ISTA-ISSS webinar

Seed pathology - the good and the bad microbes

Uncovering the seed microbiota diversity and its role for seedling health using synthetic ecology

Louna COLAERT-SENTENAC

Gontran ARNAULT, Aurélie CHARRIER, Didier DEMILLY, Audrey DUPONT, Anaïs HARDOUIN, Laurence LE CORRE, Coralie MARAIS, Anne PRÉVEAUX, Marie-Hélène WAGNER, Guillaume TCHERKEZ, Béatrice TEULAT, Élisabeth PLANCHET, Marie SIMONIN

louna.colaert-sentenac@inrae.fr

International Seed Testing Association · Uniformity in Seed Testing

isss

SUCSEED

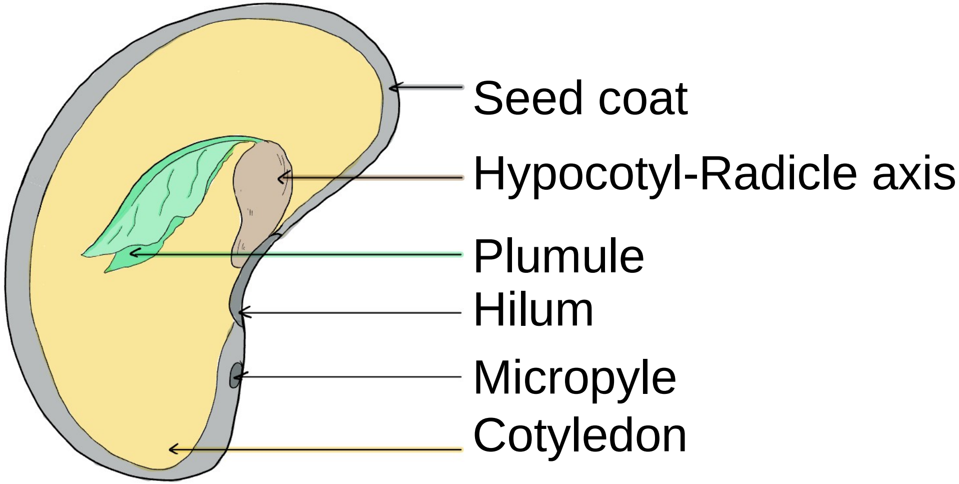
IRHS Institut de Recherche en Horticulture et Semences

université angers

L'institut agro Rennes Angers

INRAE la science pour la vie, l'humain, la terre

Seeds are not alone : the Holobiont



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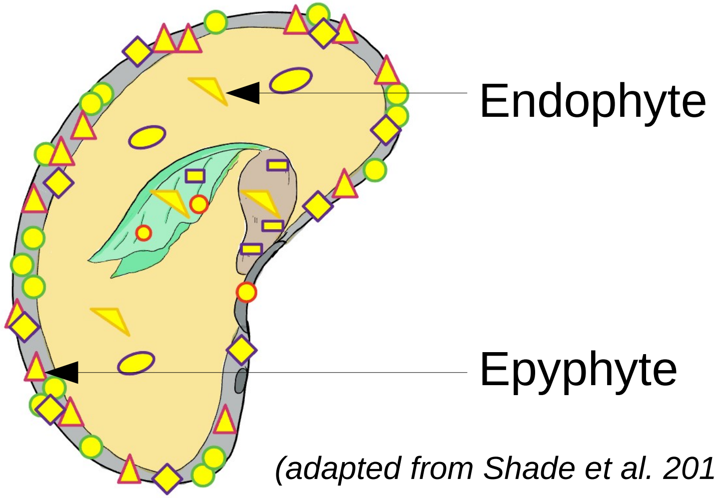
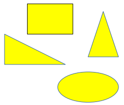


(adapted from Shade et al. 2017)



Bacteria,
Fungi,
Archaea…

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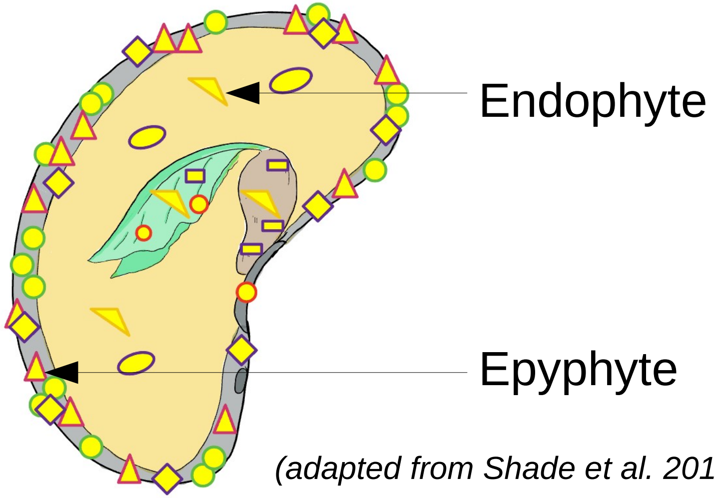

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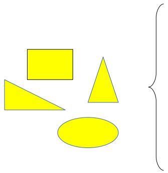

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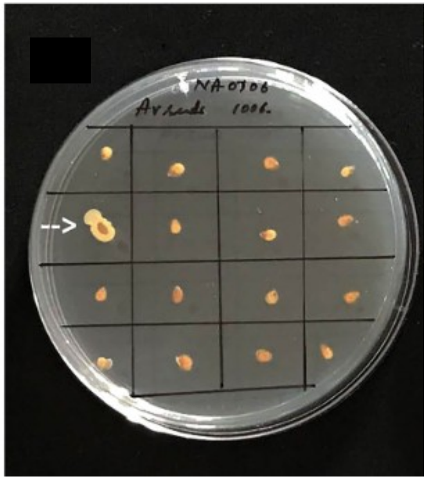
[redacted]

Surface-sterilized seeds cultivated on enriched nutrient agar
(Thomas & Shaik, PMI 2019)

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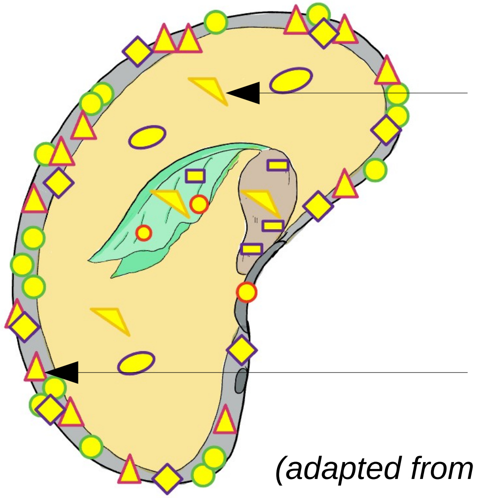
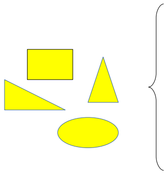


Endophyte

Epyphyte

(adapted from Shade et al. 2017)



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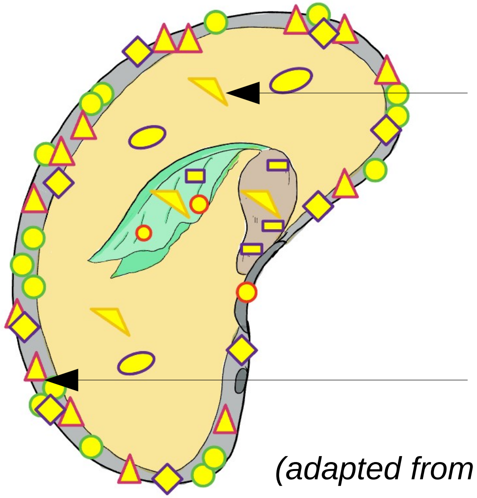

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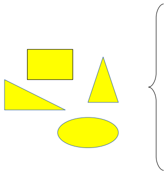

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Pathogenic

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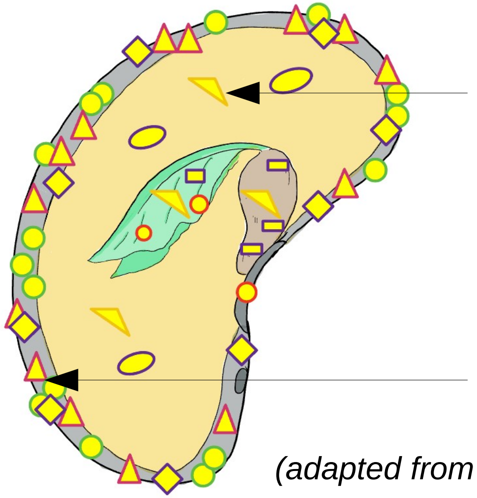

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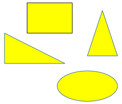

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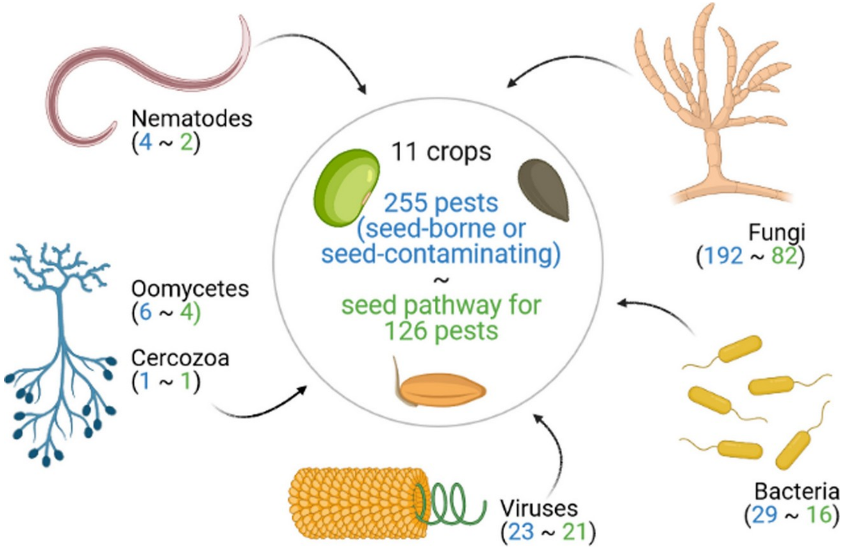

Denanté, Grimault 2022

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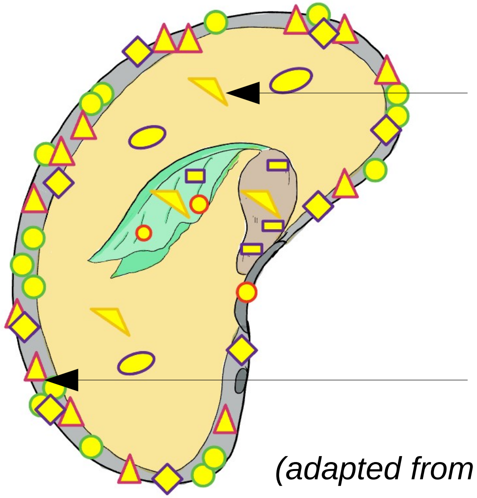

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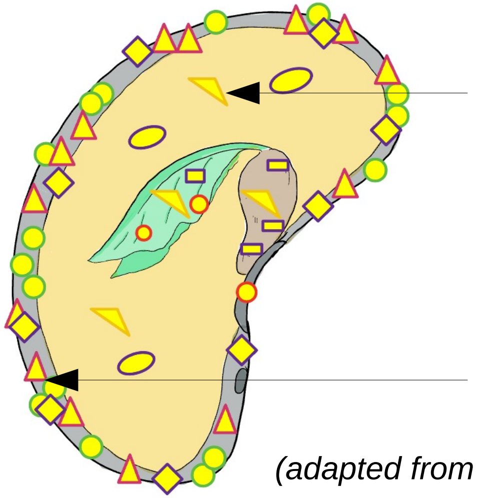

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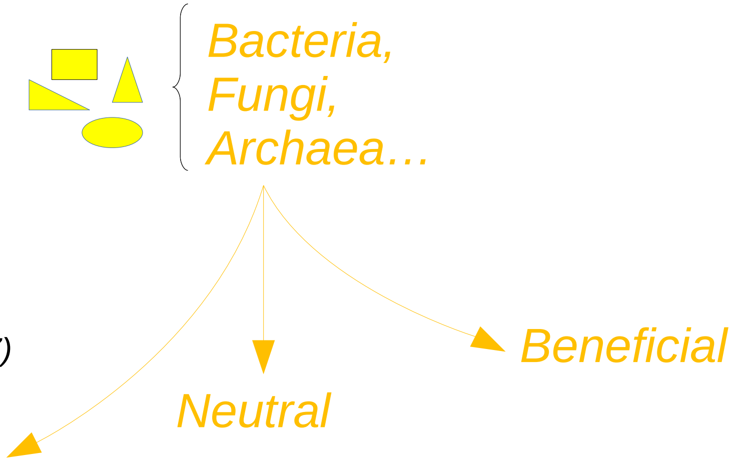

Bacteria,
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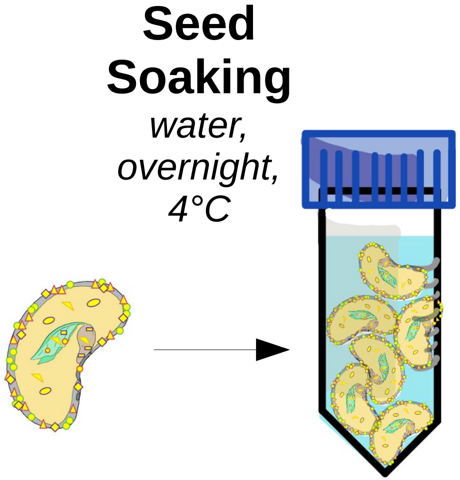
Archaea…

Pathogenic

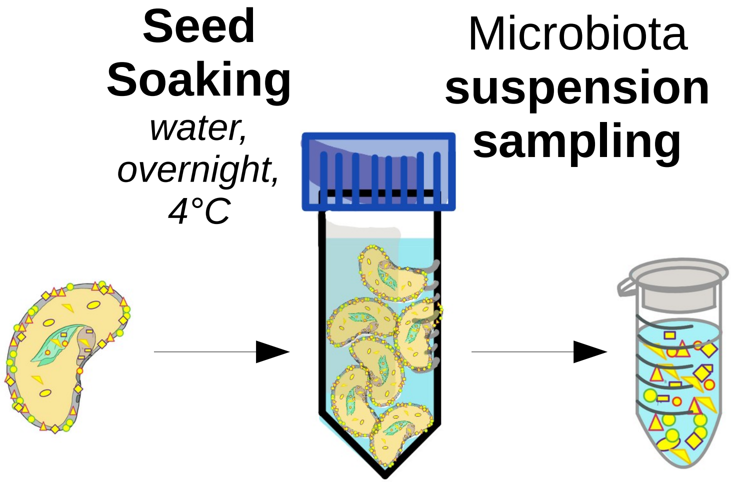
Neutral

Beneficial

Studying seed microbiota


Seed Soaking
water, overnight, 4°C

Studying seed microbiota



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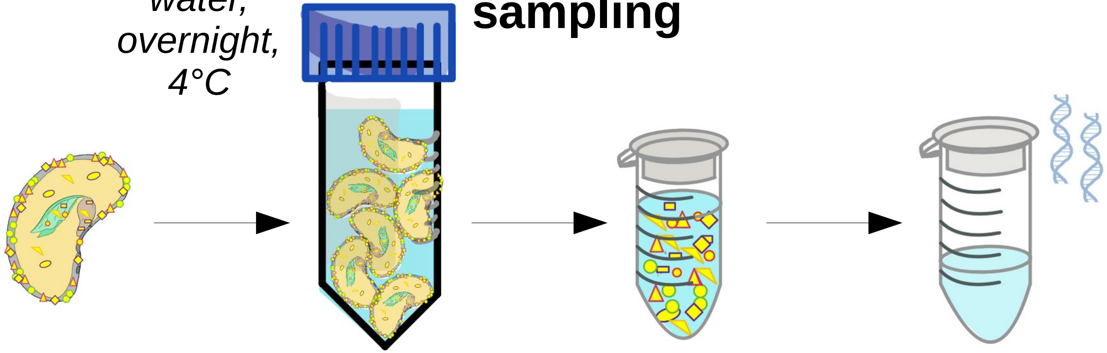
Microbiota **suspension sampling**

Studying seed microbiota



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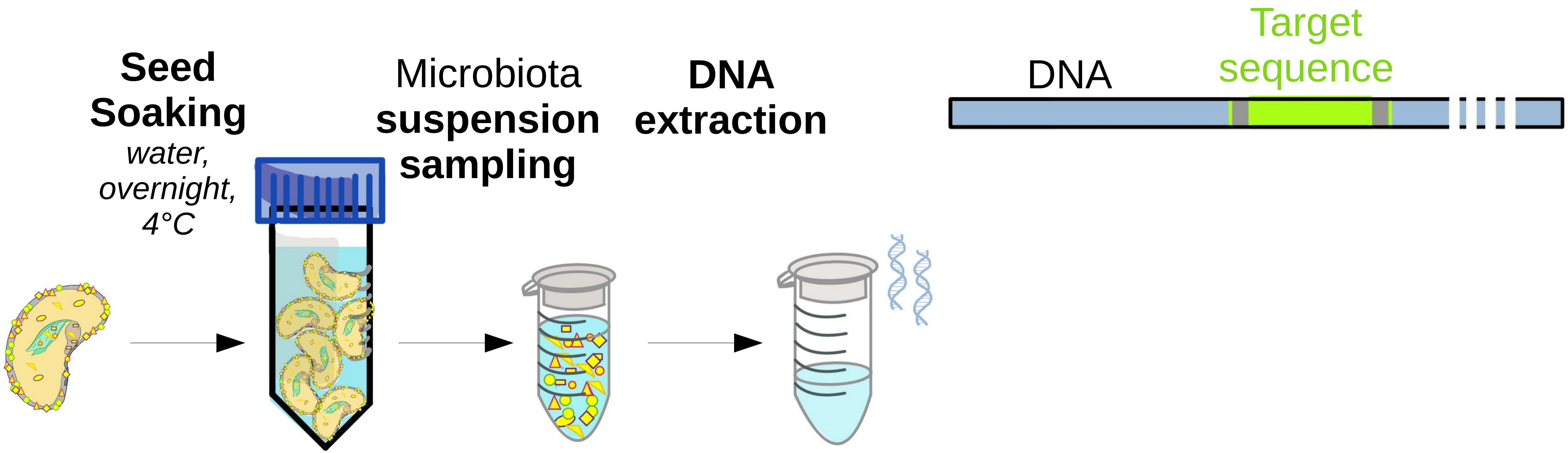
Microbiota **suspension sampling**

DNA extraction

Studying seed microbiota



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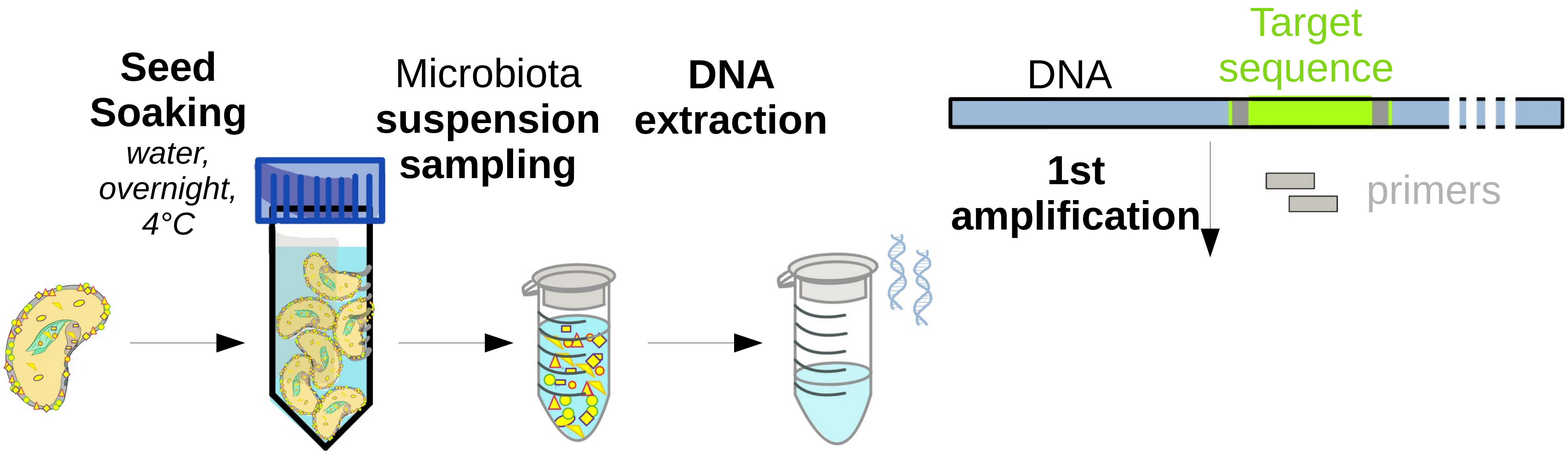
Microbiota **suspension sampling**

DNA extraction

DNA

Target sequence

Studying seed microbiota



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DNA extraction

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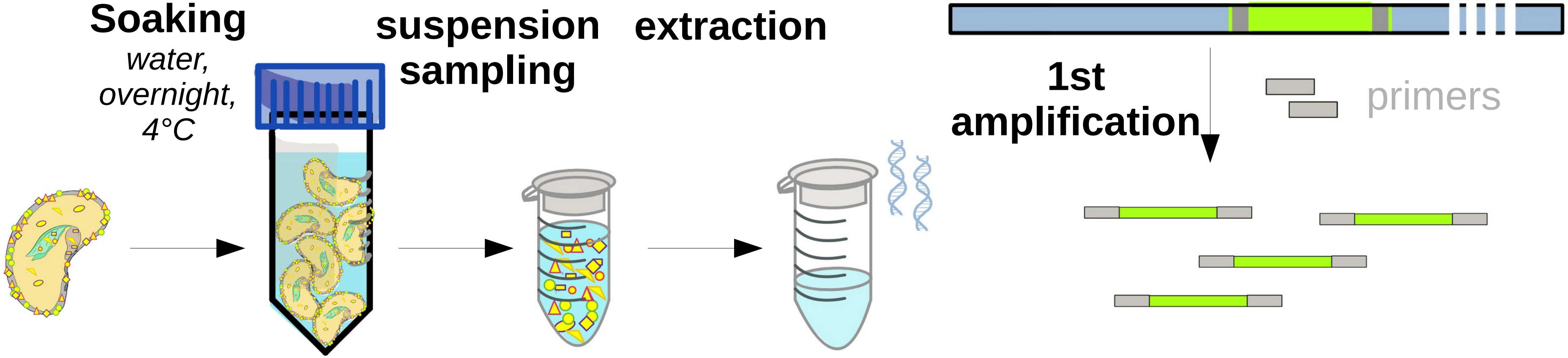
Target sequence

1st amplification

primers

Studying seed microbiota



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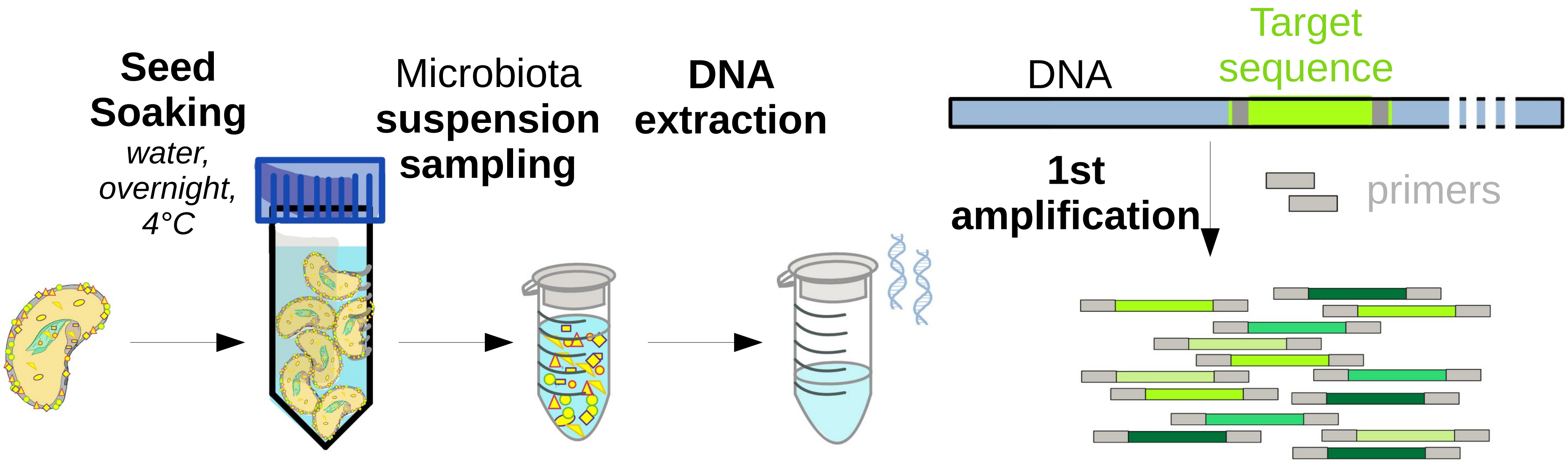

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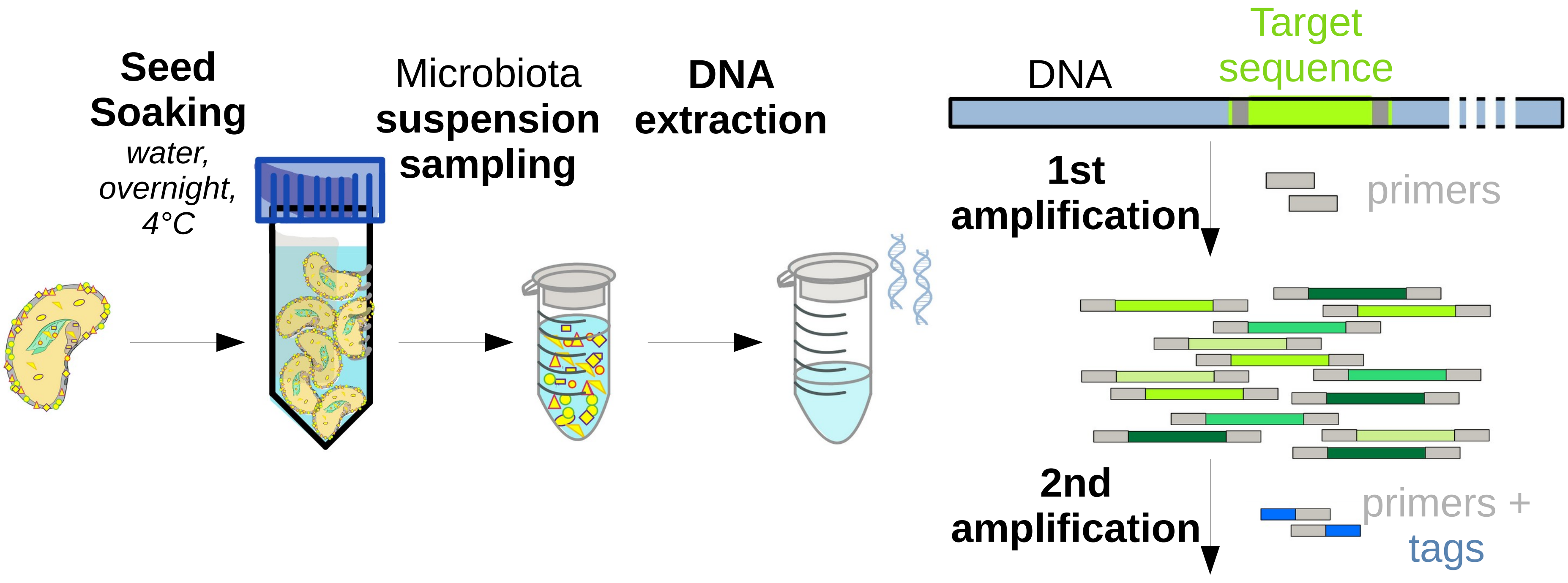
Target sequence

1st amplification

primers

ASVs: Amplicon
Sequence
Variants

Studying seed microbiota



Seed Soaking
water, overnight, 4°C

Microbiota **suspension sampling**

DNA extraction

DNA

Target sequence

1st amplification

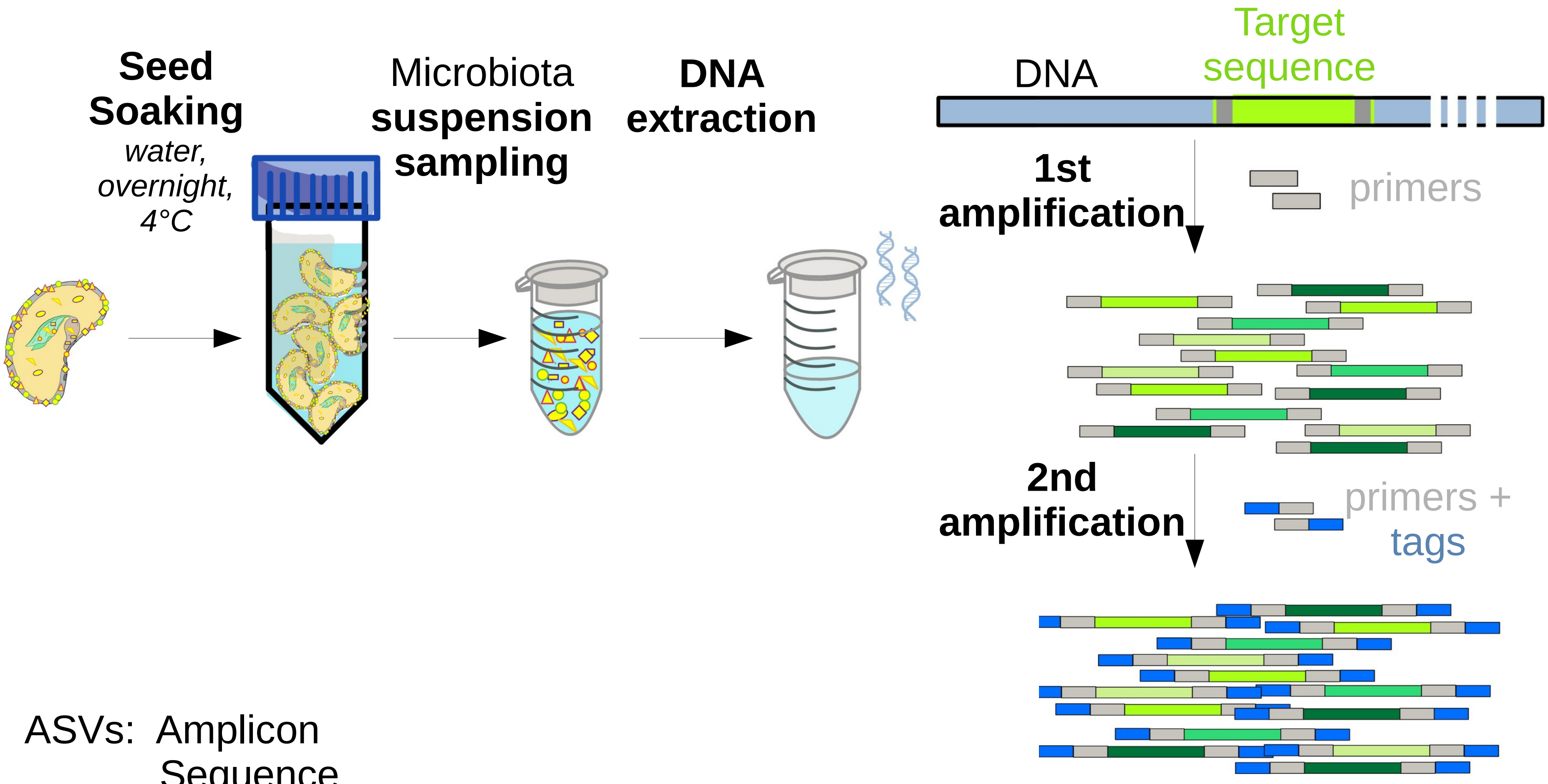
primers

2nd amplification

primers + tags

ASVs: Amplicon
Sequence
Variants

Studying seed microbiota



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Microbiota **suspension sampling**

DNA extraction

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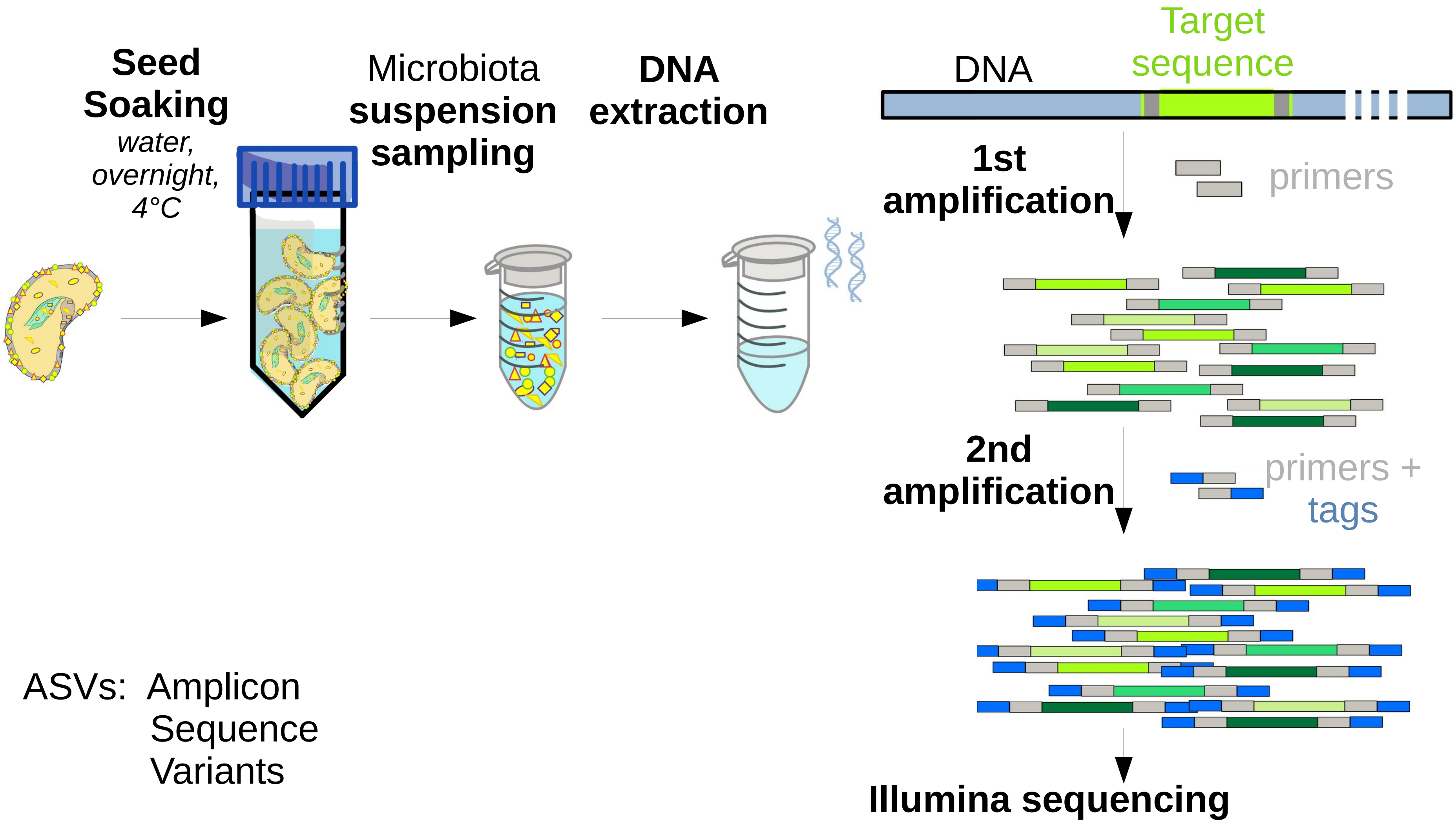
Studying seed microbiota



Seed Soaking
water, overnight, 4°C

Microbiota **suspension sampling**

DNA extraction

DNA
Target sequence

1st amplification
primers

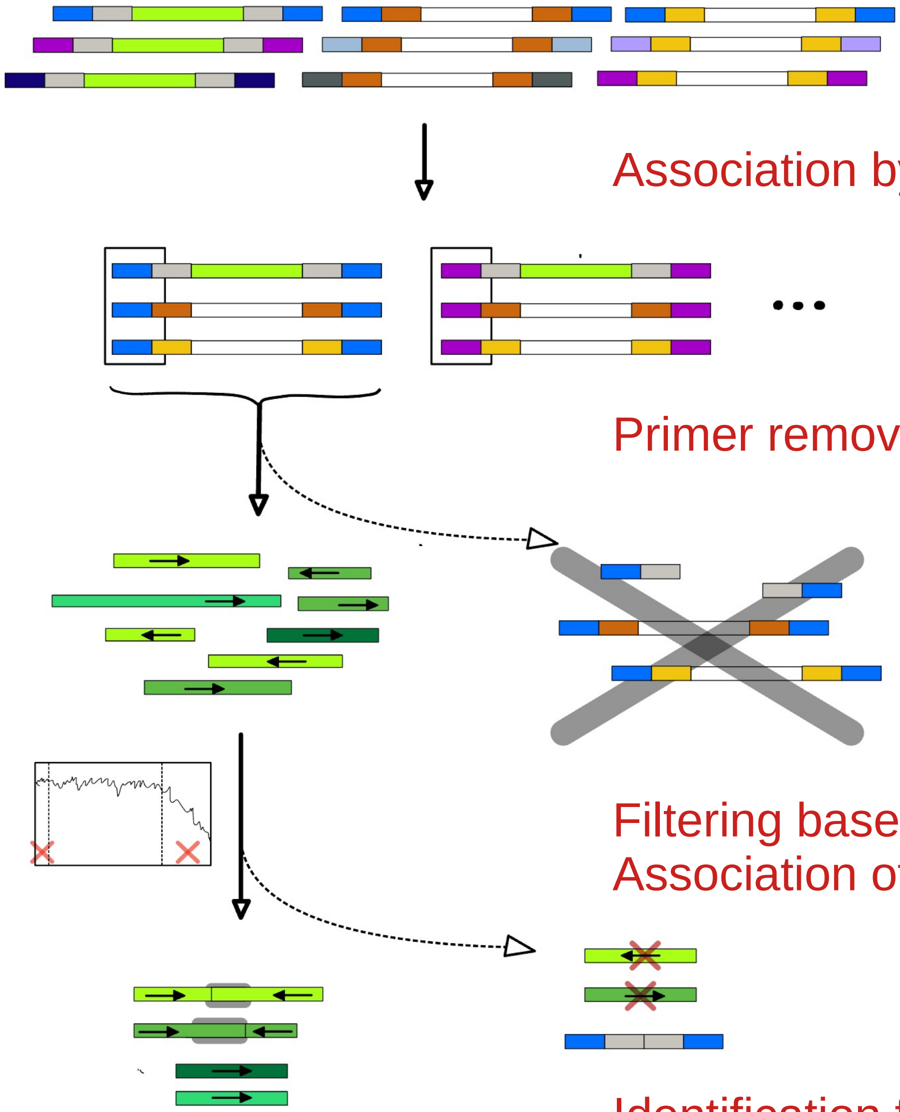
2nd amplification
primers + tags

Illumina sequencing

ASVs: Amplicon Sequence Variants

Studying seed microbiota

(Informatic workflow)



Association by tag=sample

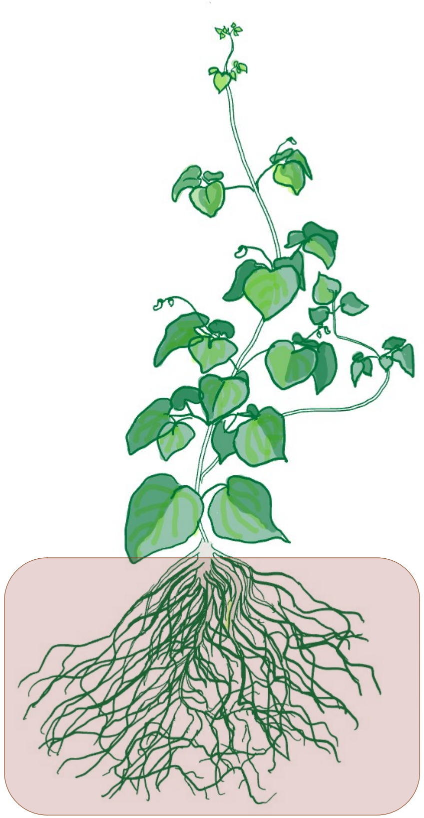
Primer removal

Filtering based on quality
Association of sequences

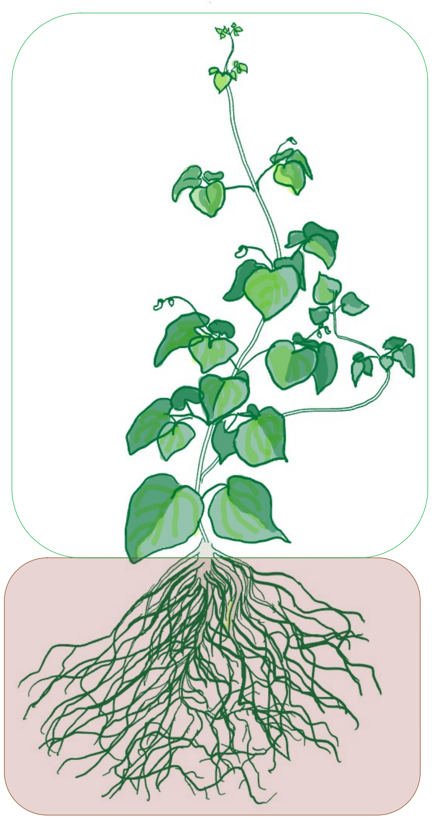
Identification from library

ASVs: Amplicon
Sequence
Variants

Seed: a neglected compartment for plant microbiota



Seed: a neglected compartment for plant microbiota

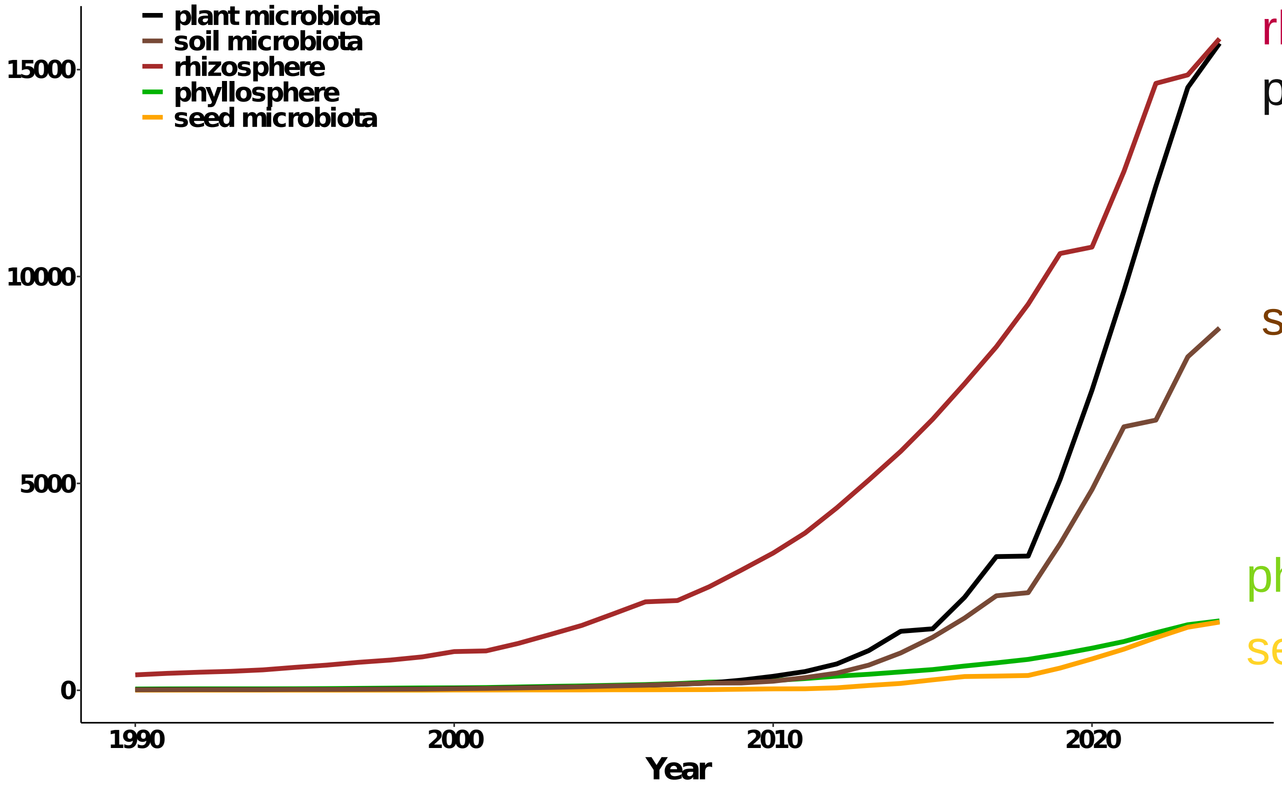


Phylosphere

Rhizosphere

Seed: a neglected compartment for plant microbiota

Cumulated number of articles published on the microbiota of plant compartments



rhizosphere

plant microbiota

soil microbiota

phylosphere

seed microbiota

based on PubMed, by Gontran Arnault

Seed: a neglected compartment for plant microbiota

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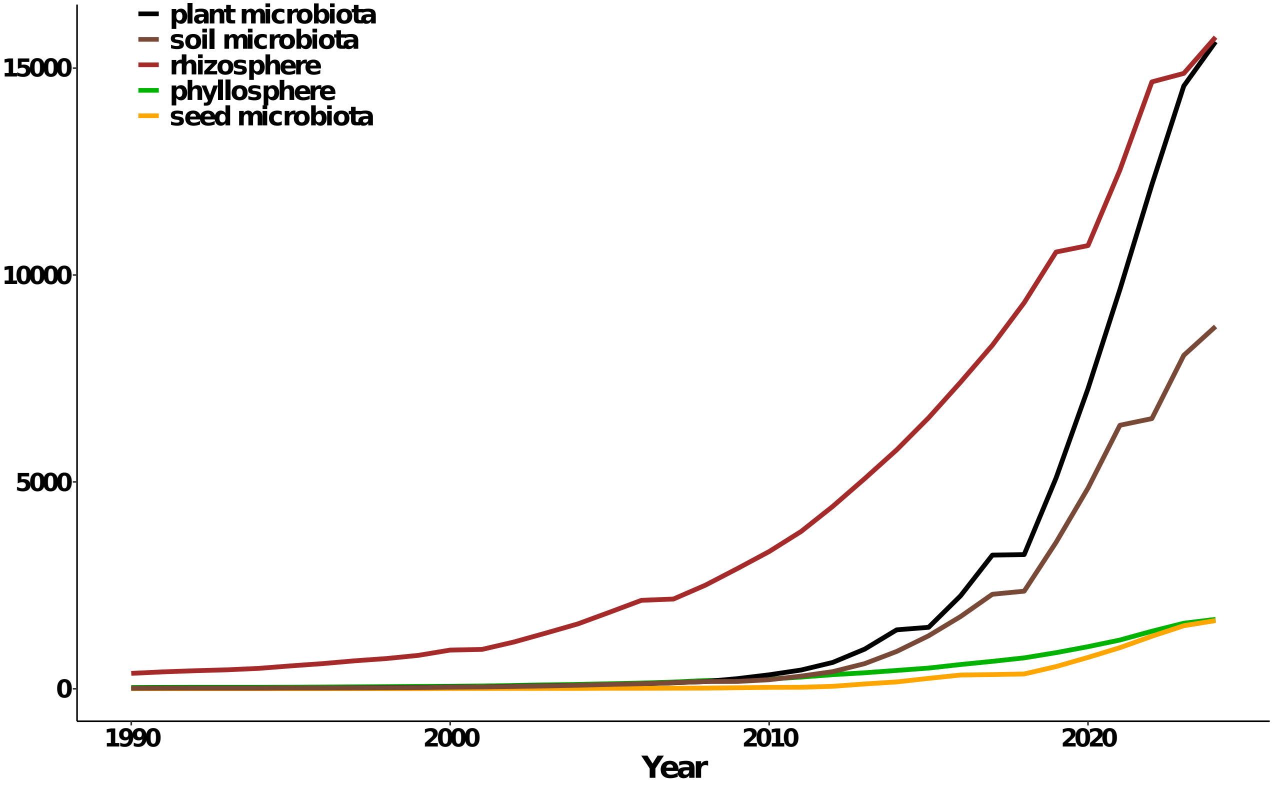

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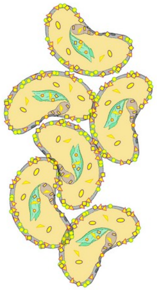
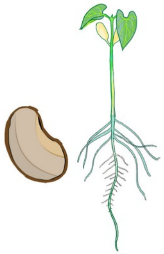
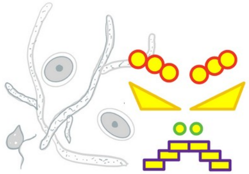
Why?



Low biomass

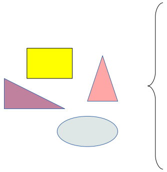


Plant DNA

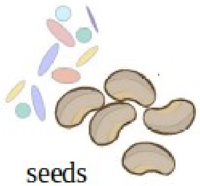


Focus on pathogens

Why is seed microbiota important?



Bacteria,
Fungi,
Archaea…



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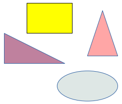

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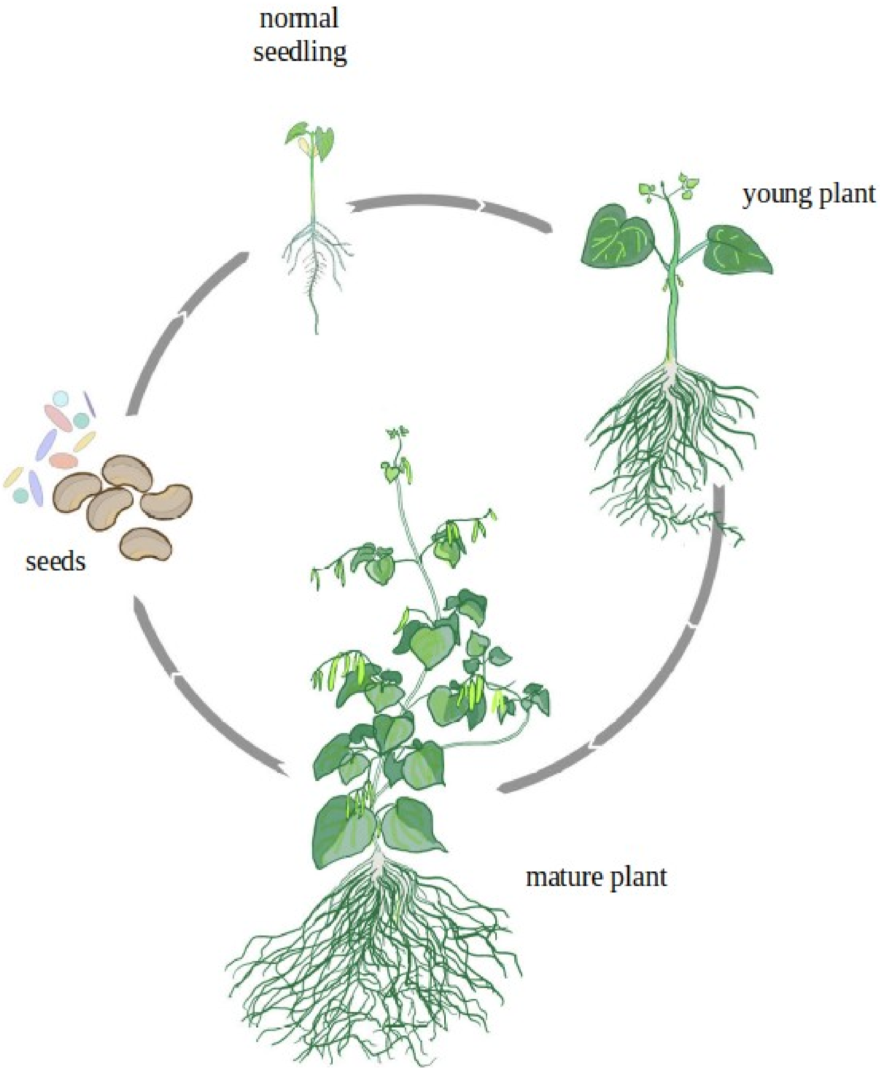
First plant inoculum



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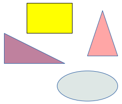

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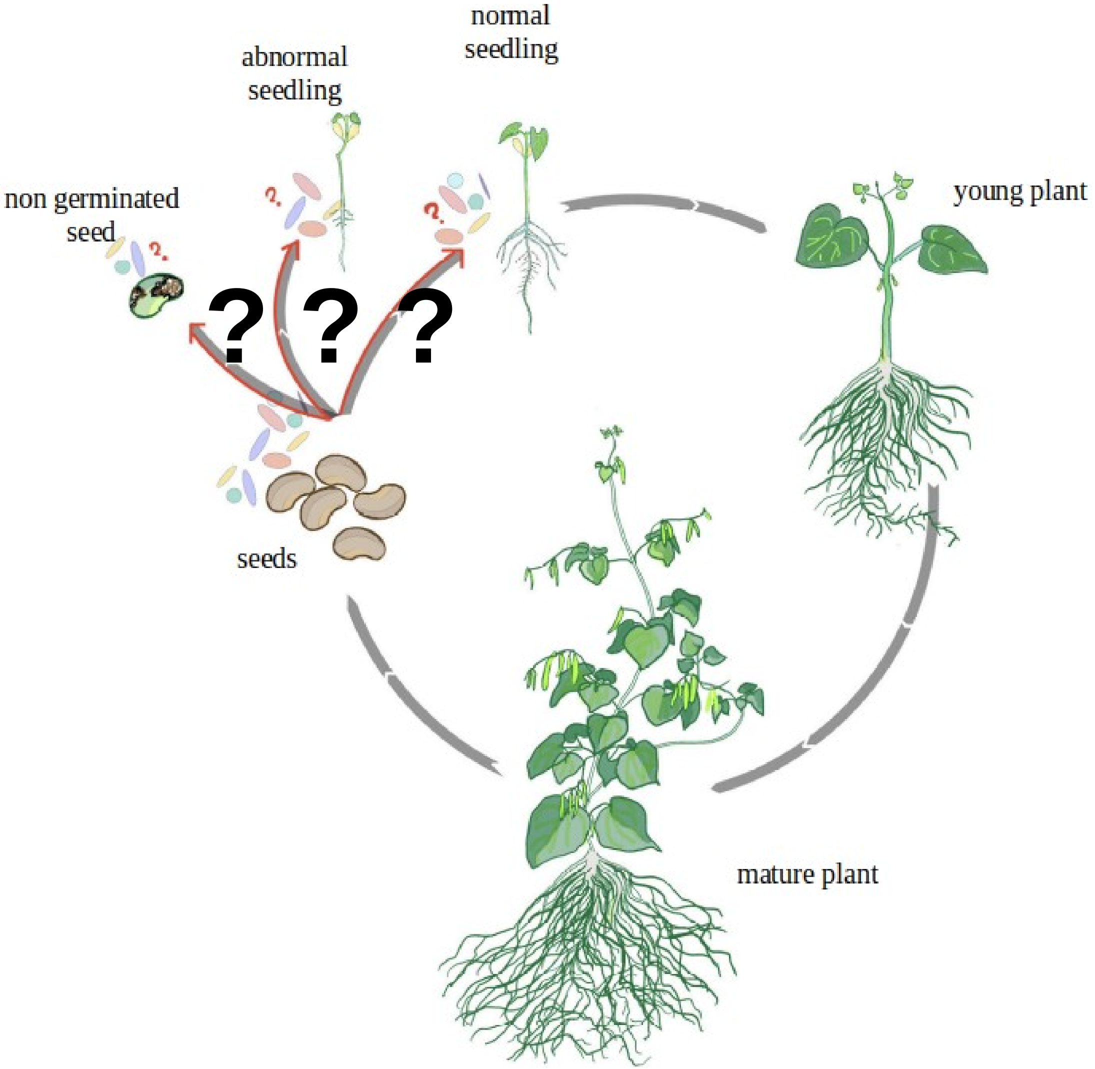
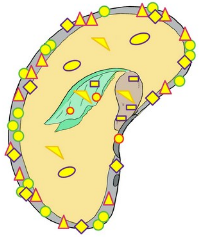


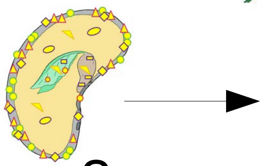
First plant inoculum

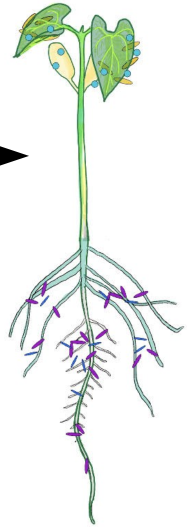
Outline

I. Seed native microbiota: Who is there?

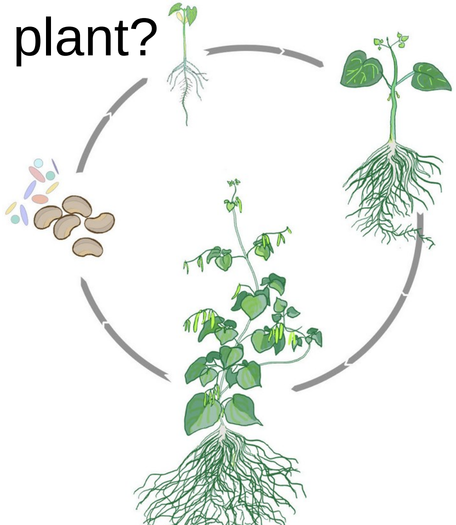


II.Seed microbiota: Does it impact seedling phenotype?
Can we use it to improve seed quality/seedling phenotype?





III.Synthetic microbiota: Is it transmitted to the plant?



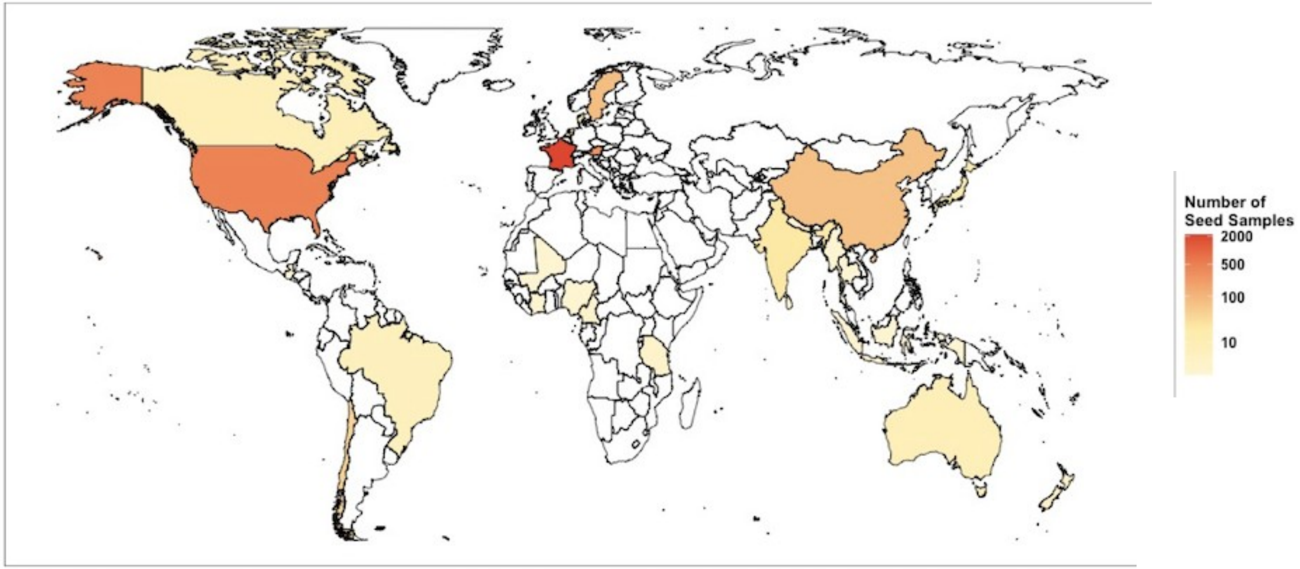
Who is there? A meta-analysis on seed batches

Simonin et al. 2022 New Phytol

Who is there? A meta-analysis on seed batches

Seed Microbiota Database

- 63 studies
- 28 countries
- **50 plant species**
- 3190 seed samples
- ASVs: 16S rRNA, gyrB, ITS region



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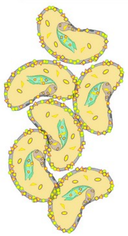

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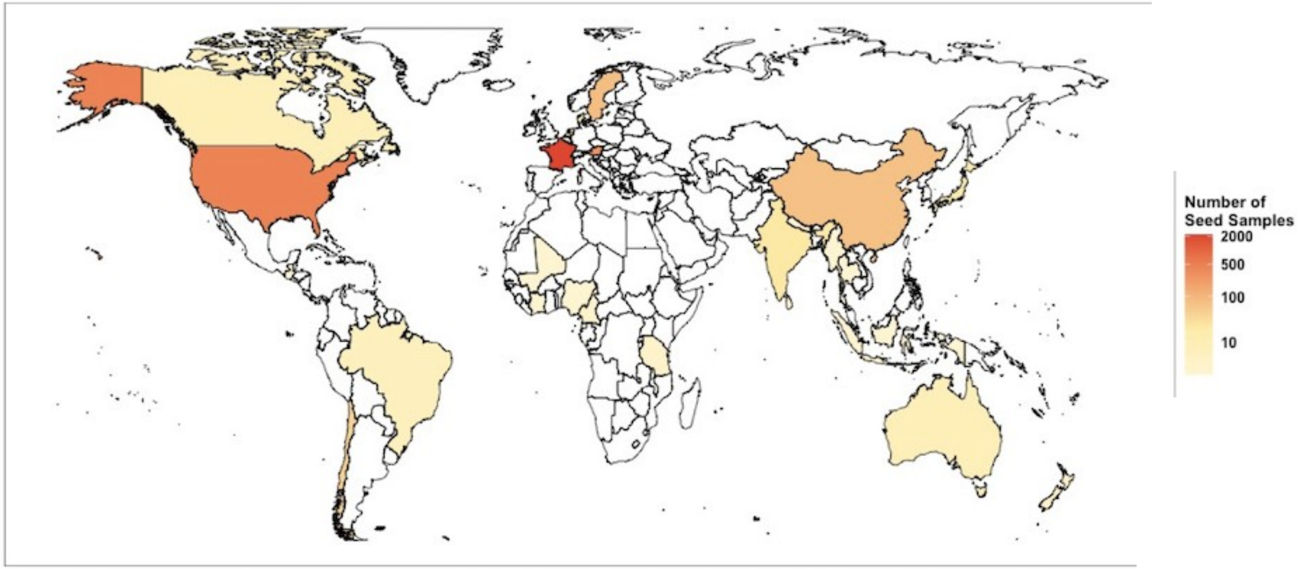

- Median per seed sample:
  48 bacterial taxa +
  52 fungal taxa
- **8200 bacterial taxa** (ASVs)
- **2100 fungal taxa** (ASVs)

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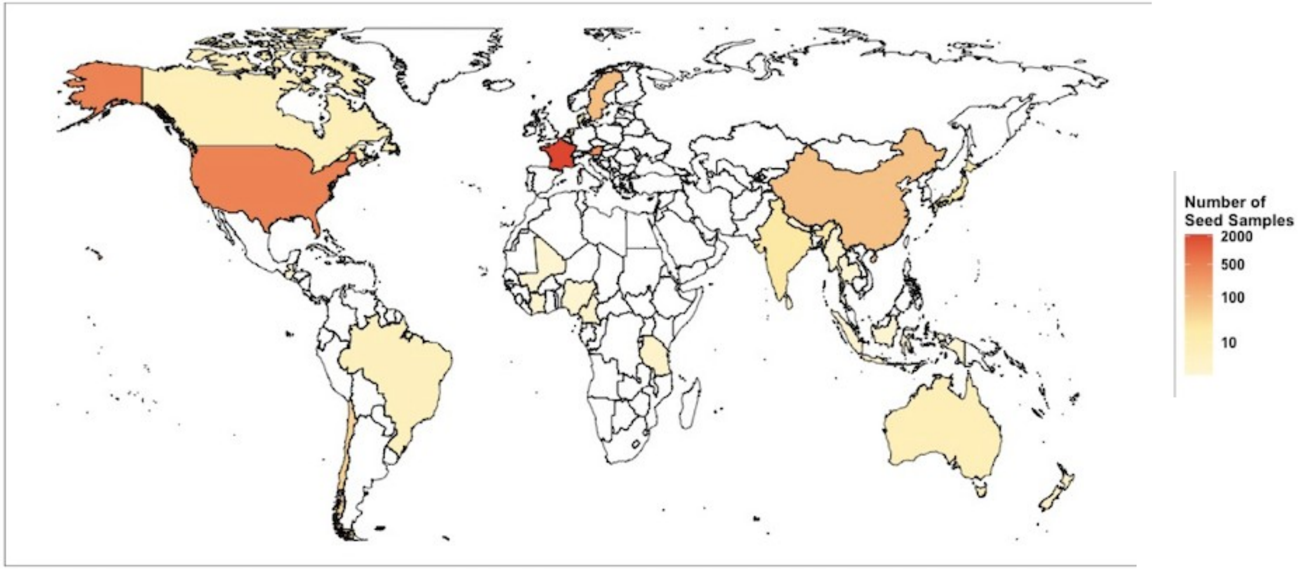

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Number of Plant Species Detected	Number of taxa (ASVs)	
	Bacteria	**Fungi**
1 species	**5257**	**1221**
2-4 species	2422	687
5-9 species	378	121
10-19 species	119	47
20-31 species	13	16

Simonin et al. 2022 New Phytol

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Core microbiota:

the **fraction of microbiota** found

- in a specified host regardless of the environmental conditions, or
- in a specific environment **regardless of the host species**

that is stable in terms of relative composition and across time.

(Shade et al. 2012)

Simonin et al. 2022 New Phytol

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- Pseudomonas
- Sphingomonas
- Rhizobium

- Cladosporium perangustum
- Capnodiales
- Alternaria metachromatica
- Vishniacozyma

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- Pantoea
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- Sphingomonas
- Rhizobium

13 most prevalent bacterial taxa represent ~**41%** of the relative abundance

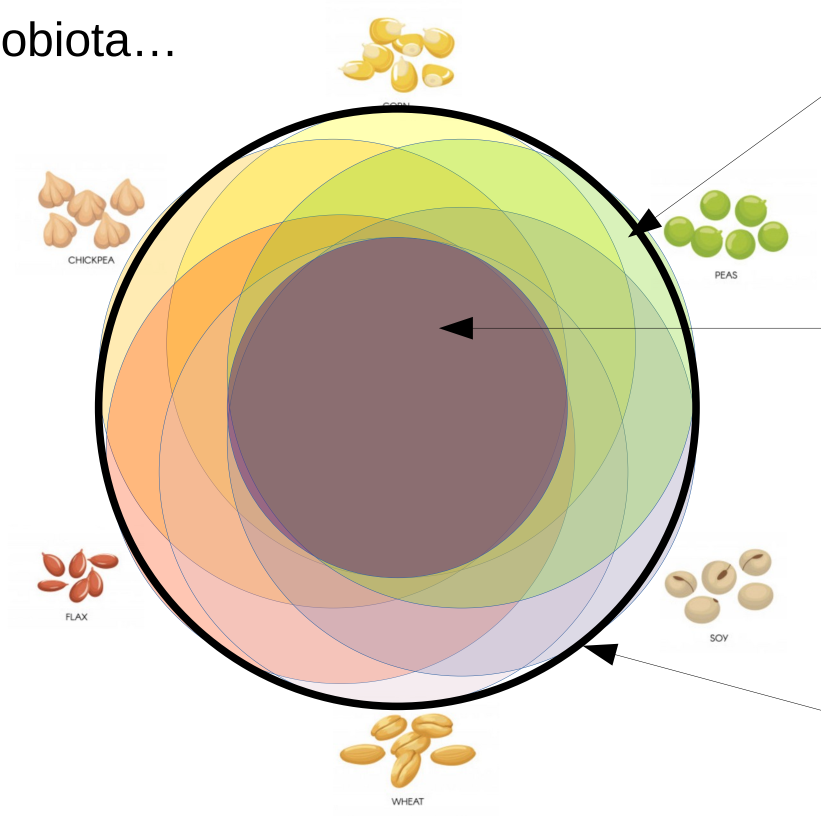
- Cladosporium perangustum
- Capnodiales
- Alternaria metachromatica
- Vishniacozyma

16 most prevalent fungal taxa represent **~47%** of the relative abundance

Simonin et al. 2022 New Phytol

Native microbiota: Who is there?

Seed microbiota…

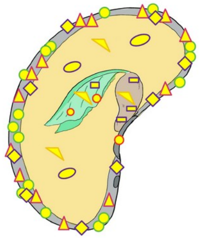


partly plant-specific

half core microbiota
(prevalent &
predominant)

Total : > 10 000 taxa

I. Seed native microbiota: Who is there?



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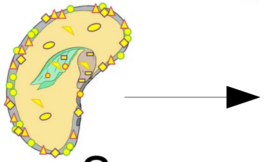
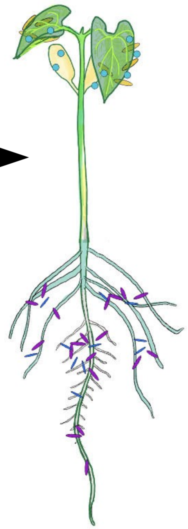
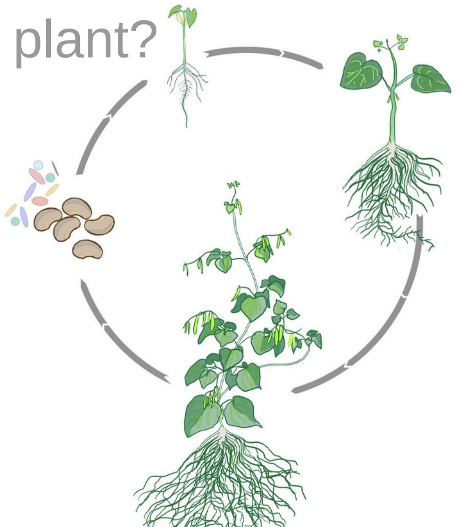




III. Synthetic microbiota: Is it transmitted to the plant?



Using seed microbiota to impact seed quality & seedling phenotype?

EUROPEAN
GREEN
DEAL

ÉCOPHYTO
RÉDUIRE ET AMÉLIORER
L'UTILISATION DES PHYTOS

SUCSEED
CULTIVER PROTÉGER autrement

"Stop the Use of Pesticides on Seeds"

Using seed microbiota to impact seed quality & seedling phenotype?

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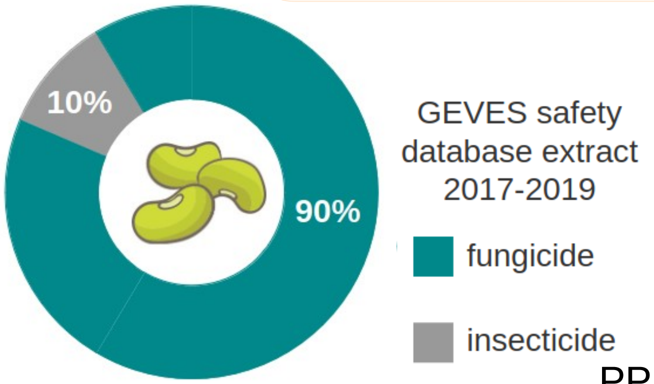

Using seed microbiota to impact seed quality & seedling phenotype?

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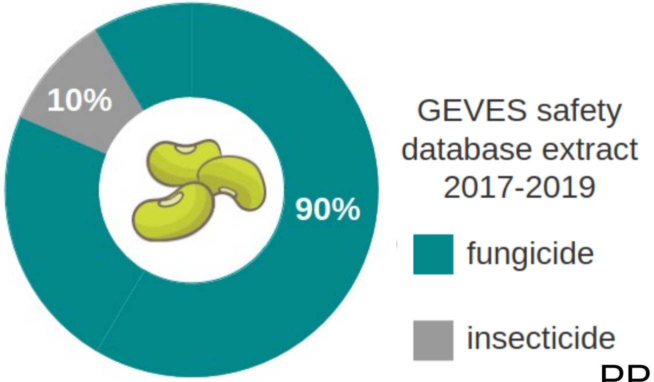
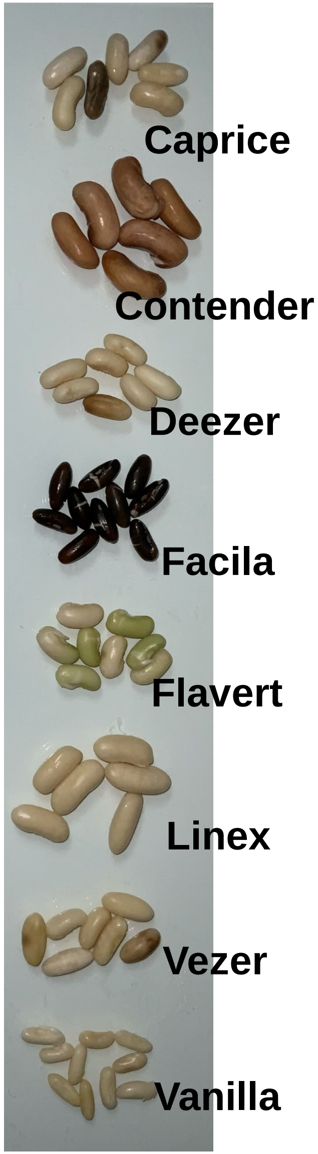

GEVES safety database extract 2017-2019

PPR SUCSEED – Coordinator M. BARRET – IRHS, Angers - 2020-2026

Focus on eight varieties of Common bean *Phaseolus vulgaris*

FNAMS experimental station (South West of France)



Does seed microbiota impact seed quality?

Seed quality: the seed ability to **germinate and emerge quickly** and **uniformly** once sown.

All germinate

germinate quickly

germinate simultaneously

germinate in various conditions

are resistant to stresses

produce normal seedlings

Does seed microbiota impact seed quality?

Seed quality: the seed ability to **germinate and emerge quickly** and **uniformly** once sown.

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germinate quickly

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germinate in various conditions

are resistant to stresses

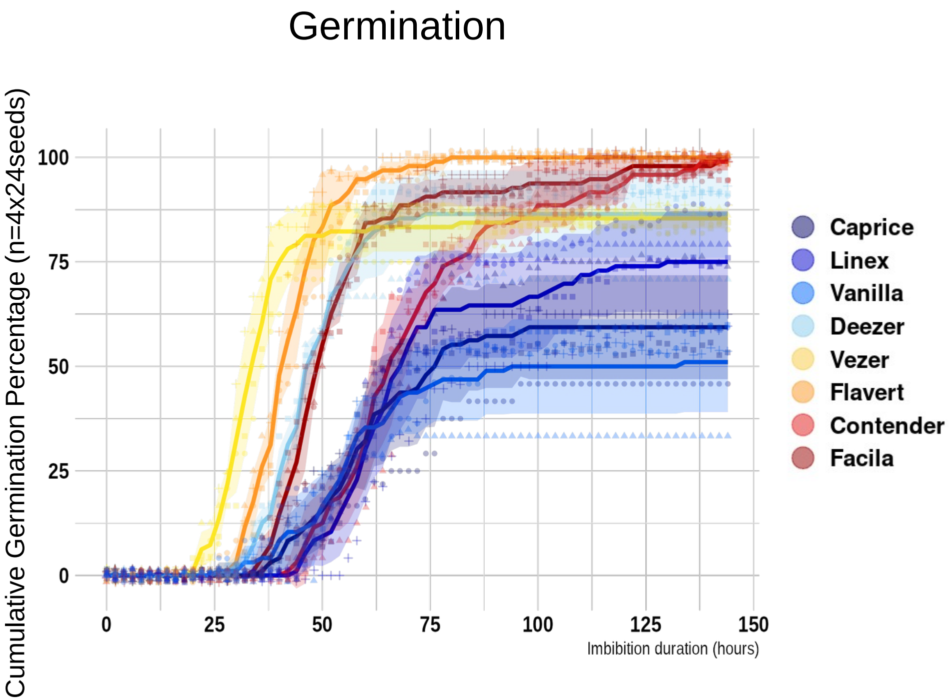
produce normal seedlings

Normal…

… and abnormal seedlings

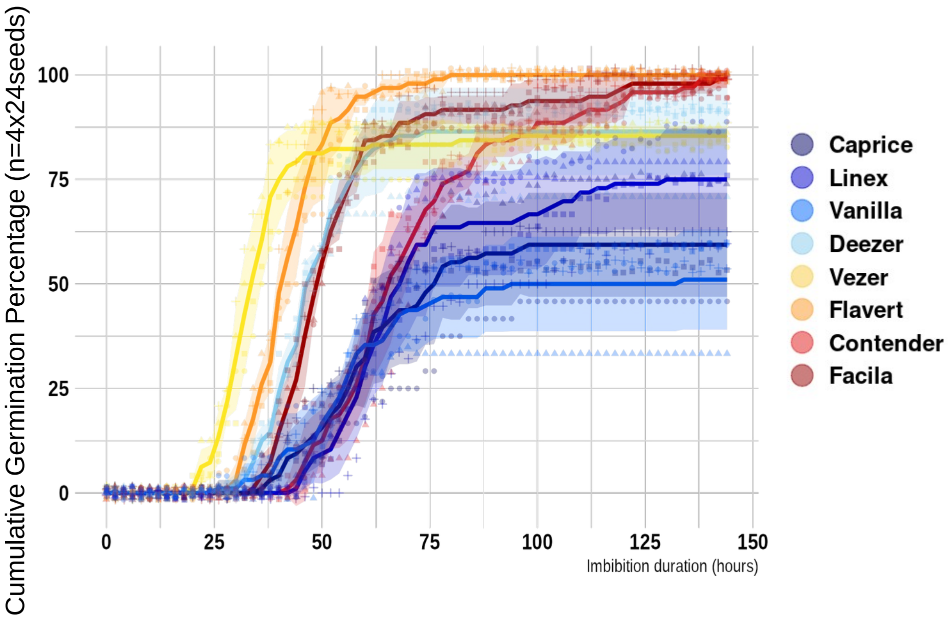
Photo: G. Arnault – IRHS, Angers

Seed quality differs between Common bean varieties

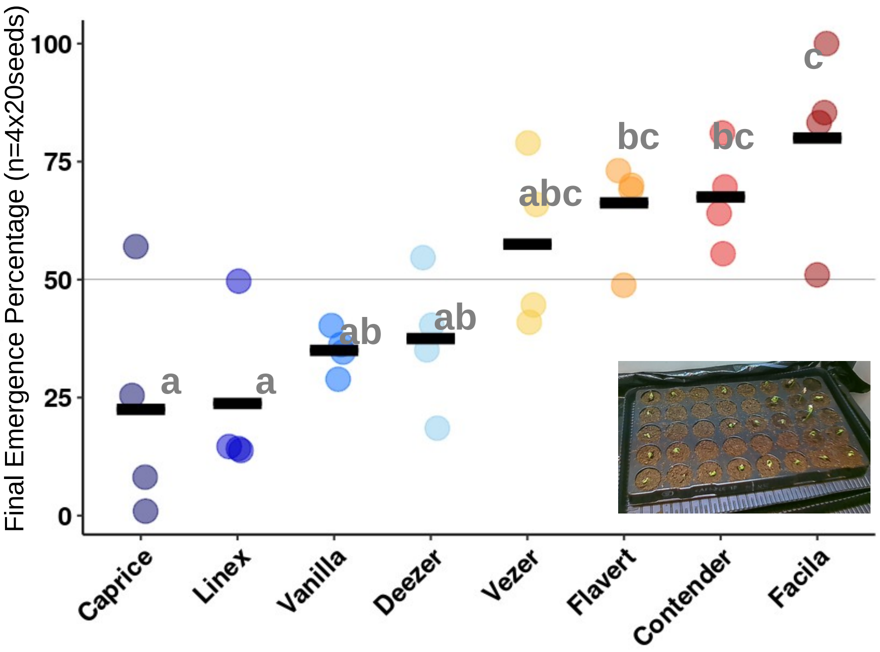


Seed quality differs between Common bean varieties

Germination

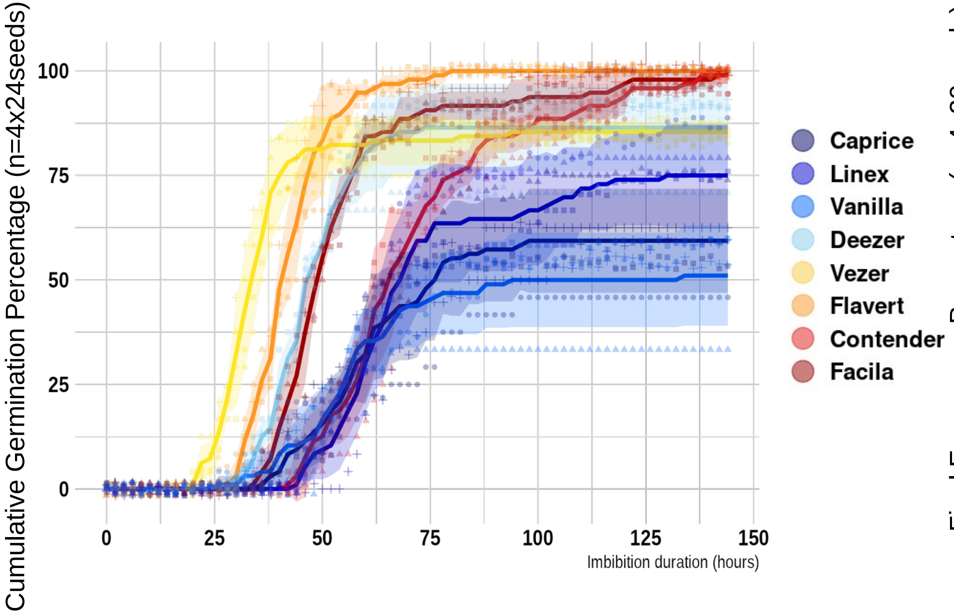


Emergence

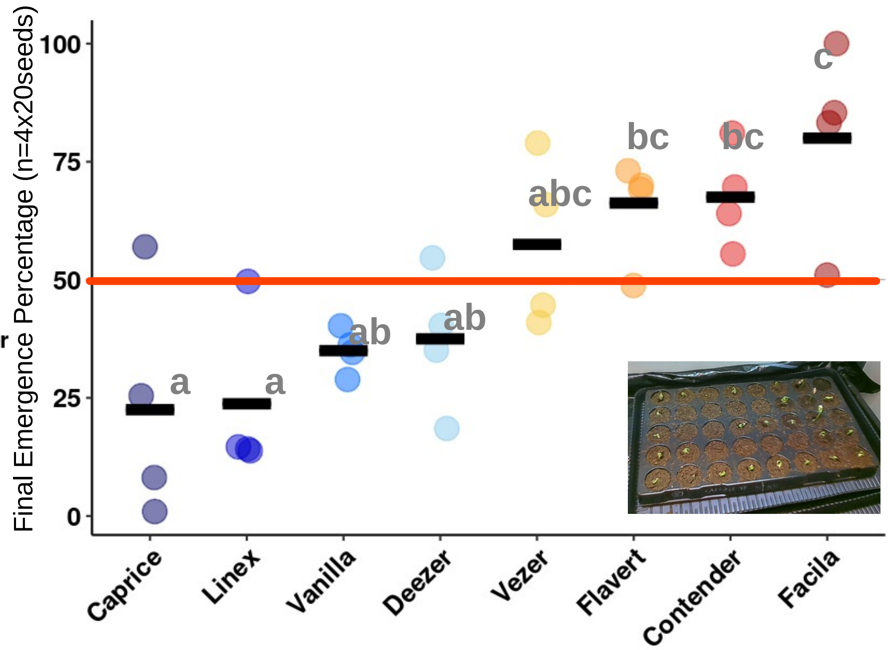


Seed quality differs between Common bean varieties

Germination



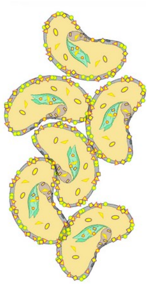
Emergence

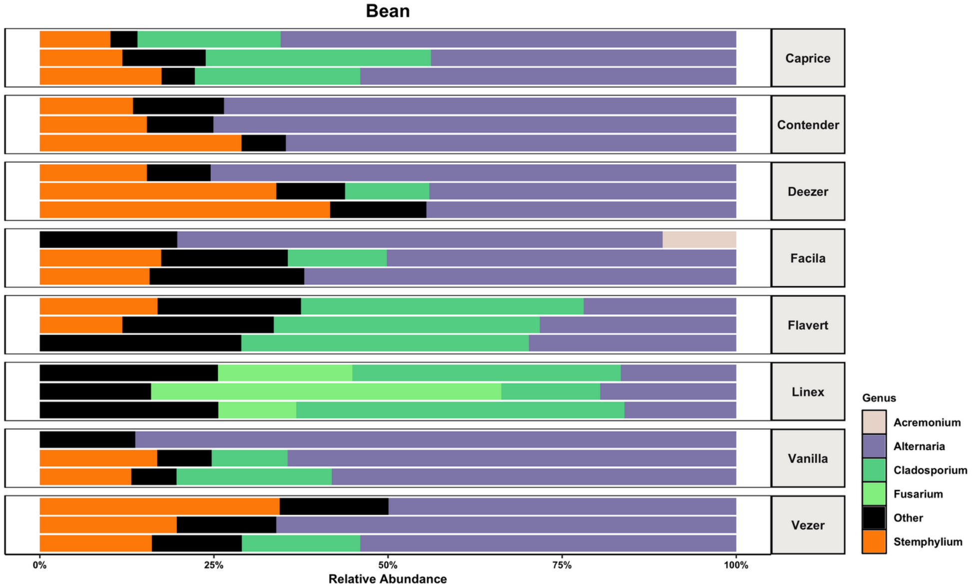


Seed microbiota differs between Common bean varieties

Seed lot

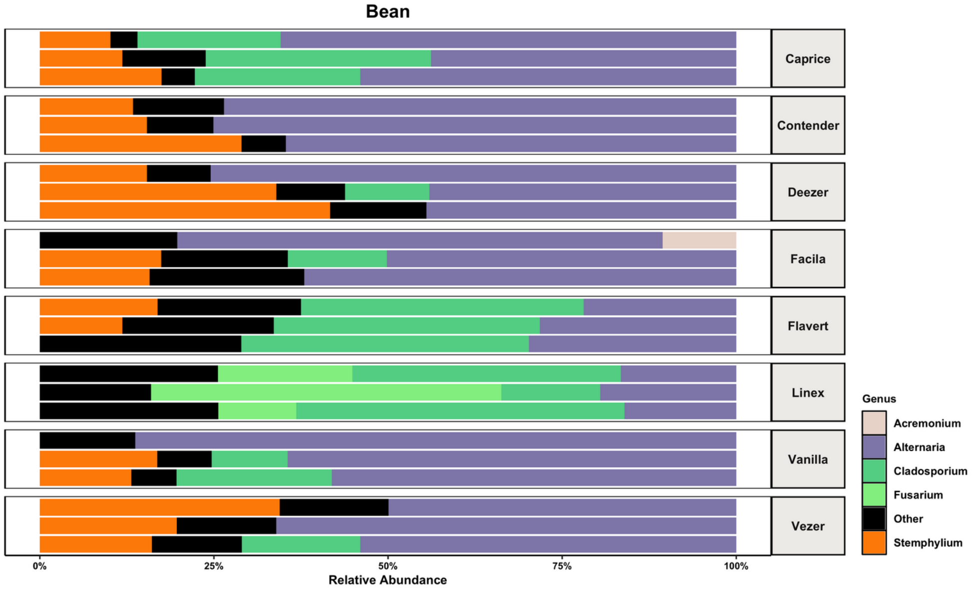
fungi





Seed microbiota differs between Common bean varieties

fungi



Seed lot

Fungal community

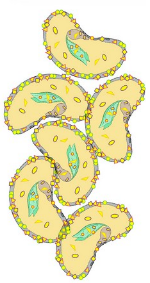
- 25-80 fungal taxa, 23 families

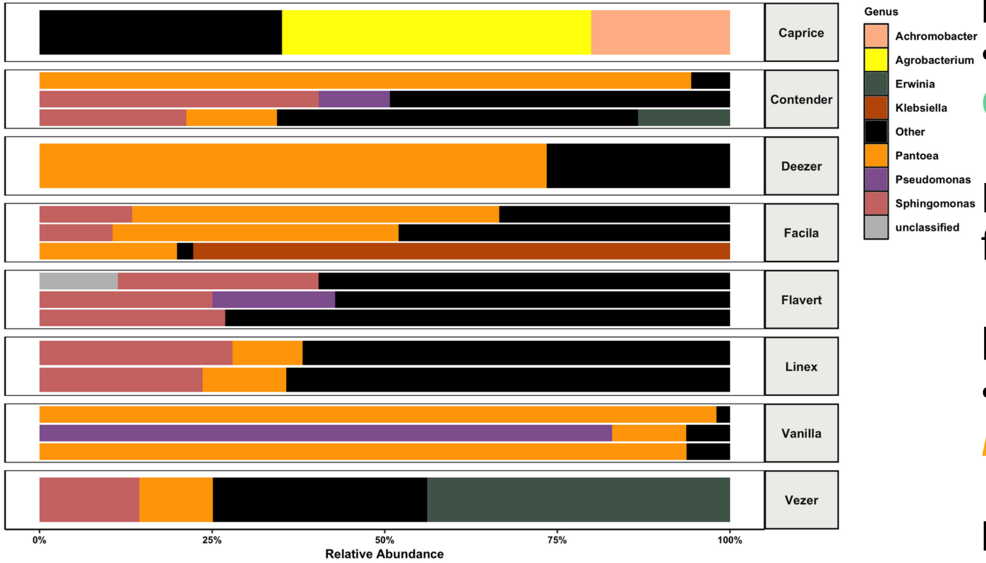
Cladosporium, Alternaria, Stemphylium

Plant variety explains for 78% of the fungal community variation

Seed microbiota differs between Common bean varieties

bacteria





Seed lot

Fungal community

- 25-80 fungal taxa, 23 families

Cladosporium, Alternaria, Stemphylium

Plant variety explains for 78% of the fungal community variation.

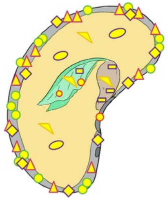
Bacterial community

- 50-250 bacterial taxa, 19 families

Pantoea, Pseudomonas, Sphingomonas

Plant variety explains for 42% of the bacterial community variation

(Single seed microbiota shows low-diversity, low-abundancy)

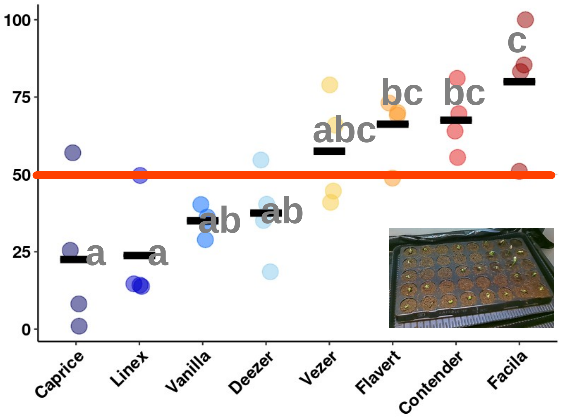


Single seed

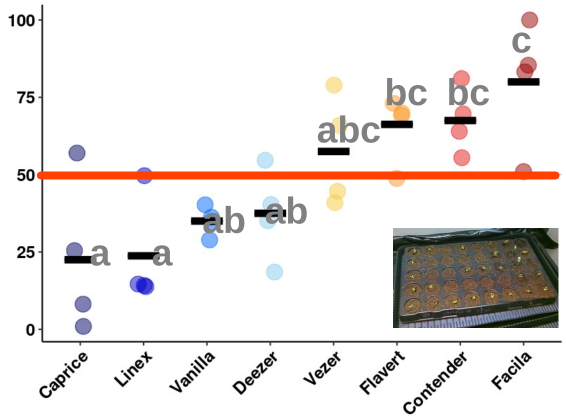
- 8 taxa (ASVs) / seed
- 1 dominant taxa
- 10^2 CFU

Chesneau et al. 2022 mBio

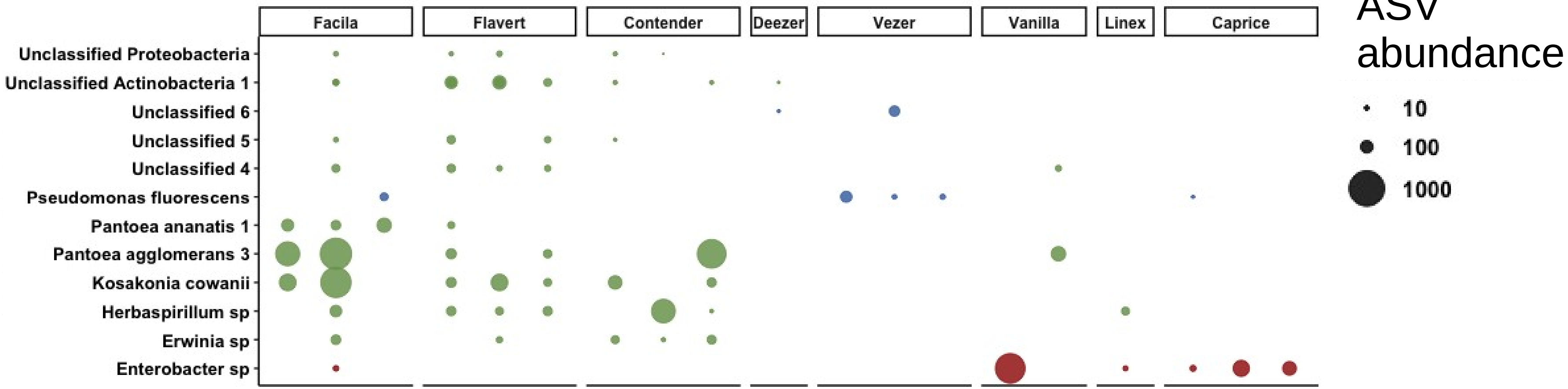
Does seed microbiota impact seed quality?



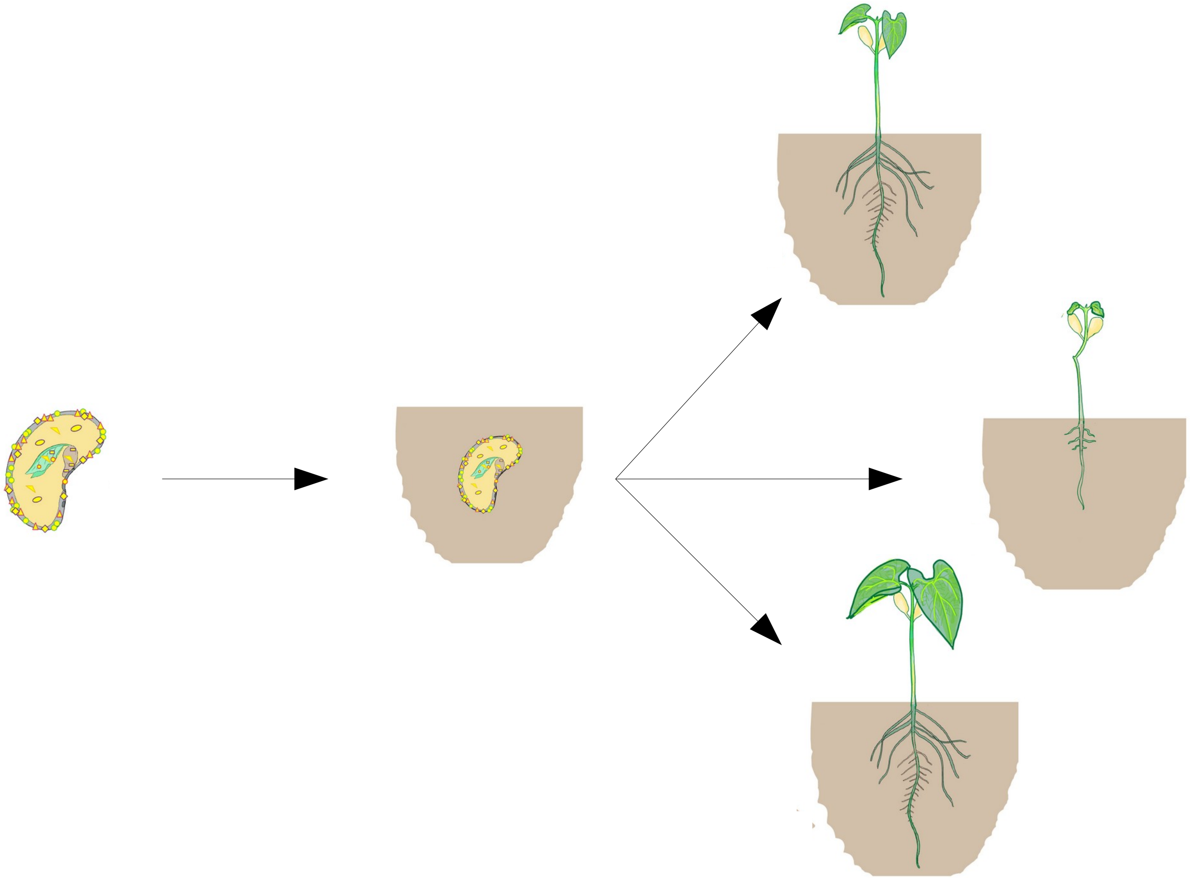
Does seed microbiota impact seed quality?



Bacterial indicator taxa for Final Emergence Percentage

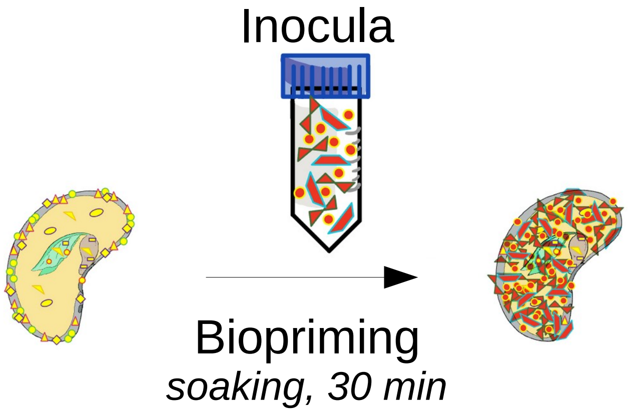


How to test causality between seed microbiota and seedling phenotype?



How to test causality between seed microbiota and seedling phenotype?

Synthetic communities (SynComs) : designed associations of microorganisms mimicking the native microbiota.



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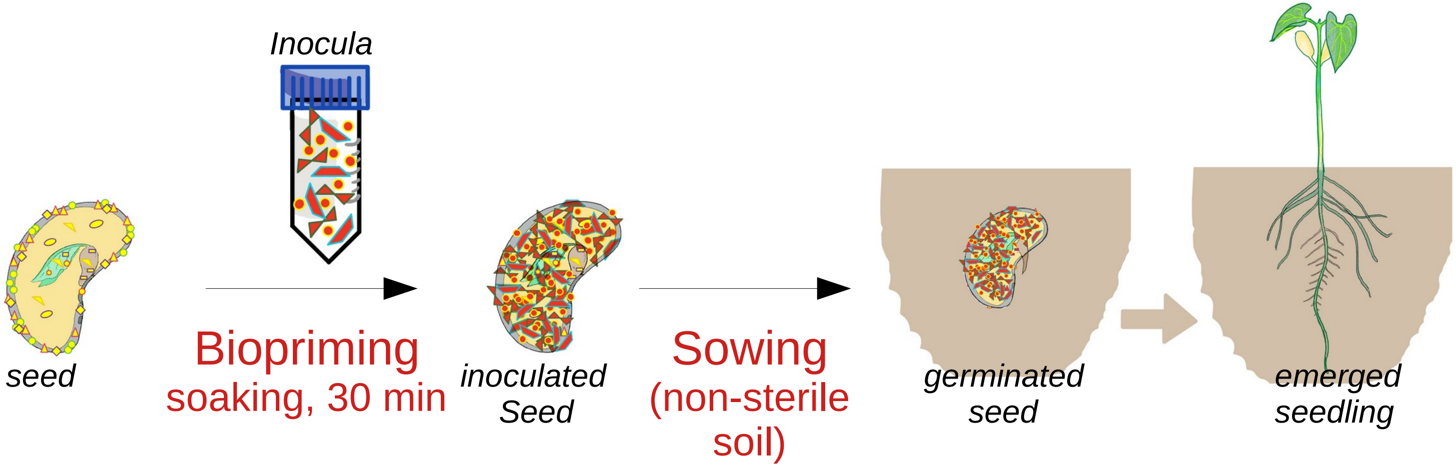

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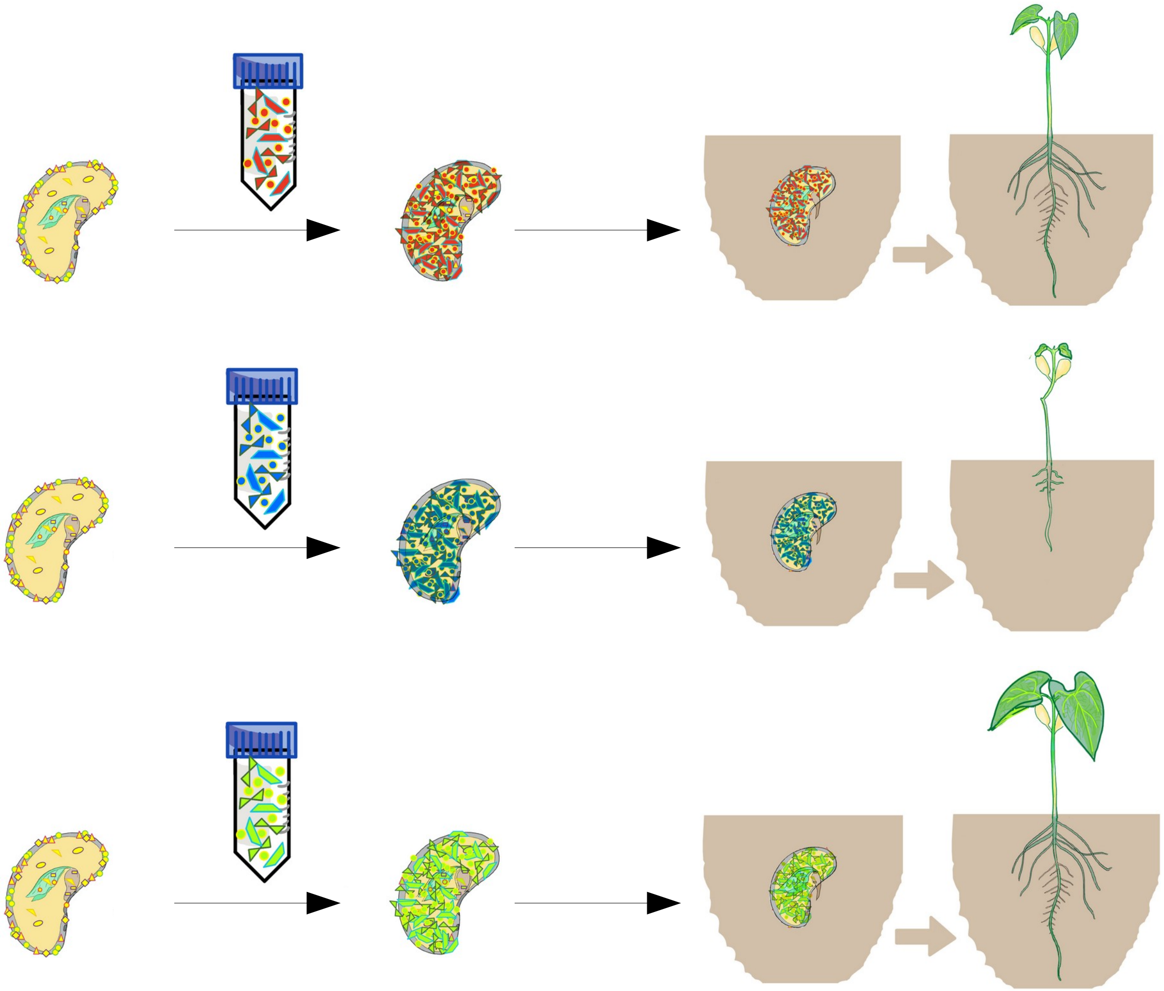
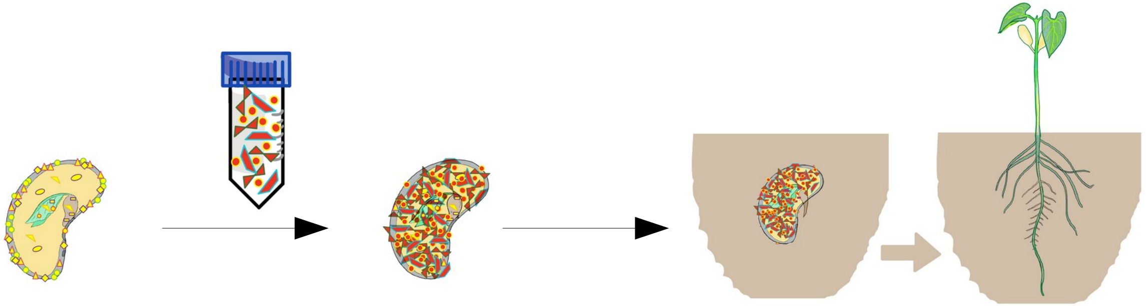
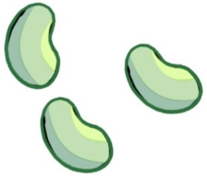


How to test causality between seed microbiota and seedling phenotype?

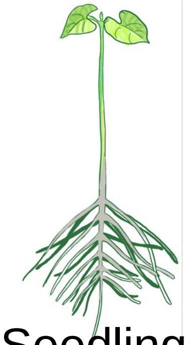


1. Isolate microorganisms from the plant microbiota
2. Select (interesting) taxa
3. Efficiently inoculate the seed

Design SynCom : 1/ Isolate and stock plant microorganisms



Seed batches



Seedlings

Grinding & Maceration (4°C, overnight)

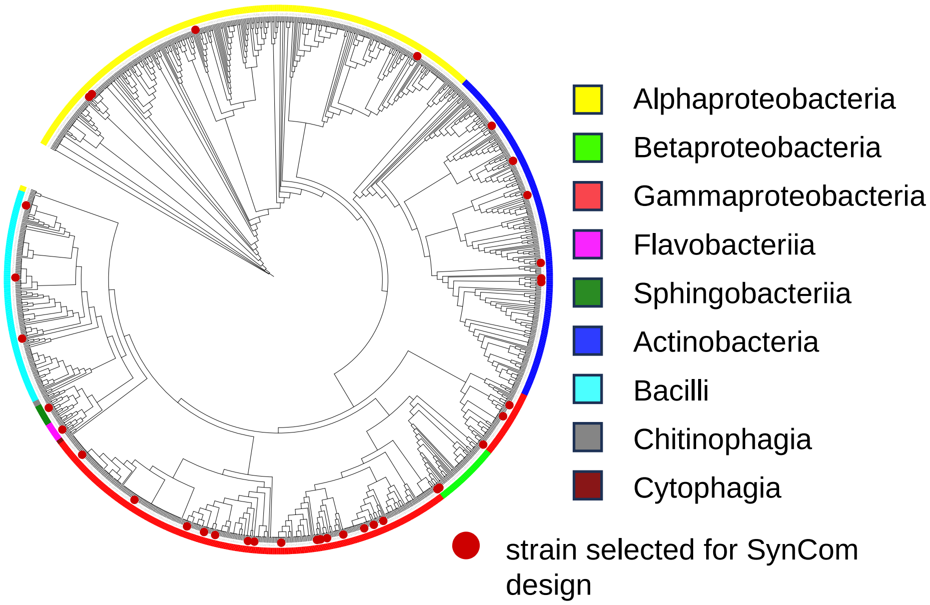
Microbiota description

Microorganisms isolation
and identification

CFBP collection (bacteria)
Mycotheque (fungi)

C. Marais, M. Simonin, M. Marchi, N. Guschinskaya, IRHS Angers (SUCSEED)

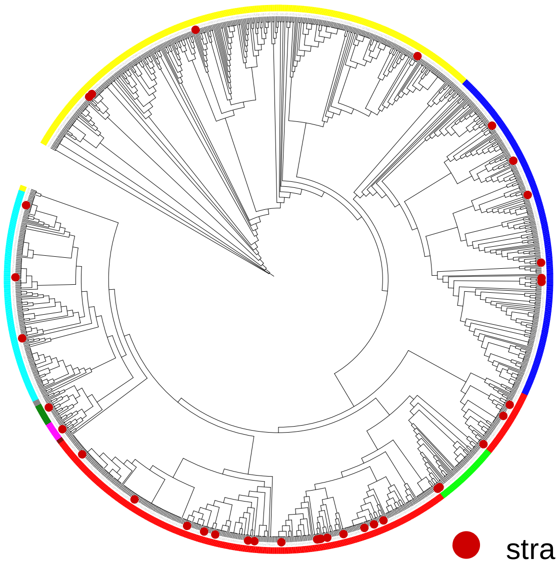
Design SynCom : 2/ Select taxa



Selection of 36 taxa from the collection
(1250 taxa,
73% of the relative abundance of seed microbiota)

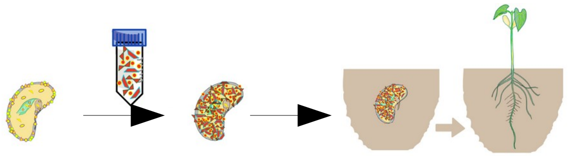
G. Arnault, C. Marais, M. Simonin, IRHS Angers

Design SynCom : 2/ Select taxa



🟨 Alphaproteobacteria
🟩 Betaproteobacteria
🟥 Gammaproteobacteria
🟪 Flavobacteriia
🟩 Sphingobacteriia
🟦 Actinobacteria
🟦 Bacilli
⬜ Chitinophagia
🟥 Cytophagia

● strain selected for SynCom design



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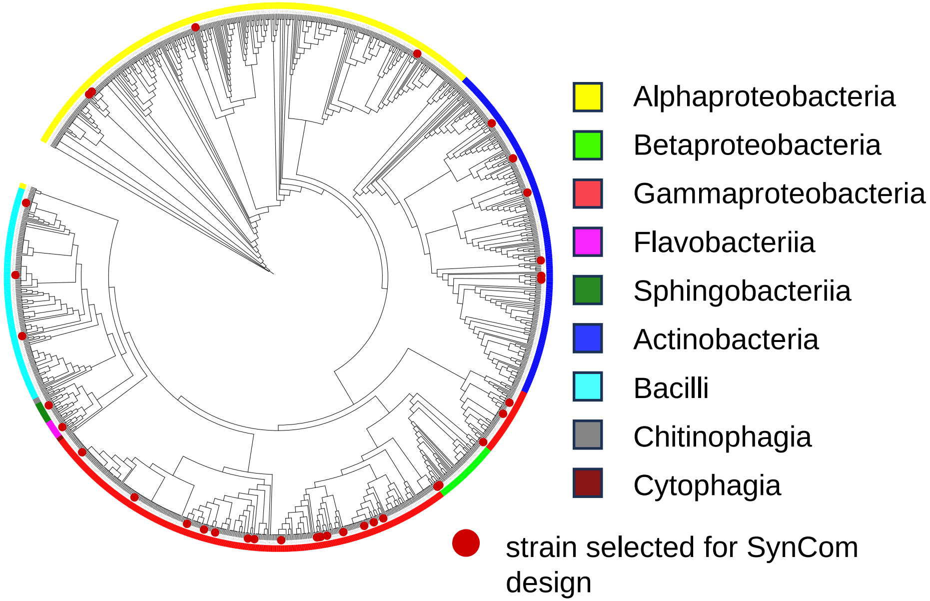

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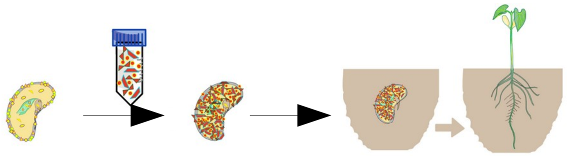

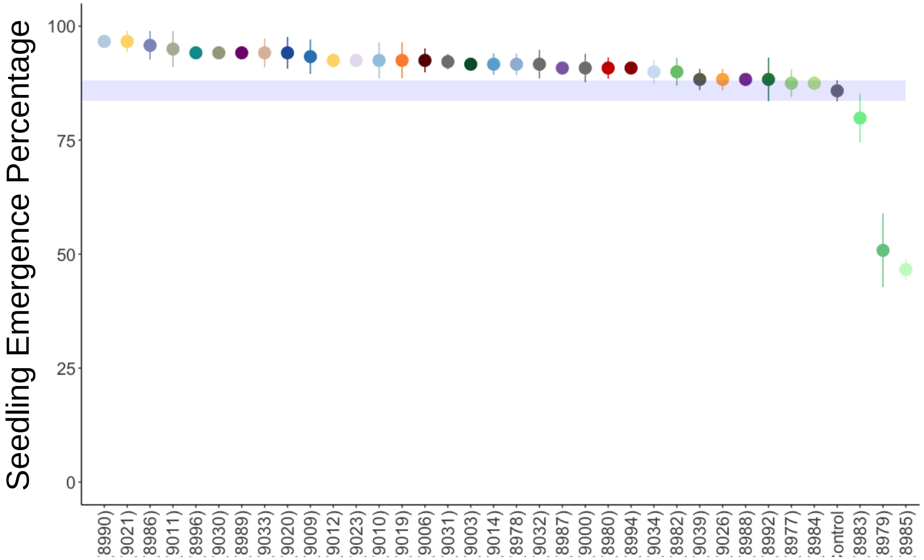

G. Arnault, C. Marais, M. Simonin, IRHS Angers

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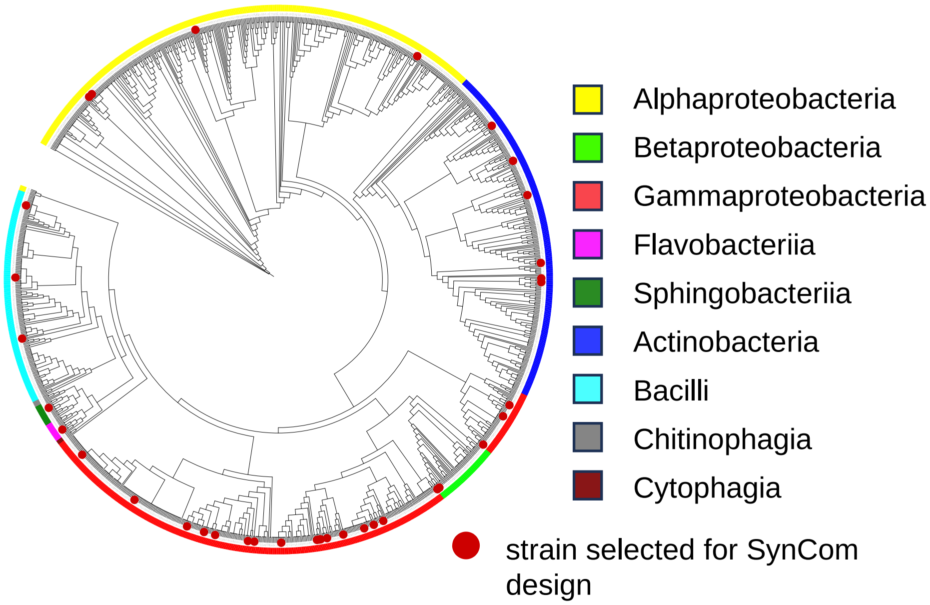

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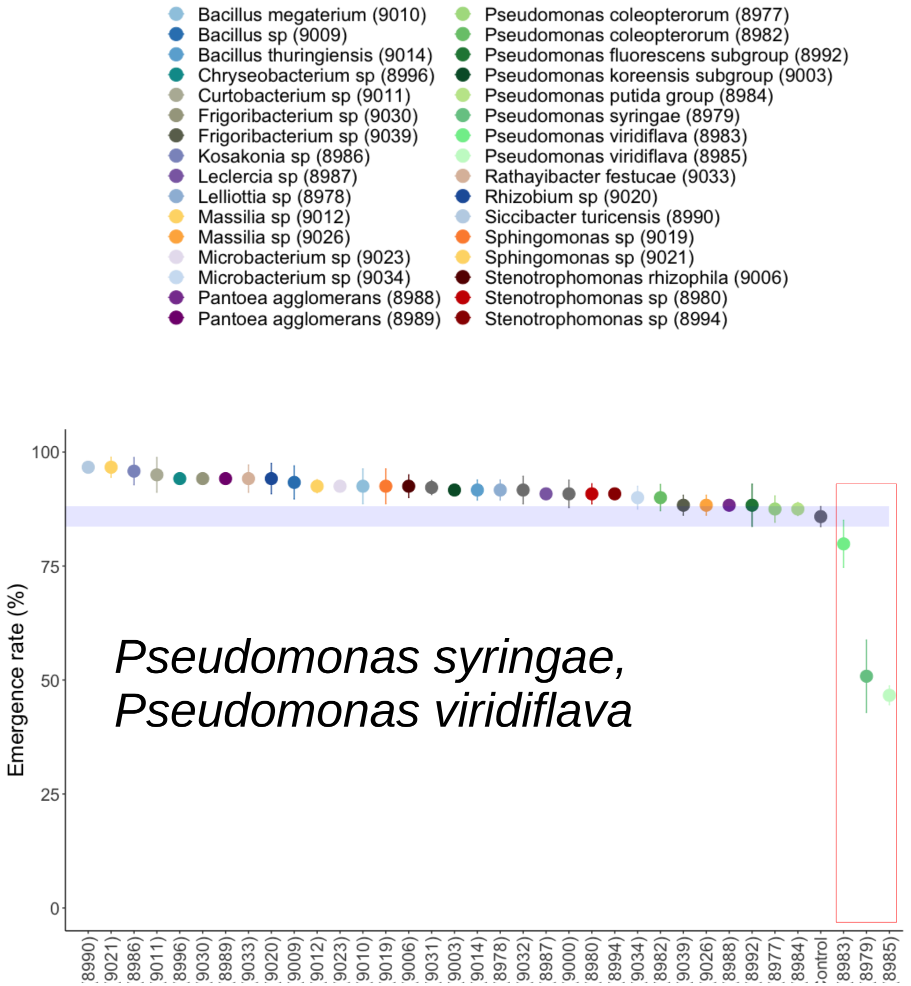
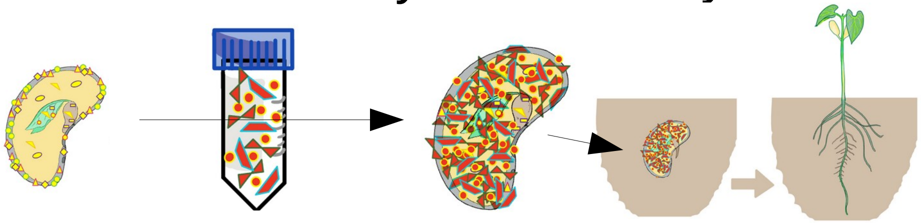


Pseudomonas syringae,
Pseudomonas viridiflava

G. Arnault, C. Marais, M. Simonin, IRHS Angers

Design SynCom : 3/ Efficiently inoculate SynCom on seeds



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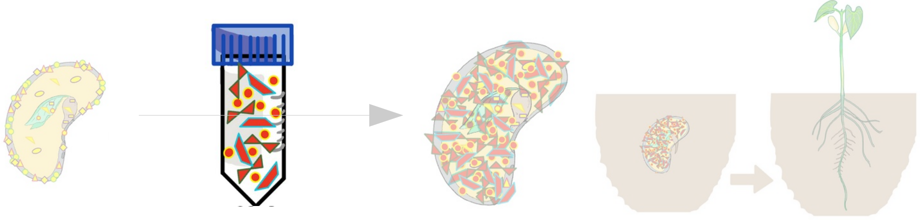

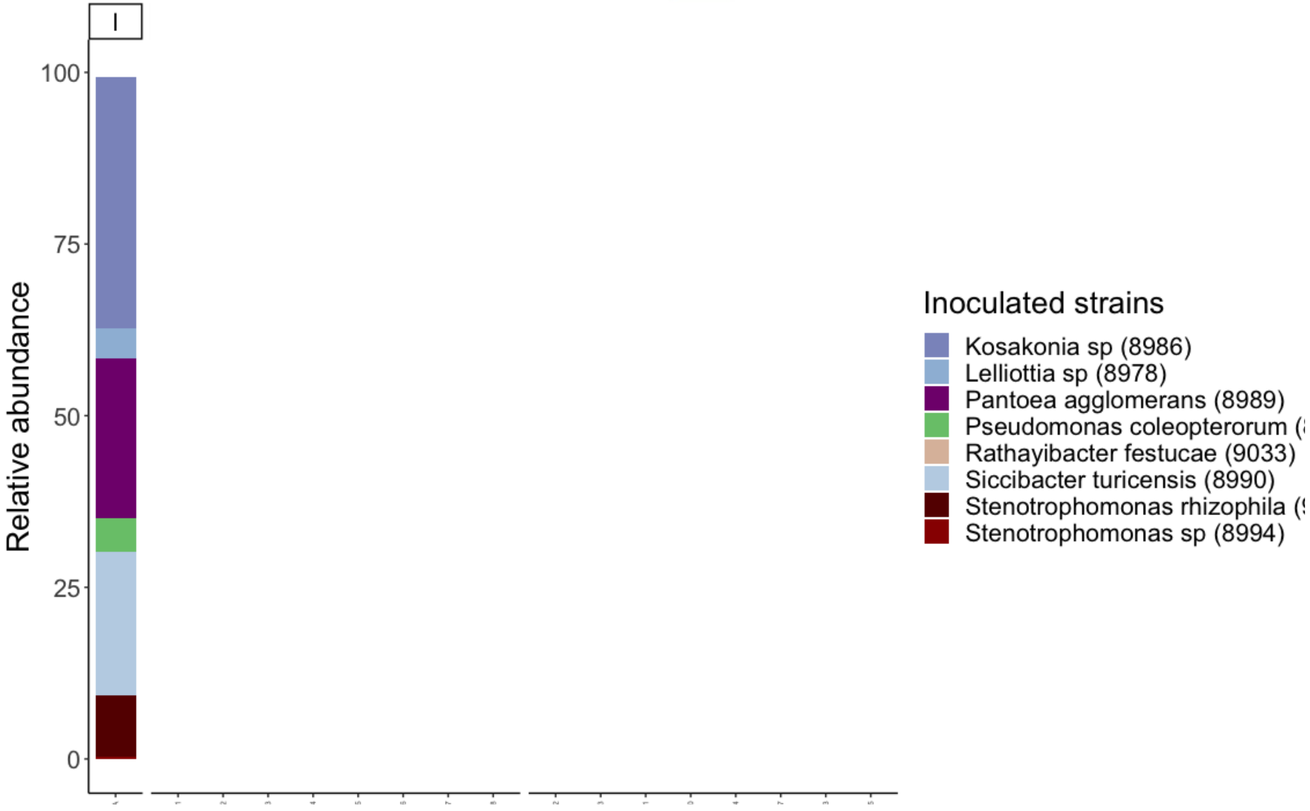

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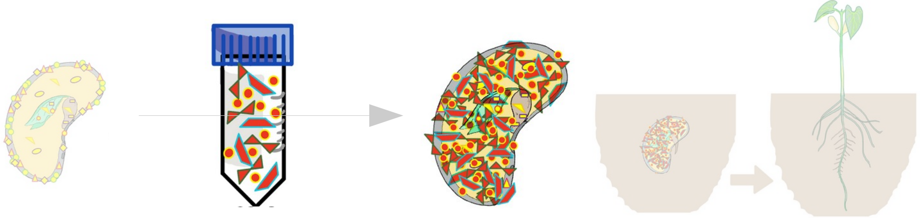
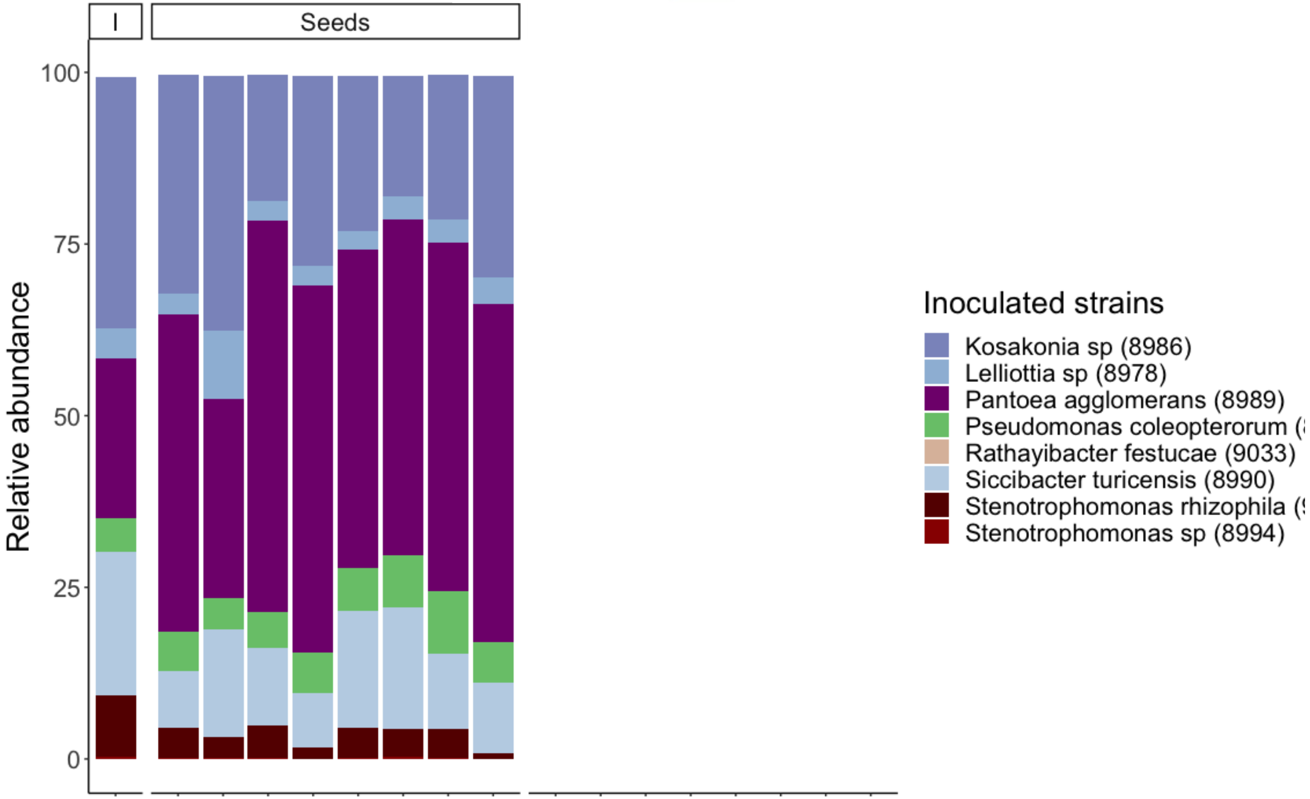
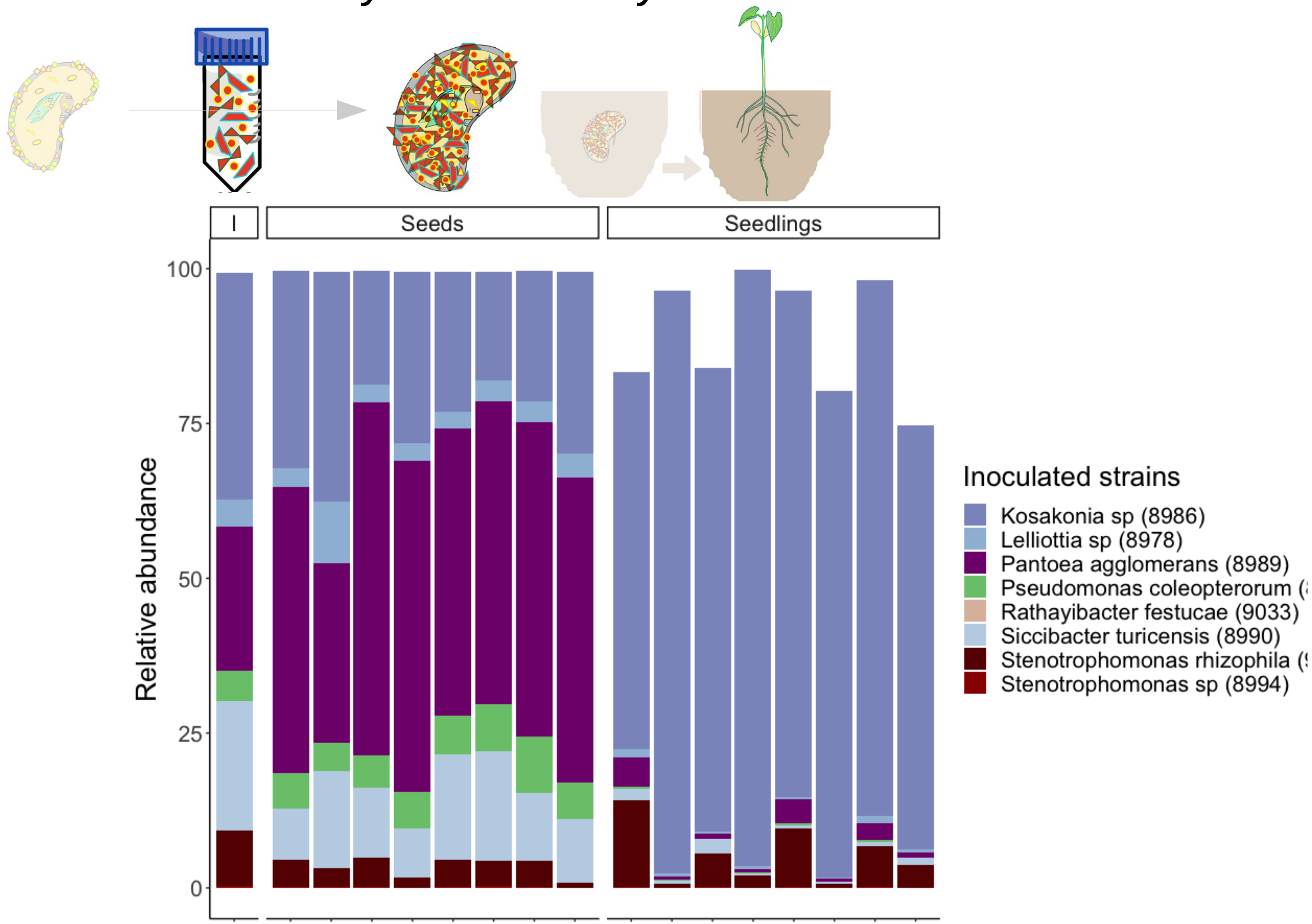




Design SynCom : 3/ Efficiently inoculate SynCom on seeds



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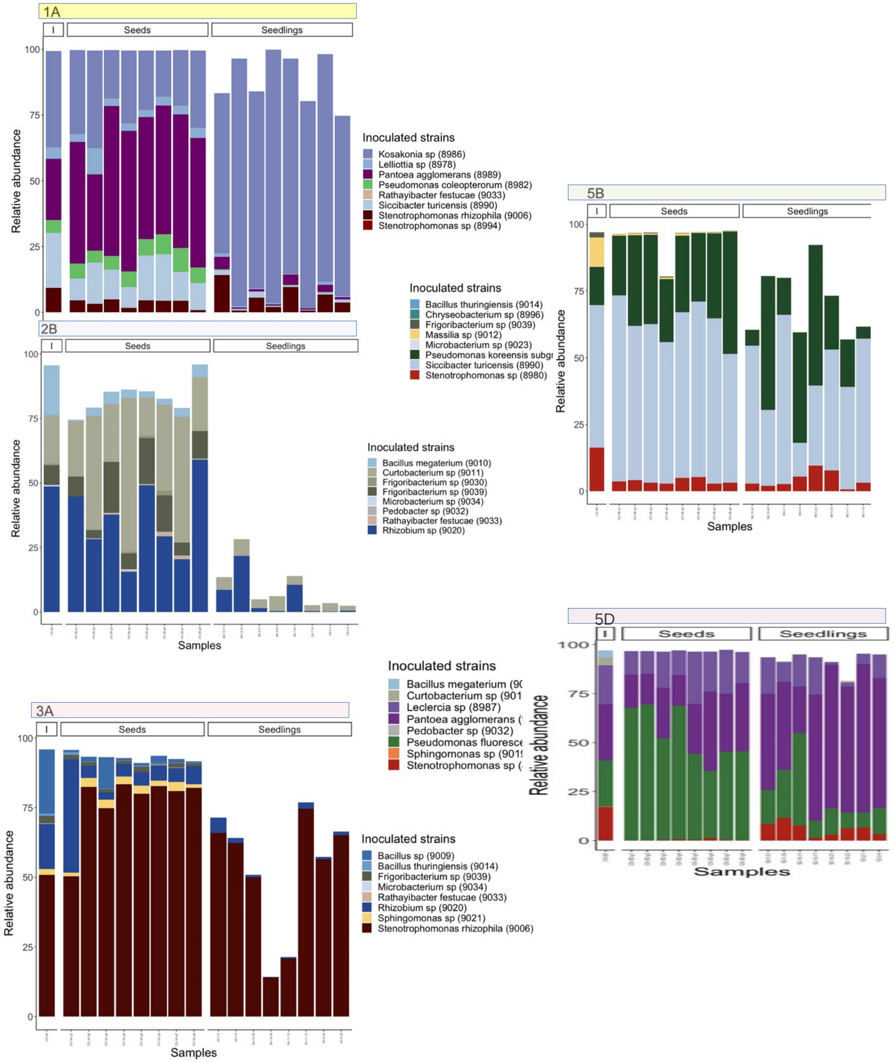

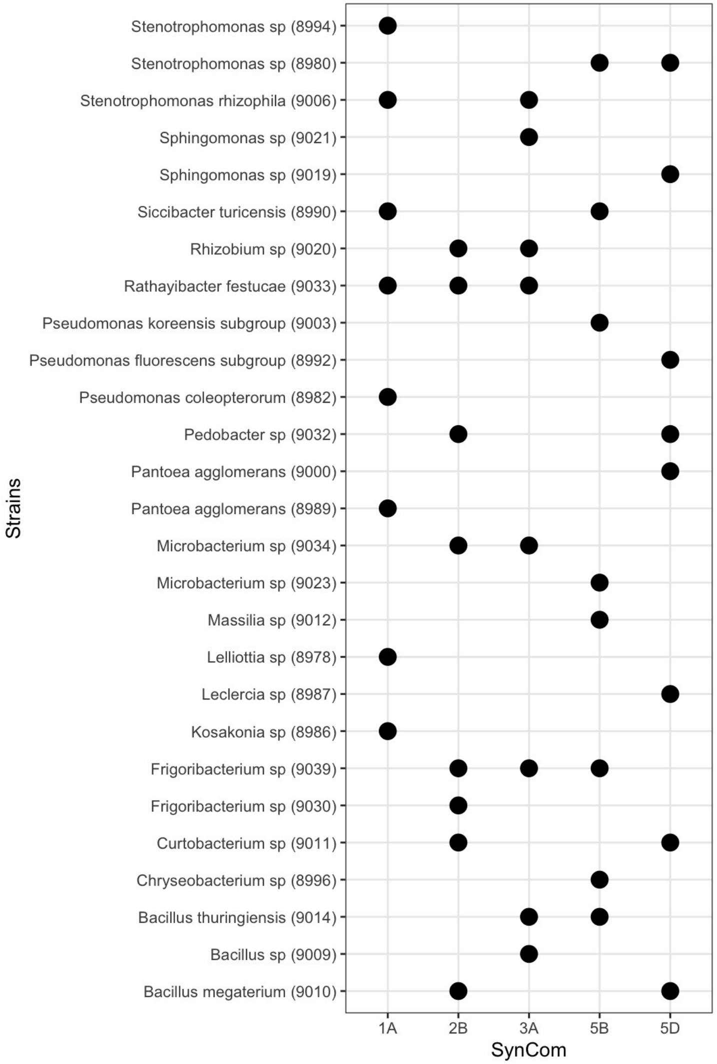

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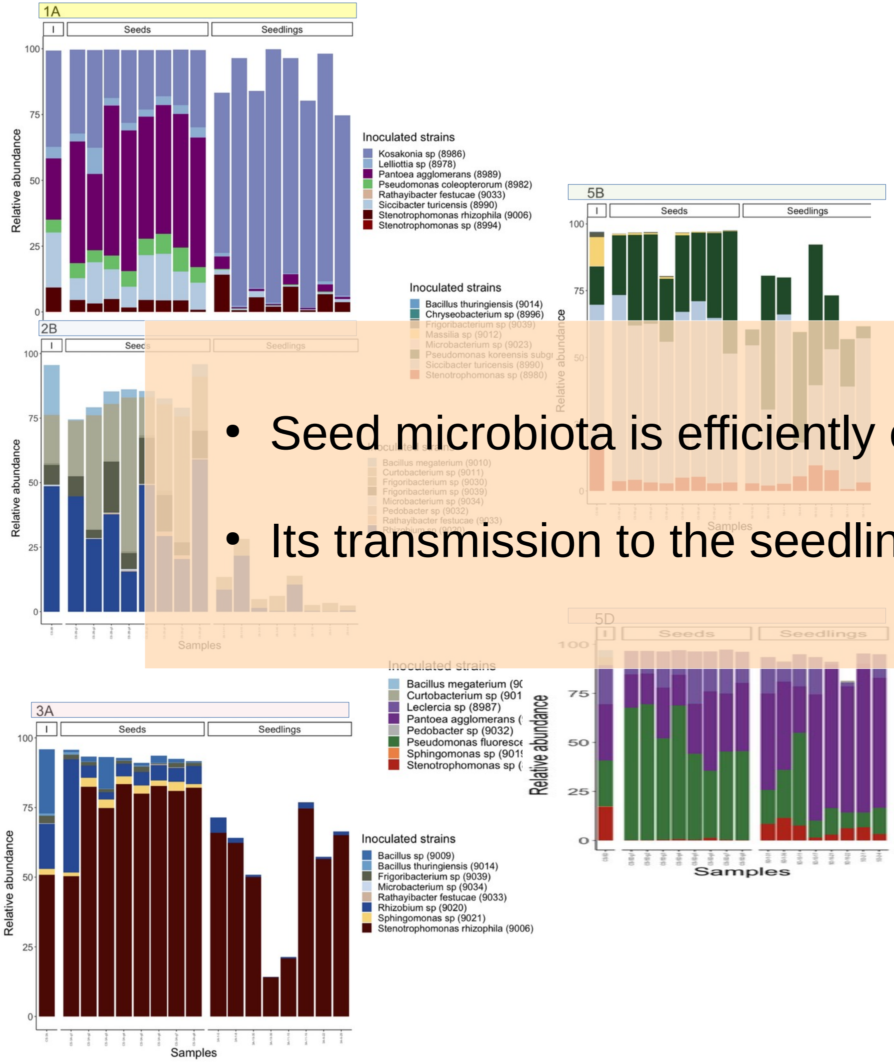
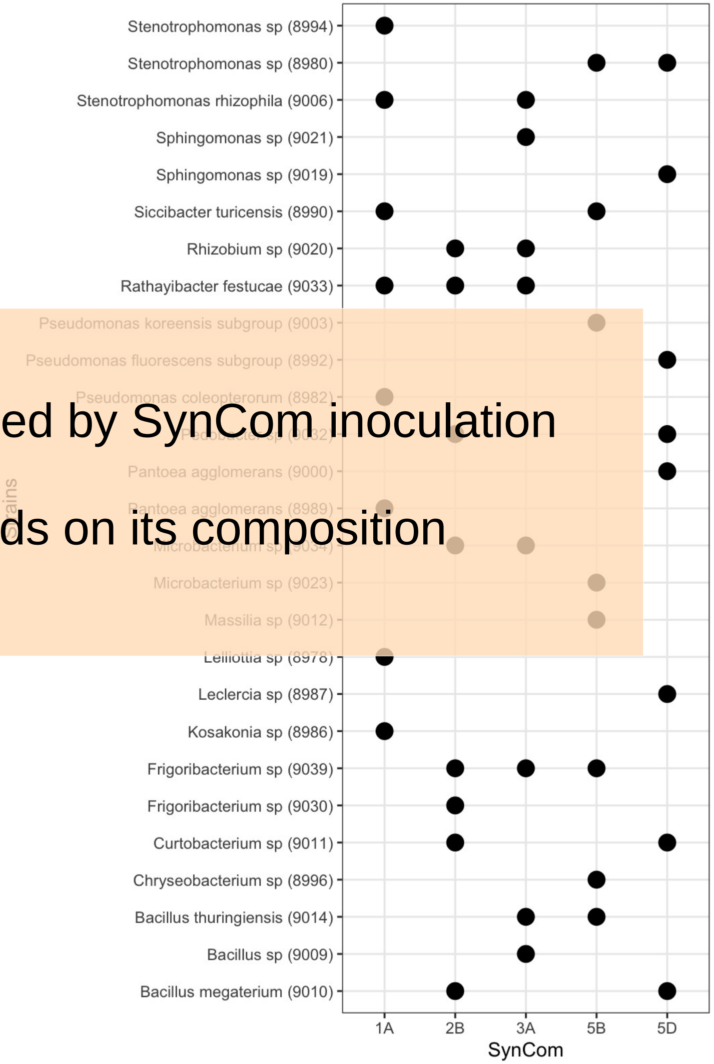
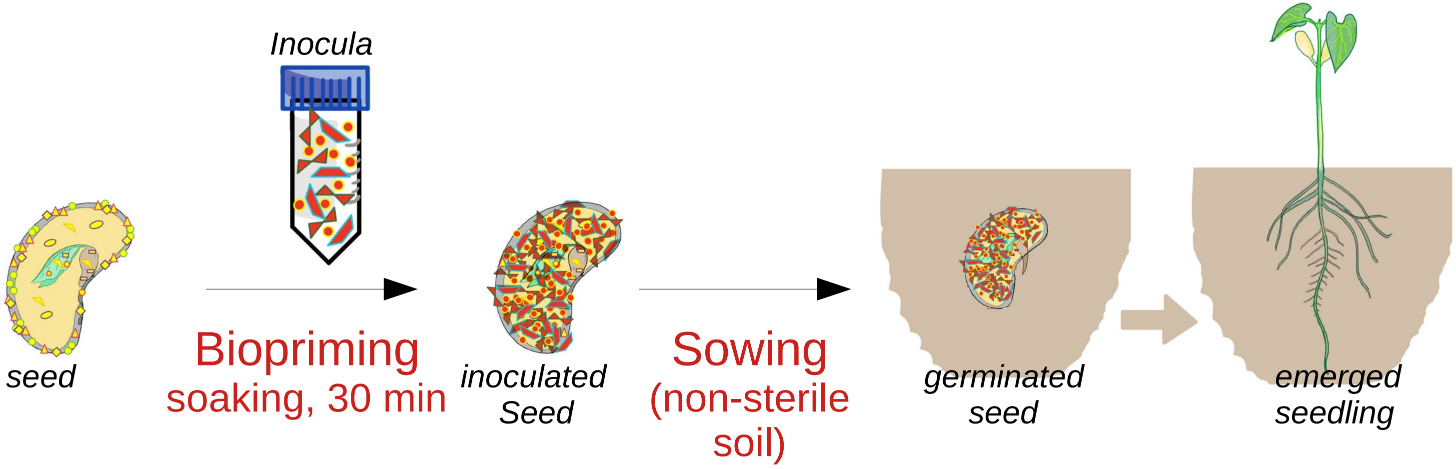


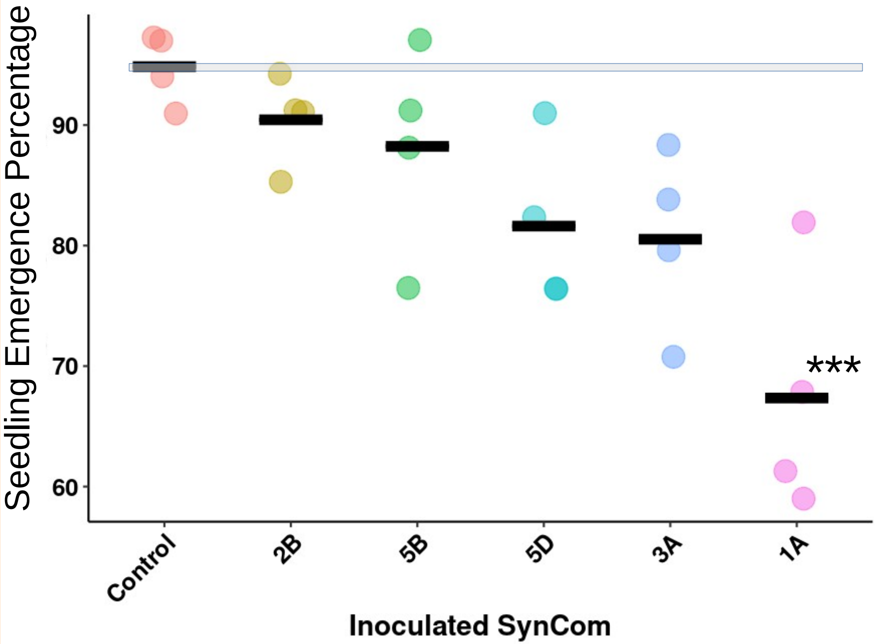


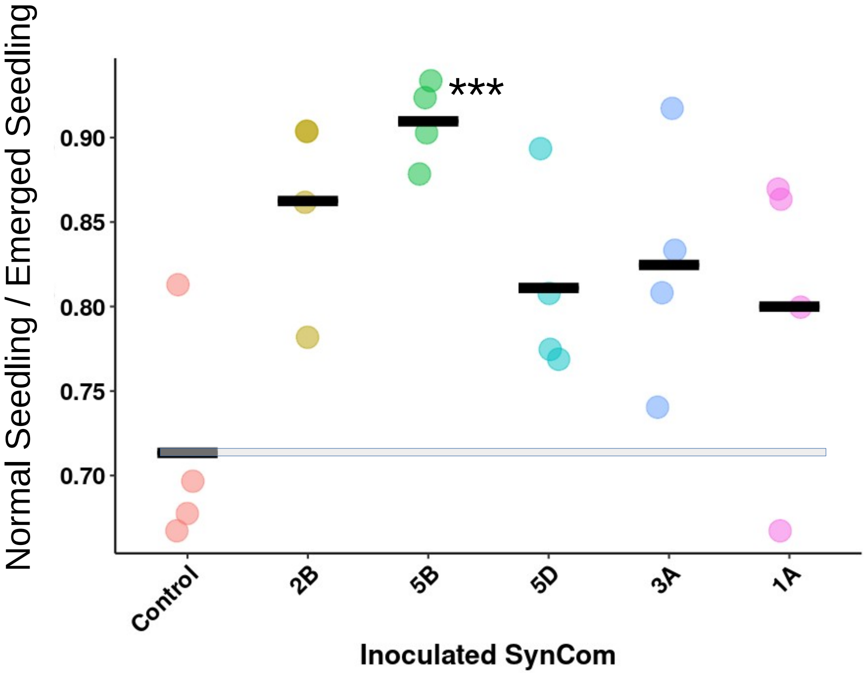
- Seed microbiota is efficiently engineered by SynCom inoculation
- Its transmission to the seedling depends on its composition

SynCom effect on seedling phenotype



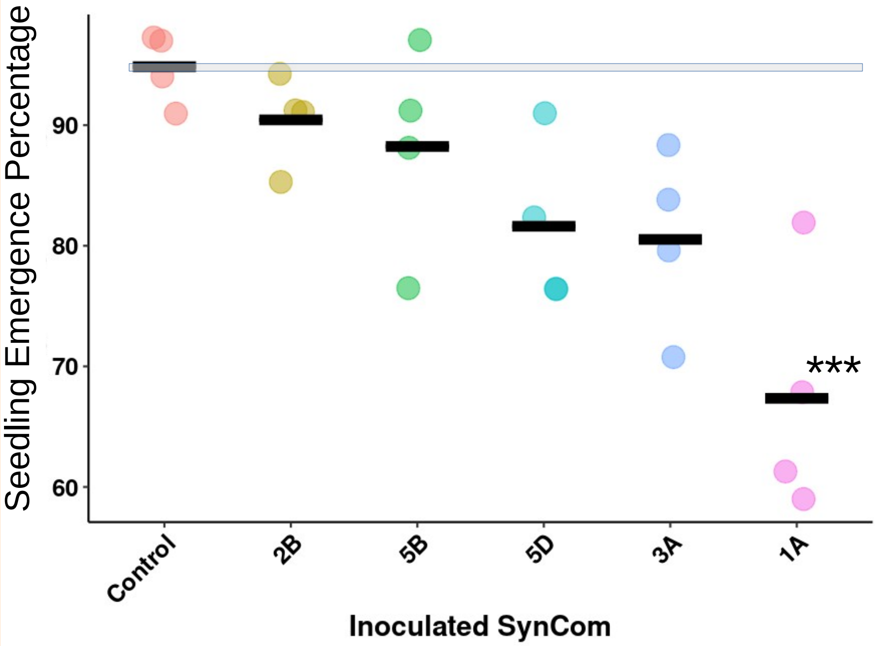
SynComs effect from seed to seedling

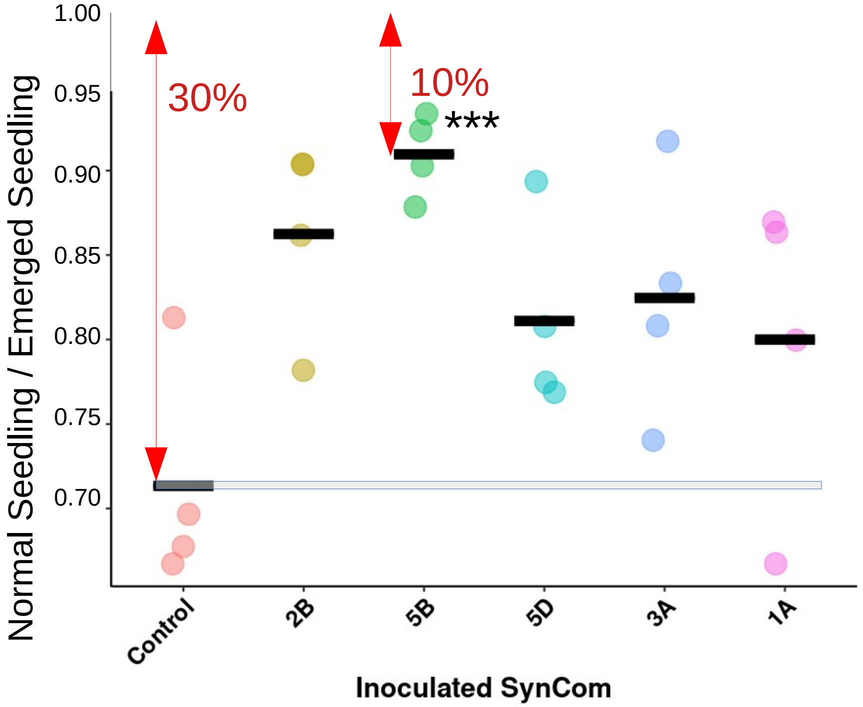




How is this effect mediated? *Coming soon*

SynComs effect from seed to seedling



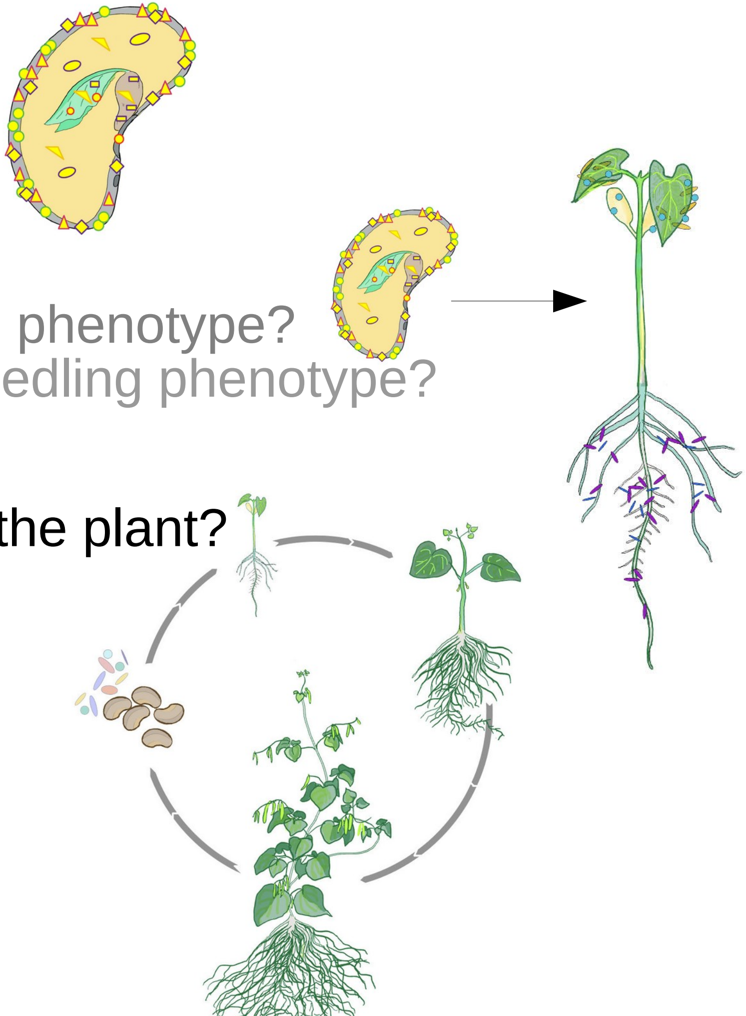


How is this effect mediated? *Coming soon*

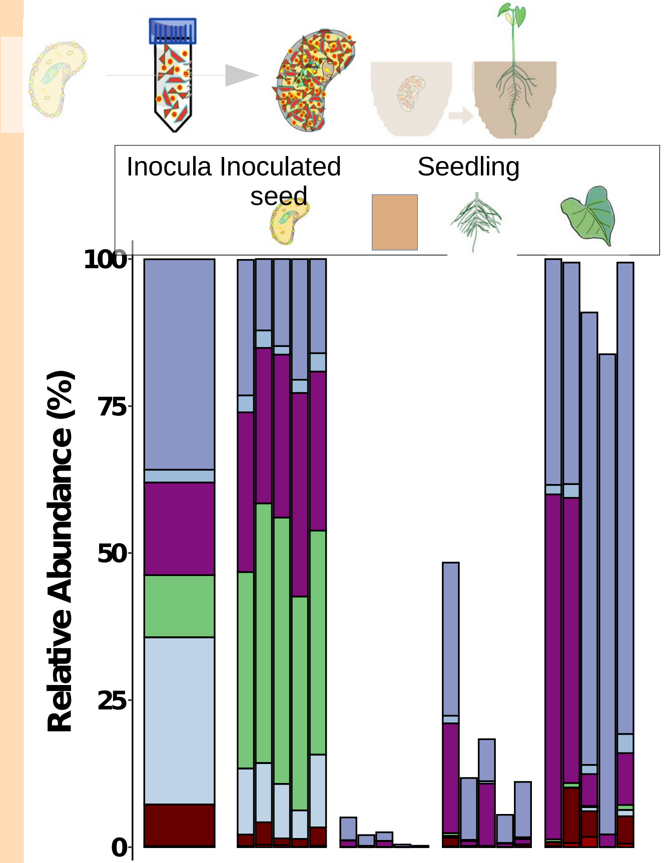
I. Seed native microbiota: Who is there?

II. Seed microbiota: Does it impact seedling phenotype?
Can we use it to improve seed quality/seedling phenotype?

III. Synthetic microbiota: Is it transmitted to the plant?



SynComs monitoring: what happens later?



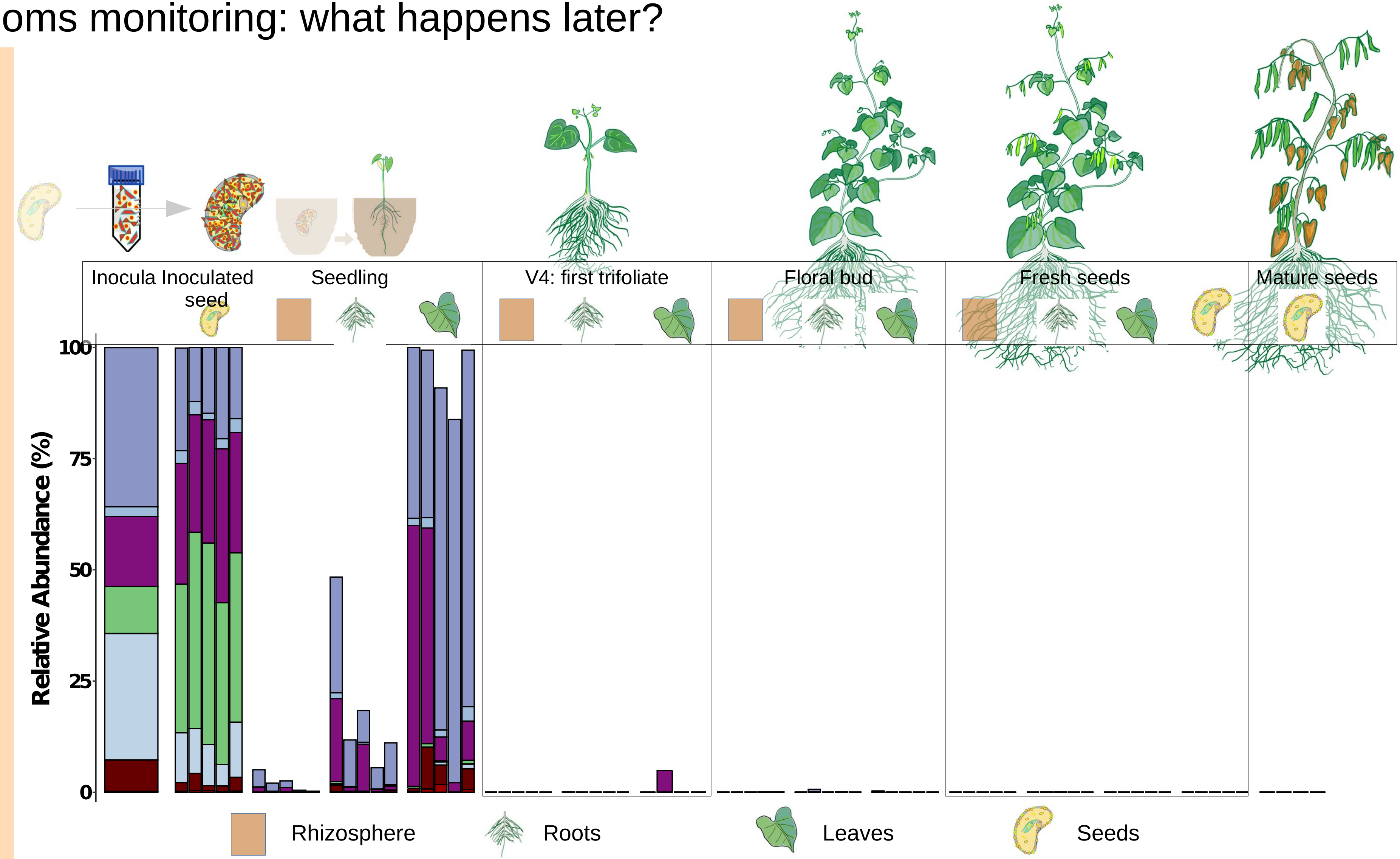
🟫 Rhizosphere

Roots

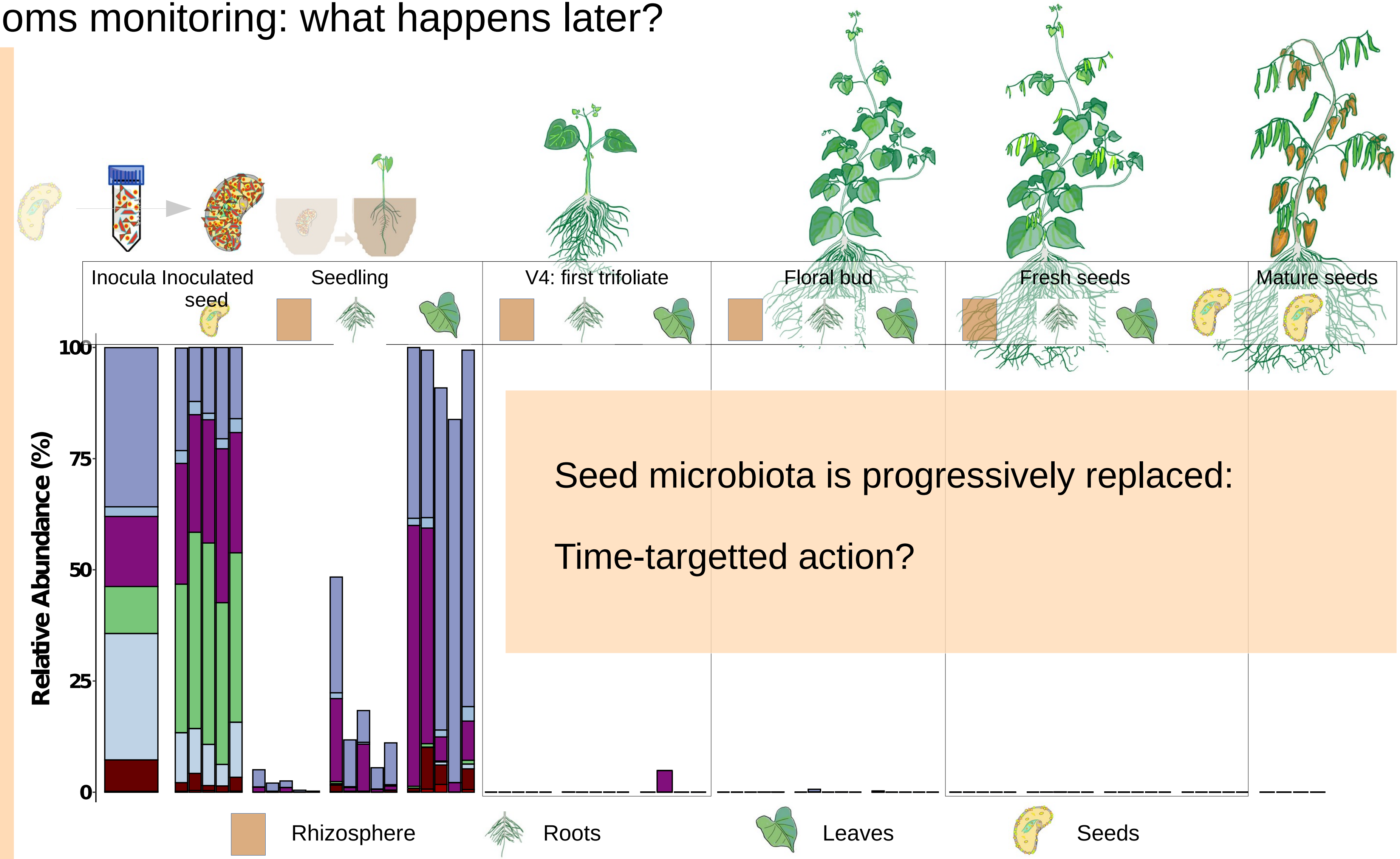
Leaves

Seeds

SynComs monitoring: what happens later?



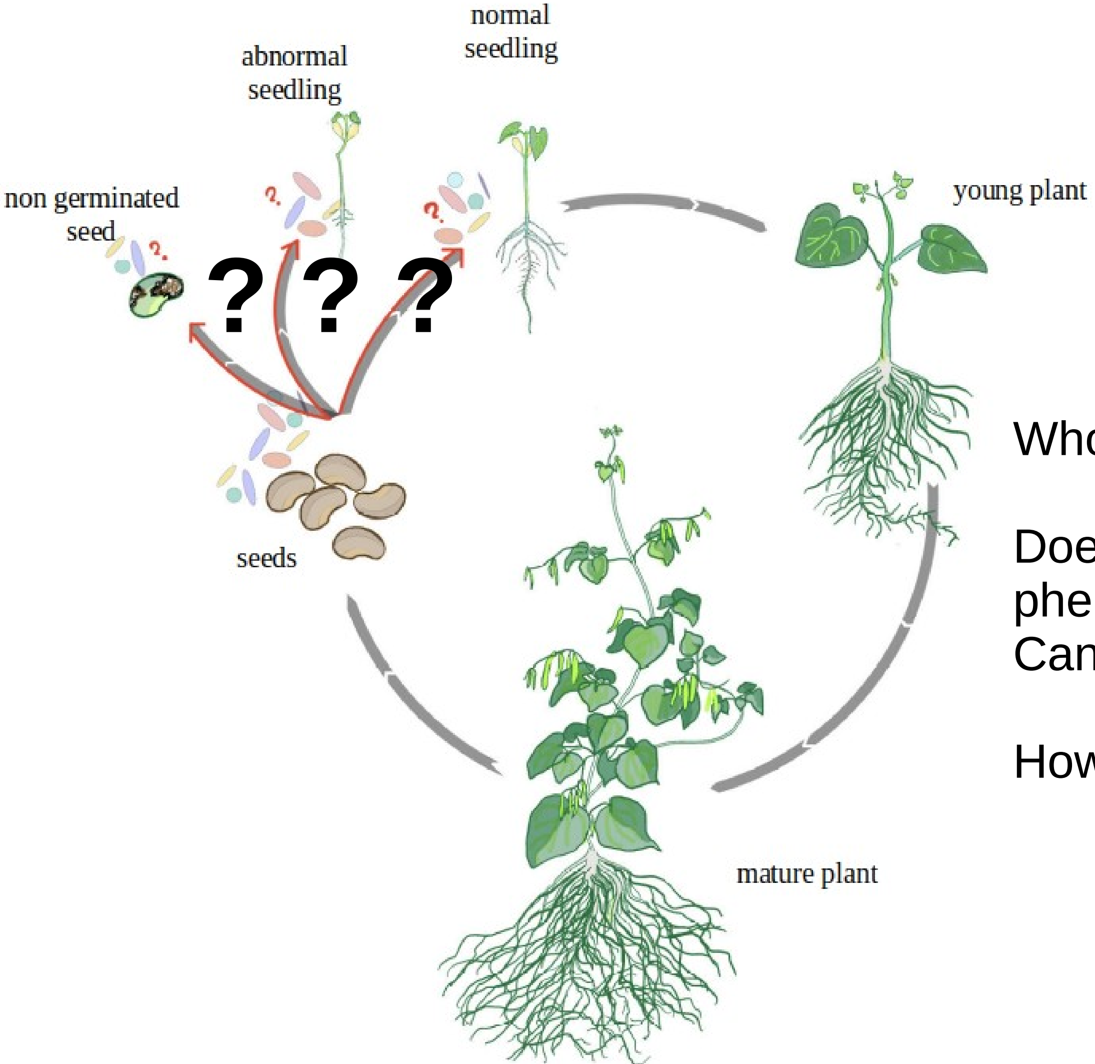
SynComs monitoring: what happens later?

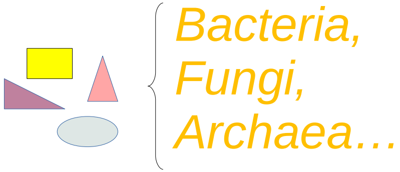


Seed microbiota is progressively replaced:

Time-targetted action?

Seed microbiota: the « good » and the « bad », and how to use it



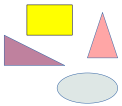


Who is there?

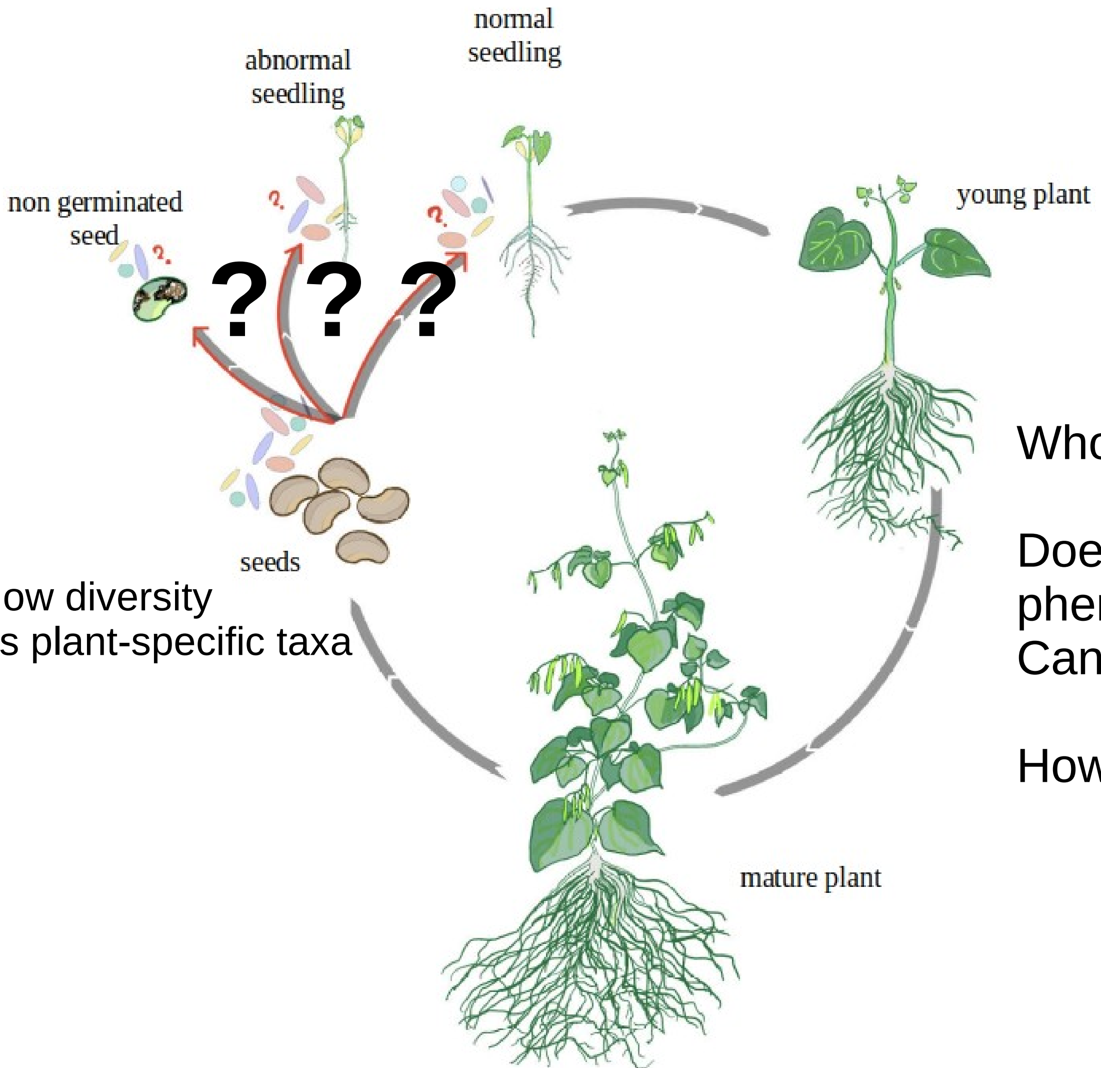
Does it impact seedling phenotype?
Can we use it?

How long does it stays?

Seed microbiota: the « good » and the « bad », and how to use it



Bacteria,
Fungi,
Archaea…



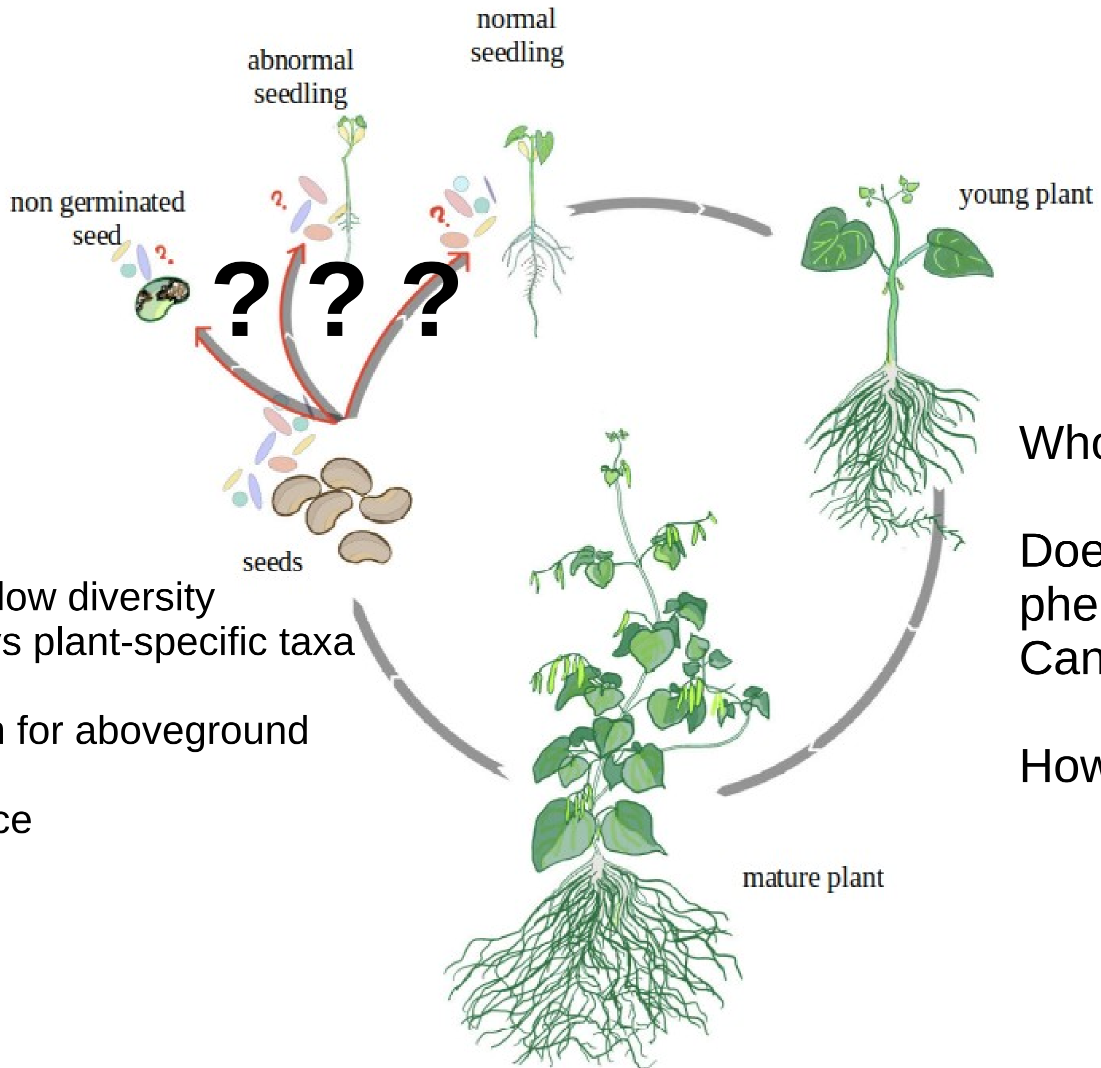
- Low abundancy, low diversity
- core microbiota vs plant-specific taxa

Who is there?

Does it impact seedling phenotype?
Can we use it?

How long does it stays?

Seed microbiota: the « good » and the « bad », and how to use it



? ? ?

Bacteria,
Fungi,
Archaea…

- Low abundancy, low diversity
- core microbiota vs plant-specific taxa

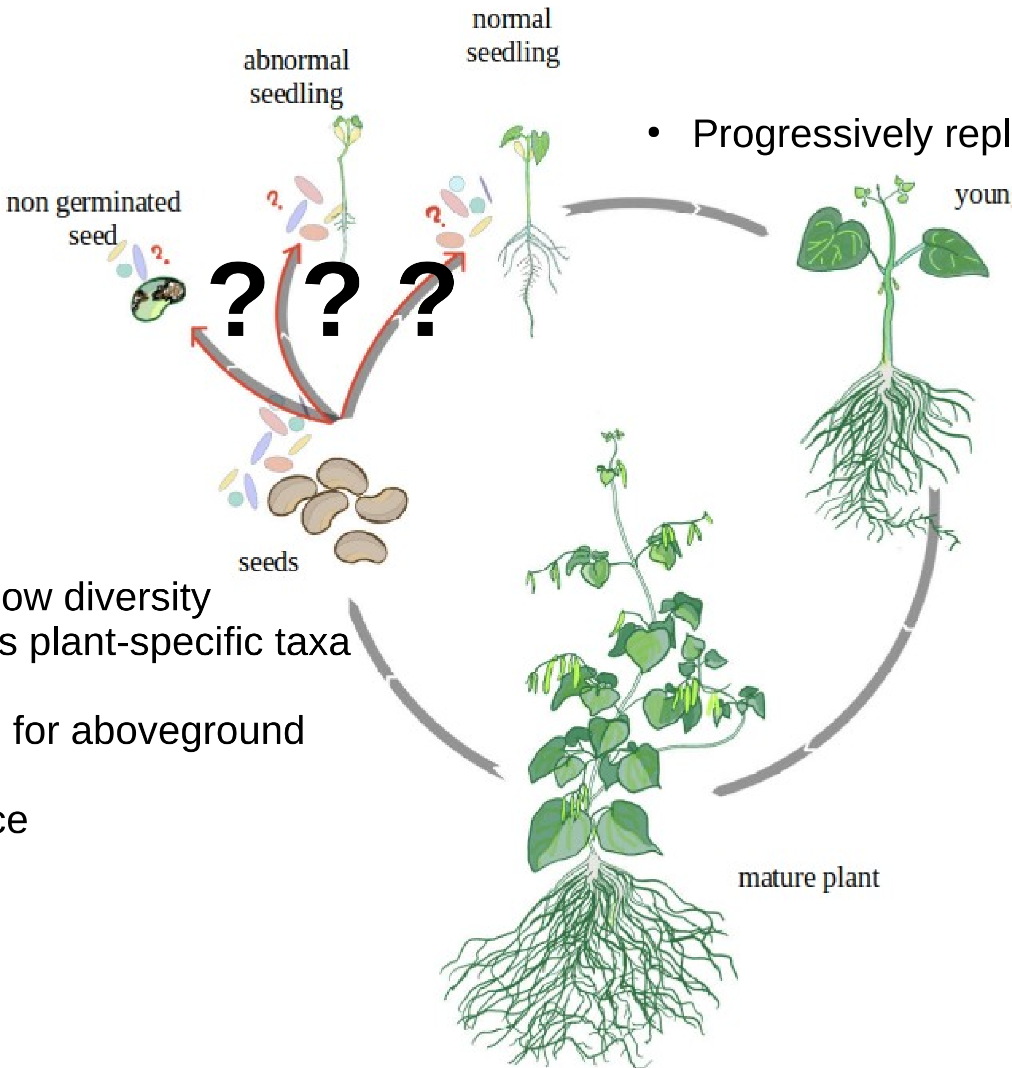
- Primary inoculum for aboveground compartments
- Role in emergence

Who is there?

Does it impact seedling phenotype?
Can we use it?

How long does it stays?

Seed microbiota: the « good » and the « bad », and how to use it



- Progressively replaced

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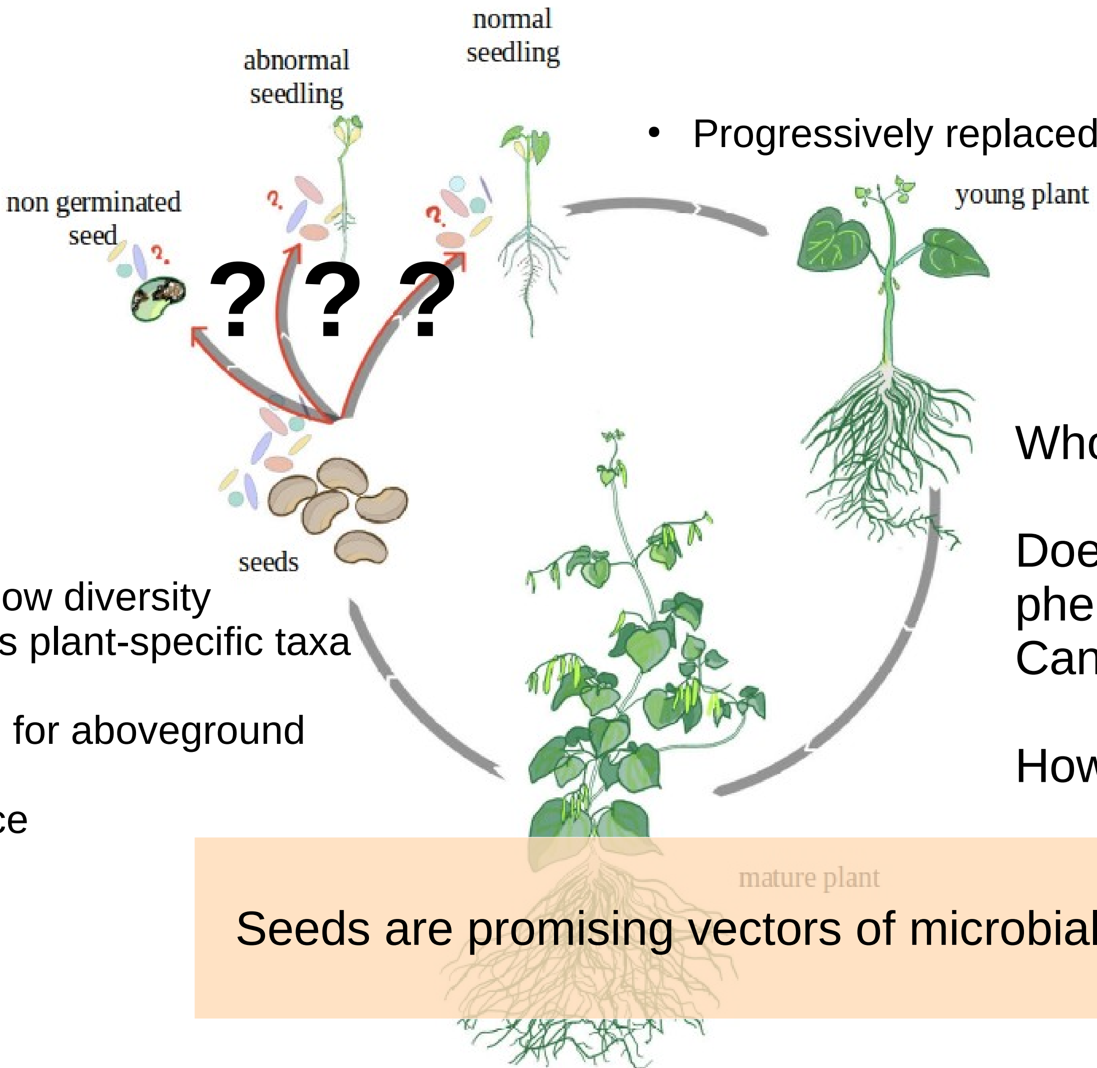

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*Bacteria,
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- Low abundancy, low diversity
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Who is there?

Does it impact seedling phenotype?
Can we use it?

How long does it stays?

Seeds are promising vectors of microbial solutions

My super-supervisors!
Marie Simonin
Béatrice Teulat
Élisabeth Planchet

Thank you kindly
for your attention !

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Béatrice Teulat

You are making all this possible…

FNAMS

GEVES
Expertise & Performance

GEVES
Aurélie Charrier
Didier Demilly
Audrey Dupont
Tituan Ganachaud
Sherif Hamdy
Laurence Le Corre
Marie-Hélène Wagner

Plateau ANAN
Plateforme Phenotic

SUCSEED
ANR-PPR SUCSEED

ISTA-ISSS comitee

and you!

IRHS
Institut de Recherche en Horticulture et Semences

université angers

L'INSTITUT agro Rennes Angers

INRAE
la science pour la vie, l'humain, la terre

Questions?

Seed are not alone: SEM on melon seeds

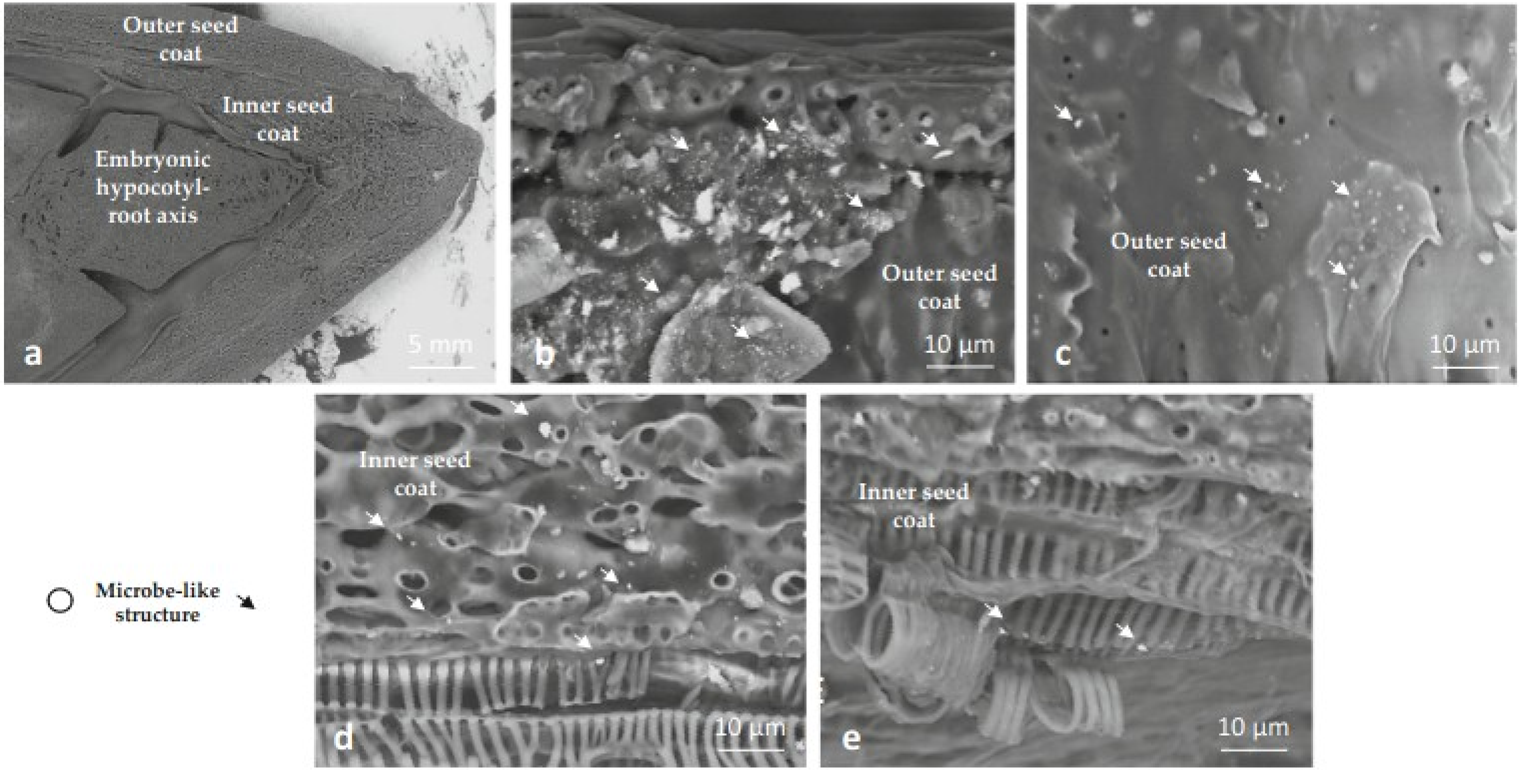


Fig. 1 SEM of seed coats of the cultivated melon *Cucumis melo reticulatus* group 'Dulce'. Within the seed sections (**a**) bacterial-like structures were detected inside the outer part of the seed coat (**b-c**), the inner seed coat (**d-e**), and nearby xylem vessels (**e**)

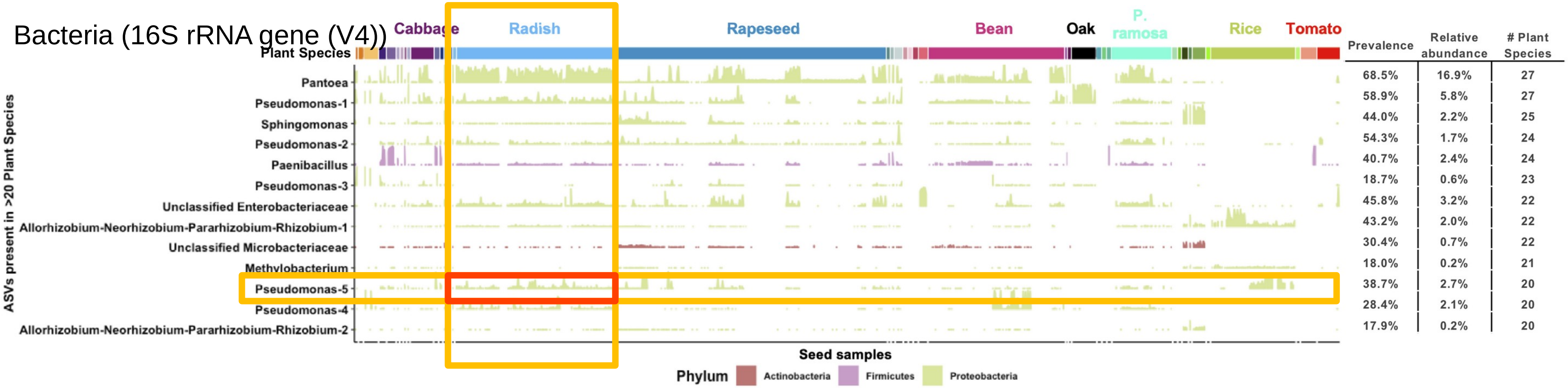
From Glassner et al, Plant Soil 2016

Who’s there? Selection criteria on seed microorganisms

- Tolerance to high osmotic pressure
- Capable of endospore formation
- Motility?

Who is there? A meta-analysis on seed batches

Bacteria (16S rRNA gene (V4))

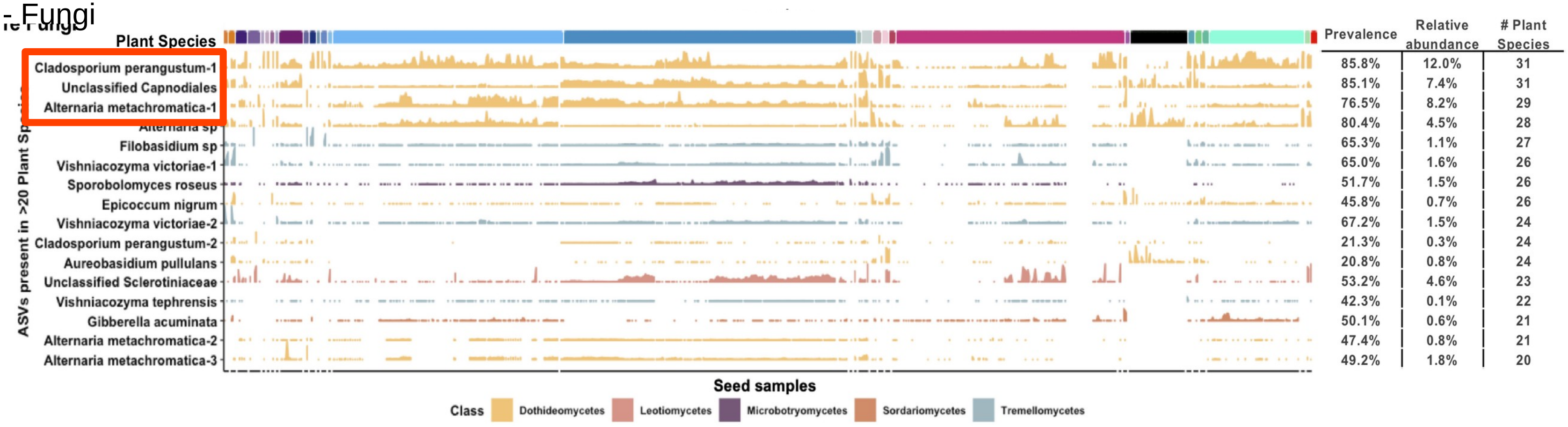


Number of Plant Species Detected	Number of ASVs	
	Bacteria	**Fungi**
1 species	5257 (64%)	1221 (58%)
2-4 species	2422 (30%)	687 (33%)
5-9 species	378 (4.6%)	121 (5.8%)
10-19 species	119 (1.5%)	47 (2.3%)
20-31 species	13 (0.2%)	16 (0.6%)

Simonin et al. 2022 New Phytol

Who is there? A meta-analysis on seed batches

ITS 1 region - Fungi



16 most prevalent fungal ASVs represent **47%** of reads

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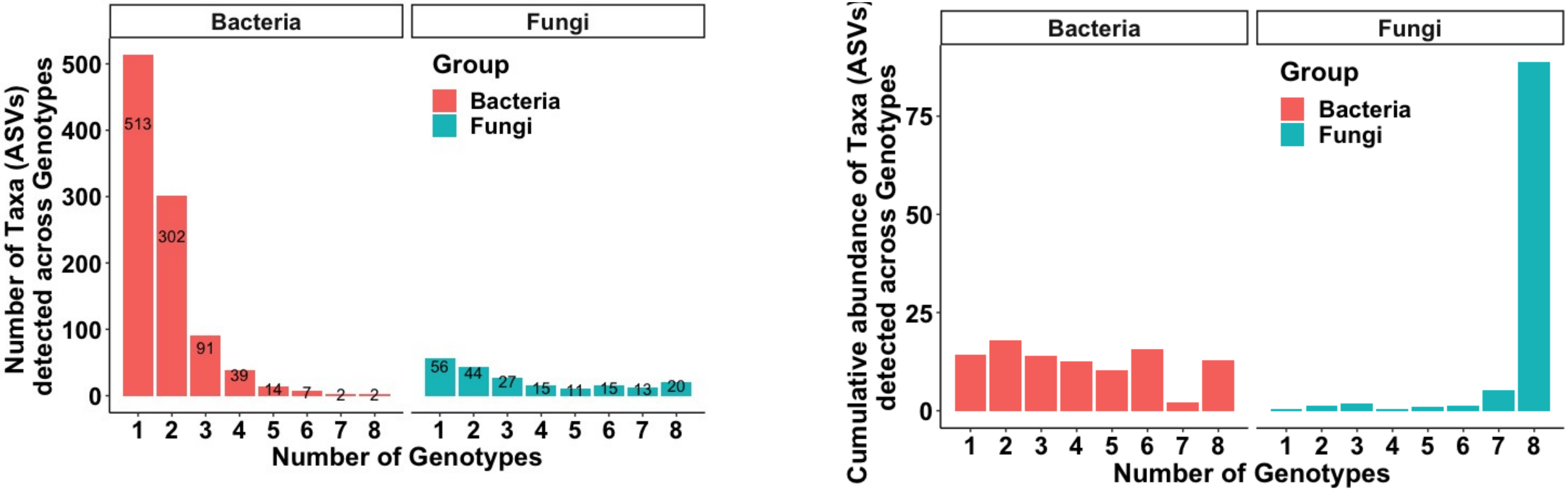
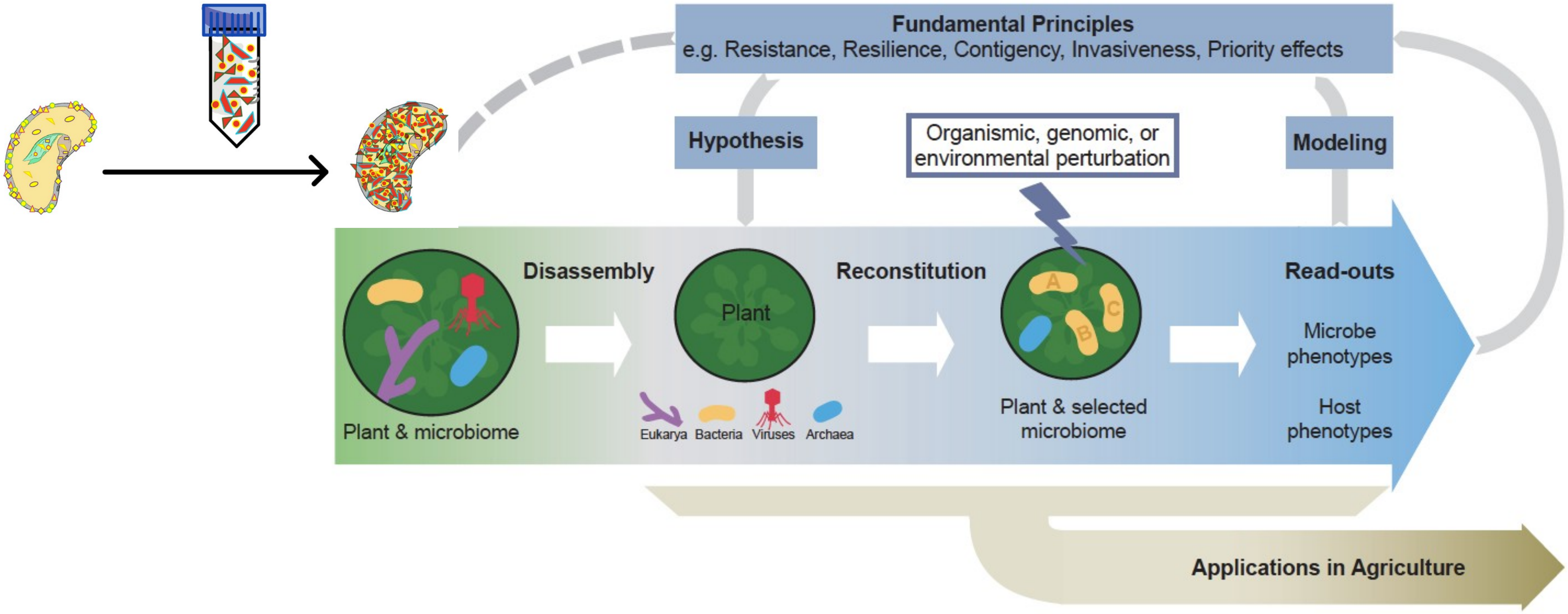
Simonin et al. 2022 New Phytol

Core and Flexible microbiota in Common bean



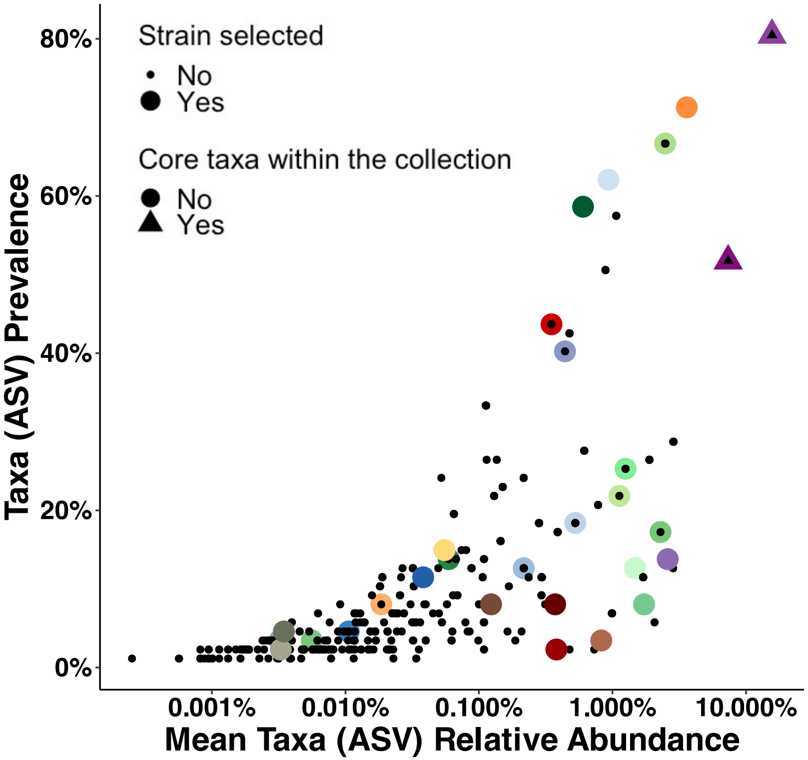
Number of shared ASVs (left) or Cumulative abundance of AVs (right) across bean varieties, for bacteria (gyrB, red) and fungi (ITS, blue) natural seed microbiota of the 8 common bean varieties.

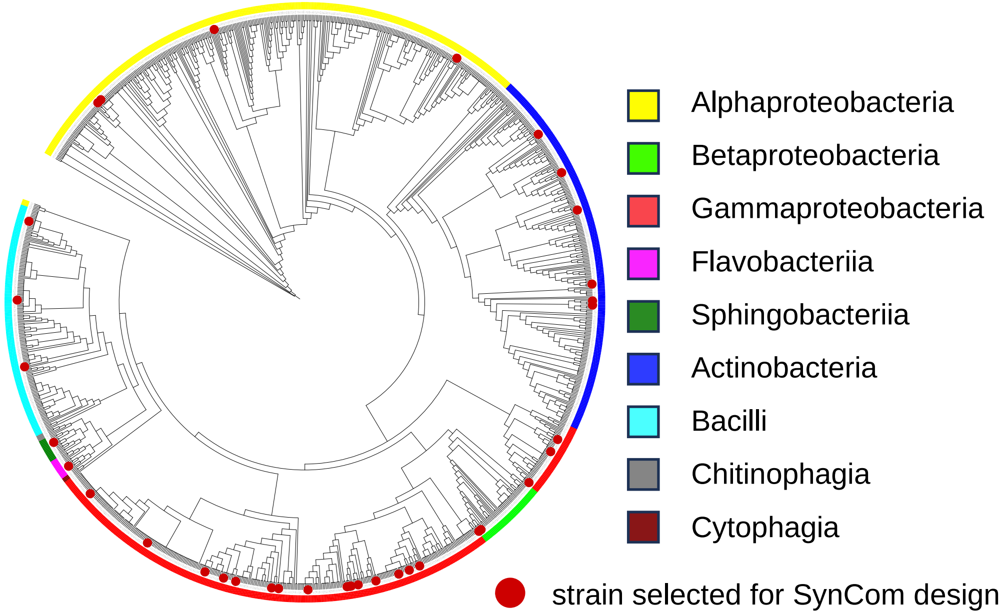
SynComs use



Vorholt et al. (2017)

Design SynCom : 2/ Select taxa

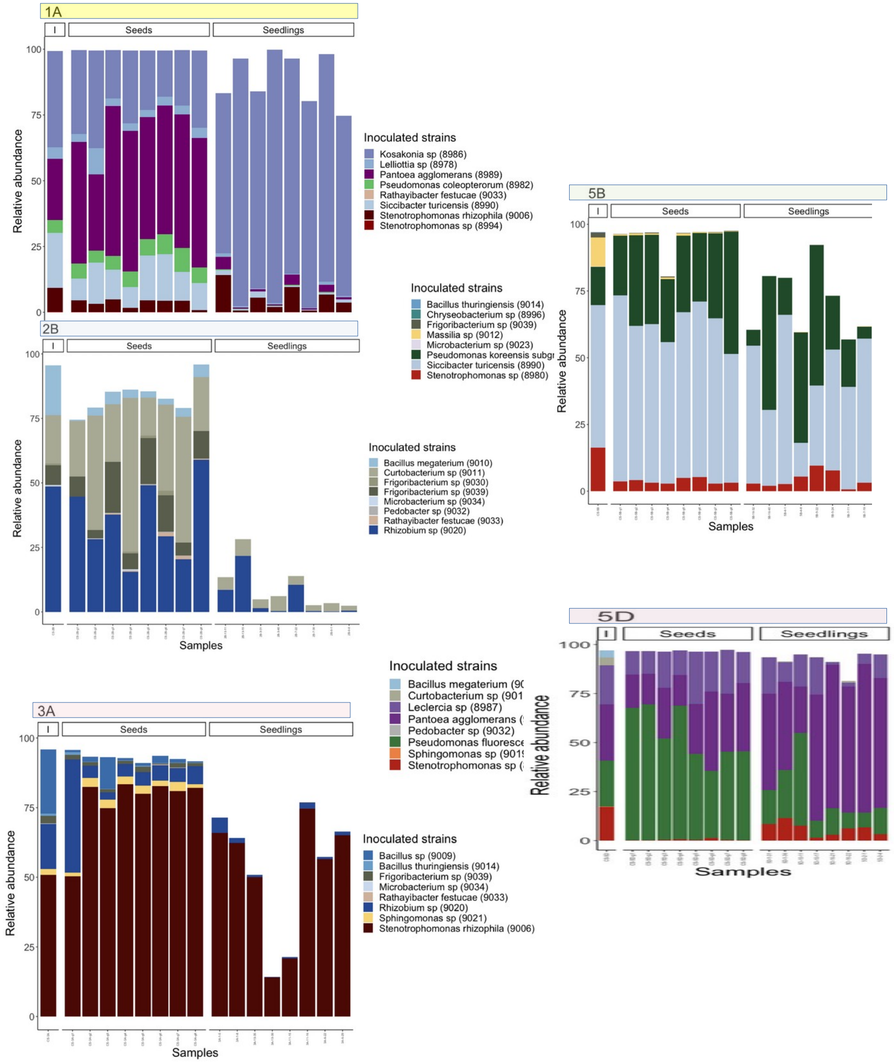


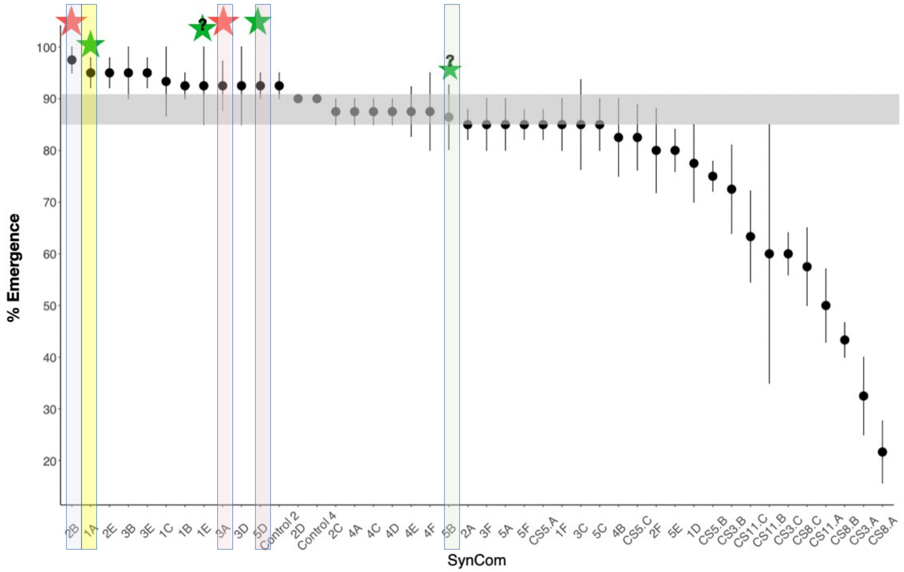


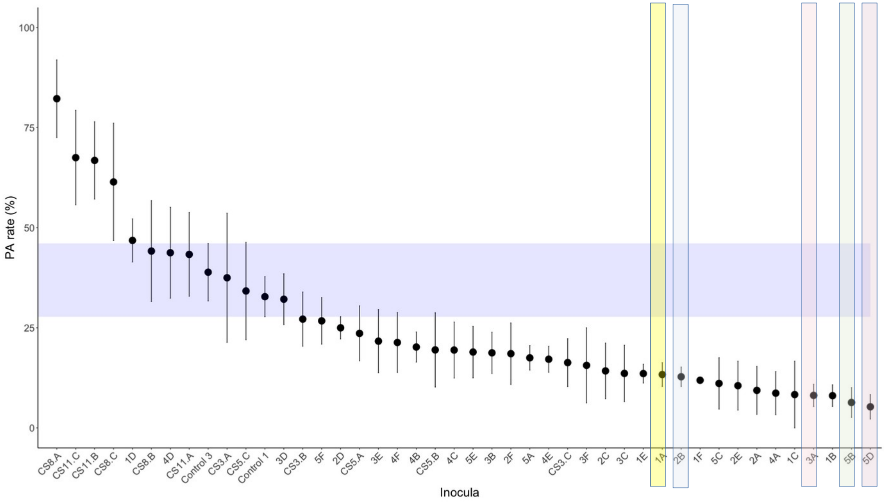
- 36 selected bacterial strains
- Prevalence & Relative abundance
- No functional screening *a priori*

Gontran Arnault, Coralie Marais, Marie Simonin

SynCom choice







SynCom effect on seedling phenotype