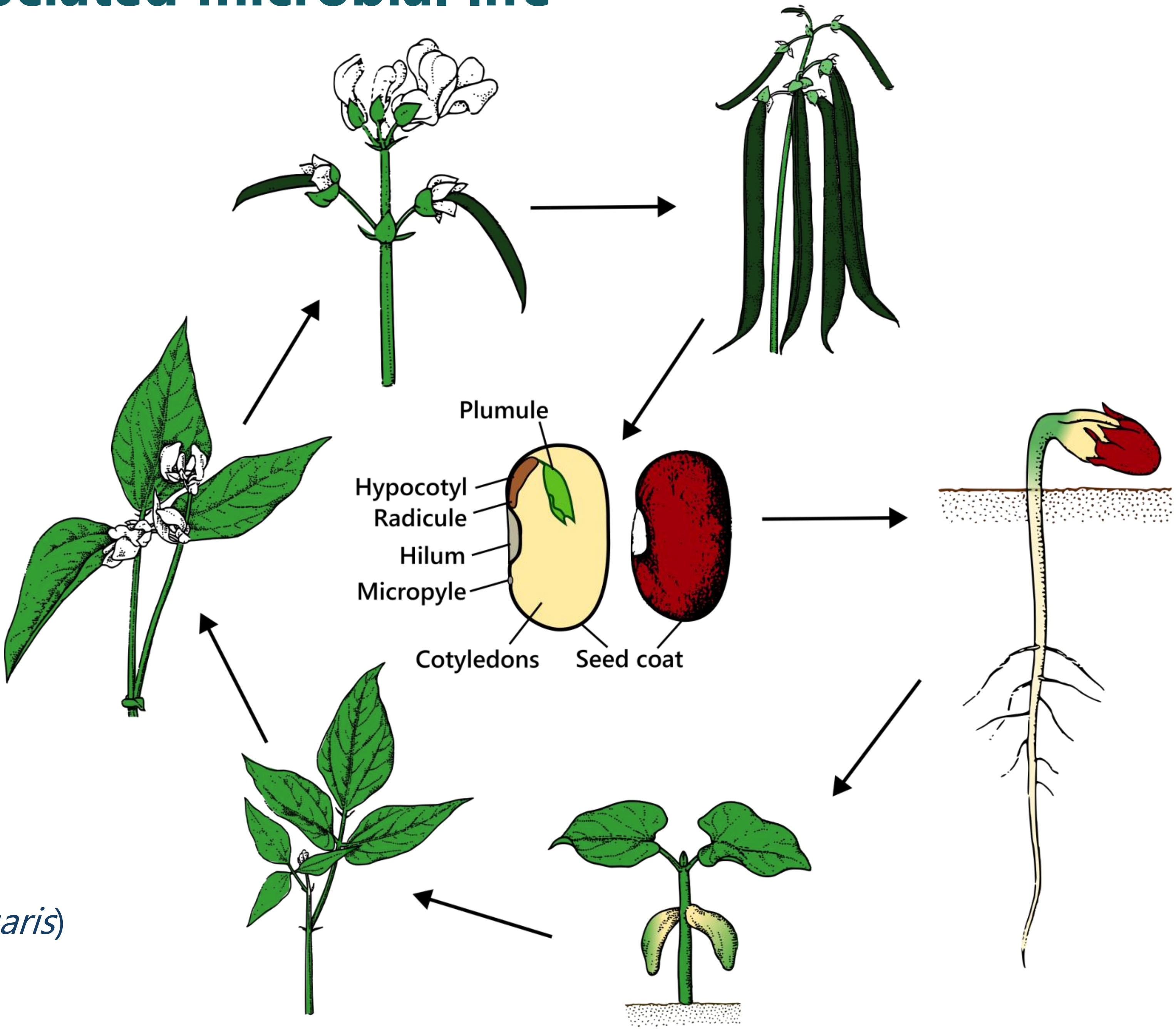
UK Research and Innovation

Overview

Seed-associated microbial life

(Rae 2018)

Seed-associated microbial life

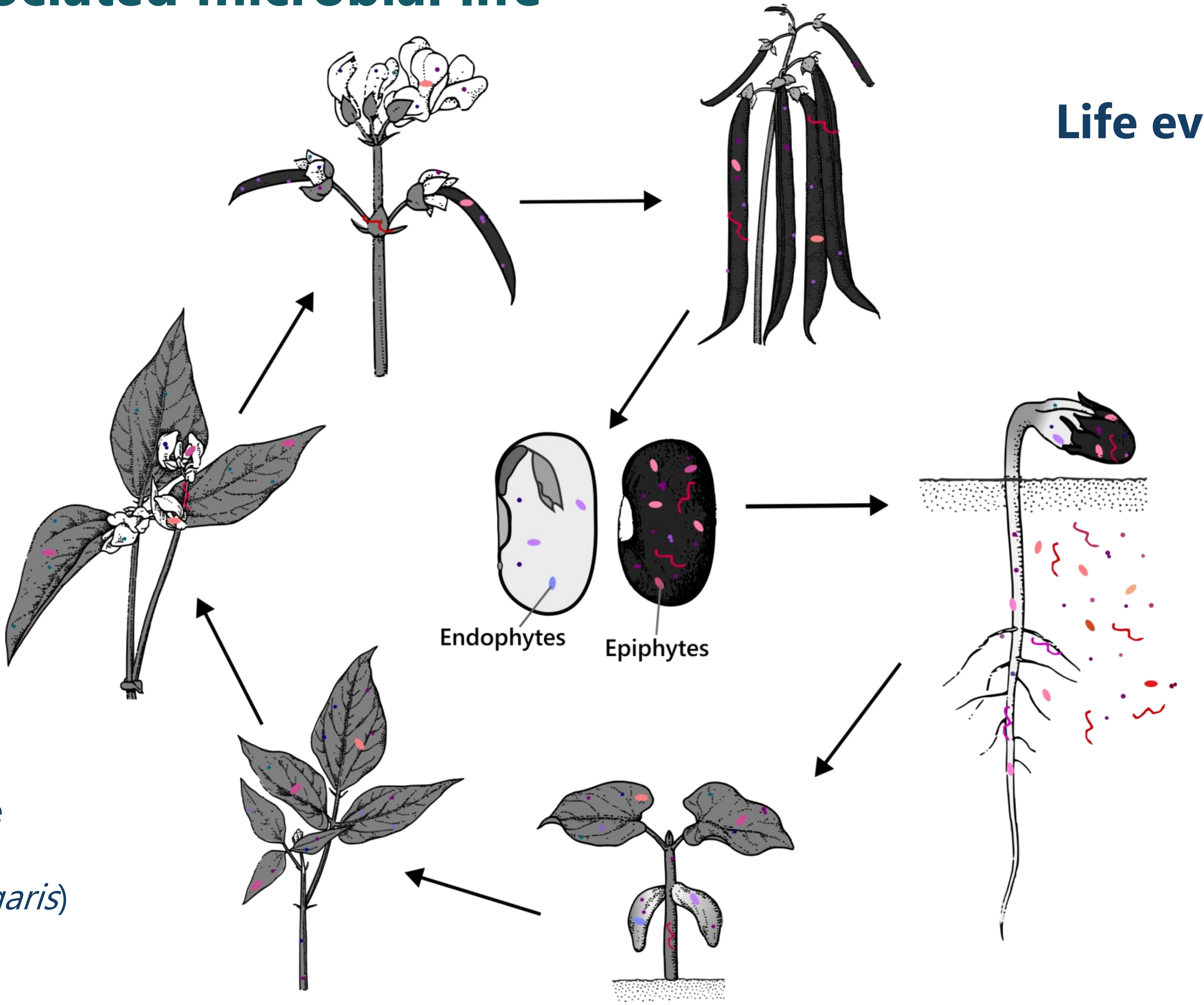


Life cycle of the common bean (*Phaseolus vulgaris*)

(Feller et al. 1995)

Seed-associated microbial life

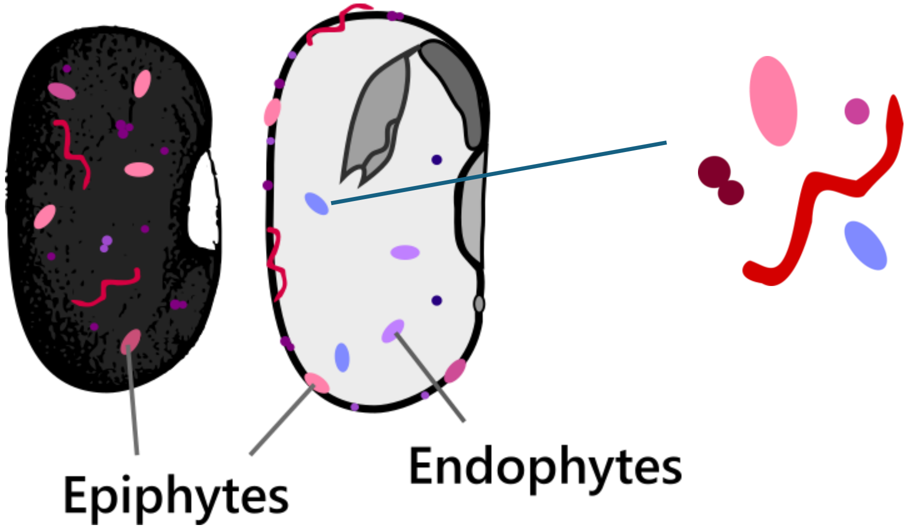
Life everywhere



Life cycle of the common bean (*Phaseolus vulgaris*)

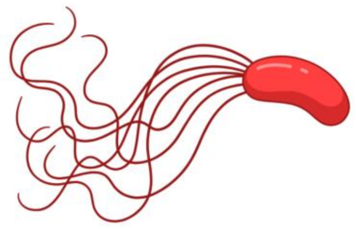
(Hassani et al. 2019; Feller et al. 1995)

Seed-associated microbial life

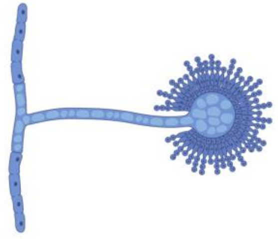


- Bacteria
- Fungi
- Oomycetes
- Protists…

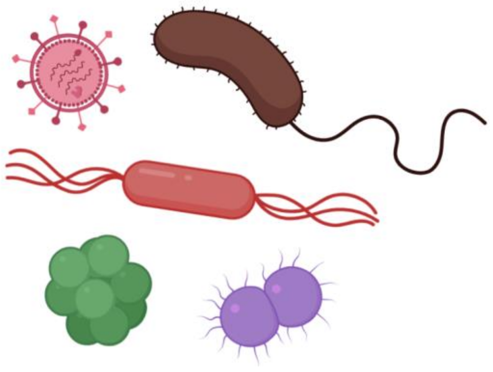
Some iconic examples of seed-associated micro-organisms



Bacterium of the *Pseudomonas* genus



Fungus of the *Aspergillus* genus



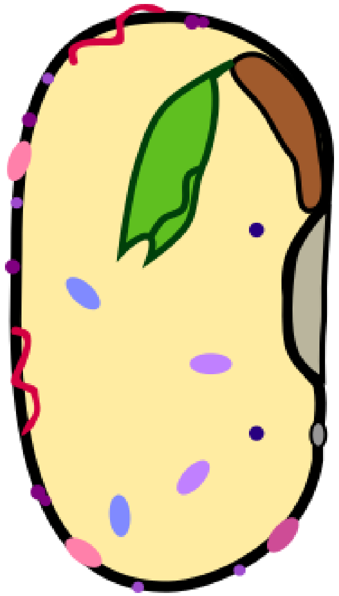
And many more…

(*Shade et al. 2017;*
Feller et al. 1995)

BioRender

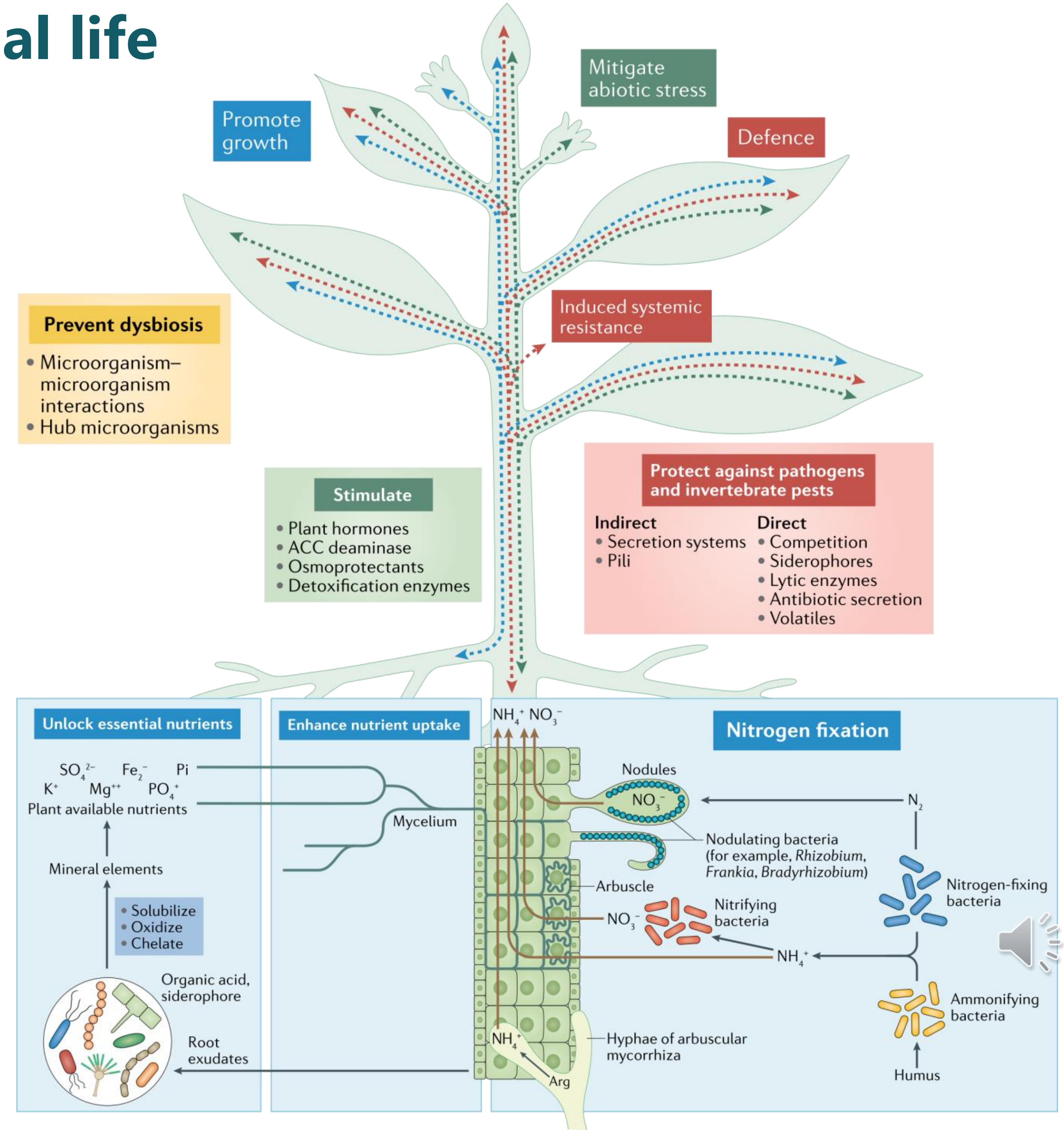
3 Seed-associated microbial life

Plant-microbe interactions



With some benefits

(Shade et al. 2017;
Feller et al. 1995)



Prevent dysbiosis

- Microorganism–microorganism interactions
- Hub microorganisms

Stimulate

- Plant hormones
- ACC deaminase
- Osmoprotectants
- Detoxification enzymes

Protect against pathogens and invertebrate pests

Indirect

- Secretion systems
- Pili

Direct

- Competition
- Siderophores
- Lytic enzymes
- Antibiotic secretion
- Volatiles

Unlock essential nutrients

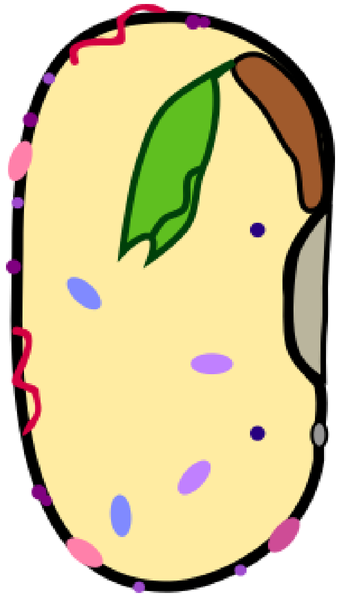
Enhance nutrient uptake

Nitrogen fixation

(Trivedi et al. 2020)

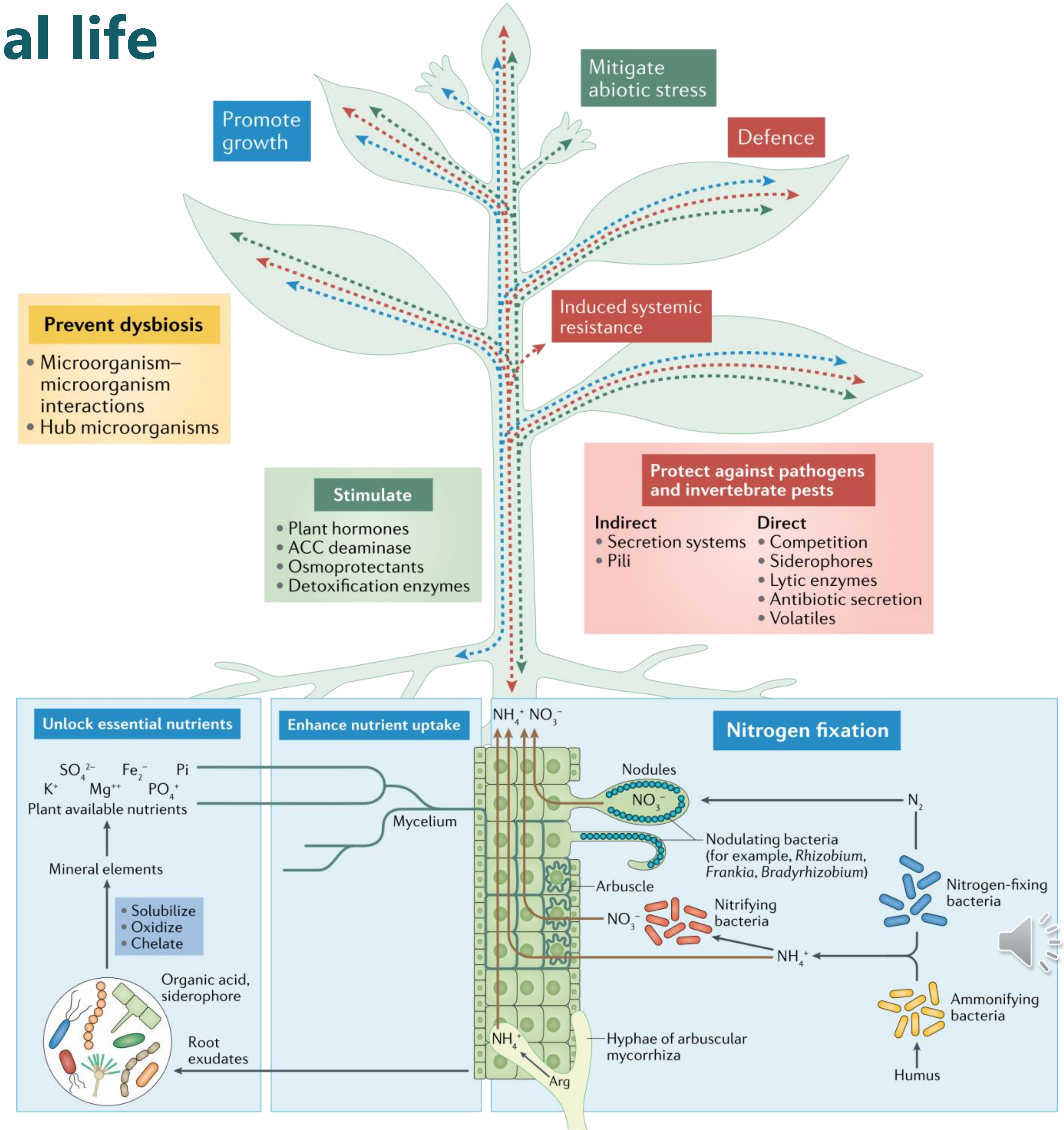
3 Seed-associated microbial life

Holobionts



With some benefits

(Shade et al. 2017;
Feller et al. 1995)

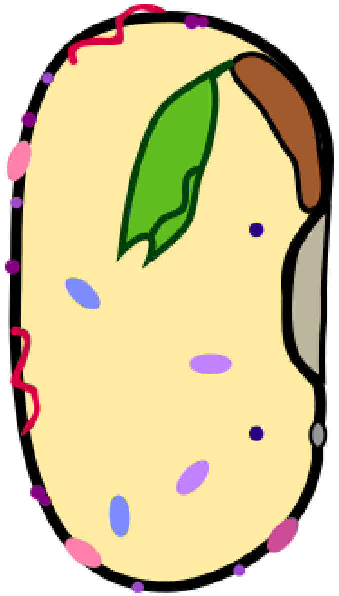


(Trivedi et al. 2020)

4

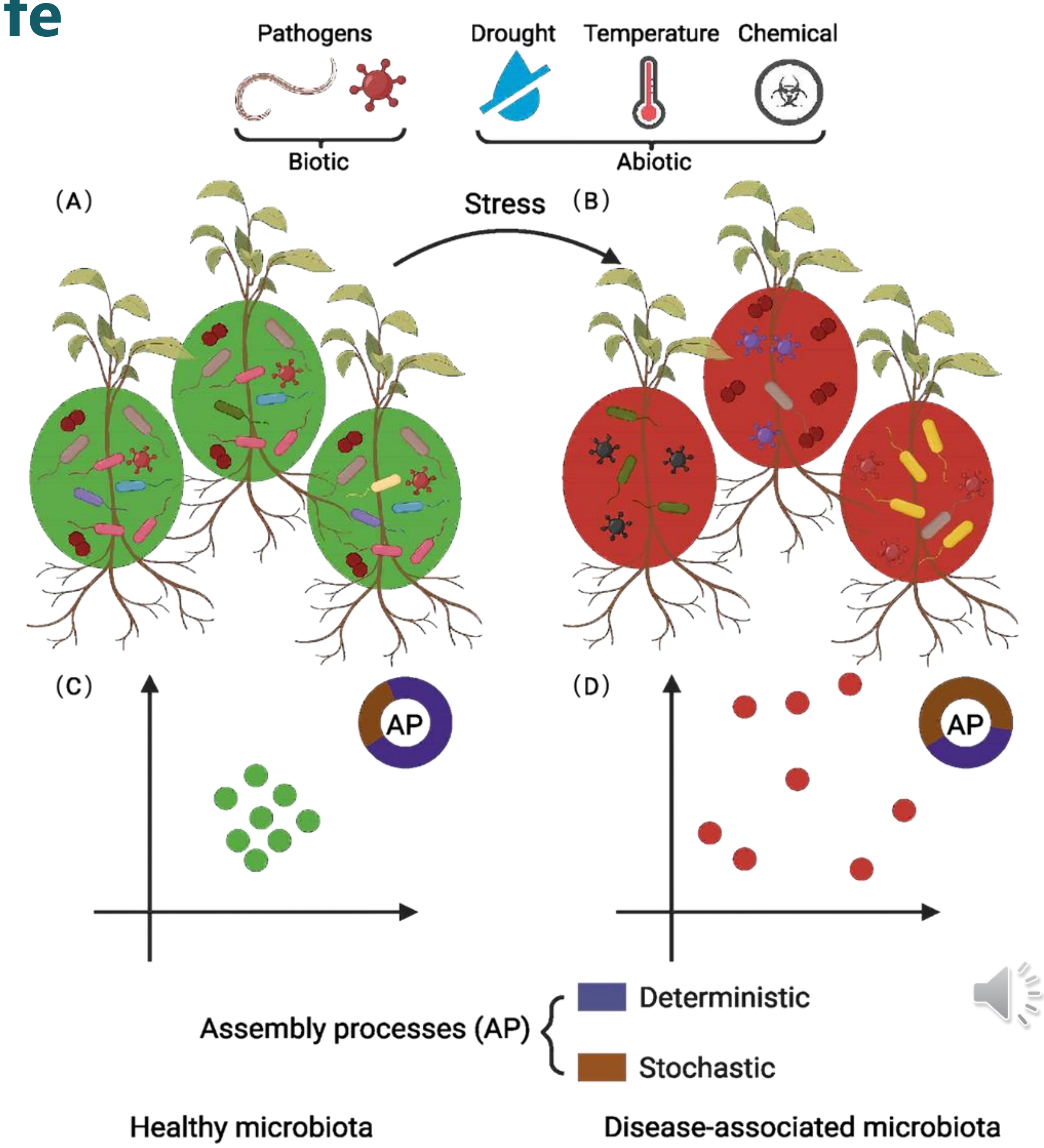
Seed-associated microbial life

Holobionts



At risk of losing balance

(*Shade et al. 2017;*
Feller et al. 1995)



(Arnault et al. 2021)

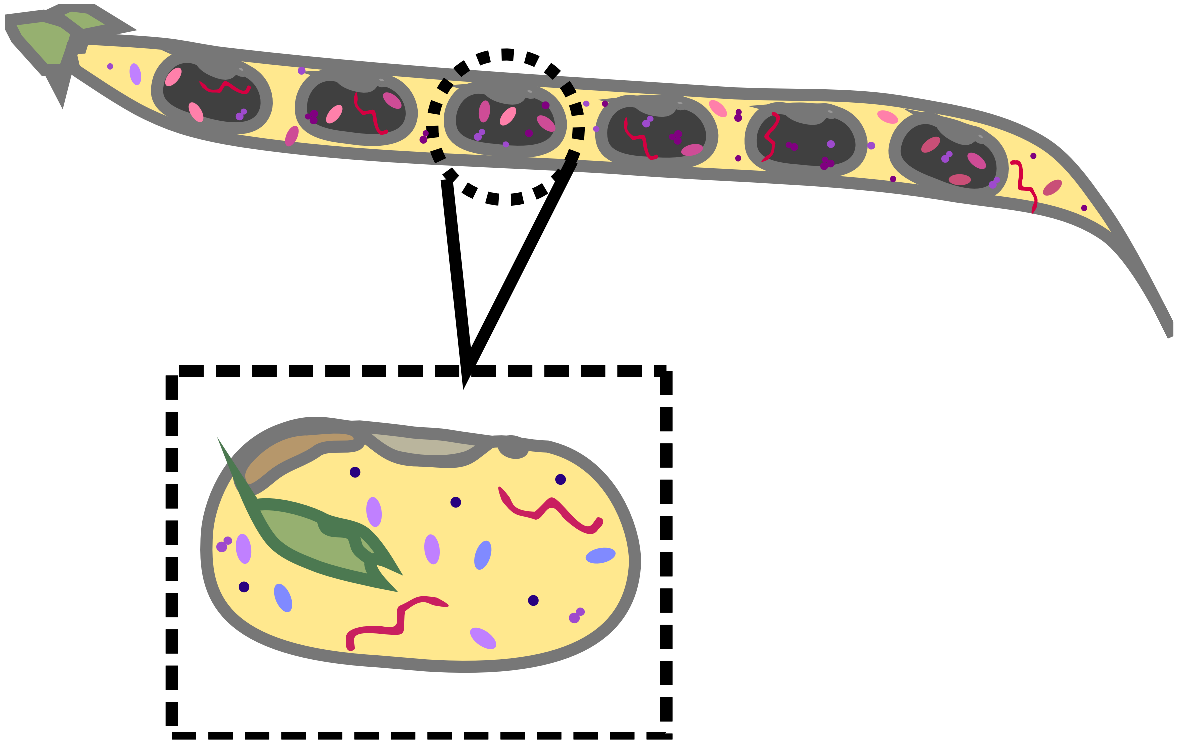
Seed associated microbial life | key takeaways

- Seeds are critical steps in the plant-microbe interaction cycle
- Microbial life is essential to plant health

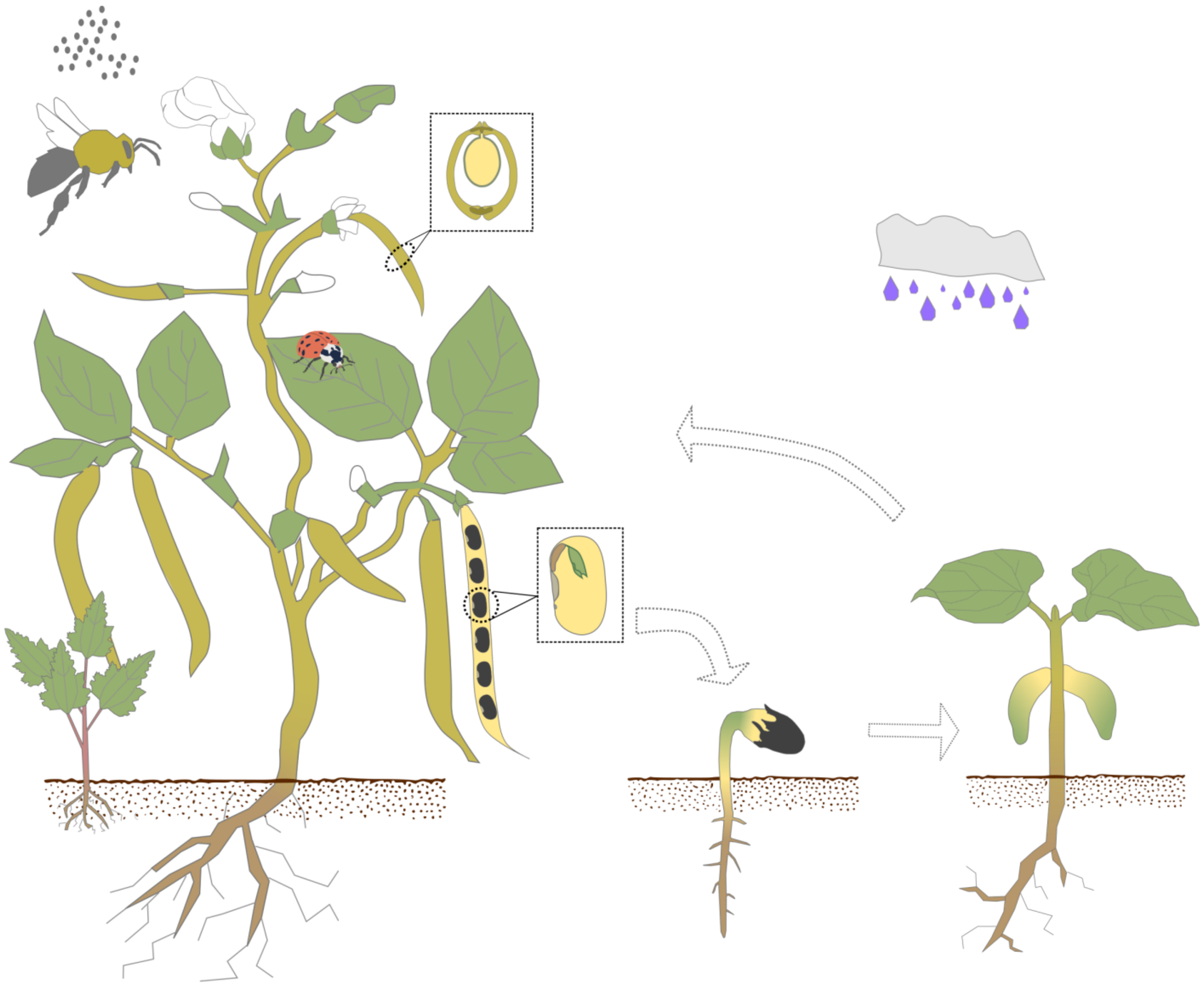
LiveSeeding

Overview

Seed microbiota assembly processes

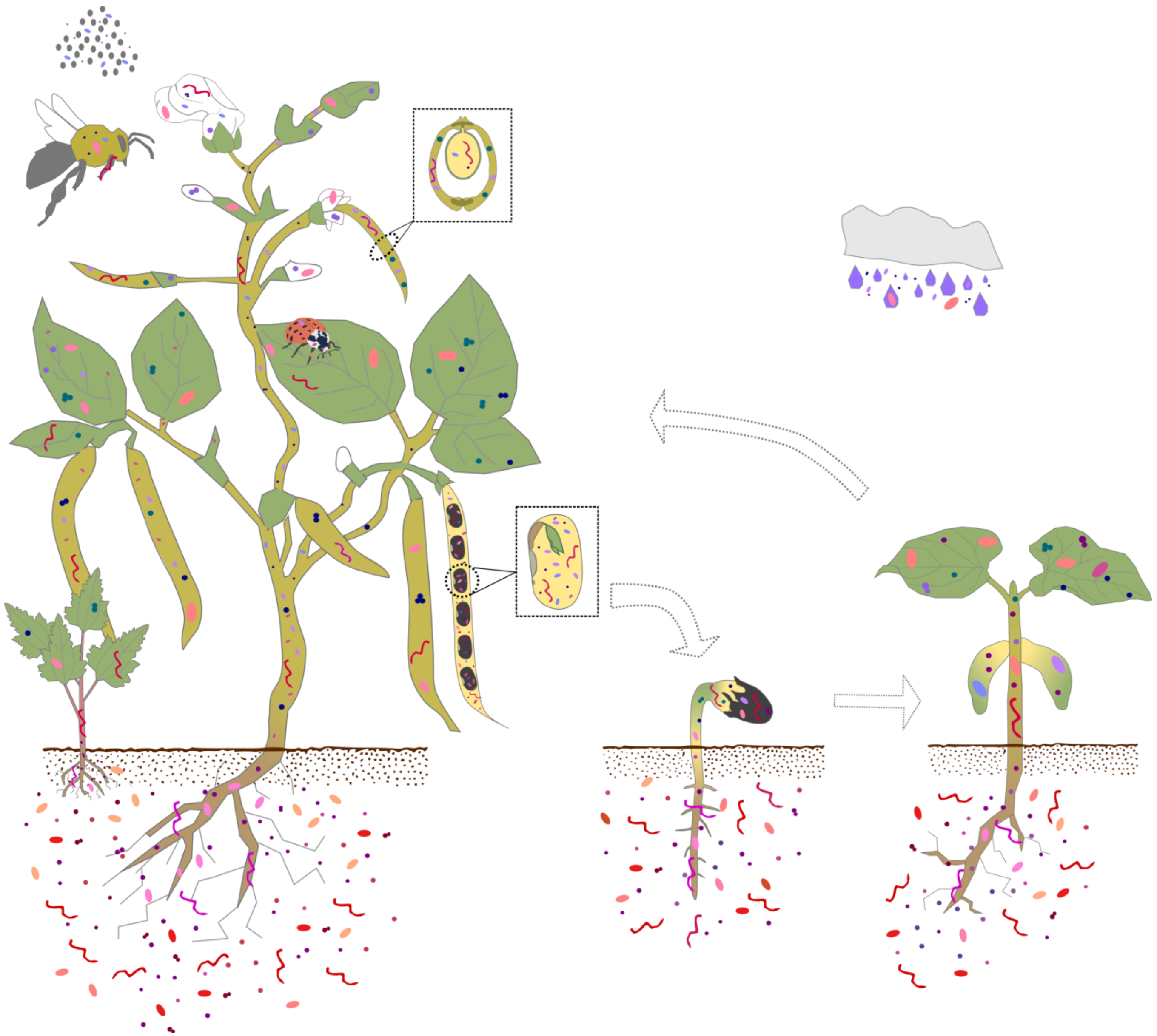


Seed microbiota assembly processes



(own work, adapted from Abdelfattah et al. 2023, Feller et al. 1995, Hill 2025 and Lonicer 1582)

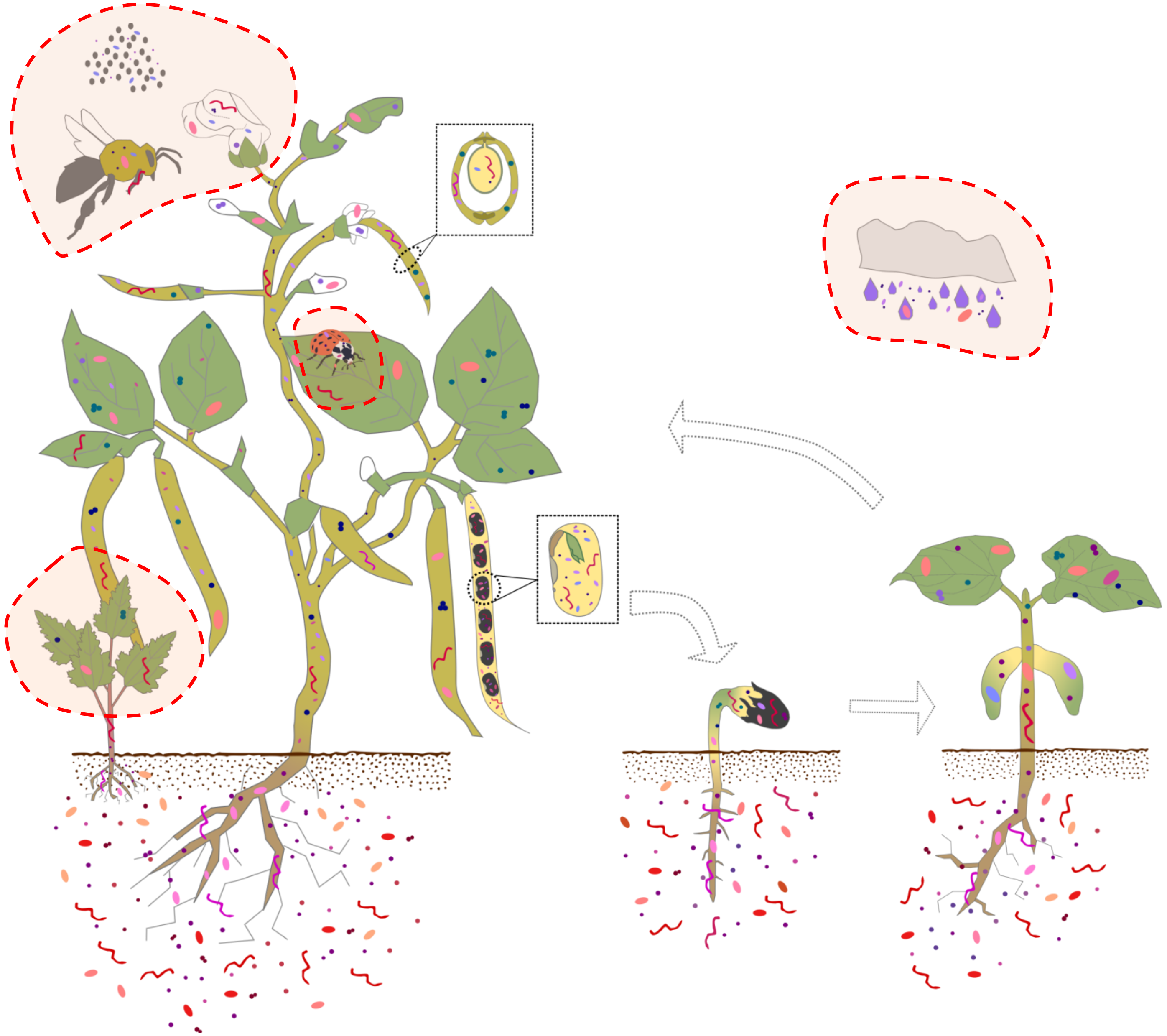
1 Seed microbiota assembly processes



(own work, adapted from Abdelfattah et al. 2023, Feller et al. 1995, Hill 2025 and Lonicer 1582)

2

Seed microbiota assembly processes

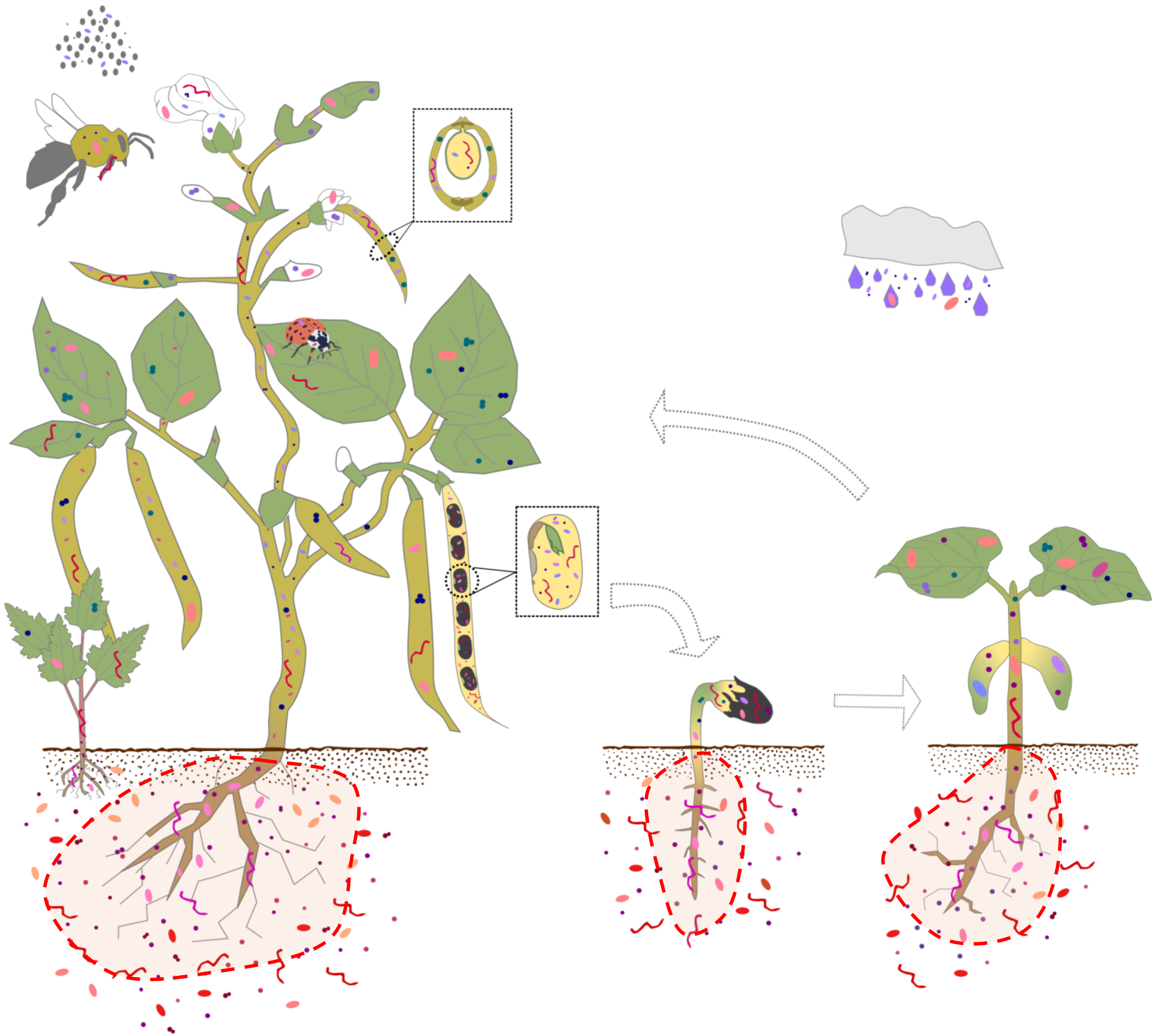


(own work, adapted from Abdelfattah et al. 2023, Feller et al. 1995, Hill 2025 and Lonicer 1582)

Environmental sources of inoculum

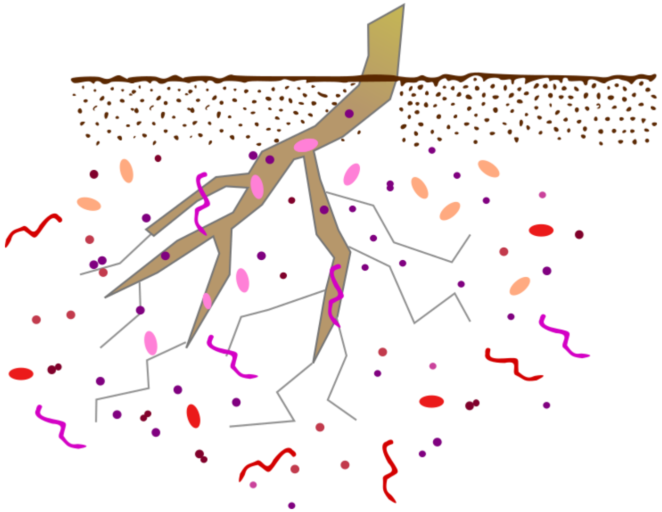
- Soil
- Precipitations
- Neighbouring plants
- Pollen and pollinators
- Other animals

3 Seed microbiota assembly processes



(own work, adapted from Abdelfattah et al. 2023, Feller et al. 1995, Hill 2025 and Lonicer 1582)

Selection by host plant

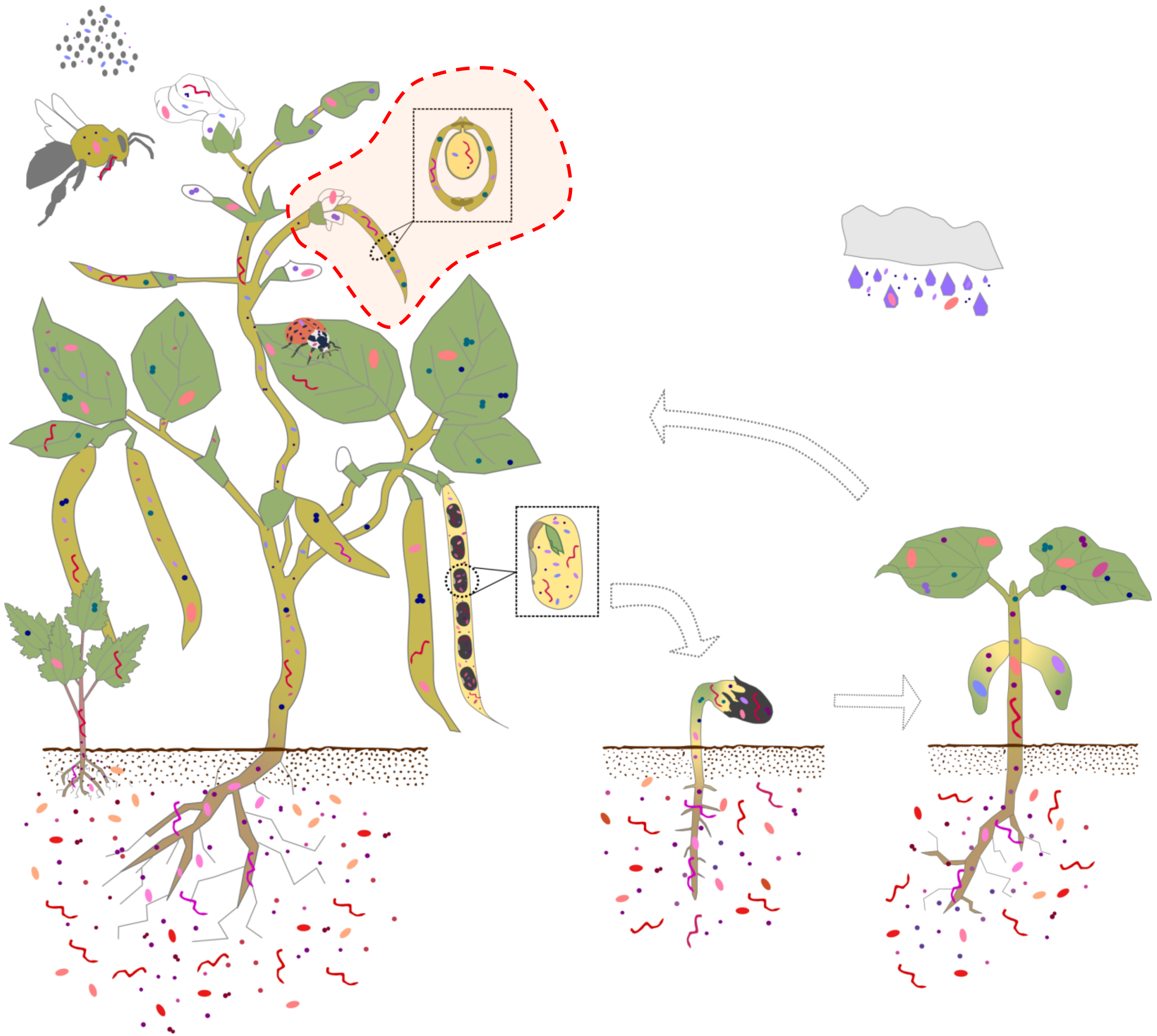


- Calibrated exudation
- Immune system

➢ **Host mediation of microbiota assembly**

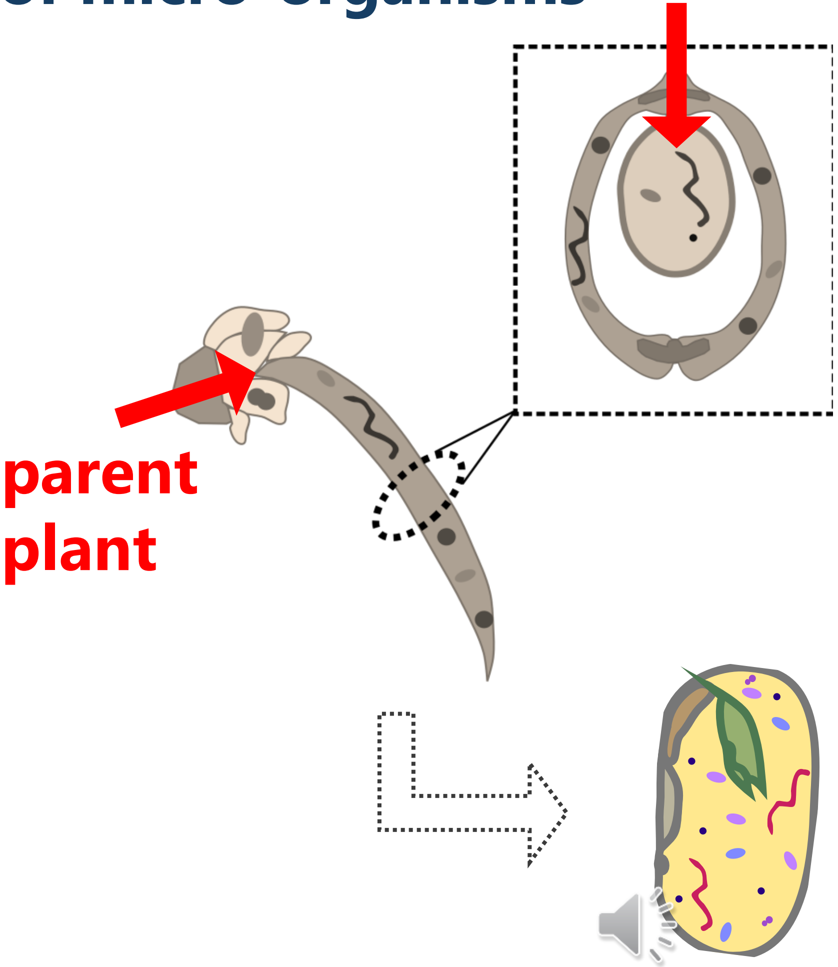
4

Seed microbiota assembly processes



(own work, adapted from Abdelfattah et al. 2023, Feller et al. 1995, Hill 2025 and Lonicer 1582)

Vertical transmission of micro-organisms



- **Interactions with seed physiological processes**

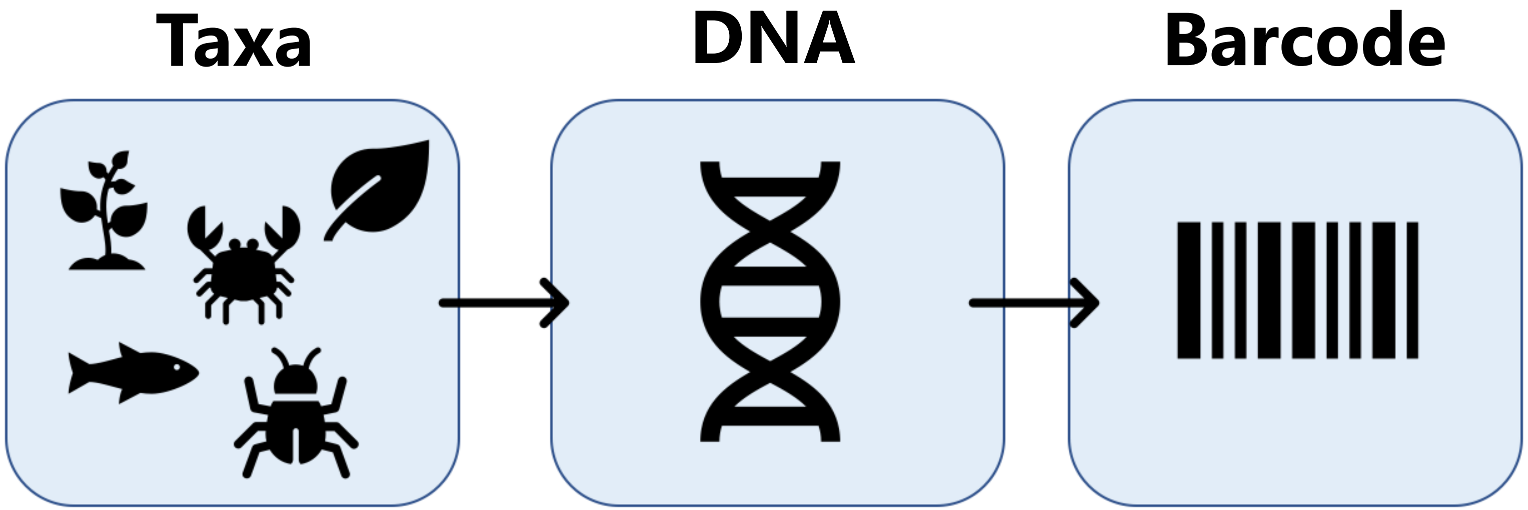
Seed microbiota assembly processes | key takeaways

- Seed quality partly relies on plant-mediated assembly of diverse microbial communities from the environment

LiveSeeding

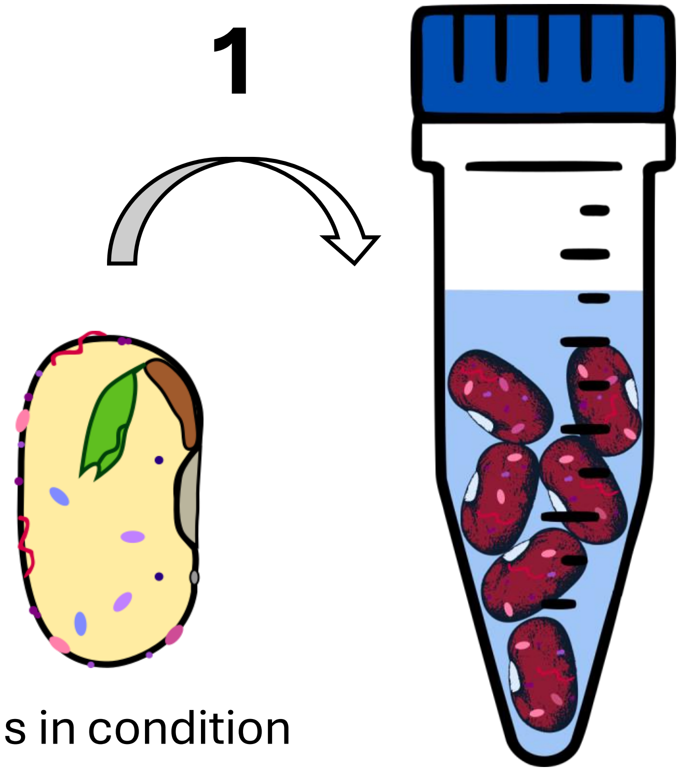
Overview

Metabarcoding : a method for studying seed microbiota



(Fruehe 2016)

1 **Metabarcoding** for analysis of seed-associated microbial communities

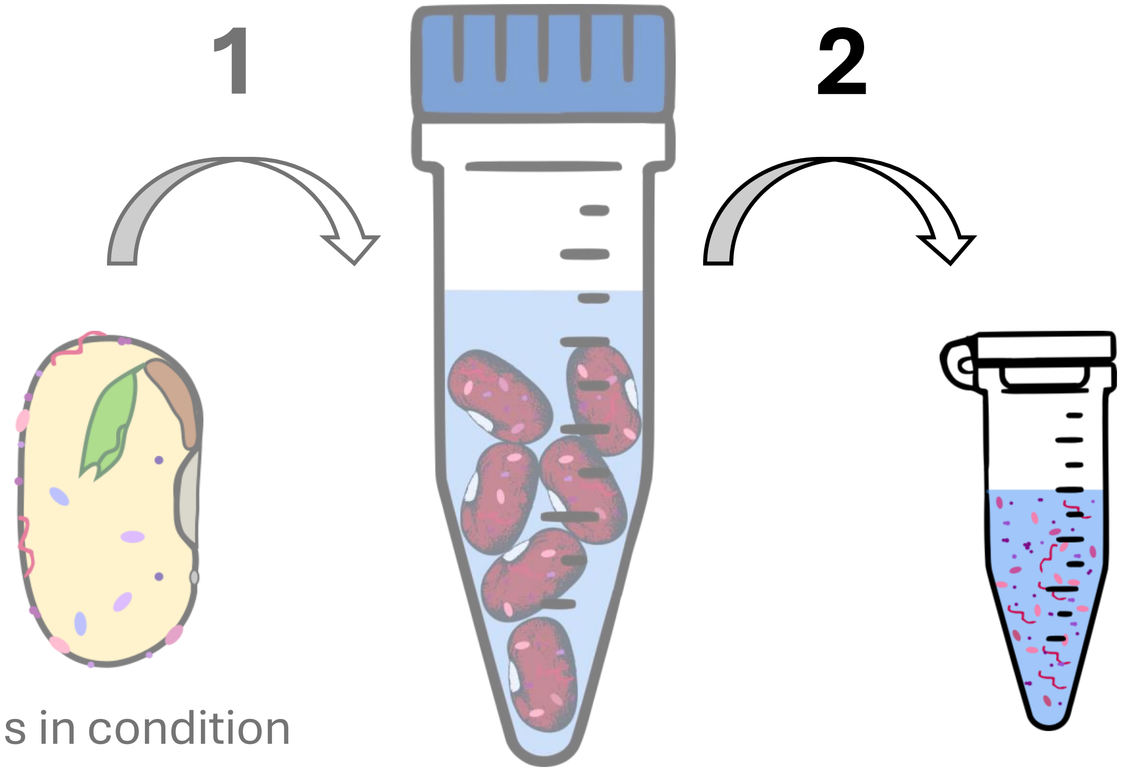


Seeds in condition
to be analysed

1. Soaking

(Feller et al. 1995)

Metabarcoding for analysis of seed-associated microbial communities



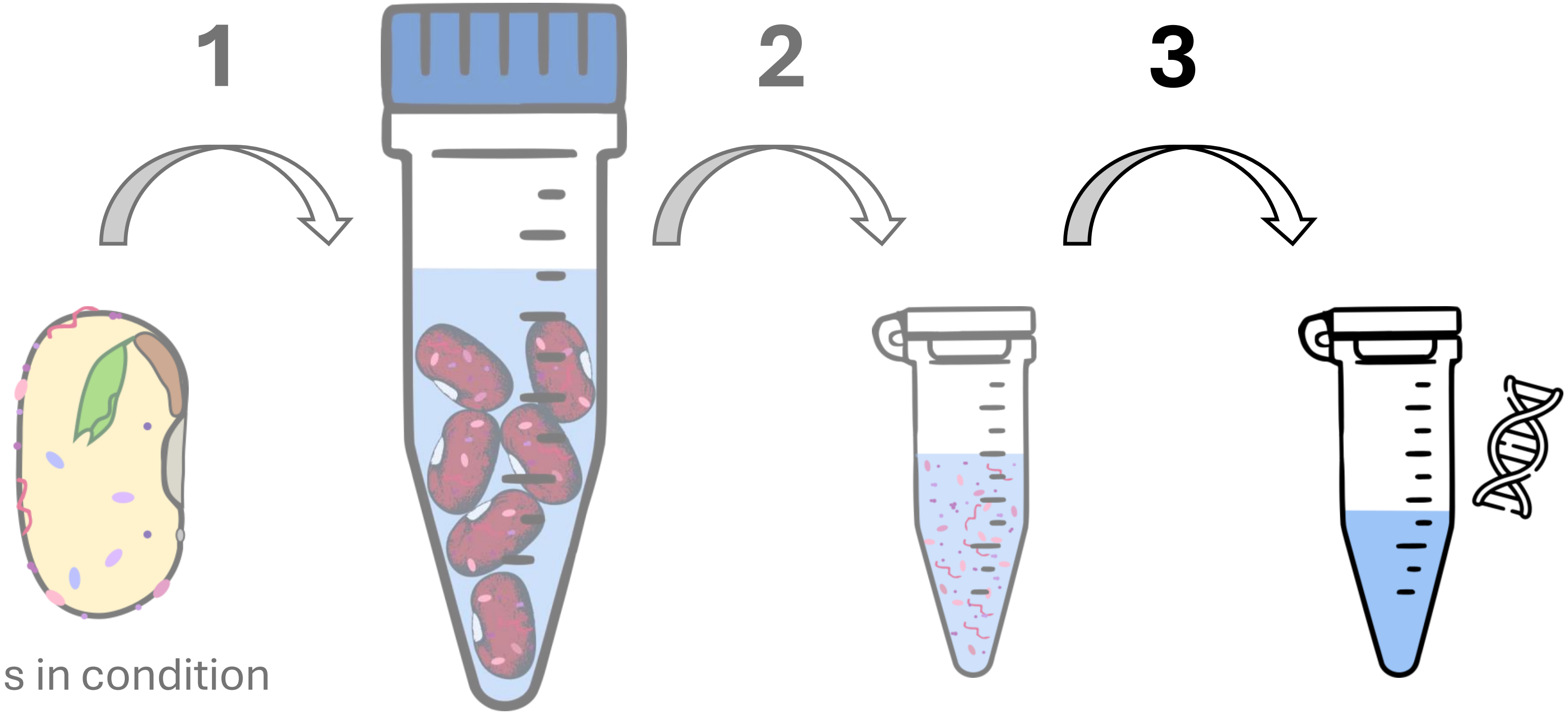
Seeds in condition
to be analysed

1. Soaking

2. Sampling of
microbial
suspension

(Feller et al. 1995)

Metabarcoding for analysis of seed-associated microbial communities



Seeds in condition
to be analysed

1. Soaking

2. Sampling of
microbial
suspension

**3. DNA
extraction**

**Extracted DNA can
come from dead
organisms**

⇩

No absolute
information on the
state of the microbial
community

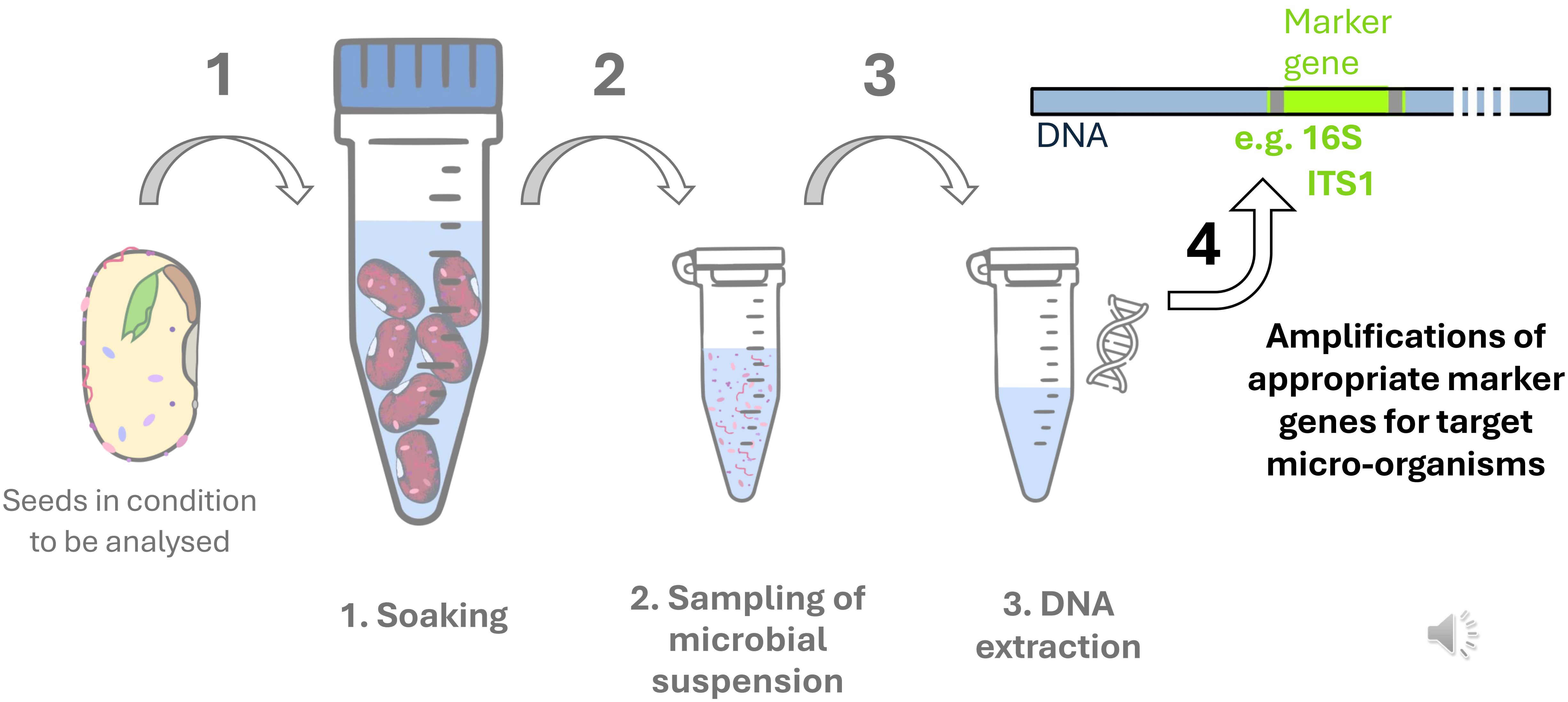
⇩

No conclusion on
quantitative dynamic

(Feller et al. 1995)

4

Metabarcoding for analysis of seed-associated microbial communities



Seeds in condition to be analysed

1. Soaking

2. Sampling of microbial suspension

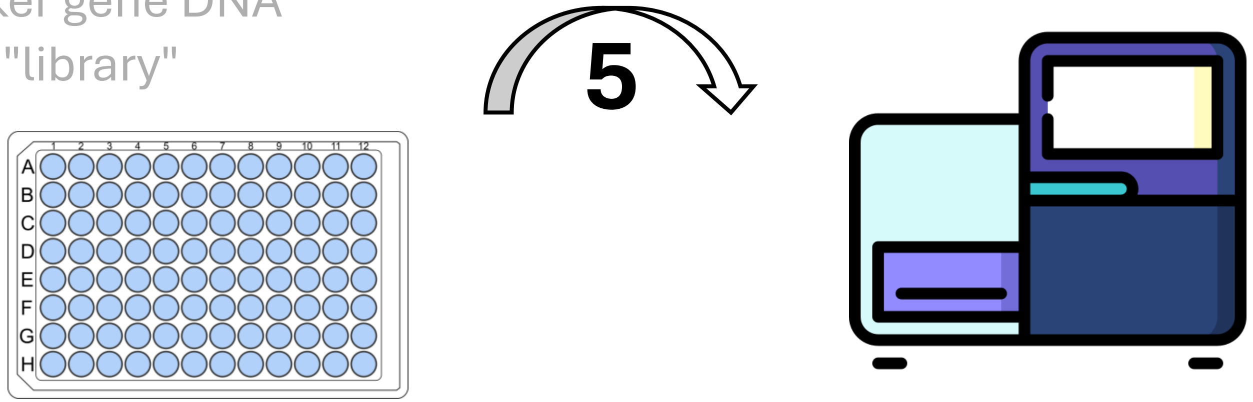
3. DNA extraction

Amplifications of appropriate marker genes for target micro-organisms

(Feller et al. 1995)

Metabarcoding for analysis of seed-associated microbial communities

5. Sequencing

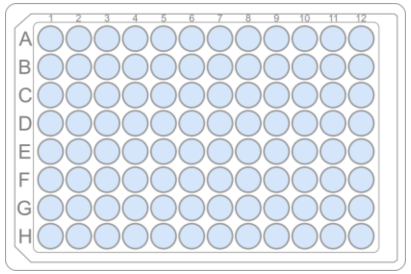


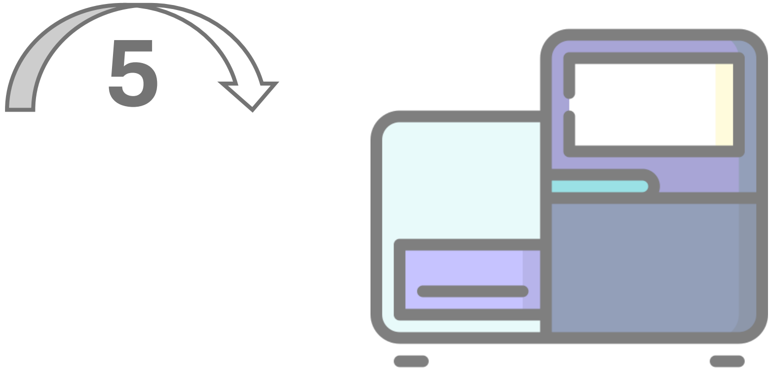
Amplified marker gene DNA segments "library"

Metabarcoding for analysis of seed-associated microbial communities

5. Sequencing

Amplified marker gene DNA segments "library"

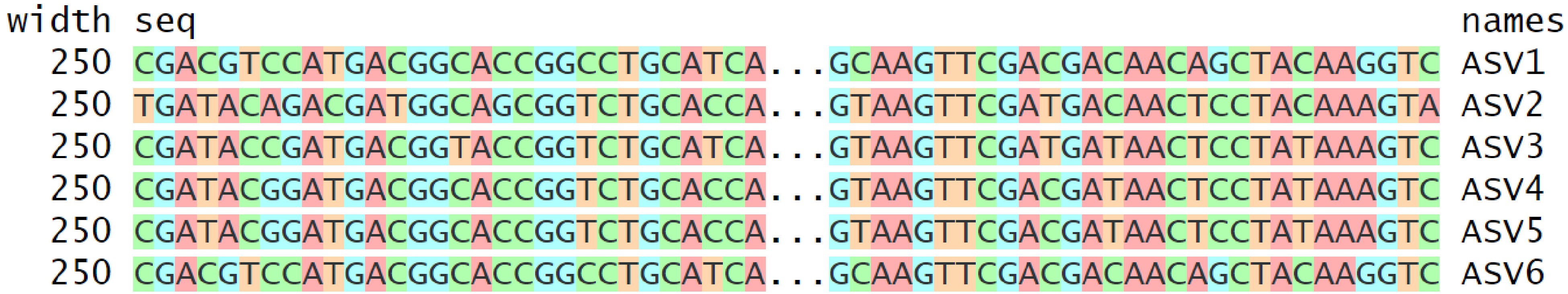




6. Cleaning

```
> cleaned_sequences
```

here from sequencing of a gyrB (bacterial marker gene) reads library



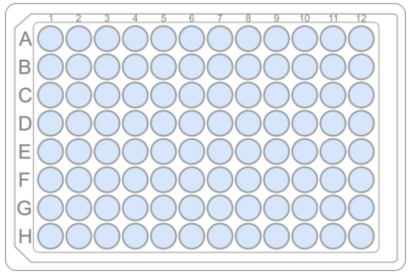
```
width seq                                                           names
  250 CGACGTCCATGACGGCACCGGCCTGCATCA...GCAAGTTCGACGACAACAGCTACAAGGTC ASV1
  250 TGATACAGACGATGGCAGCGGTCTGCACCA...GTAAGTTCGATGACAACTCCTACAAAGTA ASV2
  250 CGATACCGATGACGGTACCGGTCTGCATCA...GTAAGTTCGATGATAACTCCTATAAAGTC ASV3
  250 CGATACGGATGACGGCACCGGTCTGCACCA...GTAAGTTCGACGATAACTCCTATAAAGTC ASV4
  250 CGATACGGATGACGGCACCGGTCTGCACCA...GTAAGTTCGACGATAACTCCTATAAAGTC ASV5
  250 CGACGTCCATGACGGCACCGGCCTGCATCA...GCAAGTTCGACGACAACAGCTACAAGGTC ASV6
```

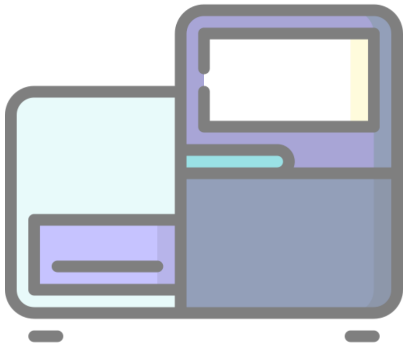
(...)

Metabarcoding for analysis of seed-associated microbial communities

5. Sequencing

Amplified marker gene DNA
segments "library"



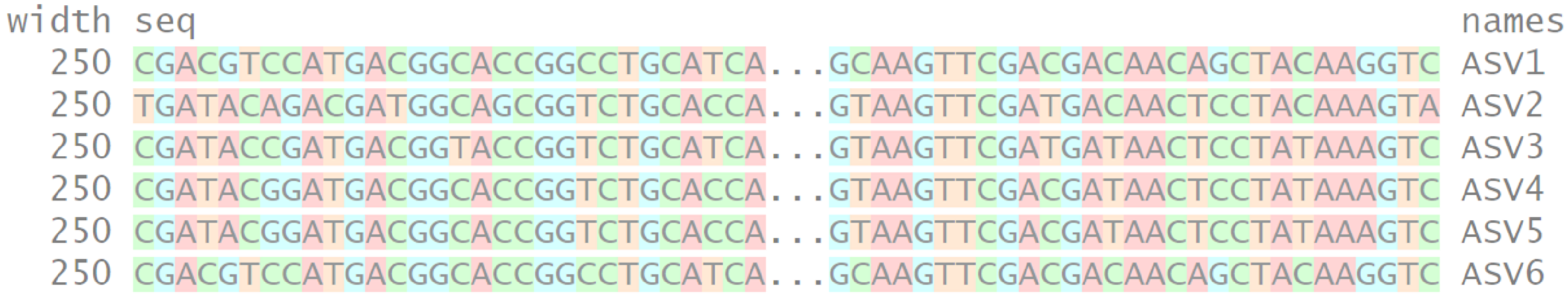


```
> cleaned_sequences
```

here from sequencing of a gyrB (bacterial marker gene) reads library

6. Cleaning

Amplicon
Sequence
Variants



```
width seq                                                              names
  250 CGACGTCCATGACGGCACCGGCCTGCATCA...GCAAGTTCGACGACAACAGCTACAAGGTC ASV1
  250 TGATACAGACGATGGCAGCGGTCTGCACCA...GTAAGTTCGATGACAACTCCTACAAAGTA ASV2
  250 CGATACCGATGACGGTACCGGTCTGCATCA...GTAAGTTCGATGATAACTCCTATAAAGTC ASV3
  250 CGATACGGATGACGGCACCGGTCTGCACCA...GTAAGTTCGACGATAACTCCTATAAAGTC ASV4
  250 CGATACGGATGACGGCACCGGTCTGCACCA...GTAAGTTCGACGATAACTCCTATAAAGTC ASV5
  250 CGACGTCCATGACGGCACCGGCCTGCATCA...GCAAGTTCGACGACAACAGCTACAAGGTC ASV6
```

(...)

7. Taxonomic attribution

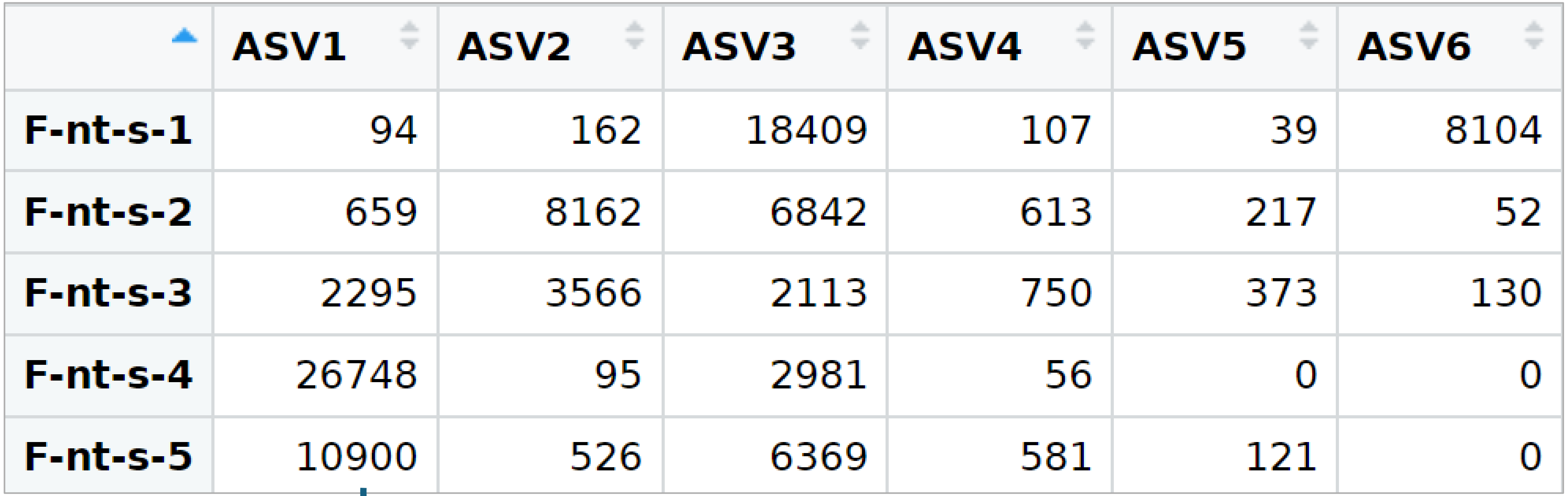
```
> assigned_taxonomy
                 ASV1                   ASV2                  ASV3
  "Xanthomonas_citri" "Pseudomonas_amygdali" "Pantoea_agglomerans"
                 ASV4                   ASV5                  ASV6
  "Kosakonia_cowanii"       "Klebsiella_cf."   "Xanthomonas_citri"
```

(...)

Metabarcoding for analysis of seed-associated microbial communities

8. Formatting as table of abundances

Taxa



Assigned species	*Xanthomonas citri*	*Pseudomonas amygdali*	*Pantoea agglomerans*	*Kosakonia cowanii*	*Klebsiella cf.*	*Xanthomonas citri*	
	ASV1	**ASV2**	**ASV3**	**ASV4**	**ASV5**	**ASV6**	(...)
F-nt-s-1	94	162	18409	107	39	8104	
F-nt-s-2	659	8162	6842	613	217	52	
F-nt-s-3	2295	3566	2113	750	373	130	
F-nt-s-4	26748	95	2981	56	0	0	
F-nt-s-5	10900	526	6369	581	121	0	
(...)							

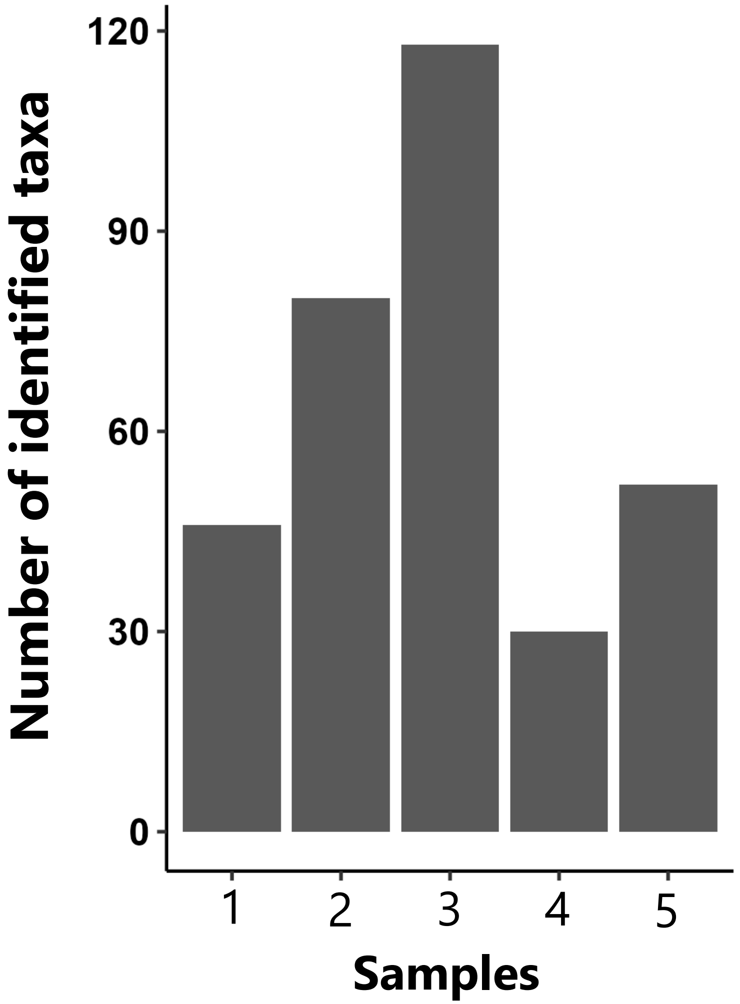
Samples

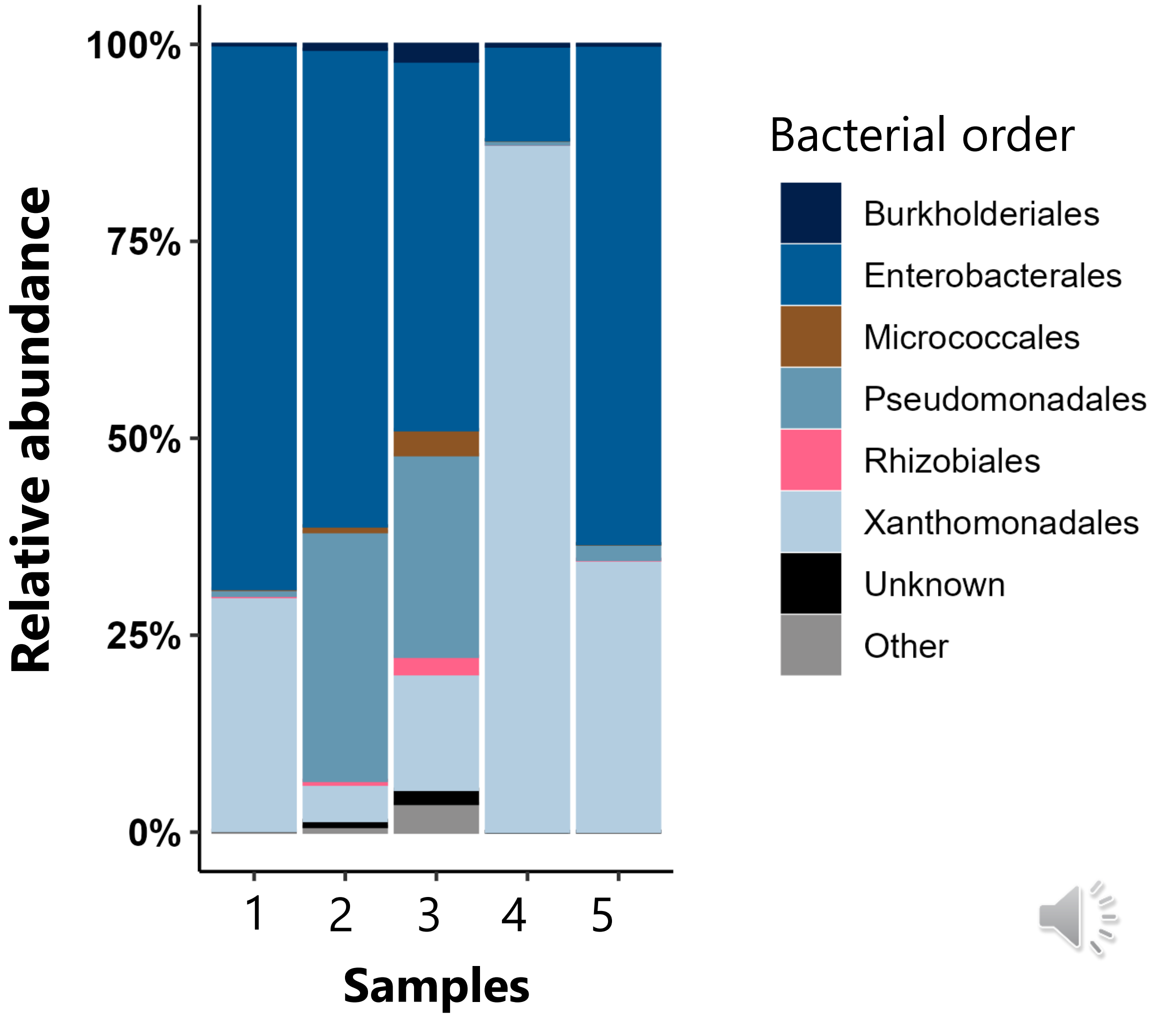
Number of reads

=> Taxon abundance in sample

Metabarcoding for analysis of seed-associated microbial communities

9. Visualisation and quantification





Example data from a 2022 experiment as part of the ITAB/Biau Germe research project *SemAVie* on microbial life for bean seed health

Metabarcoding on seed microbiota | key takeaways

- Metabarcoding analysis can extrapolate information on microbial community structure from soaked seed samples
- Taking its biases into account is essential for reliable interpretations of results

LiveSeeding

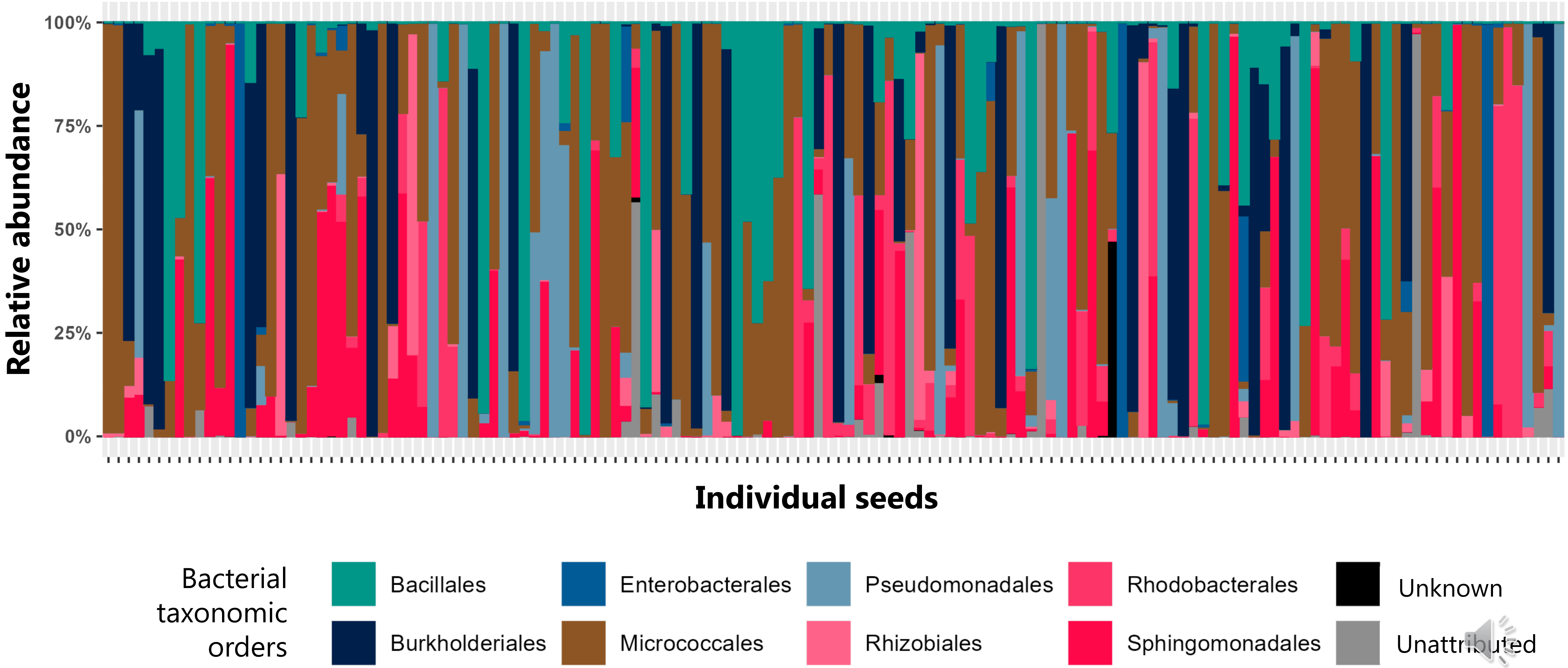
Example

What kind of bacterial communities do individual bean seeds host ?

Guillaume Chesneau et al. 2022

Common bean cv. Flavert
Collected 50 days after pollination

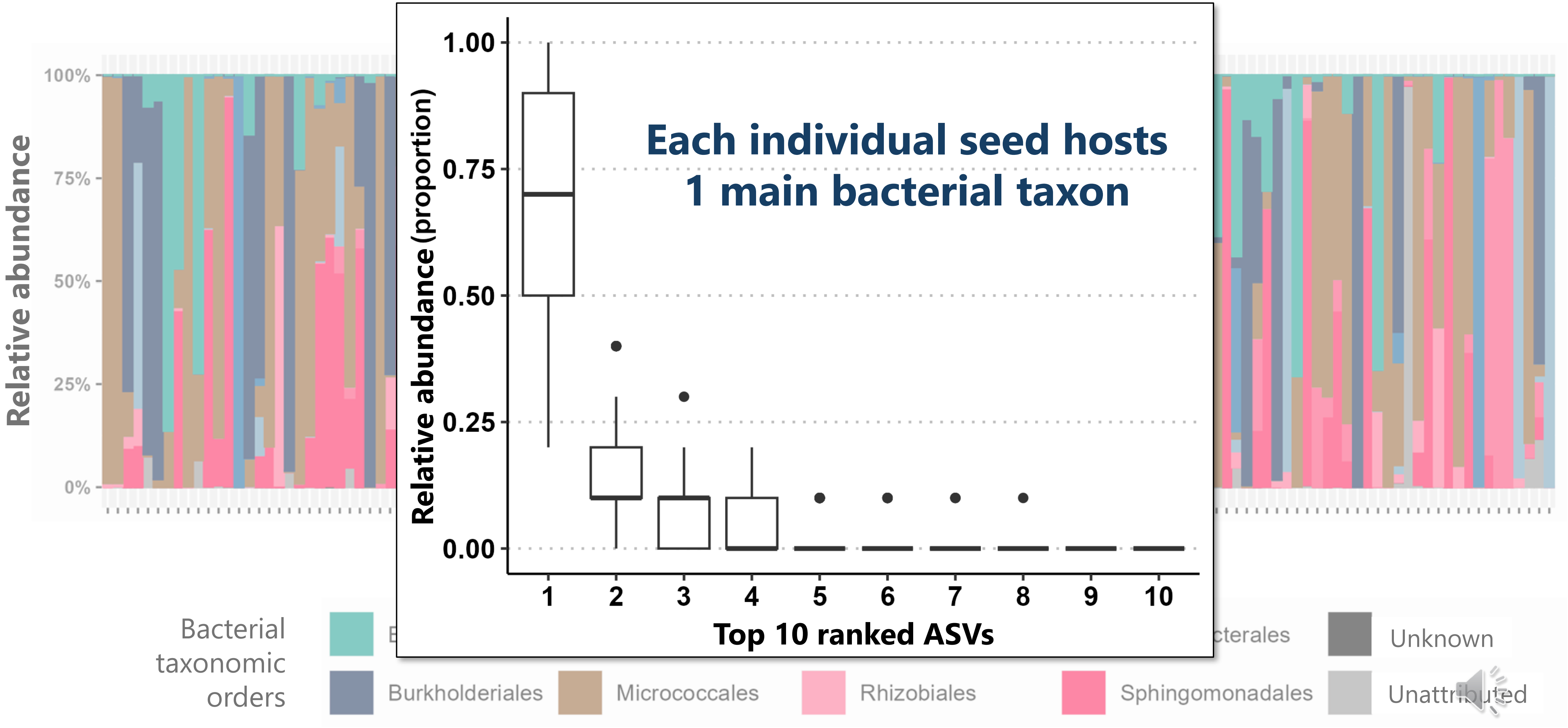
1 Bean seed microbiota : **Individual bacterial assemblies**



n = 144 seeds

(*Chesneau et al. 2022*)

② Bean seed microbiota : **Individual bacterial assemblies**



Each individual seed hosts 1 main bacterial taxon

n = 144 seeds

(*Chesneau et al. 2022*)

Single bean seed microbiota | key takeaways

- The bacterial communities of individual bean seeds are characterised by **highly variable low-richness** communities, with **1 main taxon** making up most of the composition

Example

What patterns can be observed at a statistical level in the seed microbiota of different plant species?

Marie Simonin et al. 2021

IRHS Institut de Recherche en Horticulture et Semences

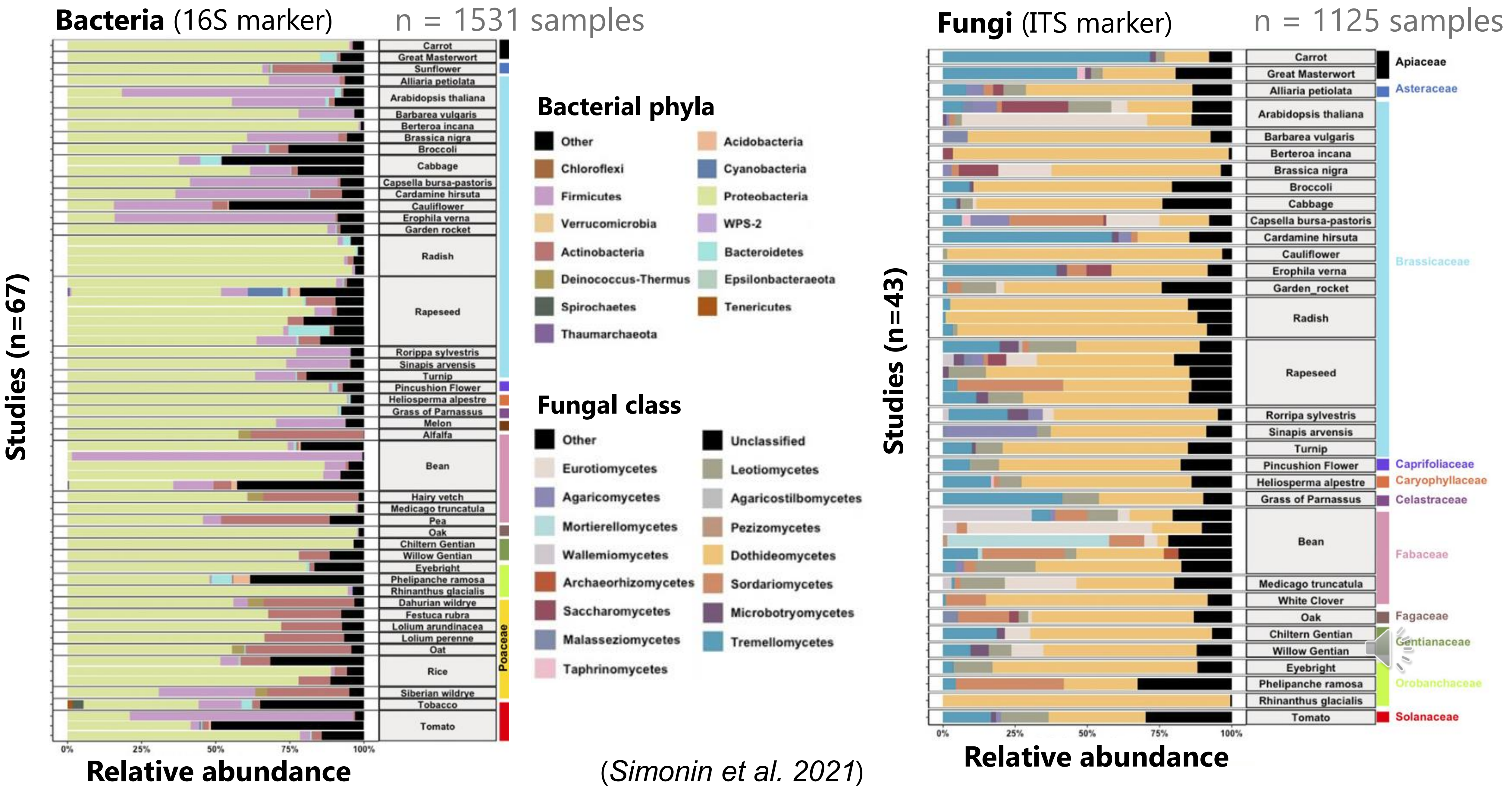
INRAE

L'INSTITUT agro Rennes Angers

université angers

LiveSeeding

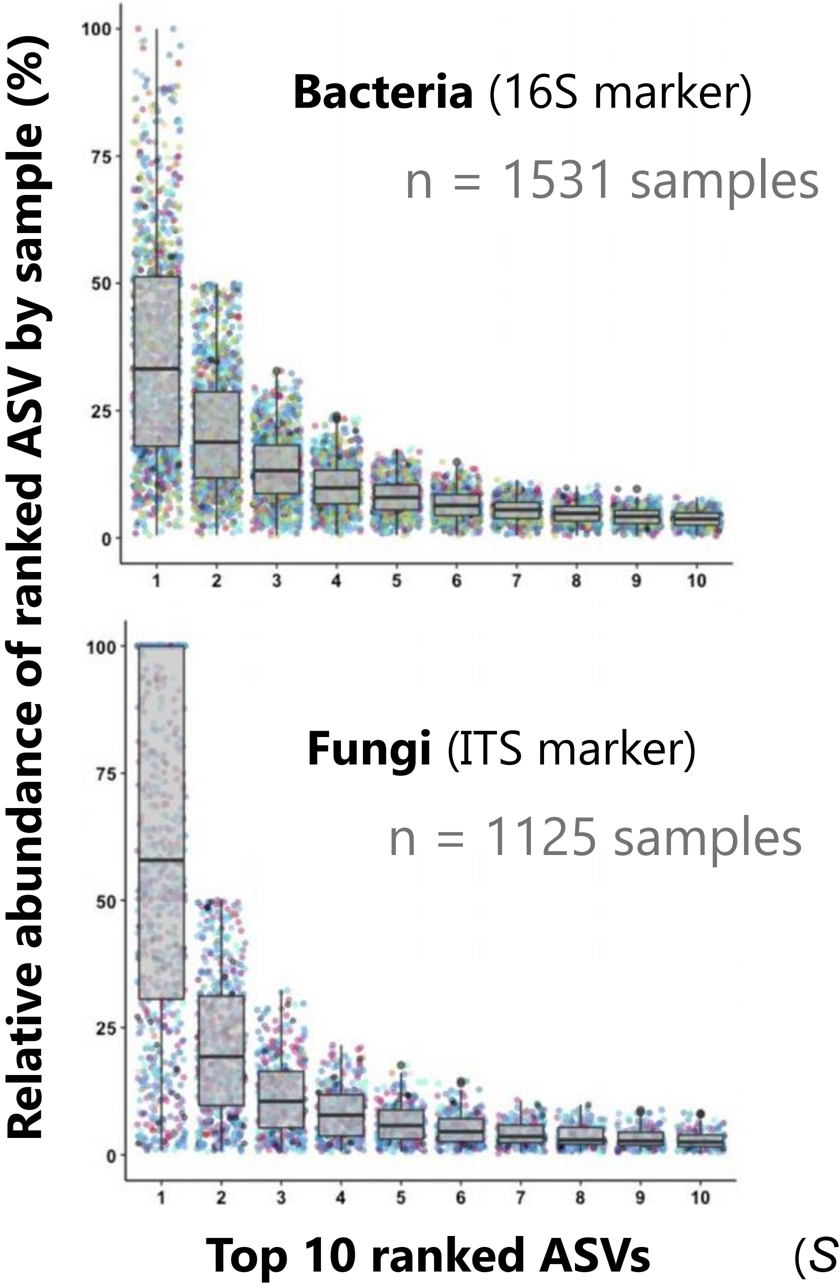
1 Seed microbiota : **Patterns across plant host species**



(*Simonin et al. 2021*)

2

Seed microbiota : **Patterns across plant host species**



- Seed microbiota are diverse and **extremely variable** in structure
- Significant **influence of the host plant** is noted on the diversity and structure of seed microbiota
- Seeds are **selective** habitats :
  - A single taxon tends to make up most of their observed composition

(*Simonin et al. 2021*)

Closing messages

Plants can be viewed as **holobionts**, defined in association with the micro-organisms inhabiting their compartments

Seeds are an essential step in these holobionts’ life cycles

Metabarcoding analyses allow for the study of seed microbial life but come with inherent **precautions**

Seed bacterial and fungal communities are **highly specific**

Plant breeding and seed multiplication may gain **robustness** by considering **associated microbial life**

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Scientific project management : Monika Messmer (FiBL-CH)

Project leader: Mariano Iossa (FIBL-EU)

Designer of training modules on the production of high-quality organic seed (modules 11 to 18) : Stephanie Klaedtke and Stéphanie Mothes (ITAB)

Authors of videos, questionnaires, exercices : module 11 Stephanie Klaedtke (ITAB), modules 12 et 13 Steven Groot (WUR & International Seed Academy), module 14 Gaspard de Tournemire and Stephanie Klaedtke (ITAB), Jan Kodde (WUR) and Valentin Gfeller (FiBL-CH) module 15 Jelena Baćanović-Šišić (Bingenheimer Saatgut AG) module 16 Mária Megyeri module 17 Györéné Kis Gyöngyi (ÖMKi) and Diego Guidotti (Aedit SRL) module 18 Maike Bender and Carl Vollenweider (Dottenfelderhof)

LiveSeeding

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Become a stakeholder

FiBL Europe

itab l'Institut de l'agriculture et de l'alimentation biologiques

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PIN

AEGILOPS Greek Network for Biodiversity and Ecology in Agriculture

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Louis Bolk Instituut

KWS

IFOAM ORGANICS EUROPE

Rete Semi Rurali

FiBL Germany

SEMENTES VIVAS

OIKOS SEEDS

WAGENINGEN UNIVERSITY & RESEARCH

crea Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria

pro specie rara

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