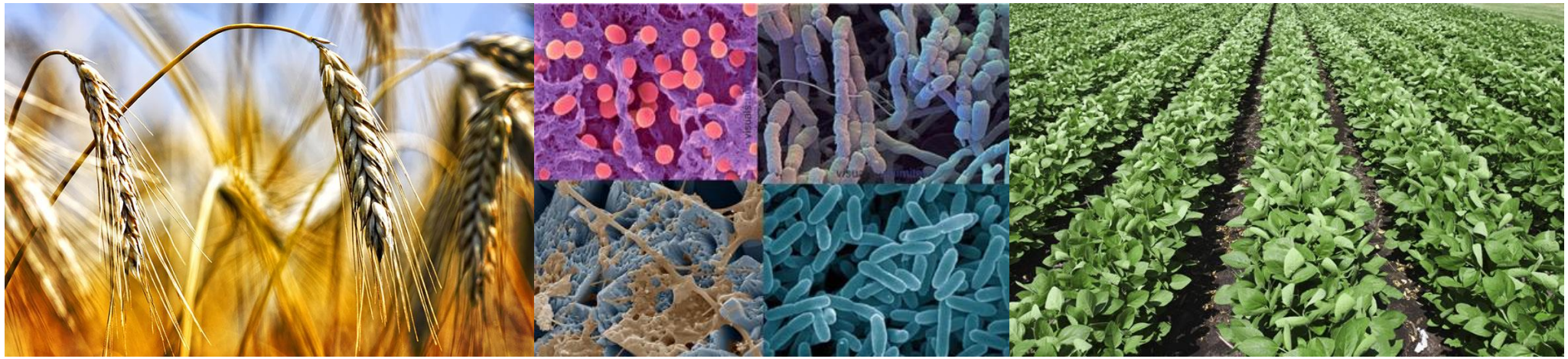
Does inoculation with introduced *Rhizoglomus irregulare* change AMF community structure in the field?



Jacynthe Masse, Geneviève Lachance, Agathe Vialle & Mohamed Hijri

Mycorhizes 2017 | Université Laval | 11 mai 2017

Institut de recherche en biologie végétale
Centre sur la biodiversité de l'Université de Montréal

PREMIER TECH

STAT2 initiative

Université de Montréal

Context: 1945 - Green revolution

USDA Photo by: Charles O'Rear - http://www.usda.gov/oc/photo/95cs2841.htm

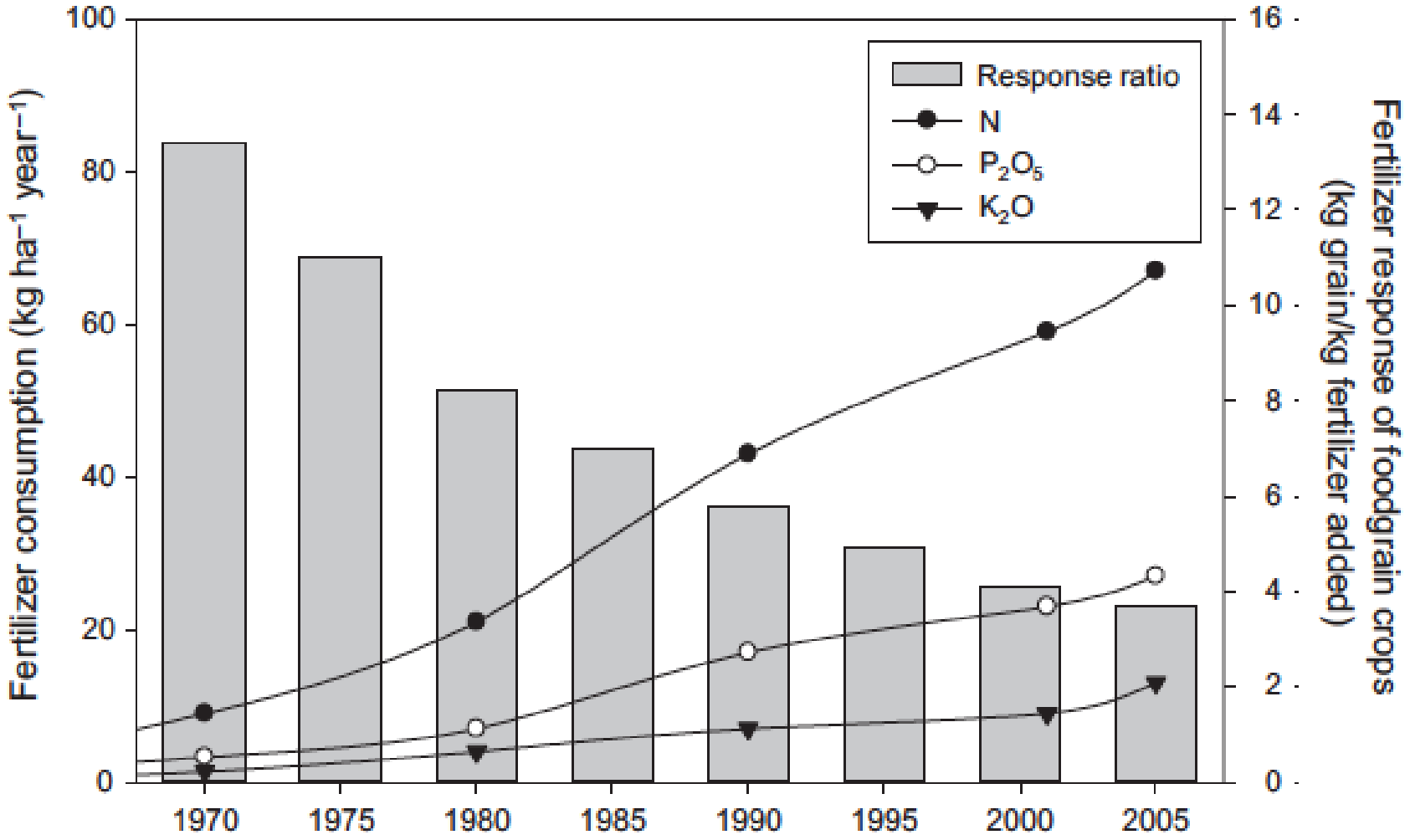
Sharp increased in fertilizers and pesticides usage

Changes in machinery

Context: 1945 - Green revolution

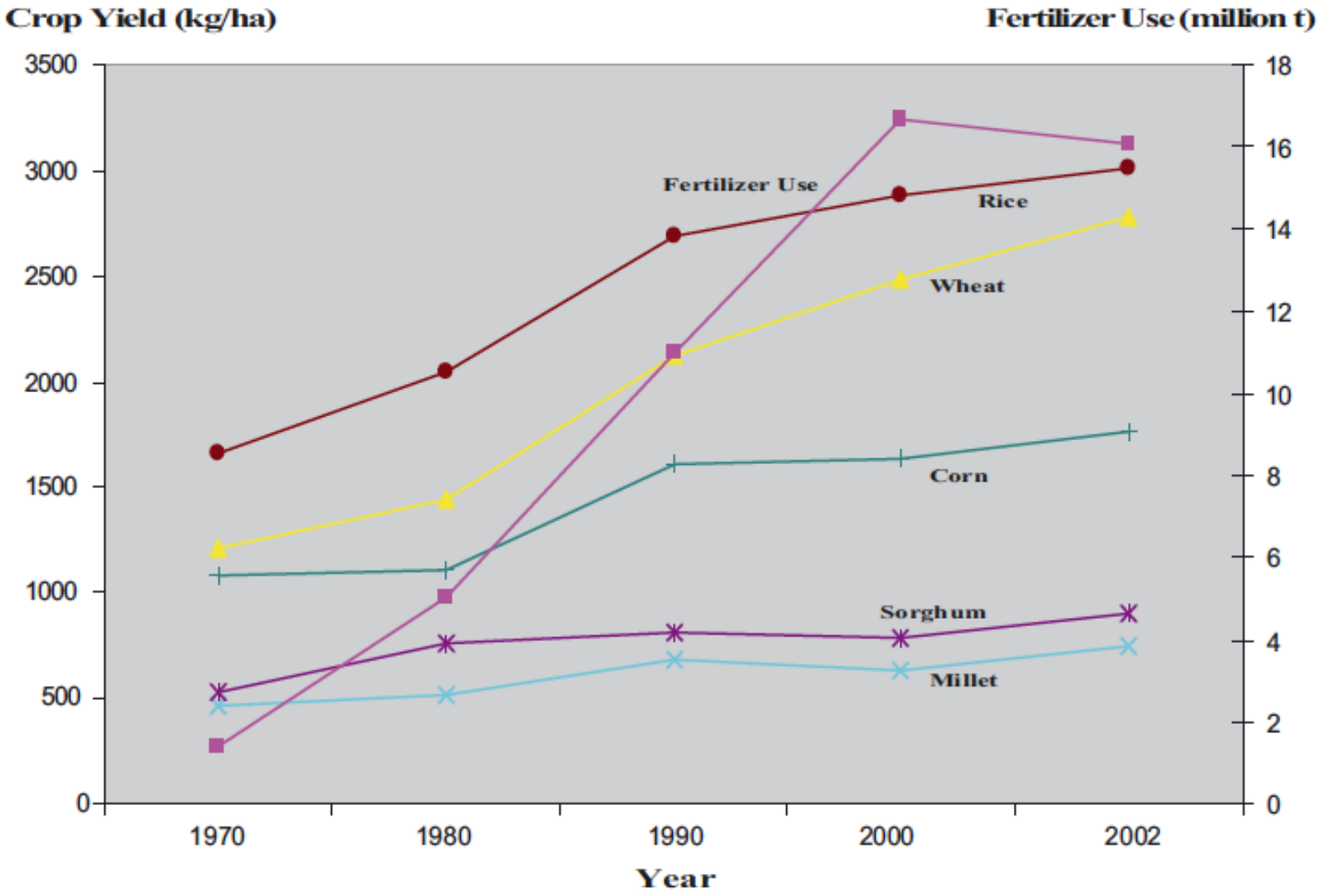
Hybrid selection

Green revolution / consequences



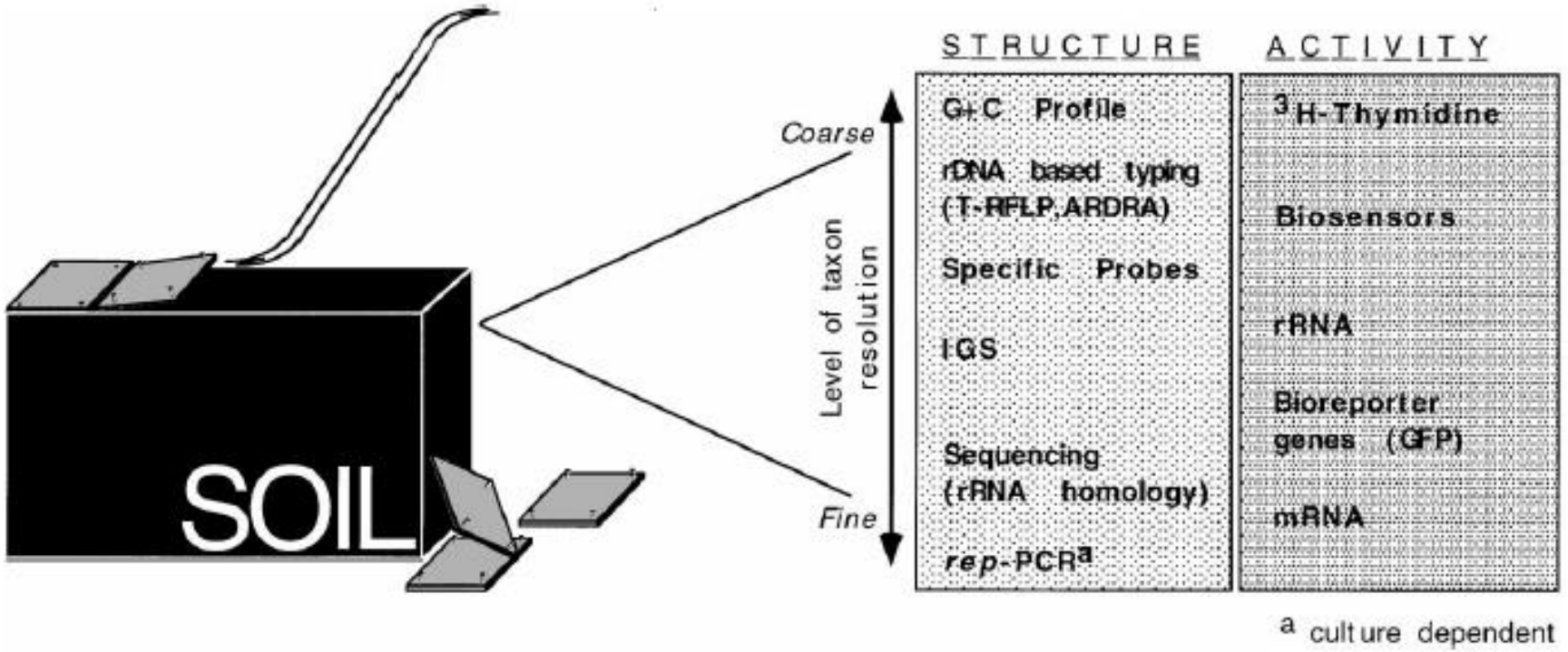
Jones et al. 2013. *Journal of Applied Ecology*

Green revolution / consequences



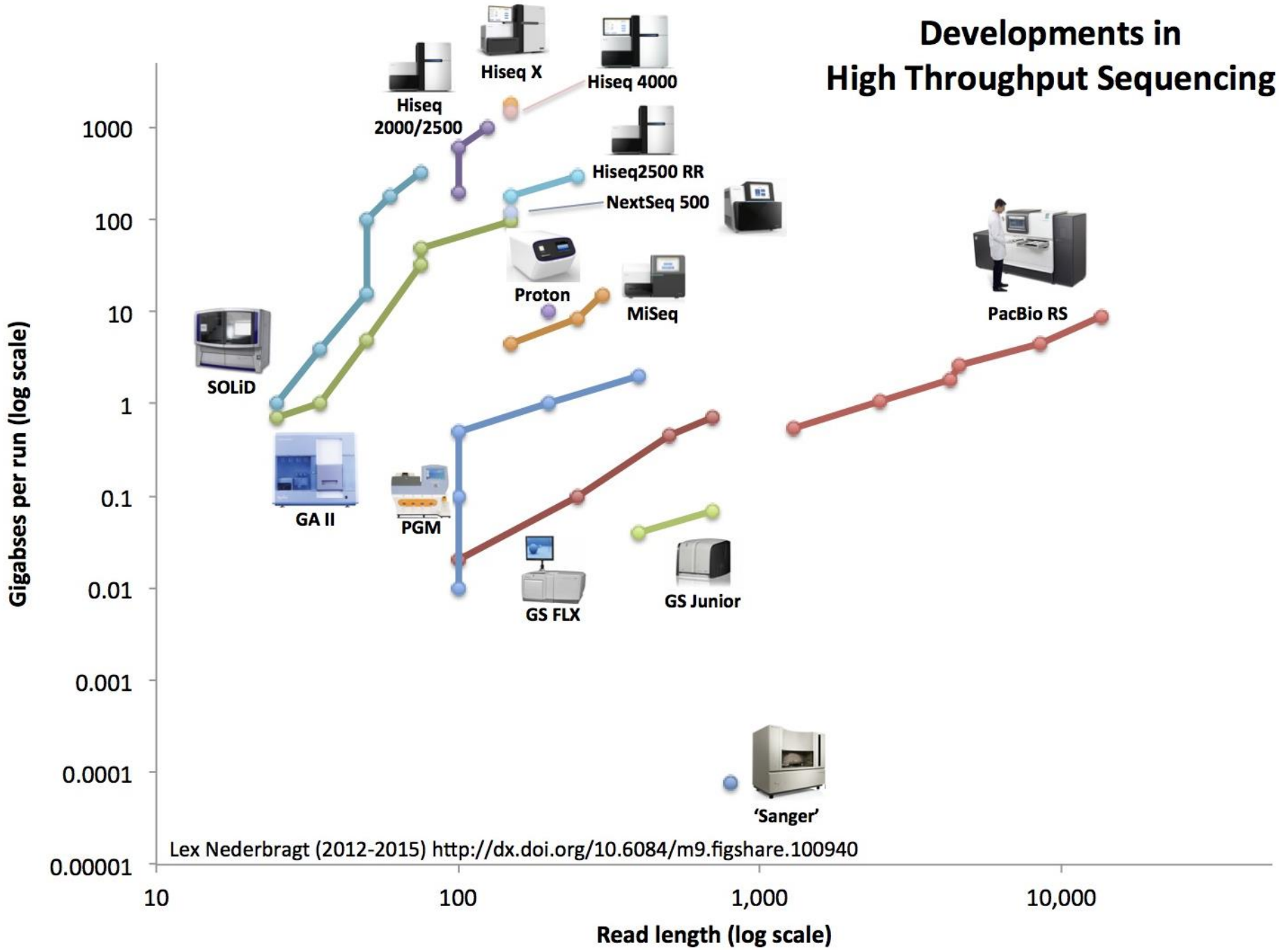
Trends in fertilizer use and crop yields in India (data compiled from FAO Production Yearbooks) – Lal 2009. *Agronomy for sustainable development*

Context: Microbial black box



Tiedje et al. 1999. *Applied Soil Ecology*

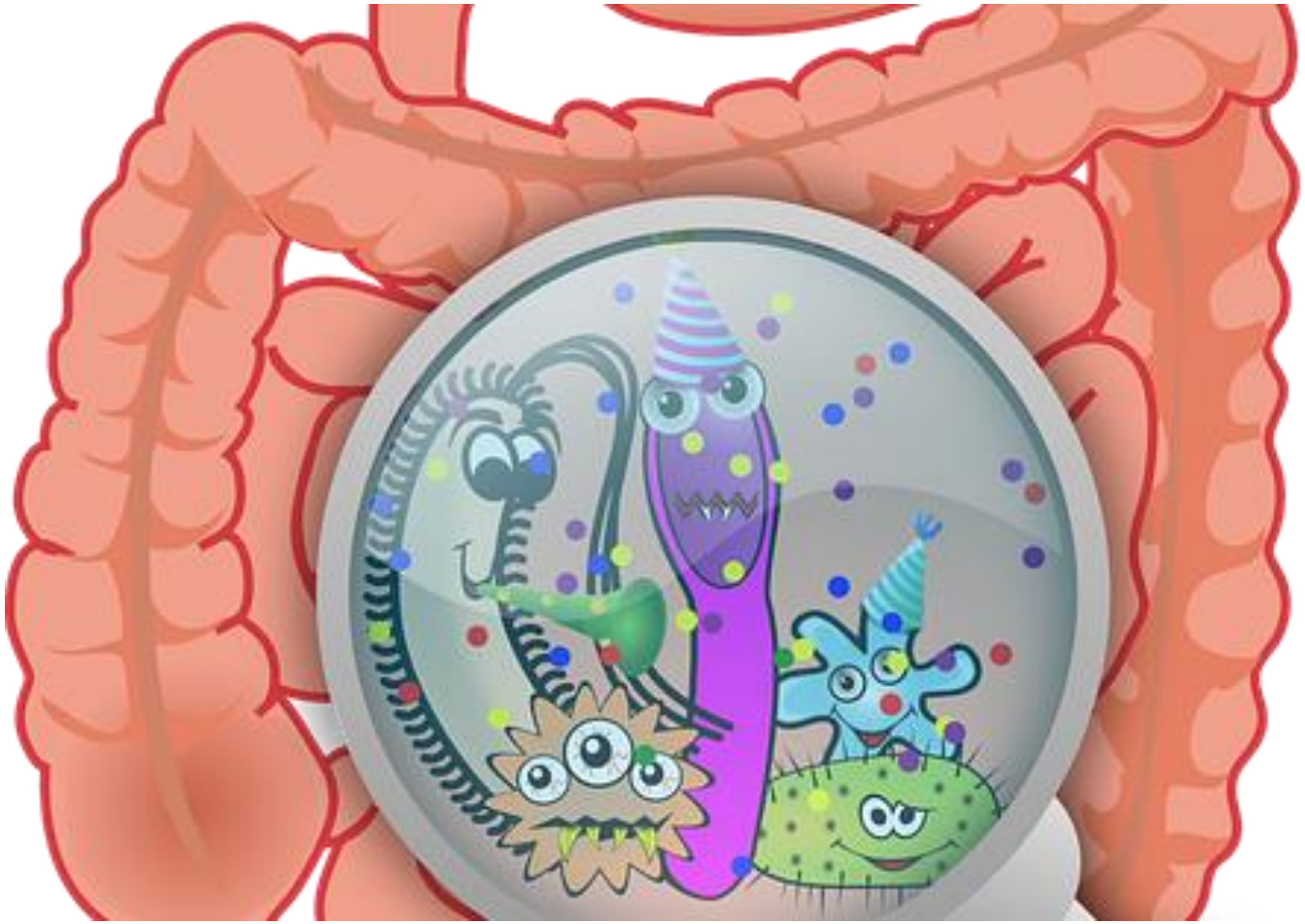
Context: Microbial black box



Towards a greener revolution

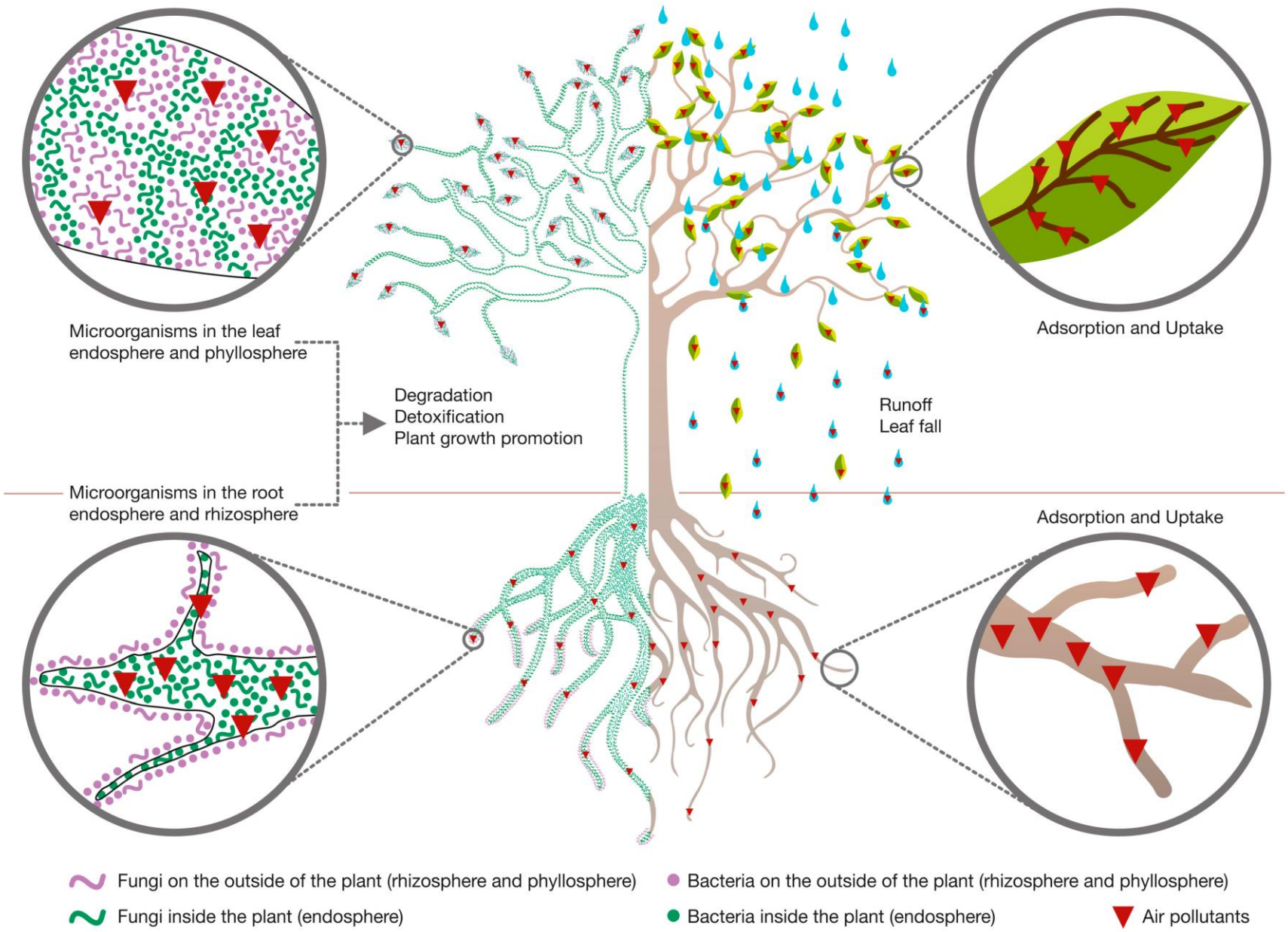
"*Improved understanding of plant-microbe interactions has the potential to* ***increase crop productivity by 20% while reducing fertilizer and pesticide requirements by 20%, within 20 years****, according to the report. These estimates rest on the recognition that all plants rely on microbial partners to secure nutrients, deter pathogens and resist environmental stress.*"

Reid and Green 2013.



https://hemtecks.wordpress.com/2016/01/17/gut-microbiota/

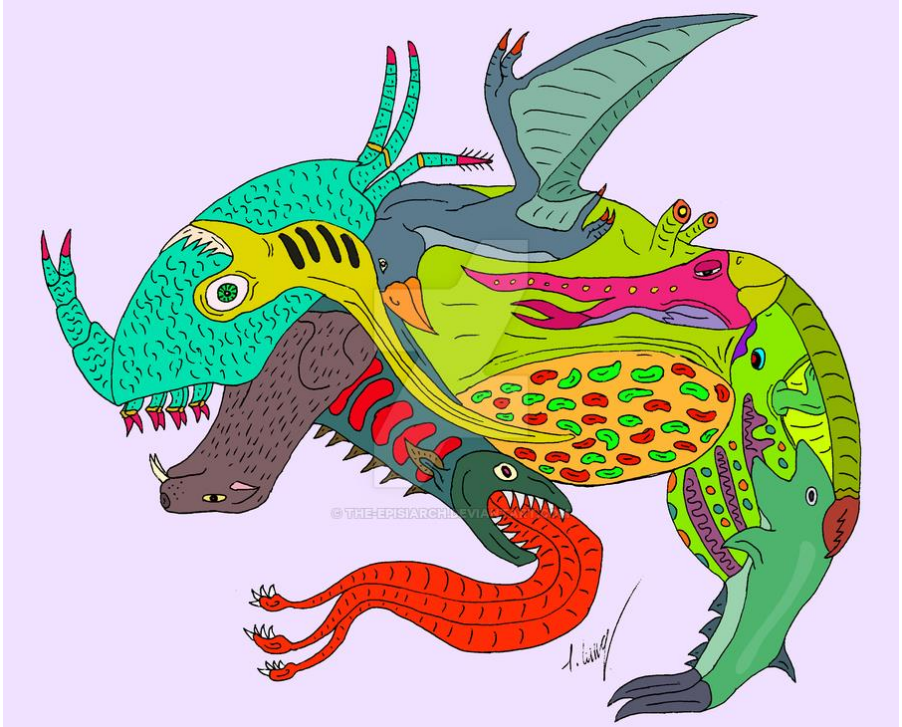
Microbiota Era / holobiont



Weyens et al. *Int. J. Mol. Sci.* **2015**

Context: holobionts

Holobionts are assemblages of different species that form ecological units: e.g. PLANTS

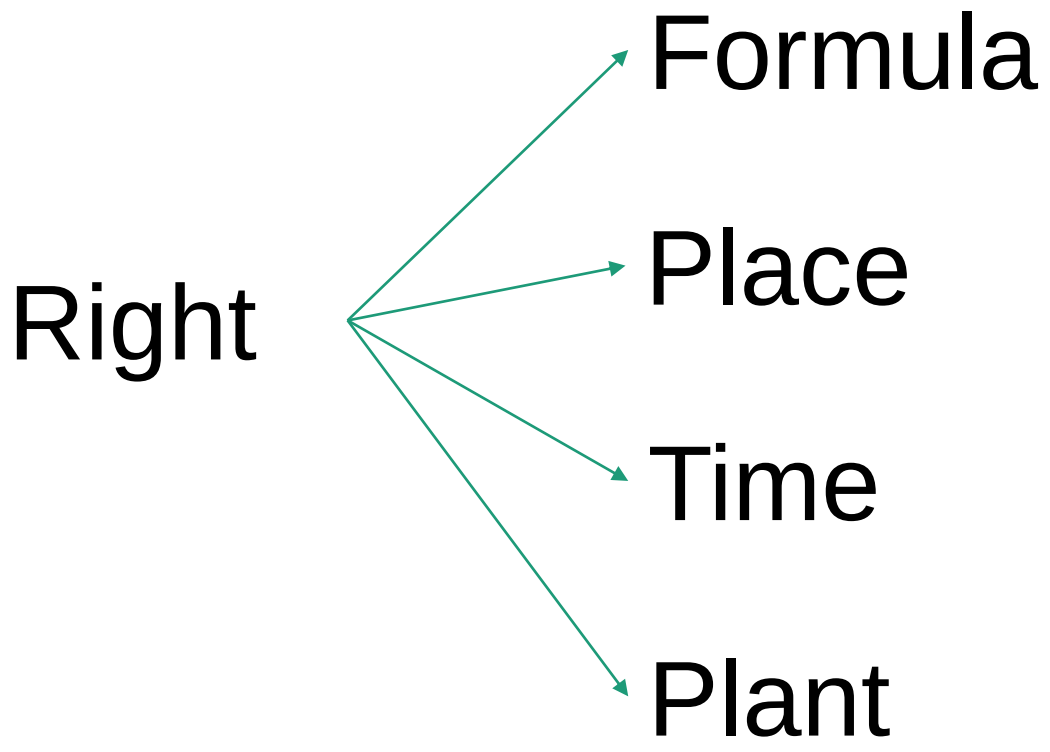


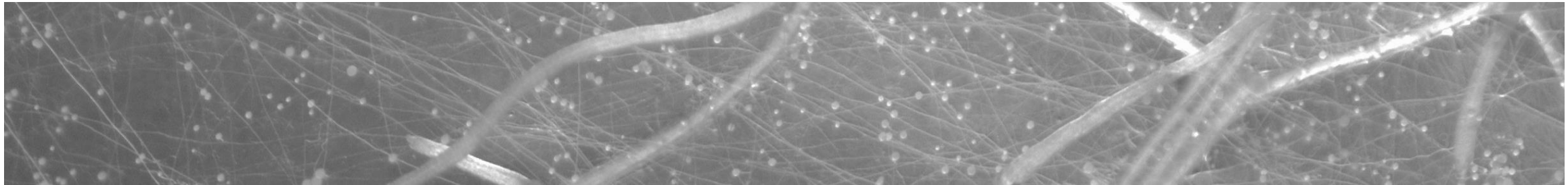
Optimized meta-organism

Enhanced N and P availability and higher levels of nutrients cycling
Improved growth
Enhanced disease supressiveness
Higher resistance to abiotic stress
Niche saturation

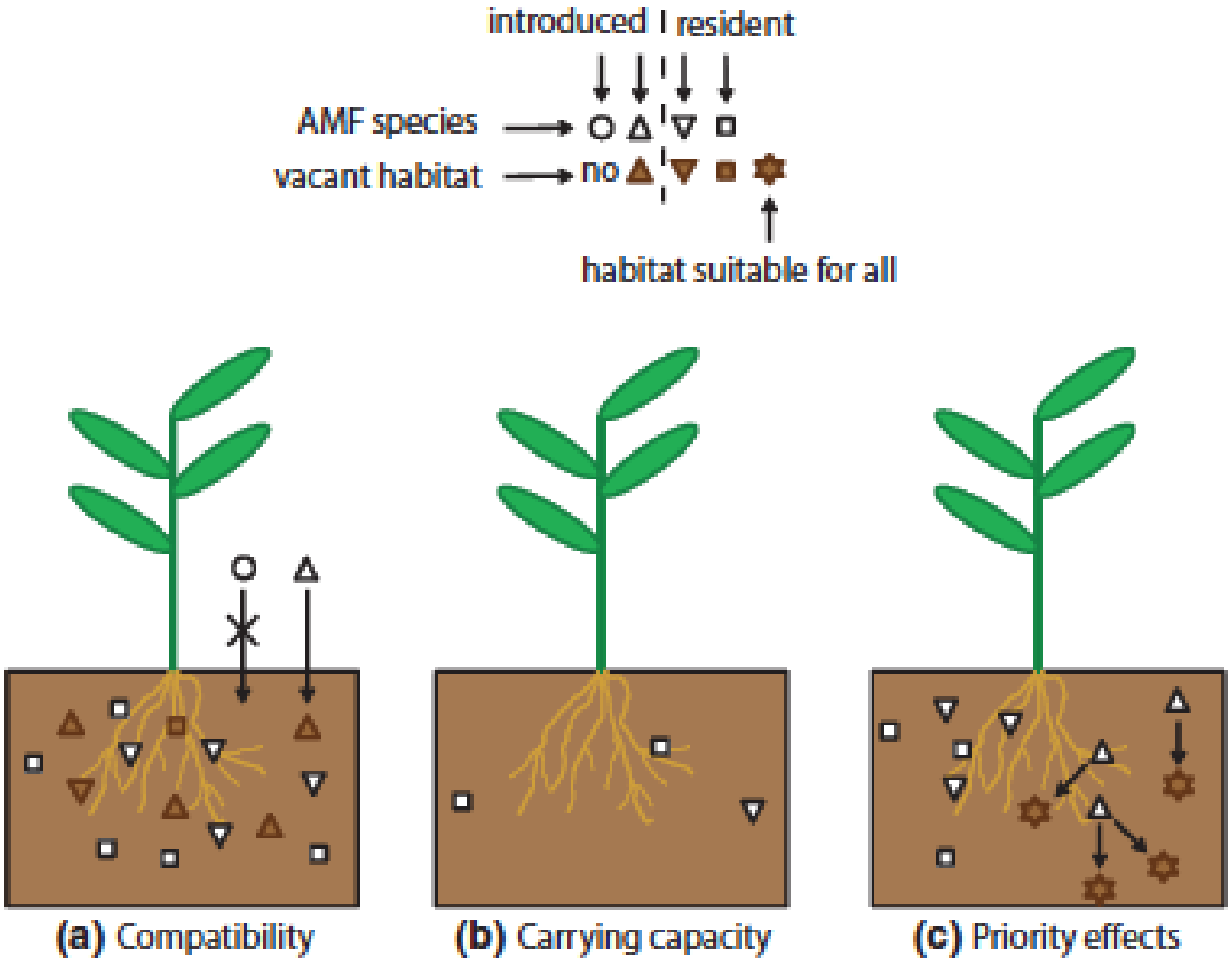
Quiza *et al* 2015. *New Phytologist*

Mycorrhizal inoculation - key questions



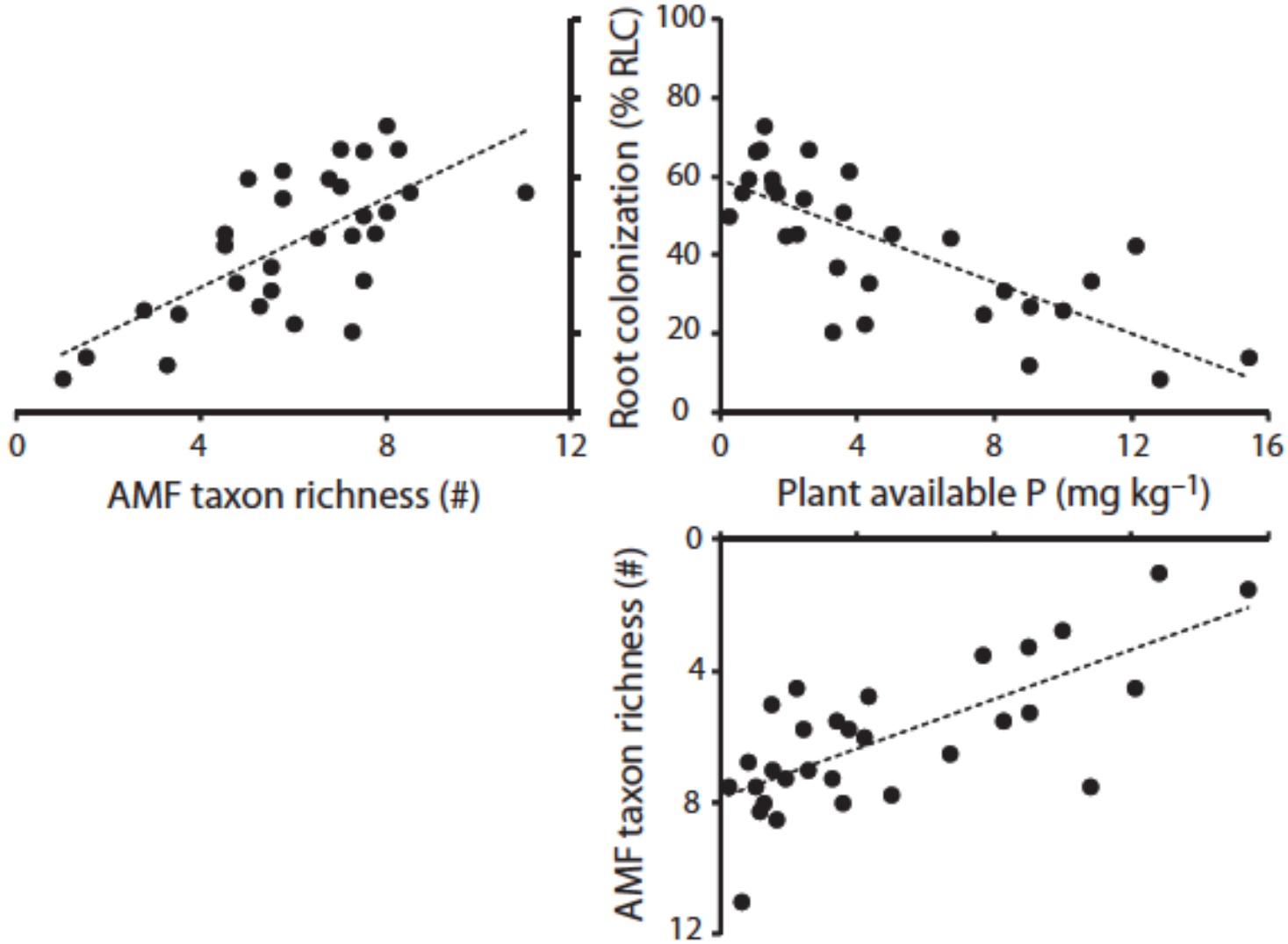


Context: Mycorrhizal inoculation



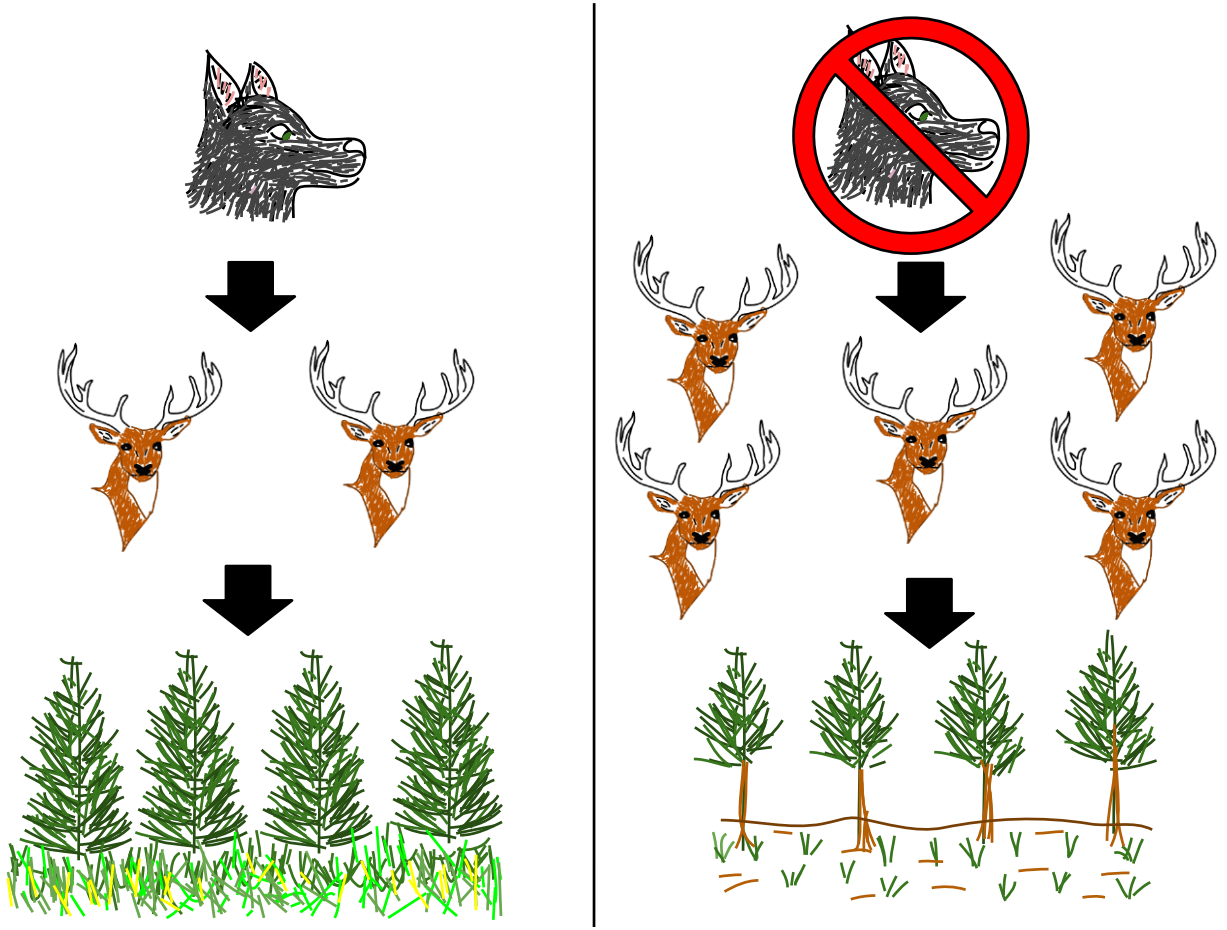
Verbruggen et al. 2013. *New Phytologist*

Context: Mycorrhizal inoculation



Verbruggen et al. 2013. *New Phytologist*

We don’t want to repeat the same errors over and over



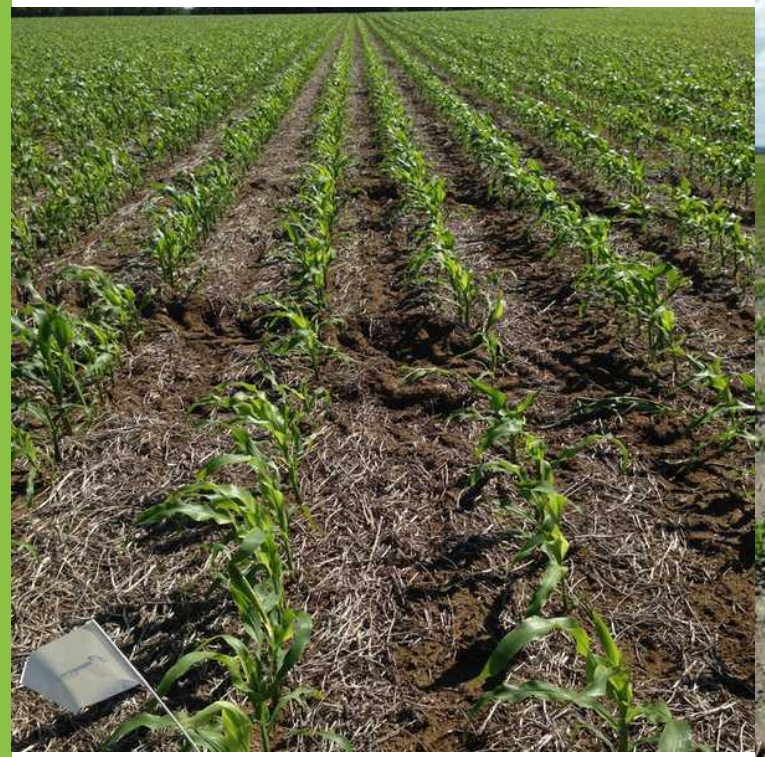
Objective

To determine whether introduced inoculants modified indigenous AMF communities in crop roots

1. Is the biodiversity (α-diversity) different in inoculated and non-inoculated fields ?

2. Is the structure of the community (β-diversity) different in inoculated and non-inoculated fields ?

Methods: Field experiment



Corn

Soybean

Wheat

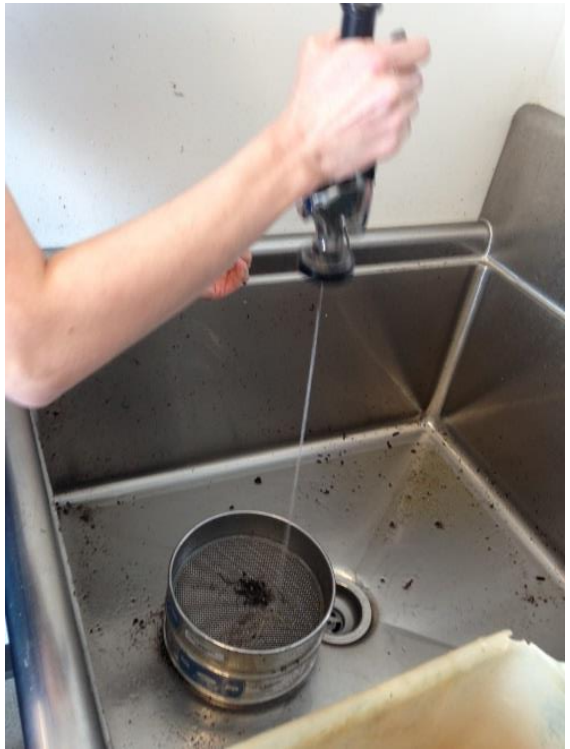
The experiment:

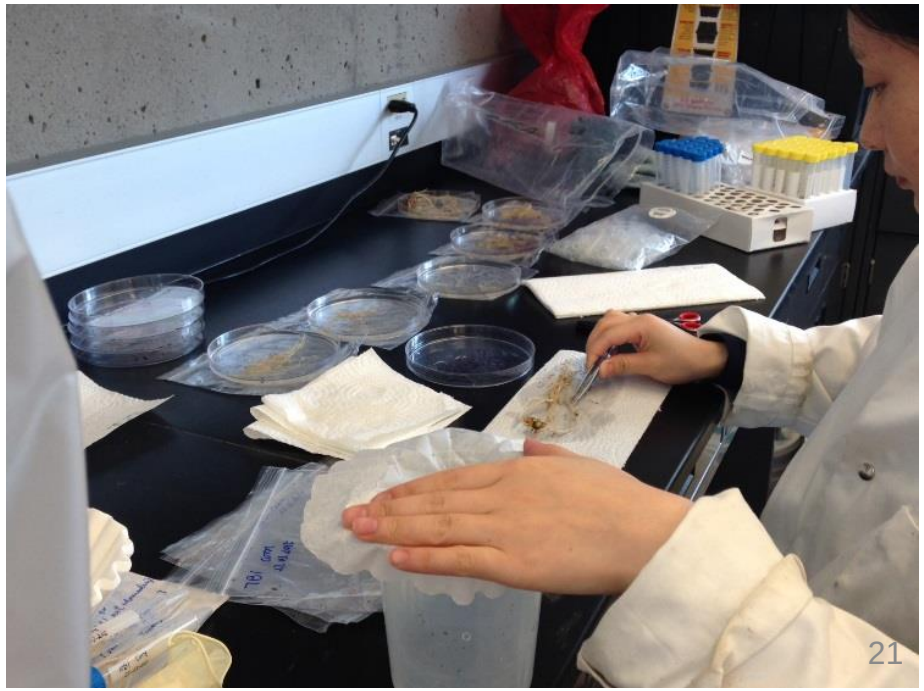
- Inoculated some part of the fields with *Rhizoglomus irregulare (Premier Tech)* while other parts were left without inoculation

- Two sampling (growing) stages

Crop	Corn		Soy	Wheat		Total of samples
Growing stage	Early stage (MV4)	Late stage (MV8)	Early	Early (Tallage)	Late (Étendard)	
Number of blocs	3 blocs	3 blocs	5 blocs	1 bloc	1 bloc	
Number of samples per blocs	7 samples/blocs	7 or 8 samples/bloc	4 samples/bloc	16 samples/bloc	17 samples/bloc	
Number of controls (non-inoculated plants)	5 controls	5 controls	6 controls	4 controls	5 controls	
Total of samples	26 samples	28 samples	26 samples	20 samples	22 samples	122 samples

Methods: Sampling

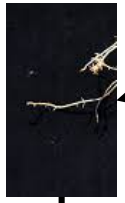


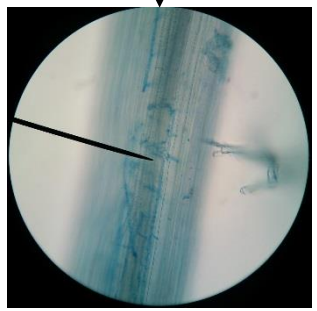


Methods: Lab experiment

1/3

2/3





Spores
Root Colonization

DNA
extraction
using kits

MiSeq
sequencing

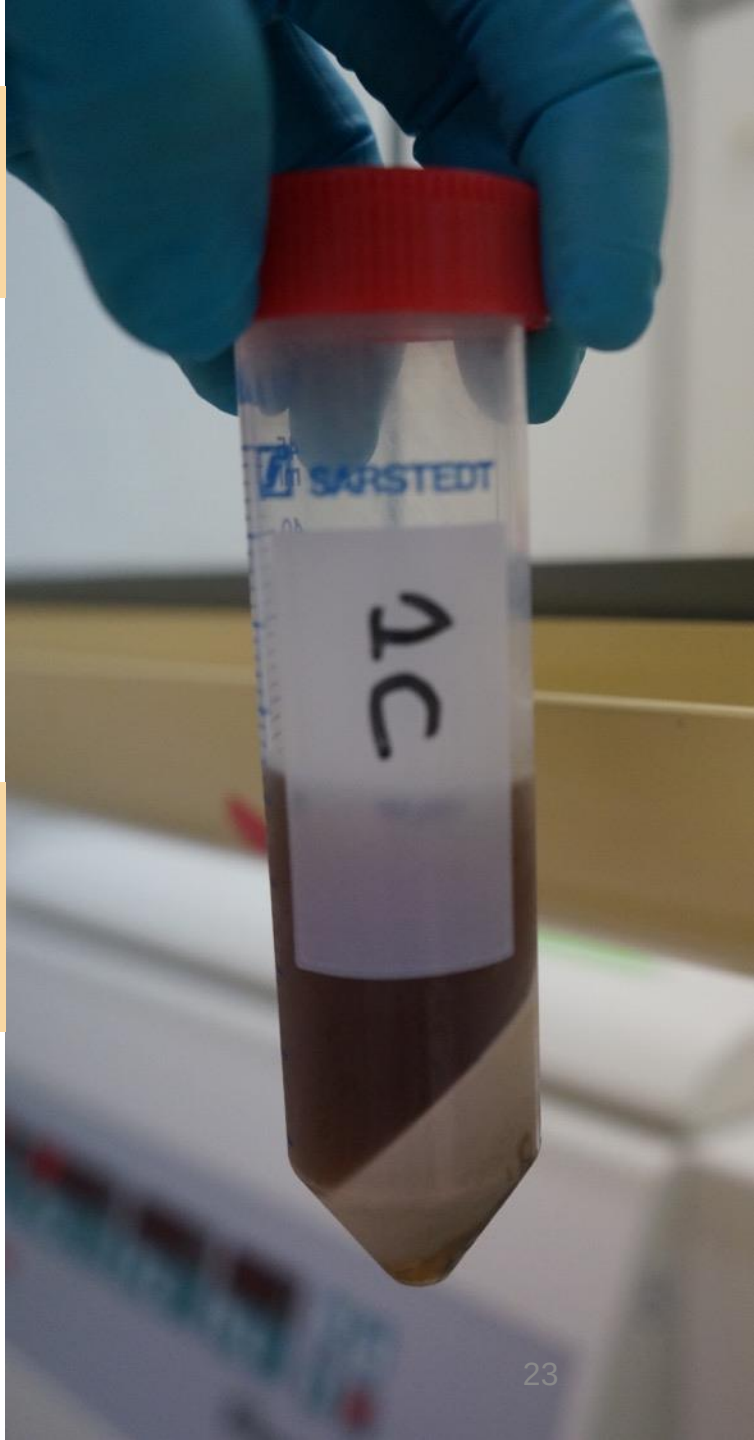
Methods: Statistics

Question 1: Is the biodiversity (α-diversity) different in inoculated and non-inoculated fields ?

- Inverse Simpson index
- Difference between inoculated and non-inoculated fields: ANOVA or non-parametric ANOVA followed by turkey post-hoc test

Question 2: Is the structure of the community (β-diversity) different in inoculated and non-inoculated fields ?

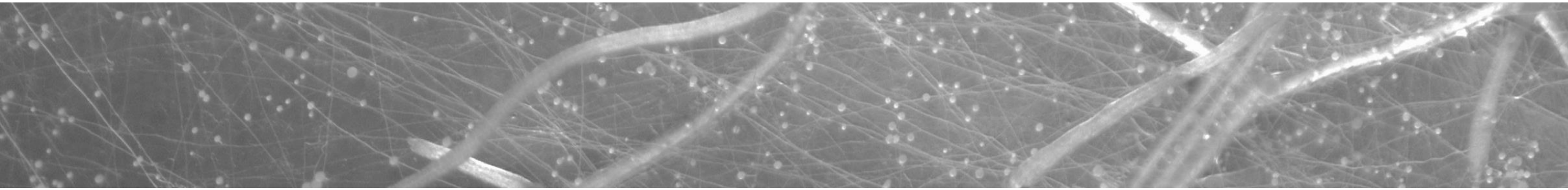
- Permanova done on relative abundance with Bray-Curtis distance matrix on hellinger transformed data
- Principle Coordinate Analysis (PCoA)

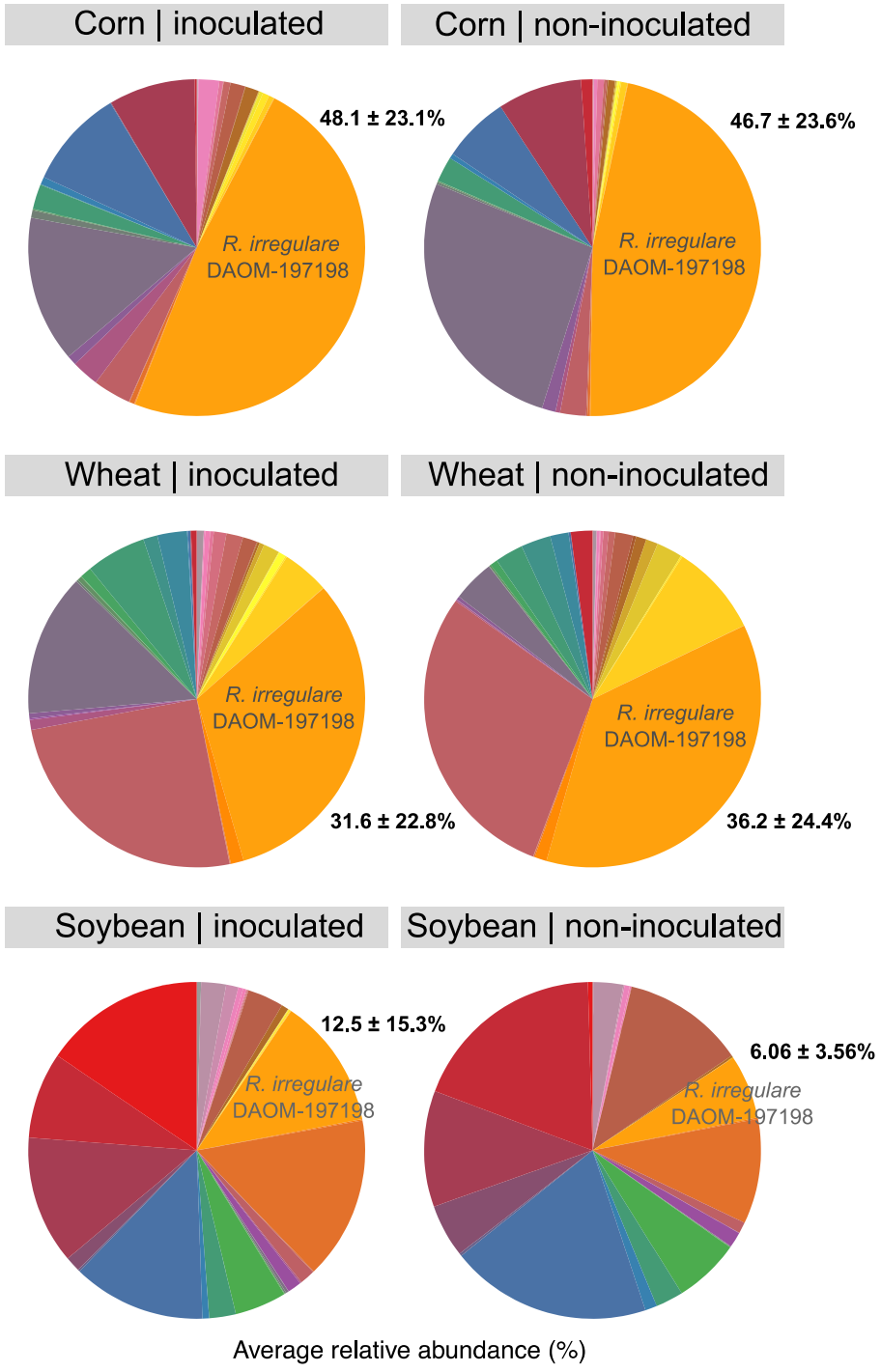


Methods: Bioinformatic

- Total of 122 samples
- Data from Genome Québec:
  - 8,181,190 reads (67,059 ± 18,564 reads per samples) were obtained from Genome Québec
- After quality trimming and bioinformatic:
  - 4,021,797 glomeromycota reads (34,671 ± 17,999 reads per sample) were left after quality trimming and bioinformatic
  - We had to drop 6 samples, because they had a low coverage and low number of reads
- Clustered to 408 OTUs (97% similarity)
  - 25% most abundant OTUs = 103 OTUs
  - Those 103 OTUs were further grouped in 46 virtual taxa (VTX) (according to the MAARJAM database)
  - The 103 OTUs (or 46 VTX) represent, on average, 99.16% of the reads sequenced in each sample

AMF community description

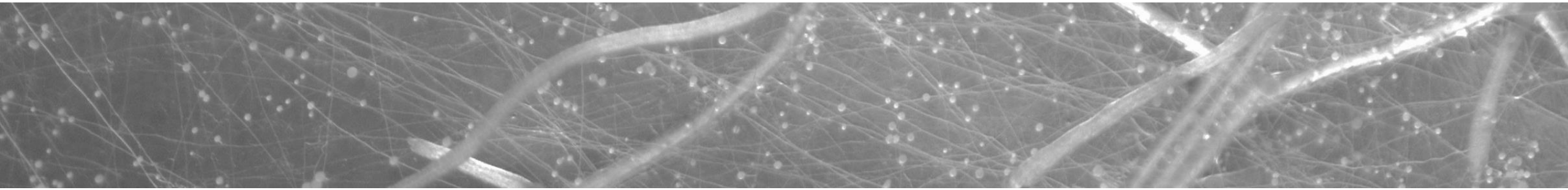


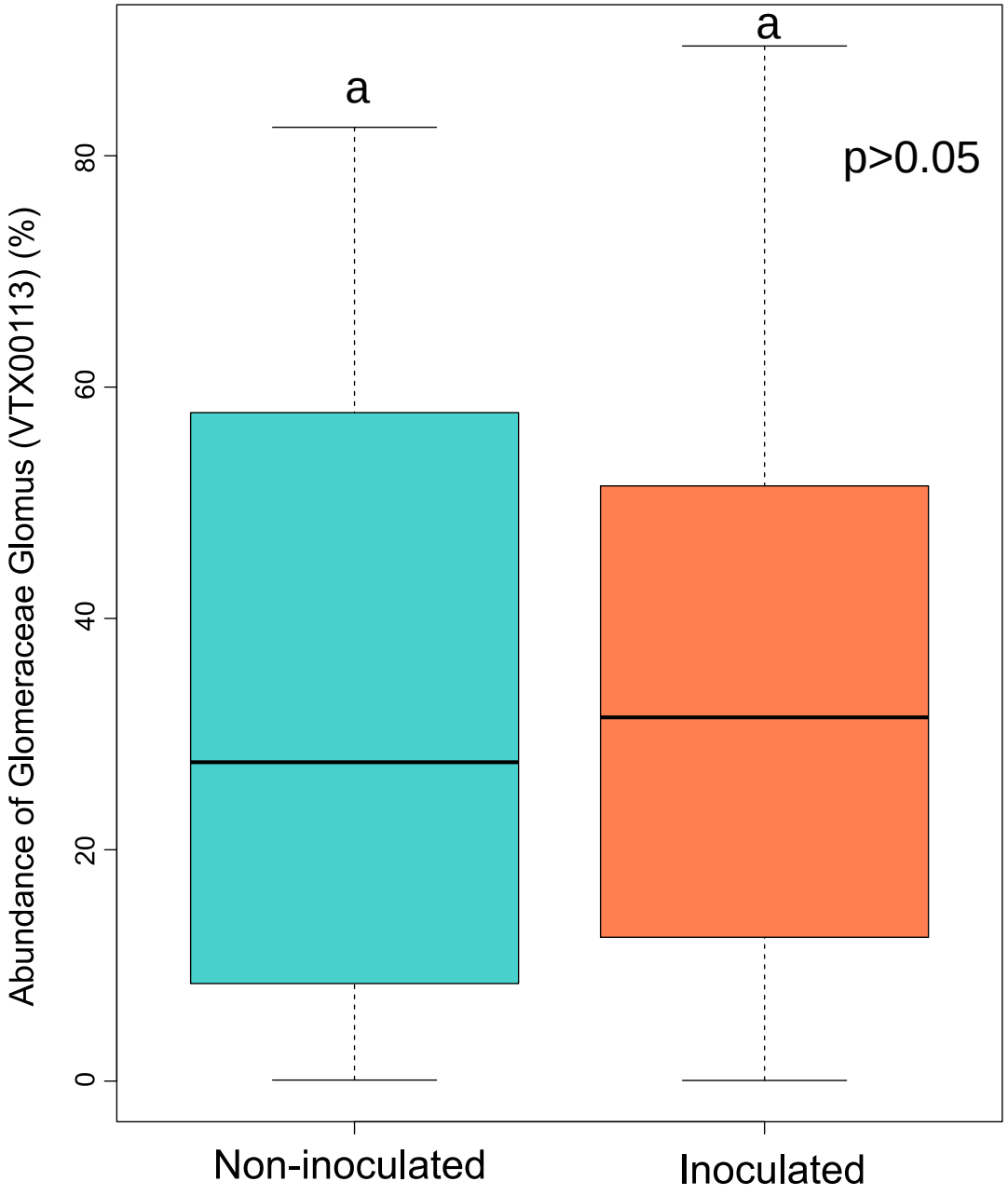


AMF taxonomy (genera and virtual taxa)

- Acaulosporaceae Acaulospora Acau10 (VTX00028)
- Acaulosporaceae Acaulospora sp. (VTX00030)
- Acaulosporaceae Acaulospora spinosa (VTX00026)
- Ambisporaceae Ambispora sp. (VTX00242)
- Archaeosporaceae Archaeospora sp. (VTX00004)
- Archaeosporaceae Archaeospora sp. (VTX00005)
- Archaeosporaceae Archaeospora sp. (VTX00009)
- Claroideoglomeraceae Claroideoglomus sp. (VTX00056)
- Claroideoglomeraceae Claroideoglomus sp. (VTX00057)
- Claroideoglomeraceae Claroideoglomus sp. (VTX00193)
- Claroideoglomeraceae Claroideoglomus sp. (VTX00225)
- Claroideoglomeraceae Claroideoglomus sp. (VTX00278)
- Claroideoglomeraceae Claroideoglomus sp. (VTX00402)
- Diversisporaceae Diversispora sp. (VTX00060)
- Diversisporaceae Diversispora sp. (VTX00061)
- Diversisporaceae Diversispora sp. (VTX00062)
- Diversisporaceae Diversispora sp. (VTX00380)
- Gigasporaceae Scutellospora sp. (VTX00255)
- Glomeraceae Glomus sp. (VTX00064)
- Glomeraceae Glomus sp. (VTX00067)
- Glomeraceae Glomus sp. (VTX00086)
- Glomeraceae Glomus sp. (VTX00093)
- Glomeraceae Glomus sp. (VTX00096)
- Glomeraceae Glomus sp. (VTX00108)
- **Glomeraceae Glomus sp. (VTX00113)**
- **Glomeraceae Glomus sp. (VTX00114)**
- Glomeraceae Glomus sp. (VTX00115)
- Glomeraceae Glomus sp. (VTX00125)
- Glomeraceae Glomus sp. (VTX00130)
- Glomeraceae Glomus sp. (VTX00137)
- Glomeraceae Glomus sp. (VTX00143)
- Glomeraceae Glomus sp. (VTX0155)
- Glomeraceae Glomus sp. (VTX00156)
- Glomeraceae Glomus sp. (VTX00160)
- Glomeraceae Glomus sp. (VTX00165)
- Glomeraceae Glomus sp. (VTX00172)
- Glomeraceae Glomus sp. (VTX00199)
- Glomeraceae Glomus sp. (VTX00212)
- Glomeraceae Glomus sp. (VTX00214)
- Glomeraceae Glomus sp. (VTX00219)
- Glomeraceae Glomus sp. (VTX00222)
- Glomeraceae Glomus sp. (VTX00247)
- Glomeraceae Glomus sp. (VTX00287)
- Glomeraceae Glomus sp. (VTX00309)
- Glomeraceae Glomus sp. (VTX00366)
- Glomeraceae Glomus sp. (VTX00418)

Preliminary question:
is *Rhizoglomus irregulare* more abundant in the roots of inoculated and non-inoculated soils

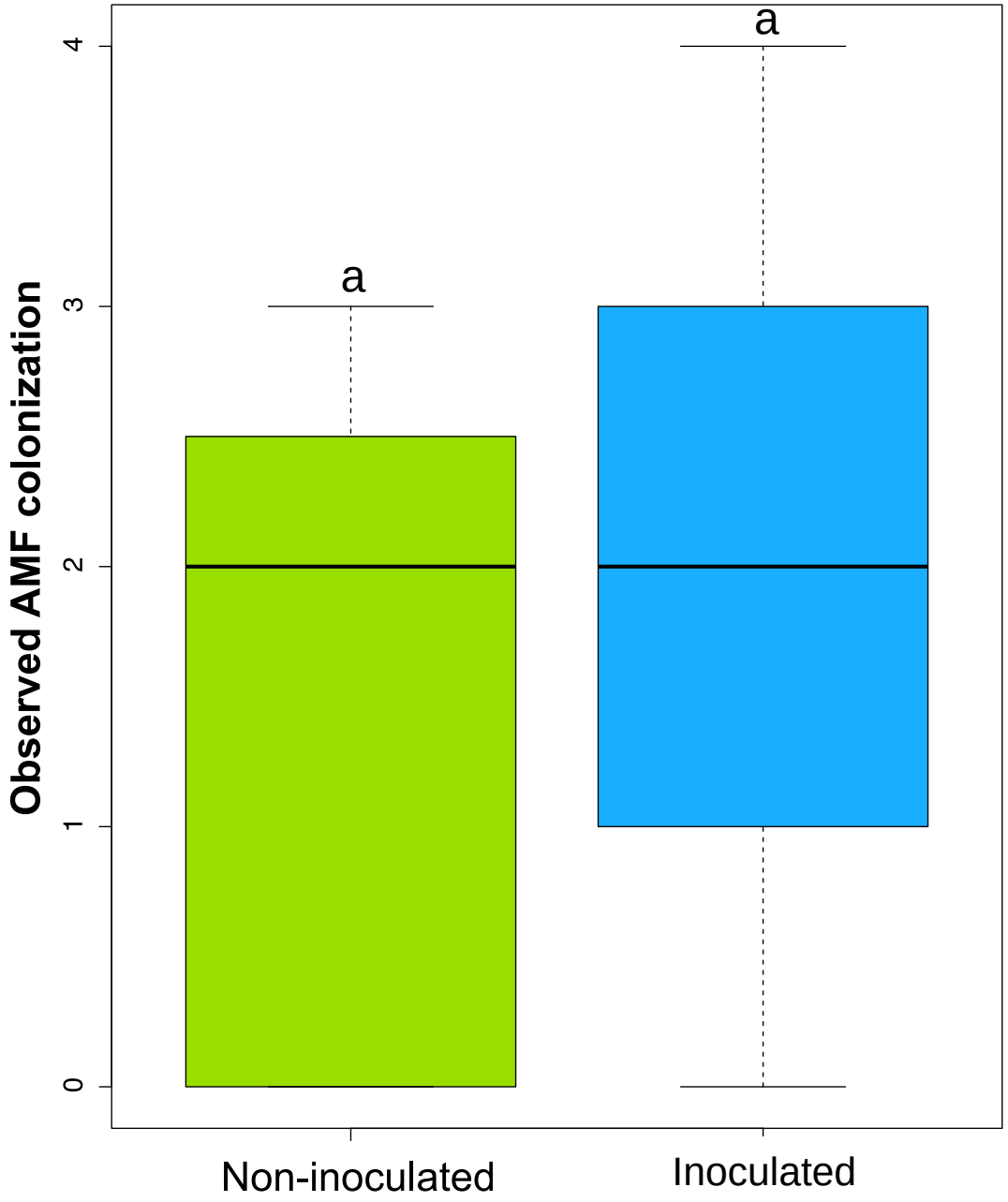


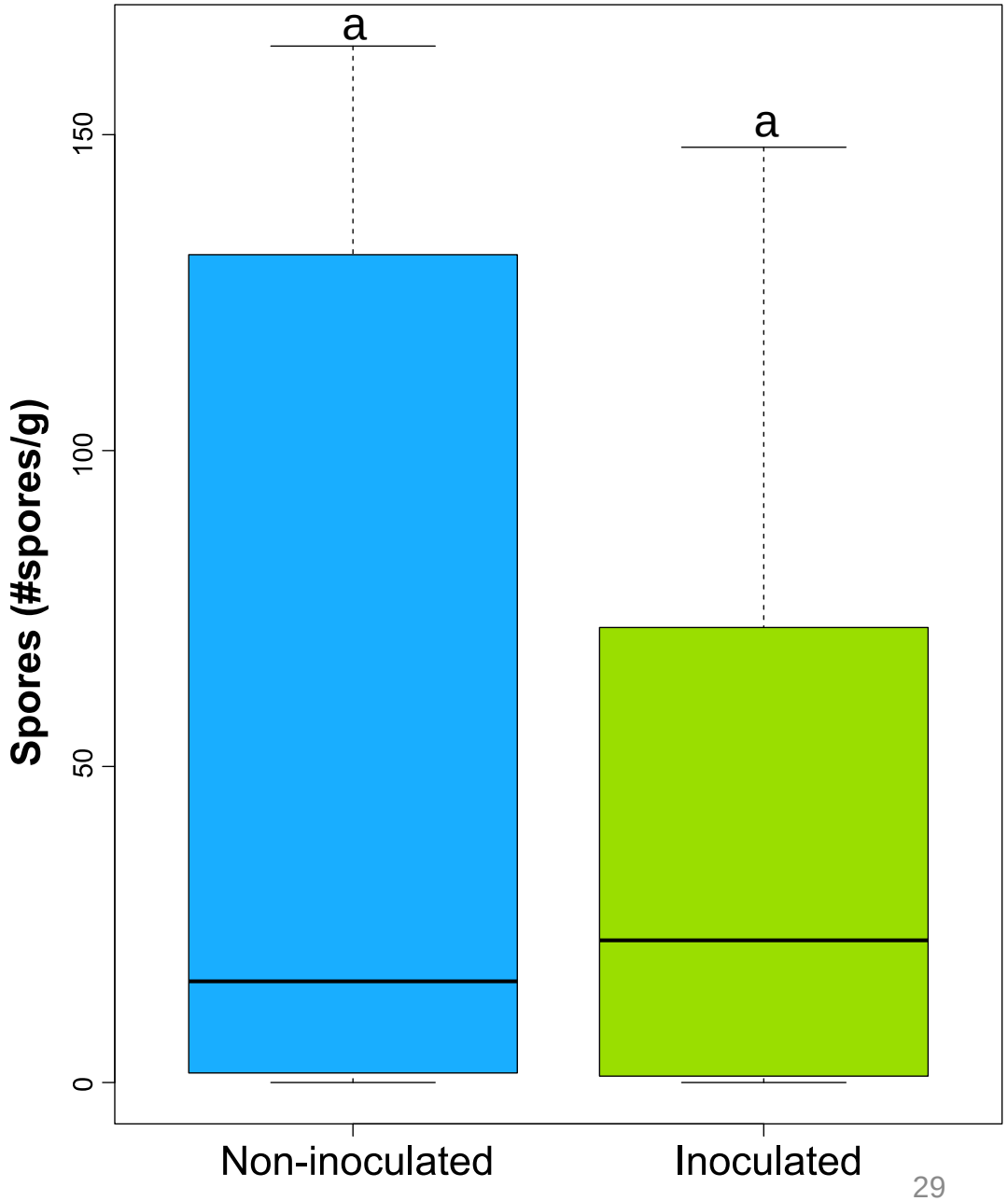


No significant differences in the relative abundance of *Rhizoglomus irregulare* in the roots of inoculated and non-inoculated soils

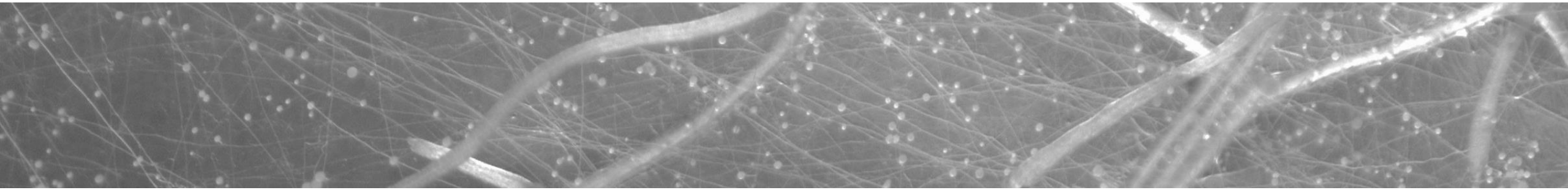
($F(1,114)=2.004$; $p=0.173$)

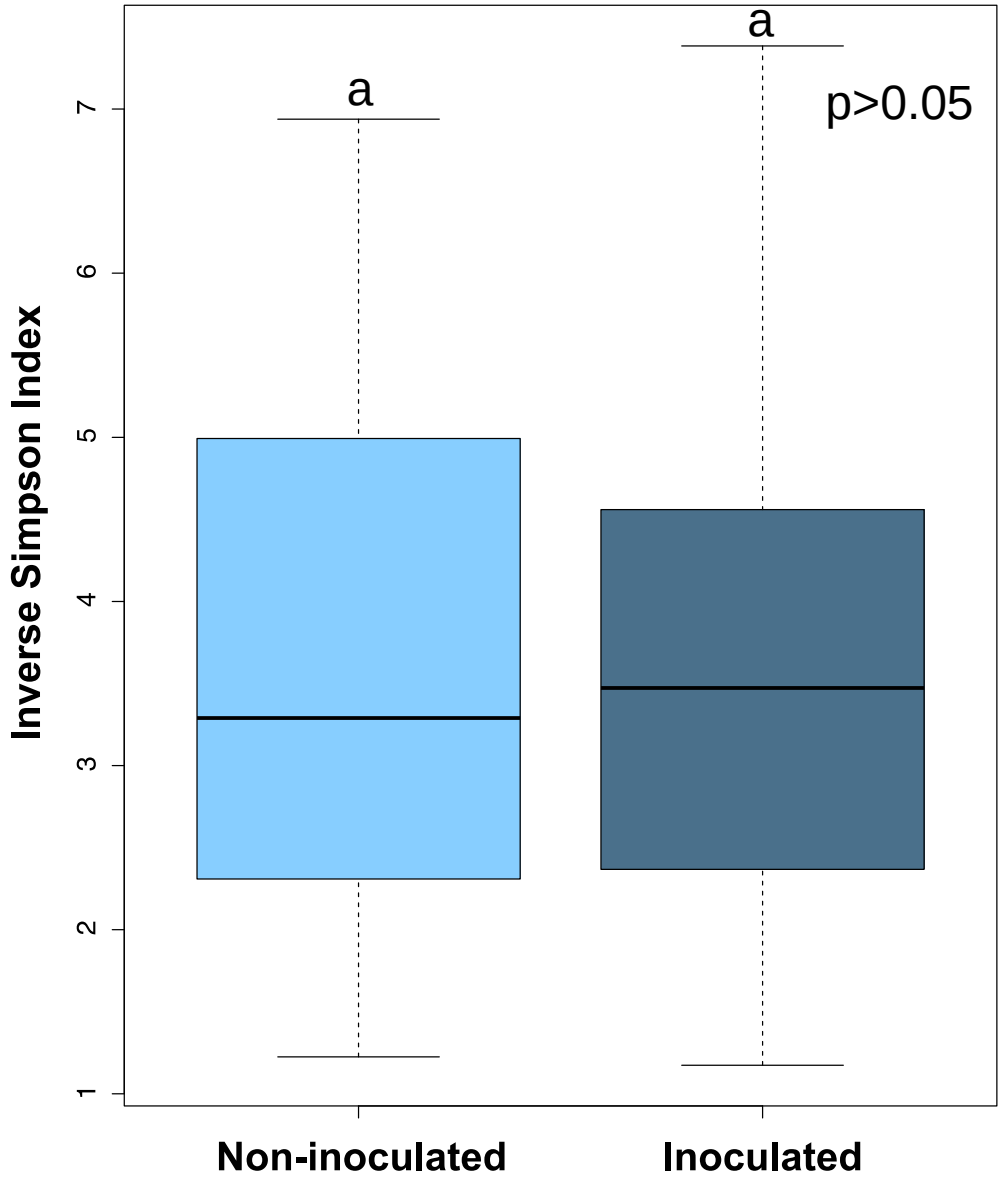
p>0.05


Observed AMF colonization
0
1
2
3
4
a
a
Non-inoculated
Inoculated


Spores (#spores/g)
0
50
100
150
a
a
Non-inoculated
Inoculated

Question 1:
Is the biodiversity (α-diversity) different in inoculated and non-inoculated fields ?



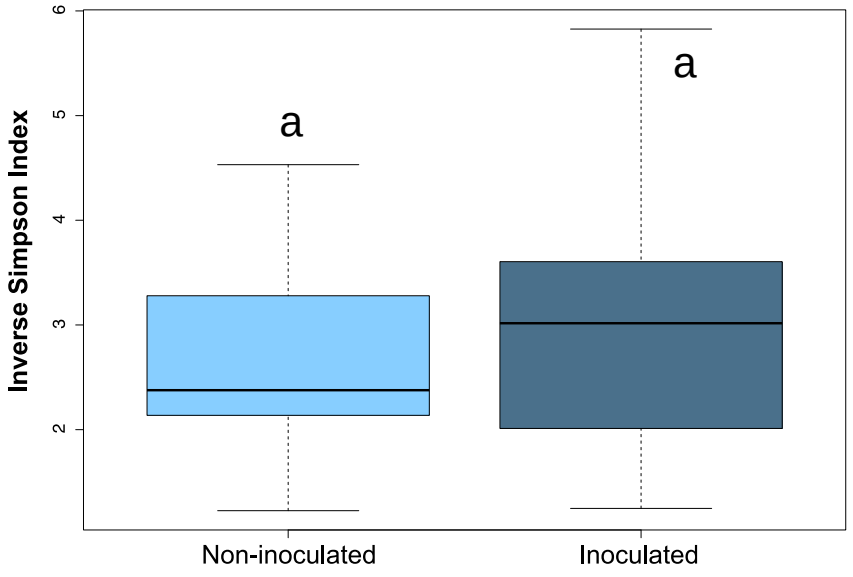


No significant differences in the α-diversity the roots of inoculated and non-inoculated soils

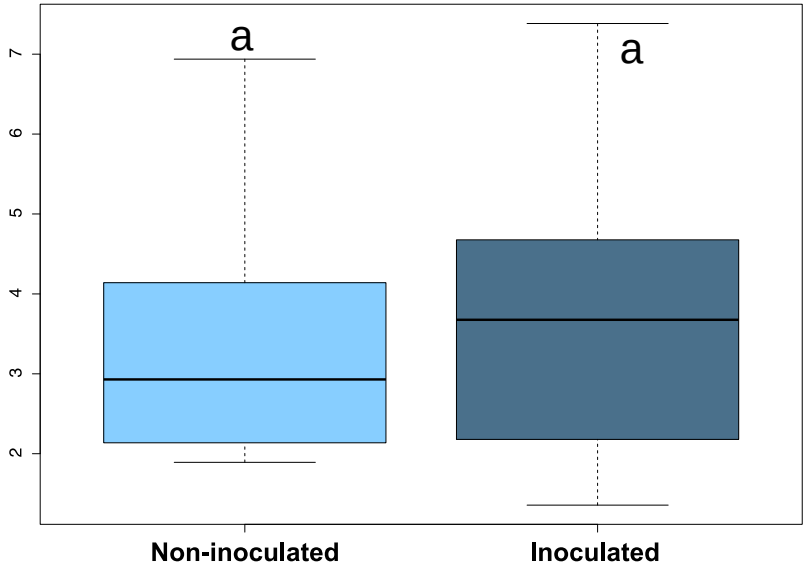
($F(1,106)=0.0001$; $p=0.992$)

p>0.05

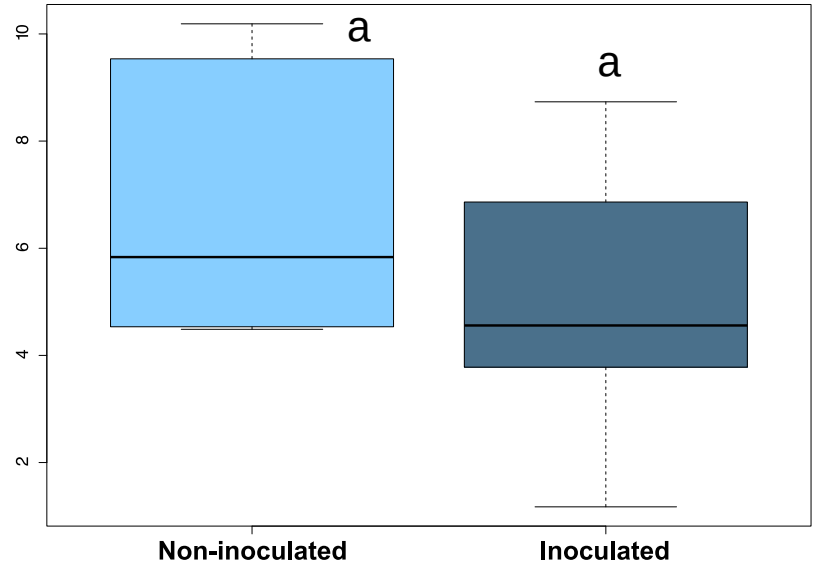
Corn

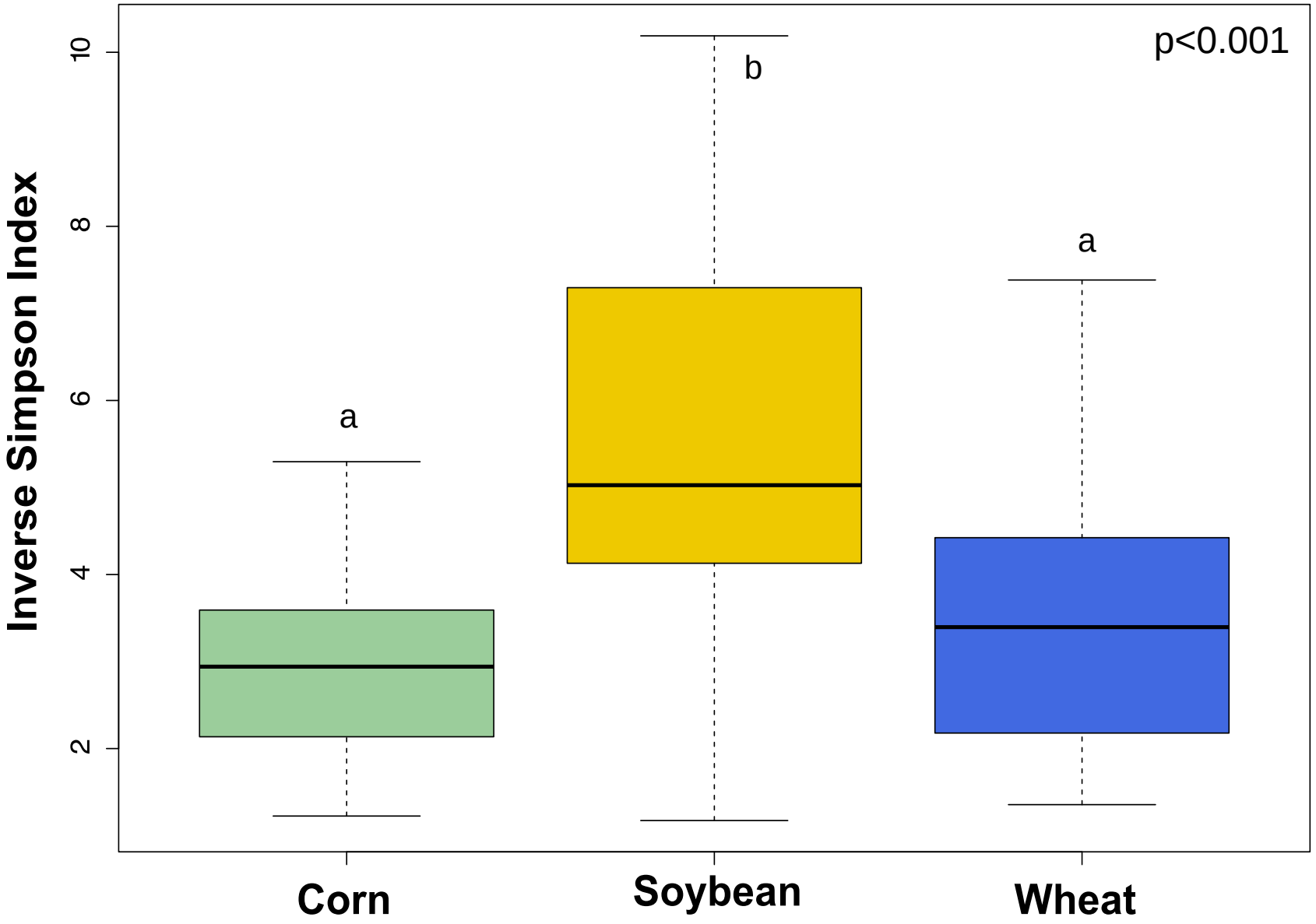


Wheat

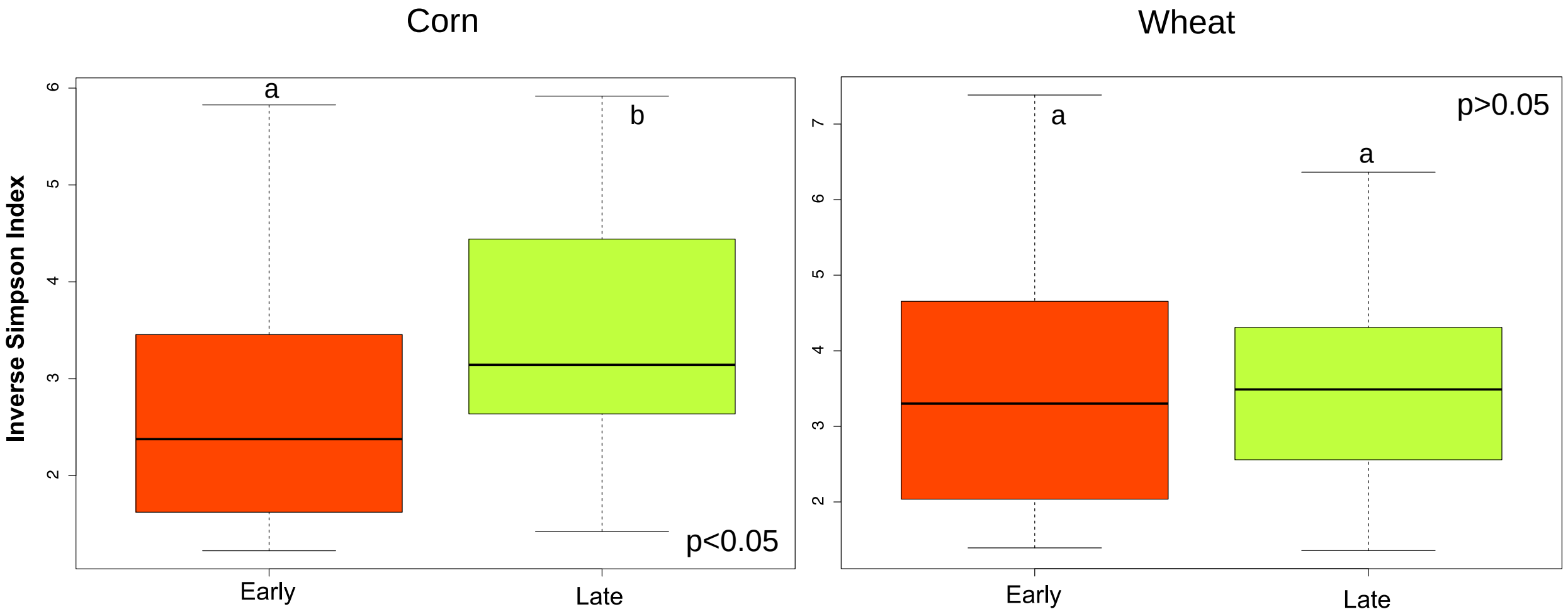


Soybean

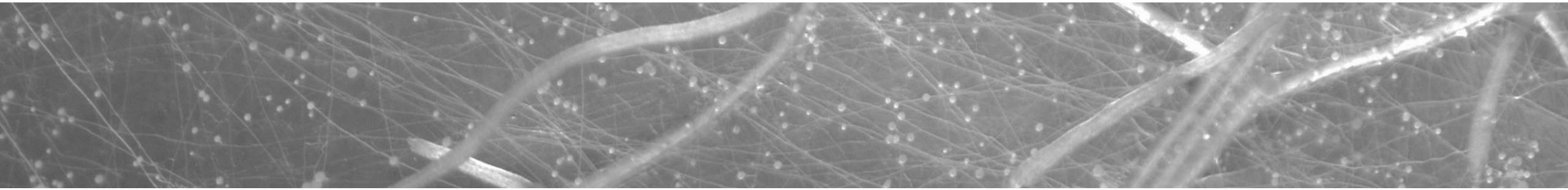


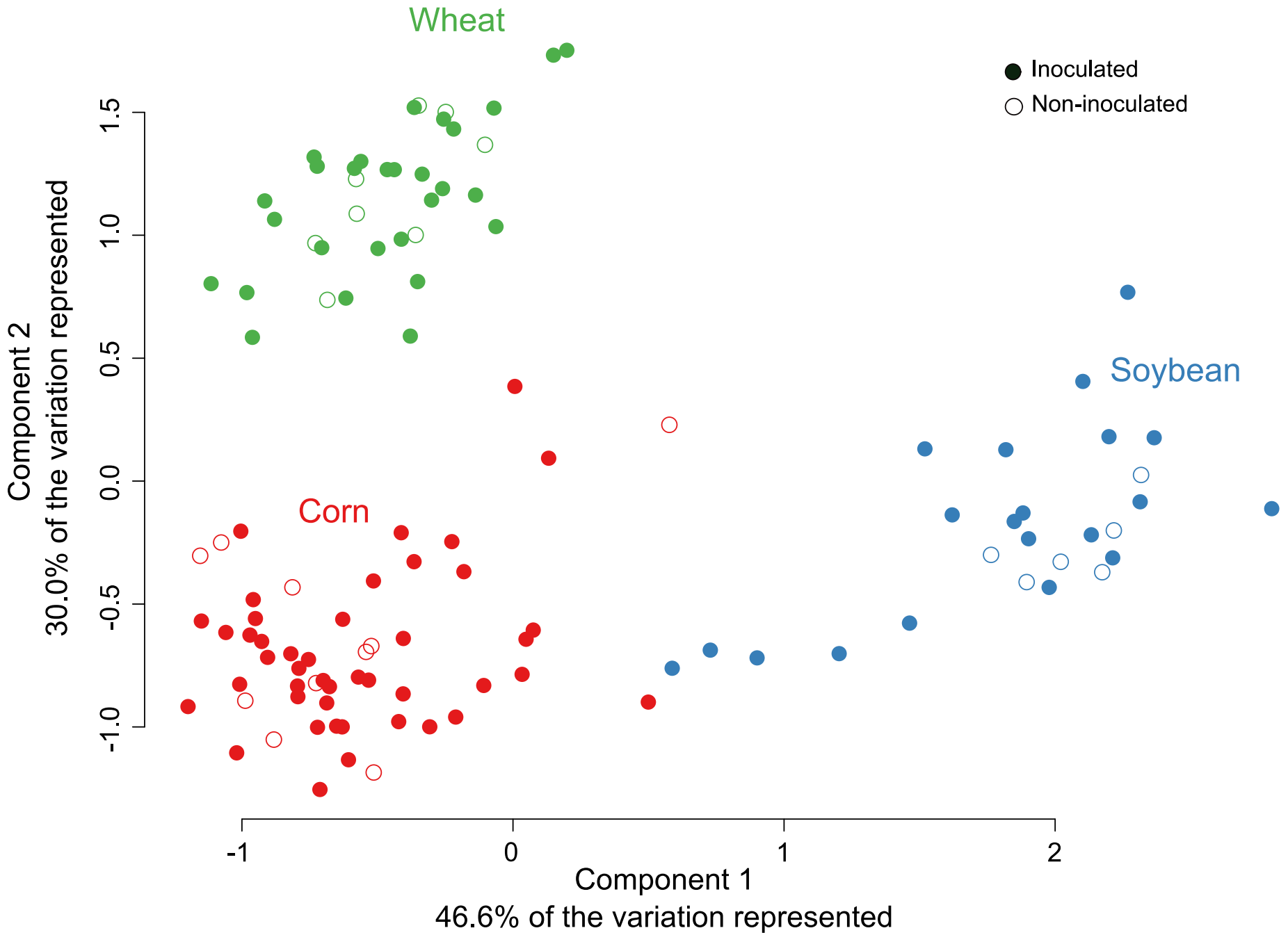


No significant differences in the biodiversity of AMF inhabiting the roots of inoculated and non-inoculated soils ($F(1,106)=14.7$; $p<0.001$)


Corn
Wheat
Inverse Simpson Index
2
3
4
5
6
7
a
b
a
a
p<0.05
p>0.05
Early
Late
Early
Late

Question 2:
Is the structure of the community (β-diversity) different in inoculated and non-inoculated fields ?




Wheat
Inoculated
Non-inoculated
Soybean
Corn
Component 2
30.0% of the variation represented
1.5
1.0
0.5
0.0
-0.5
-1.0
-1
0
1
2
Component 1
46.6% of the variation represented

Results: summary

Preliminary question: is *Rhizoglomus irregulare* more abundant in the roots of inoculated and non-inoculated soils

- **No significant differences** in the relative abundance of *Rhizoglomus irregulare,* root colonization, number of spores in the roots of inoculated and non-inoculated soils

Question 1: Is the biodiversity (α-diversity) different in inoculated and non-inoculated fields ?

- AMF biodiversity **is not different** in inoculated and non-inoculated soils
- Soybean's roots harbour significantly higher biodiversity than corn and wheat's roots
- Within corn roots, biodiversity at the latter stage is higher than at the early stage

Results: summary

Question 2: Is the structure of the community (β-diversity) different in inoculated and non-inoculated fields ?

- **No** and it is true for all three crops tested
- The structure of the AMF community is different under the different crops
- The structure of the AMF community is different in early and late growing stage

Future research

What can we do next?

- Reach a better understanding of the interactions among plants, soils and roots (and climate)
- Keep answering the mycorrhizal inoculation key questions: What's the right formula, right place (soil capacity), right time (competition pathways) and right plant
- Tracability of the inoculum over years
- Based on the results of the taxonomy of AMF community: could we inoculate the soil with a consortium of fungi or even fungi and bacteria to boost soil productivtiy

Acknowledgements

- Geneviève Lachance, Serge Gagné, Line Roy-Arcand et MartinTrépanier (Premier Tech)
- Agathe Vialle (Biopterre)
- Farmers that contributed to field trials

jacynthe.masse@umontreal.ca

Future research

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