

# How to prioritize core microbiome members?

Nejc STOPNISEK

NIOO-KNAW, the Netherlands

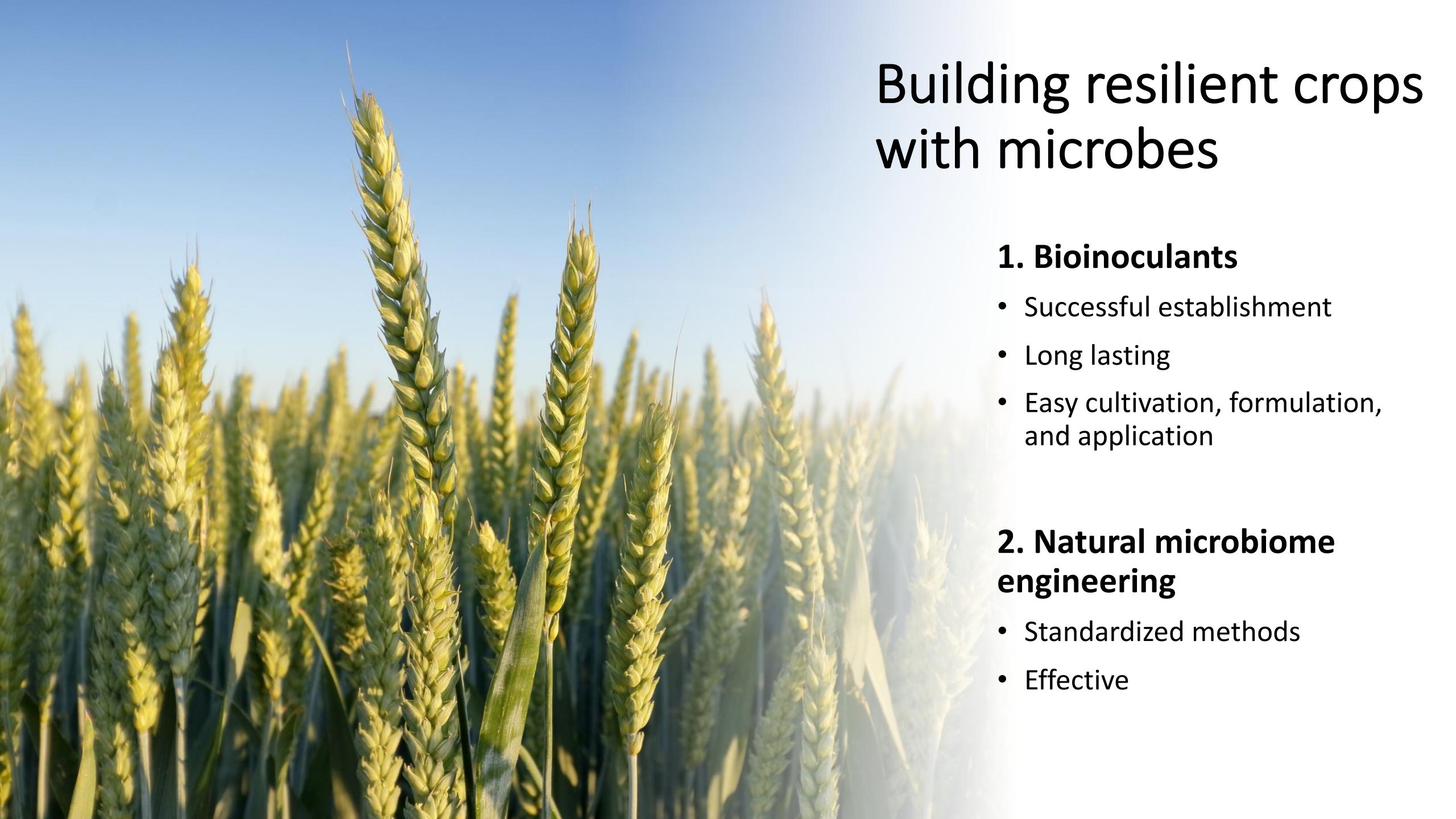
National laboratory for health, food and environment, Slovenia



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NATIONAL LABORATORY OF  
HEALTH, ENVIRONMENT AND FOOD

A close-up photograph of a wheat field. The foreground is filled with green wheat ears, some of which are in sharp focus, showing their texture and color. The background consists of a vast expanse of similar wheat fields stretching to a clear, pale blue sky.

# Building resilient crops with microbes

## 1. Bioinoculants

- Successful establishment
- Long lasting
- Easy cultivation, formulation, and application

## 2. Natural microbiome engineering

- Standardized methods
- Effective

## Core microbiomes for sustainable agroecosystems

Hirokazu Toju<sup>1,2\*</sup>, Kabir G. Peay<sup>10,3</sup>, Masato Yamamichi<sup>10,4</sup>, Kazuhiko Narisawa<sup>5</sup>, Kei Hiruma<sup>2,6</sup>, Ken Naito<sup>7</sup>, Shinji Fukuda<sup>2,8,9,10</sup>, Masayuki Ushio<sup>1,2</sup>, Shinji Nakaoka<sup>2,11</sup>, Yusuke Onoda<sup>12</sup>, Kentaro Yoshida<sup>2,13</sup>, Klaus Schlaeppi<sup>14,15</sup>, Yang Bai<sup>16,17</sup>, Ryo Sugiura<sup>2,18</sup>, Yasunori Ichihashi<sup>2,19,22</sup>, Kiwamu Minamisawa<sup>20</sup> and E. Toby Kiers<sup>21</sup>



# Building resilient crops with microbes

## 1. Bioinoculants

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How to identify plant beneficial microbes with application potentials?

# How to identify plant beneficial microbes with application potentials?

## Culture collection approach



- Diversity restricted to culturing media and conditions.
- Ability to test interactions and benefits.
- Often restricted to controlled conditions.

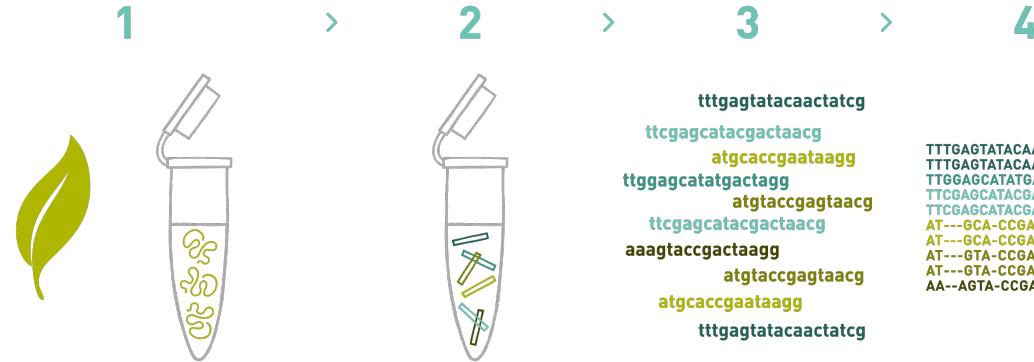
# How to identify plant beneficial microbes with application potentials?

## Culture collection approach



- Diversity restricted to culturing media and conditions.
- Ability to test interactions and benefits.
- Often restricted to controlled conditions.

## Amplicon sequencing



- Time and cost most effective method.
- Challenging to refer beneficial organisms from 16S rRNA/ITS amplicon data alone.

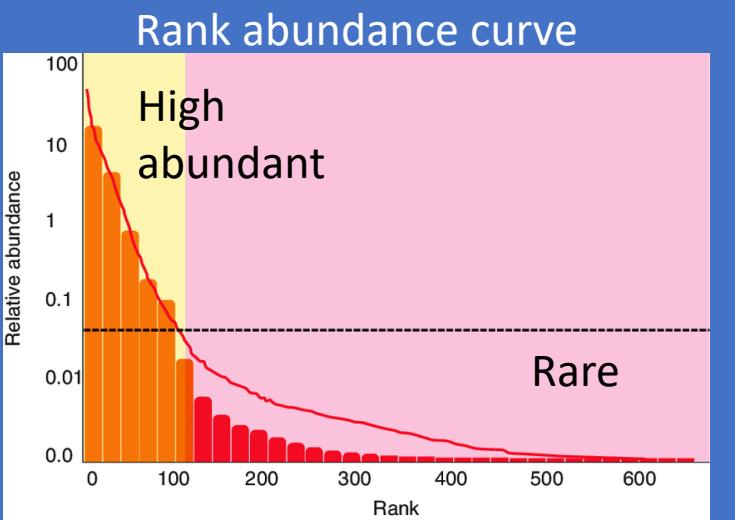
Concept of core microbiomes

# The concept of core (host) microbiome

- Persistence
- Adaptation
- Stability
- Members of the core have been shown to improve host fitness

# How to define the core?

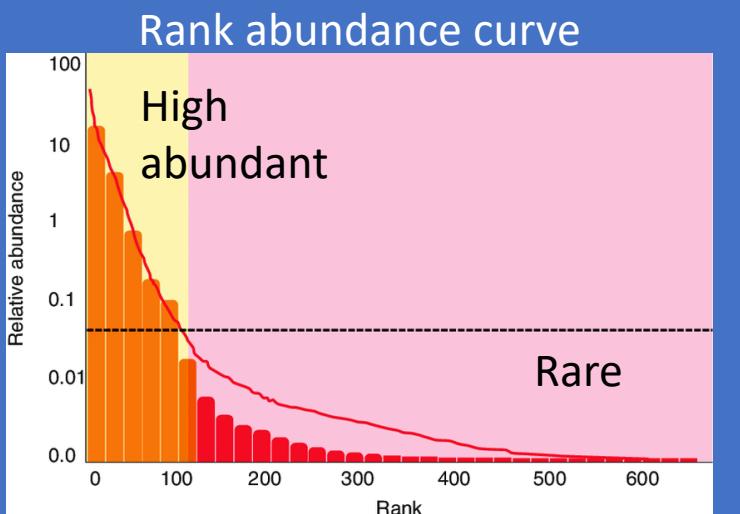
Abundance



# How to define the core?

## Abundance

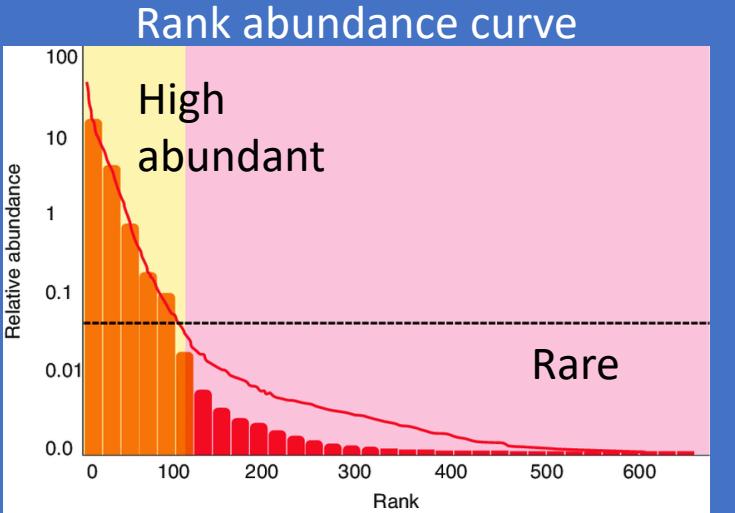
- + Informs about the most abundant taxa with a host
- Many beneficial taxa are “rare” and not included



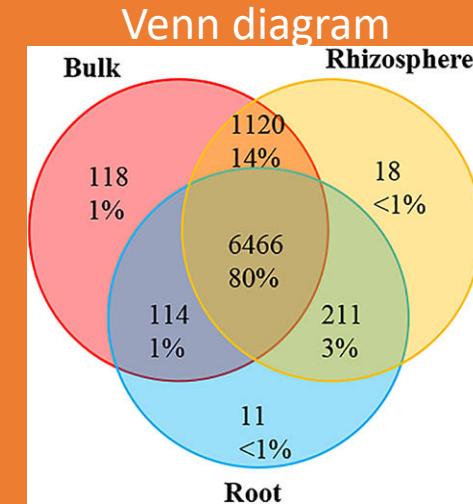
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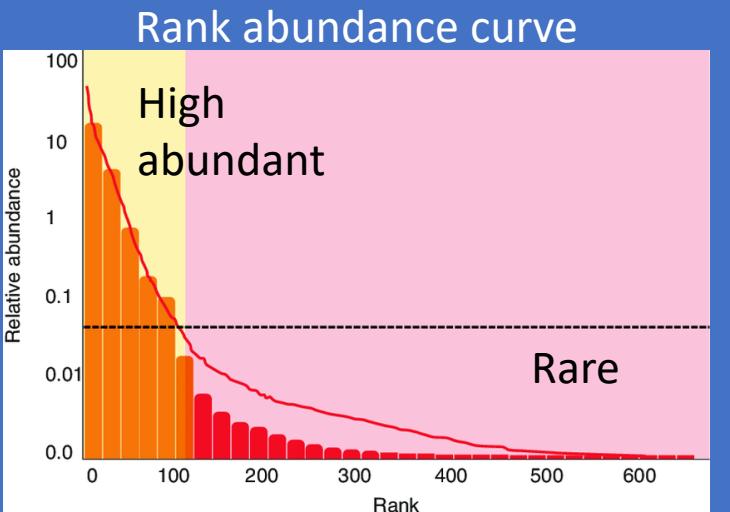
## Detection



# How to define the core?

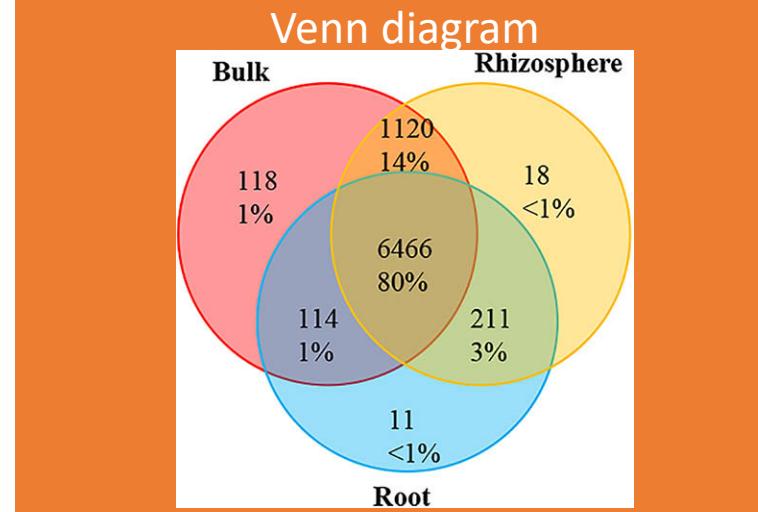
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## Detection

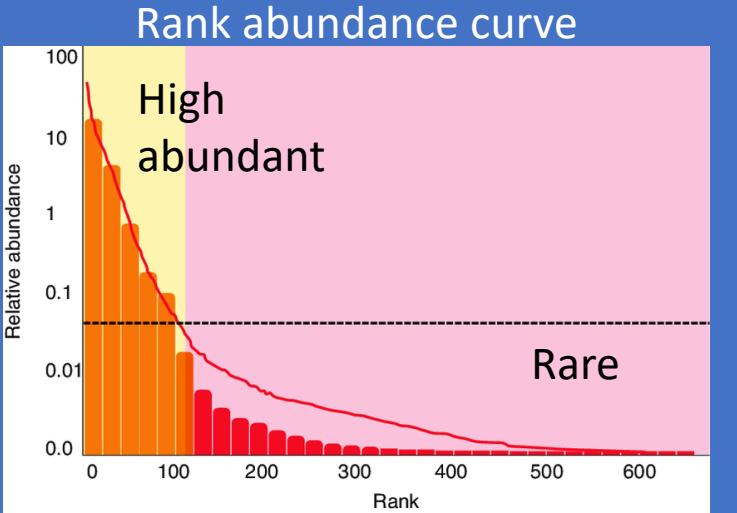
- + Taxa consistently detected on a host
- Overestimation
- Including transient/condition specific taxa



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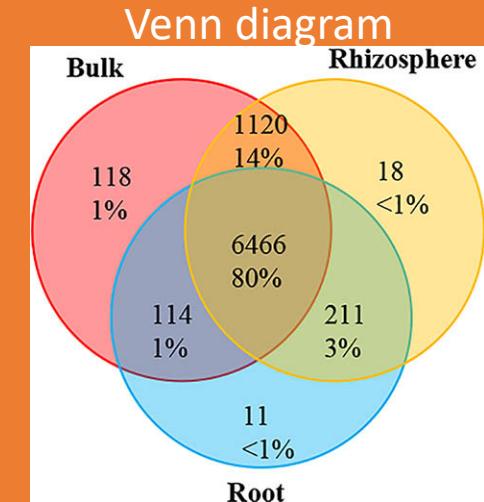
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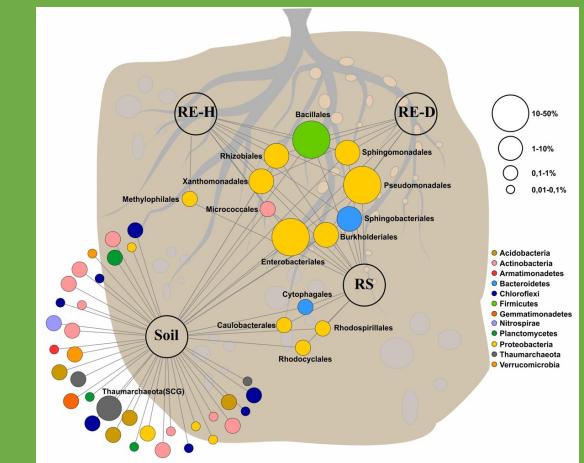
## Detection

- + Taxa consistently detected on a host
- Overestimation
- Including transient/condition specific taxa



## Combination

- + both abundance and detections are considered
- Differential abundance
- Network-analysis



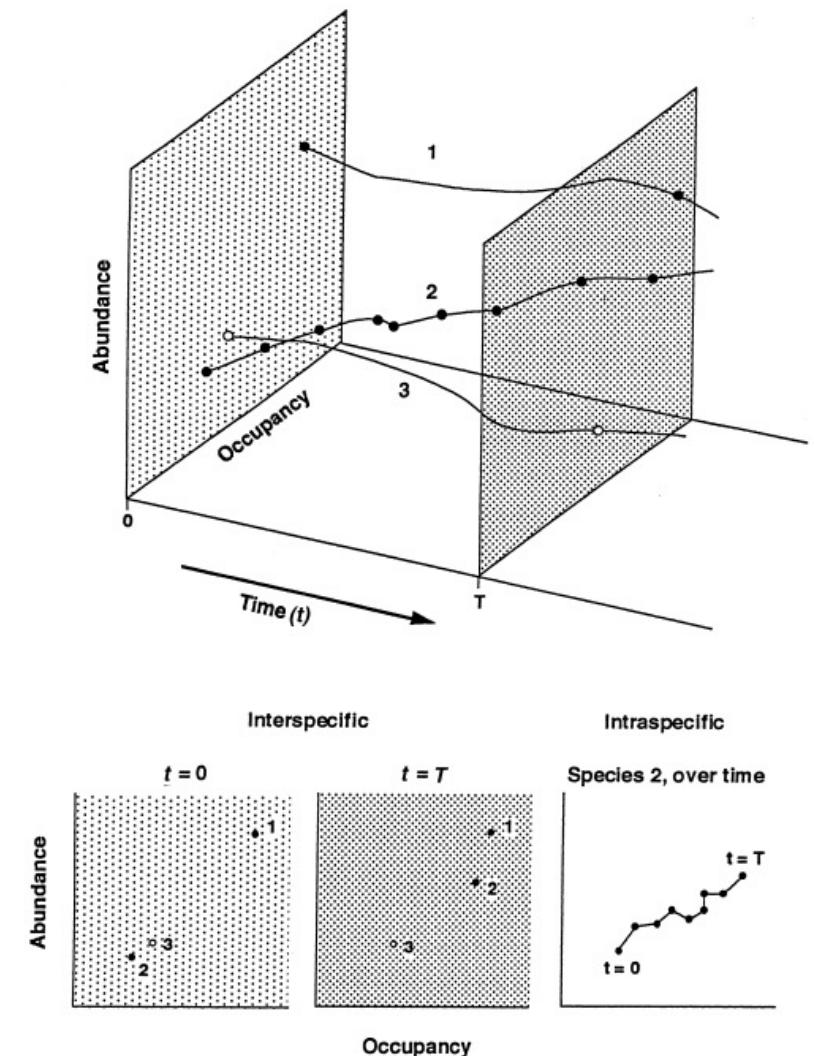
# Abundance-occupancy distribution

A widely used approach in macroecology to explore patterns in species distribution.

Abundance = proportional contribution across samples

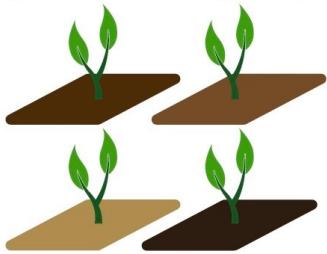
Occupancy = frequency of detection across samples

A species mean relative abundance (log transformed) is plotted against the proportion of discrete samples in which it occurs which provides rich information for interpreting diversity patterns at population and community levels.



# Proposed method to prioritize core microbiome

## ① Spatial study design



## ②

Abundance	Site 1		Site 2	
	Rep1	Rep2	Rep1	Rep2
OTU1	11	0	0	13
OTU2	10	7	15	40
OTU3	0	0	0	32
OTU4	17	4	0	23
OTUn	65	9	24	18

## Temporal study design

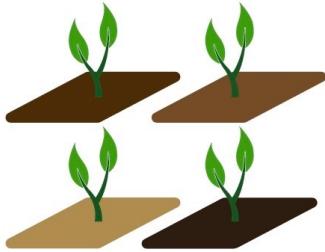


## Abundance

Abundance	Time 1		Time 2	
	Rep1	Rep2	Rep1	Rep2
OTU1	43	7	0	0
OTU2	0	0	31	27
OTU3	0	0	3	65
OTU4	15	8	0	20
OTUn	3	24	32	11

# Proposed method to prioritize core microbiome

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## Temporal study design



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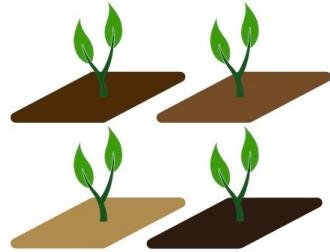
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Which taxa to prioritize?

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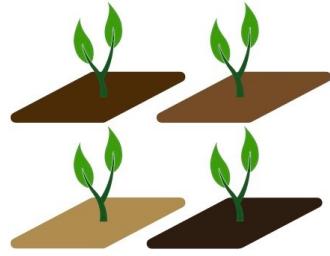
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## Which taxa to prioritize?

Presence in all samples (occupancy=1) is very conservative and biased towards more abundant taxa. We should consider detection consistency within a location/time point but also replication consistency.

# Proposed method to prioritize core microbiome

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## ③

Site-specific occupancy	Site 1		Site 2	
	OTU1	OTU2	OTU3	OTU4
OTU1	0.5	0.5	0	0
OTU2	1	1	0	0.5
OTU3	0	0.5	0	0
OTU4	1	0.5	1	0
OTUn	1	1	1	1

Replication consistency	Site 1		Site 2	
	OTU1	OTU2	OTU3	OTU4
OTU1	0	0	0	0
OTU2	1	1	0	0
OTU3	0	0	0	0
OTU4	1	0	1	0
OTUn	1	1	1	1

$$\text{Index}_{\text{OTUi}} = \frac{\sum (\text{Site-specific occupancy}) + \sum (\text{Replication consistency})}{2}$$

## Temporal study design



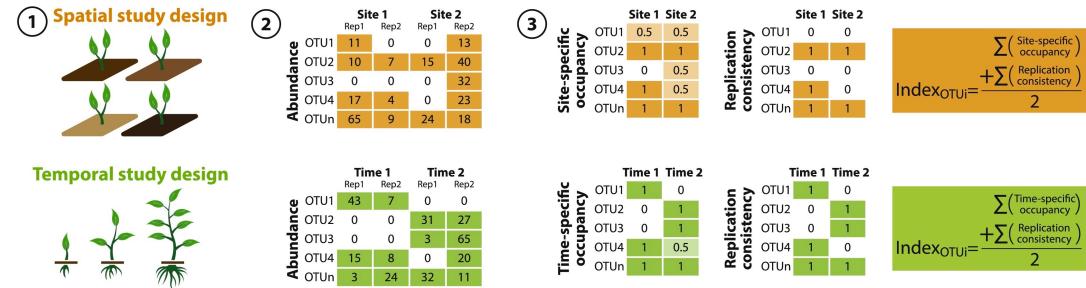
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OTU1	1	0	0	0
OTU2	0	1	0	1
OTU3	0	1	0	1
OTU4	1	0.5	1	0
OTUn	1	1	1	1

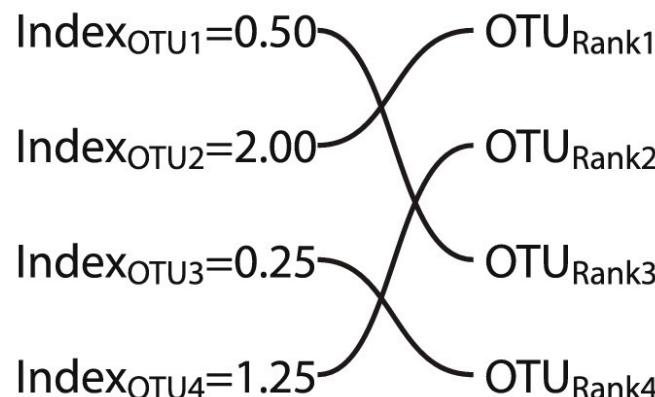
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$$\text{Index}_{\text{OTUi}} = \frac{\sum (\text{Time-specific occupancy}) + \sum (\text{Replication consistency})}{2}$$

# Proposed method to prioritize core microbiome

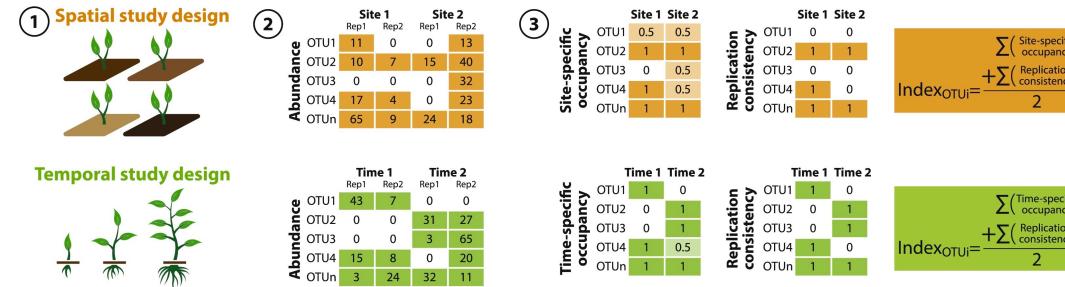


## ④ Ranking OTUs by prevalence

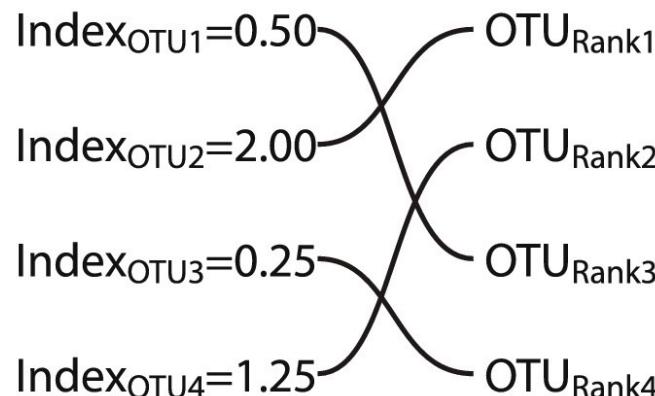


At what threshold are these OTUs considered as members of a core?

# Proposed method to prioritize core microbiome



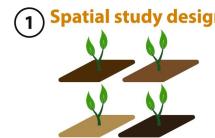
## 4 Ranking OTUs by prevalence



At what threshold are these OTUs considered as members of a core?

1. Contribution to beta-diversity
2. Assembled by deterministic processes

# Proposed method to prioritize core microbiome



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③ Site-specific occupancy

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	OTU1	OTU2	OTU3	OTU4
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OTU2	1	1	0	0.5
OTU3	0	0.5	0	0
OTU4	1	0.5	1	1
OTUn	1	1	0	0

Time-specific occupancy

	Time 1		Time 2	
	OTU1	OTU2	OTU3	OTU4
OTU1	1	0	0	0
OTU2	0	1	0	1
OTU3	0	0	1	0.5
OTU4	1	0	1	1
OTUn	1	1	0	0

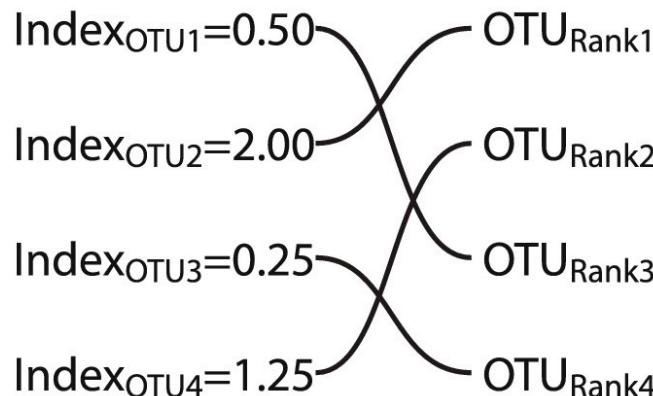
Replication consistency

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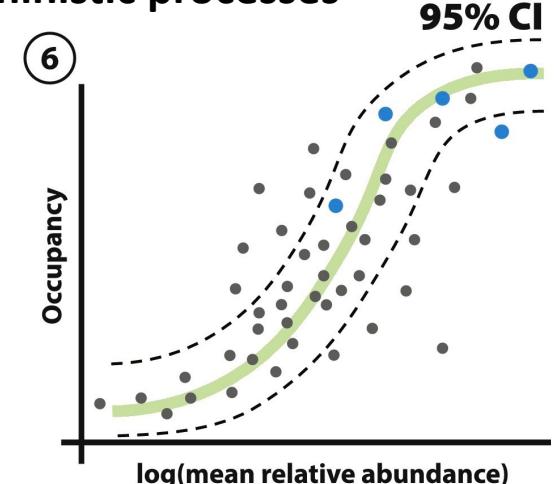
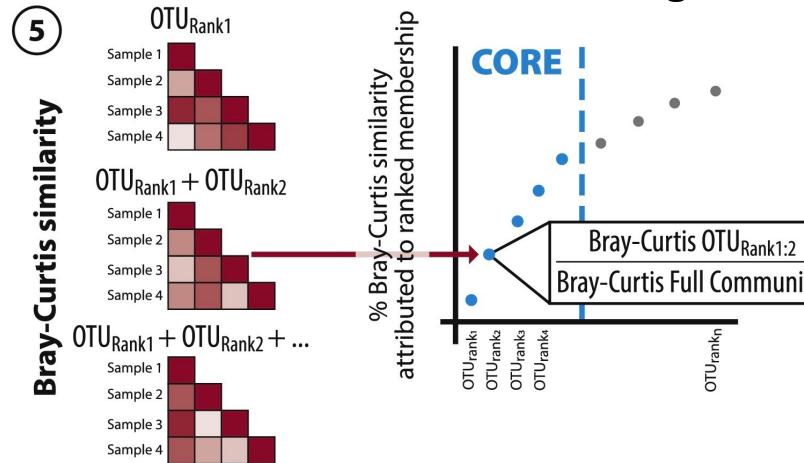
$$\text{Index}_{\text{OTUi}} = \frac{\sum (\text{Site-specific occupancy}) + \sum (\text{Replication consistency})}{2}$$

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## ④ Ranking OTUs by prevalence

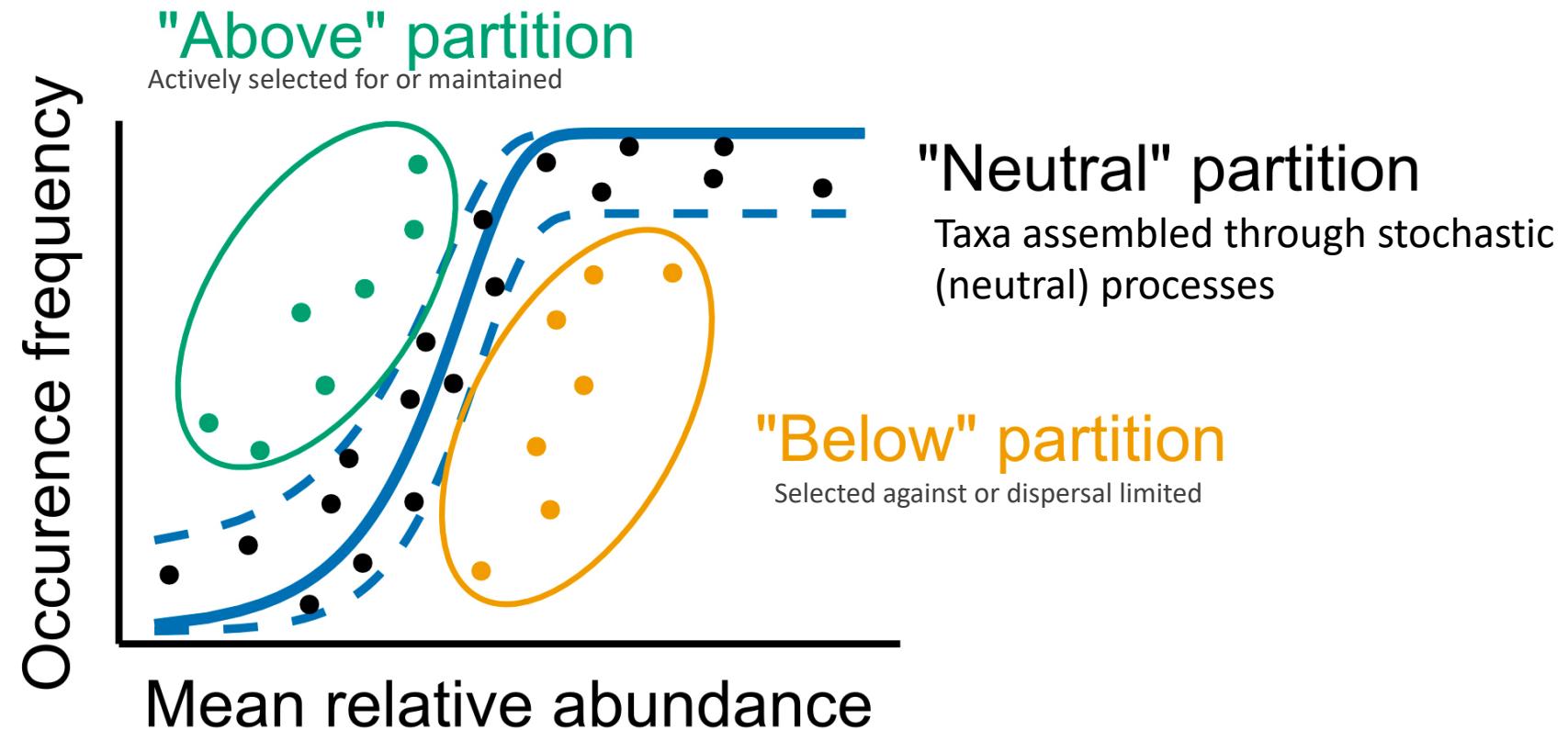


## Selecting OTUs with highest contribution to beta-diversity and/or those assembled through deterministic processes



# Community assembly processes

**Sloan neutral model** = most abundant taxa exhibit highest occupancies, and the rare taxa exhibit lowest occupancies  
(assuming unlimited dispersal and no fitness differences among taxa)



# Application of the framework – Example I

Article | Open Access | Published: 12 September 2019

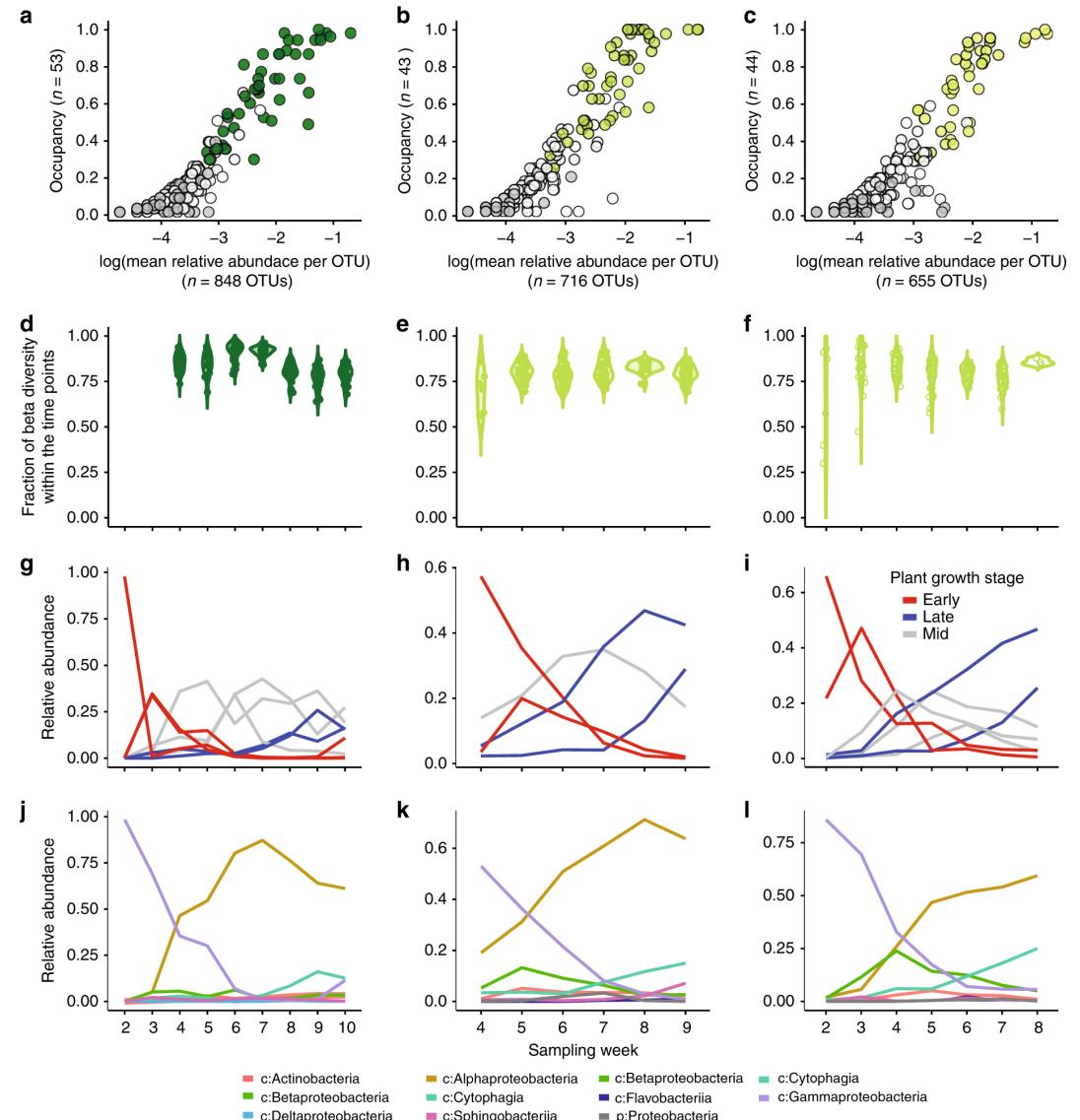
## Assembly and seasonality of core phyllosphere microbiota on perennial biofuel crops

Keara L. Grady, Jackson W. Sorensen, Nejc Stopnisek, John Guttar & Ashley Shade 

Nature Communications 10, Article number: 4135 (2019) | Cite this article

4644 Accesses | 34 Citations | 59 Altmetric | Metrics

- 61 “core” taxa
- Stable year to year
- Temporal variation within a growing season
- Phylogenetically closely related to known phyllosphere microbiota

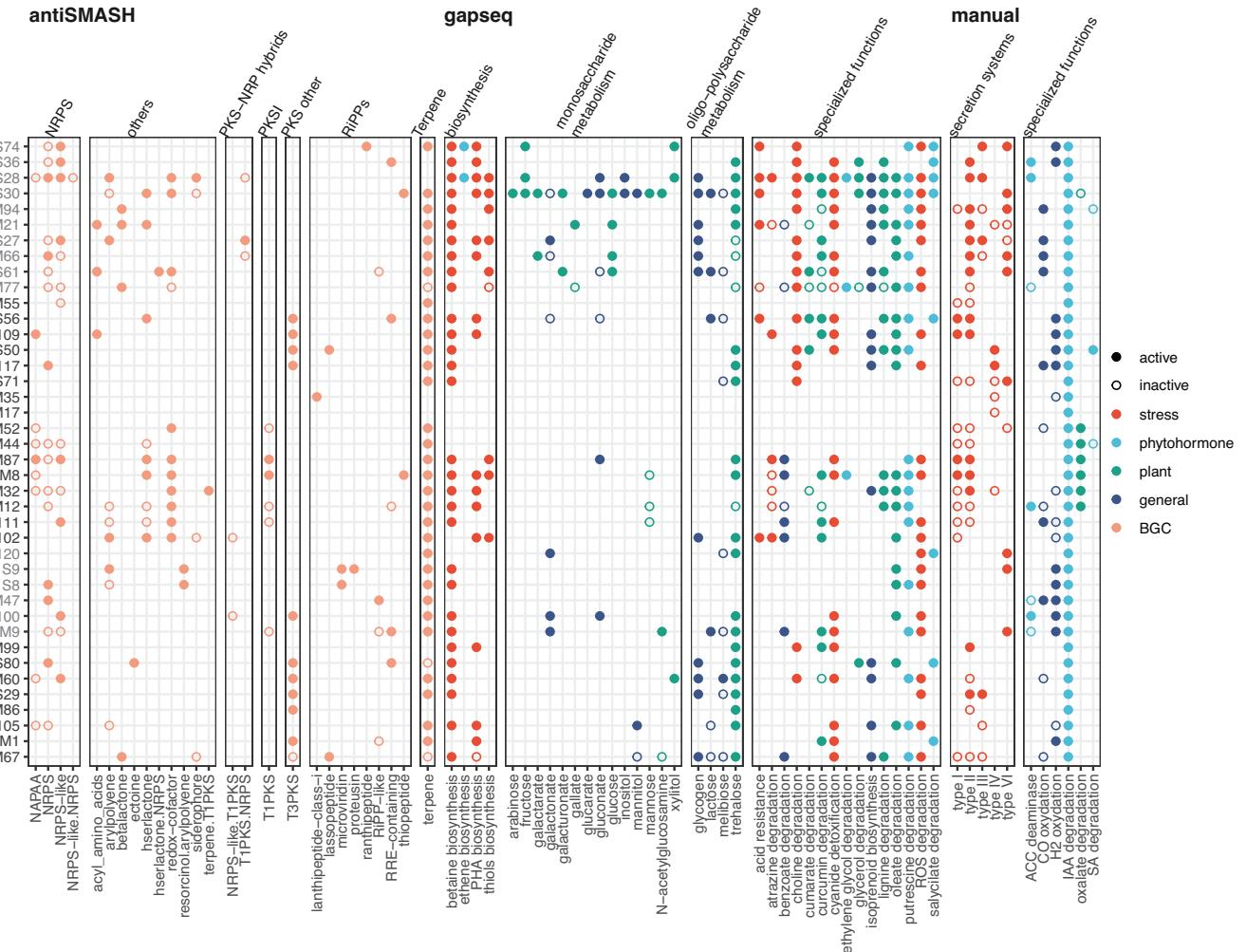
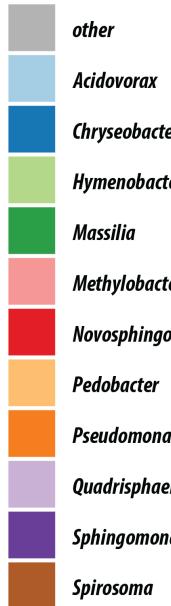
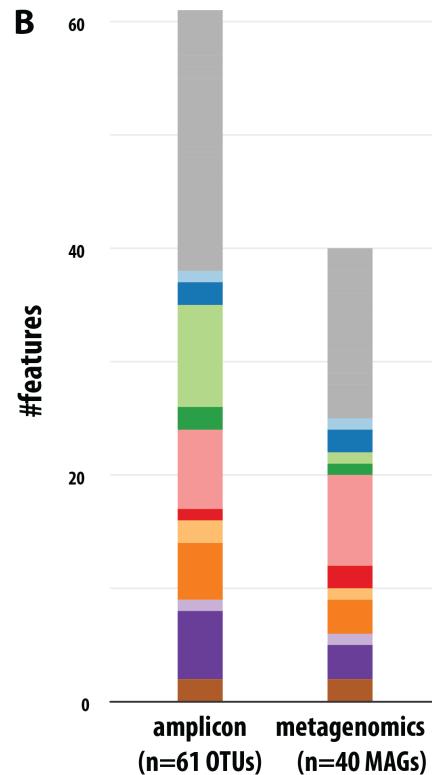


# Multi-omics confirms that prioritized taxa are important players adapted to host lifestyle

## Life on the leaf: Seasonal activities of the phyllosphere microbiome of perennial crops

Adina C. Howe, Nejc Stopnisek, Shane K. Dooley, Fan Yang, Keara L. Grady, Ashley Shade

doi: <https://doi.org/10.1101/2021.04.20.440608>



# Application of the framework – Example II

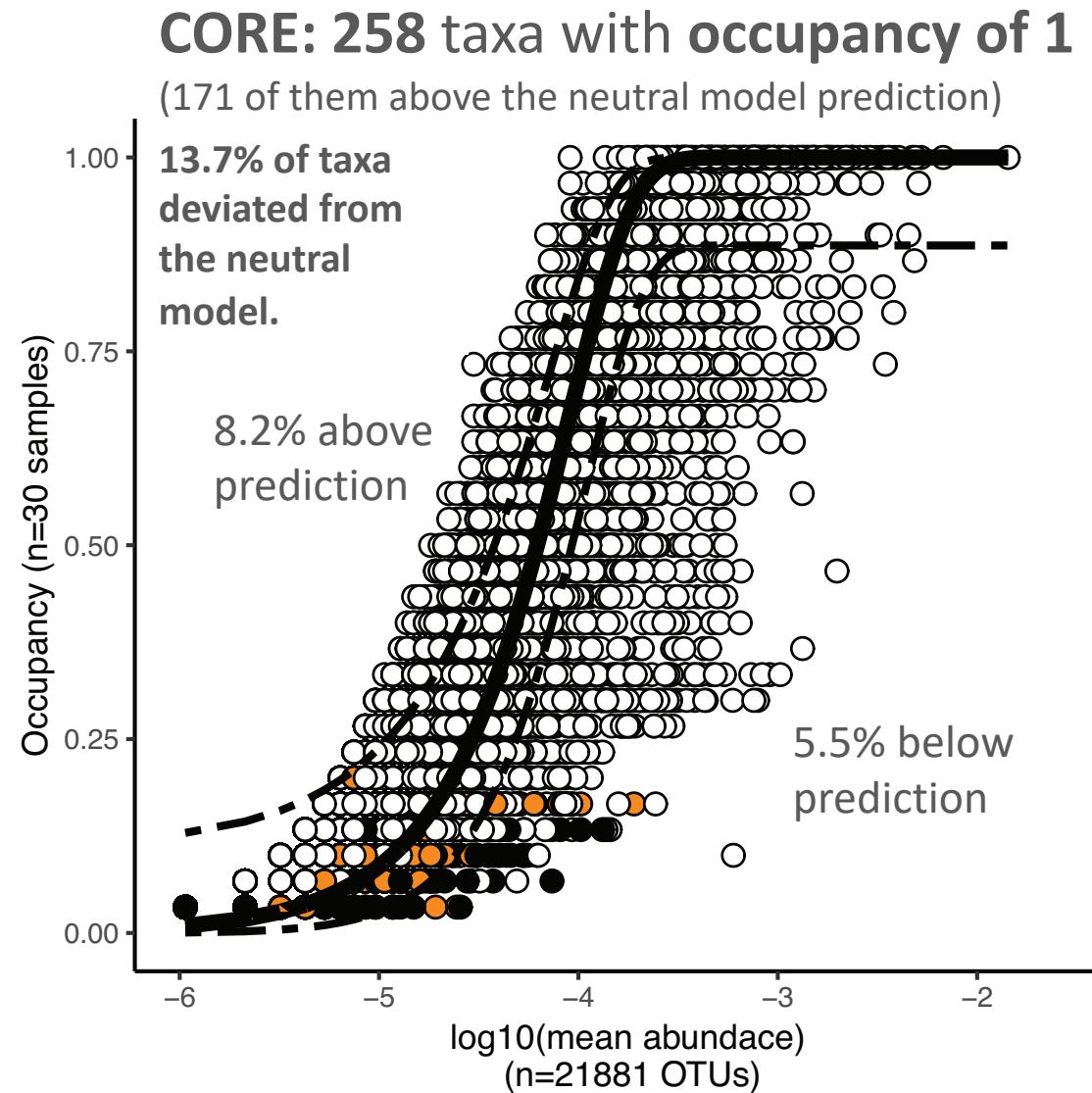
Article | Open Access | Published: 26 March 2021

## Persistent microbiome members in the common bean rhizosphere: an integrated analysis of space, time, and plant genotype

Nejc Stopnisek & Ashley Shade 

The ISME Journal (2021) | Cite this article

1525 Accesses | 33 Altmetric | Metrics



# Summary

Members of the core microbiome can be discovered and prioritized from amplicon sequencing datasets using the abundance-occupancy distributions.

Neutral models can improve hypotheses about the drivers of core microbiome assembly, to interpret mechanisms that select for core taxa, and to inform discussion and interpretation of the core.

Abundance-occupancy distributions allow researchers to discover and prioritize members of the core microbiome based on their underlying ecology.

**A systematic first step towards understanding the functional importance of the core for the plant**