



Influence of terroir on the microbial assemblages associated to common bean seed

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Introduction

- Plants have evolved in association with diverse microbial assemblages. These have an effect on plant growth and health.
 - Rhizosphere
 - Phyllosphere
 - Seeds?
- Seed-associated microbial assemblages composed of 50 to 1000 bacterial and fungal taxa



Among them: Seed-borne plant pathogens...



Fungal:

Anthracoze (*Colletotrichum lindemuthianum*)



Viral: BCMV

BCMNV...



Bacterial blights:

Pseudomonas savastanoi pv. *phaseolicola*

Xanthomonas axonopodis pv. *phaseoli* (EU quarantine)



... but not only plant pathogens!

Debate on plant health and its management following the European project *Farm Seed Opportunities* (FP6, 2007-09)



Seed-borne pathogens not isolated from their microbial communities and their production environment!



Seed-associated microbial assemblages

- Are they affected by the terroir? Might they be a factor of plant adaptation?
- Or are communities linked to particular plant genotypes in a stable manner?
- **Assessment of the relative influence of the bean genotype and terroir on the structure of the seed microbiota**



Materials and Methods: Cultivars and sites

'Rognon de Coq'

'Roi des Belges'

'St. Esprit à œil rouge'

'Flageolet Chevrier'

'Calima'
(Commercial control variety)

BZH

Brittany (F)

Organic vegetable and seed producer

LUX

Luxembourg

Organic seed producer

3 field replicates; 2 years



Materials and Methods: Bean cultivars

- Genetic diversity among and within bean cultivars (Perugia university)

Characterization of 16-18 plants

Molecular markers: 11 SSR markers covering all the species linkage groups



Materials and Methods: Microbial communities

- Composition of the microbial community (bacteria and fungi) associated to seed lots harvested in 2013 (INRA Angers)

eDNA barcoding:

Identifying different taxa in one environmental sample by sequencing molecular markers

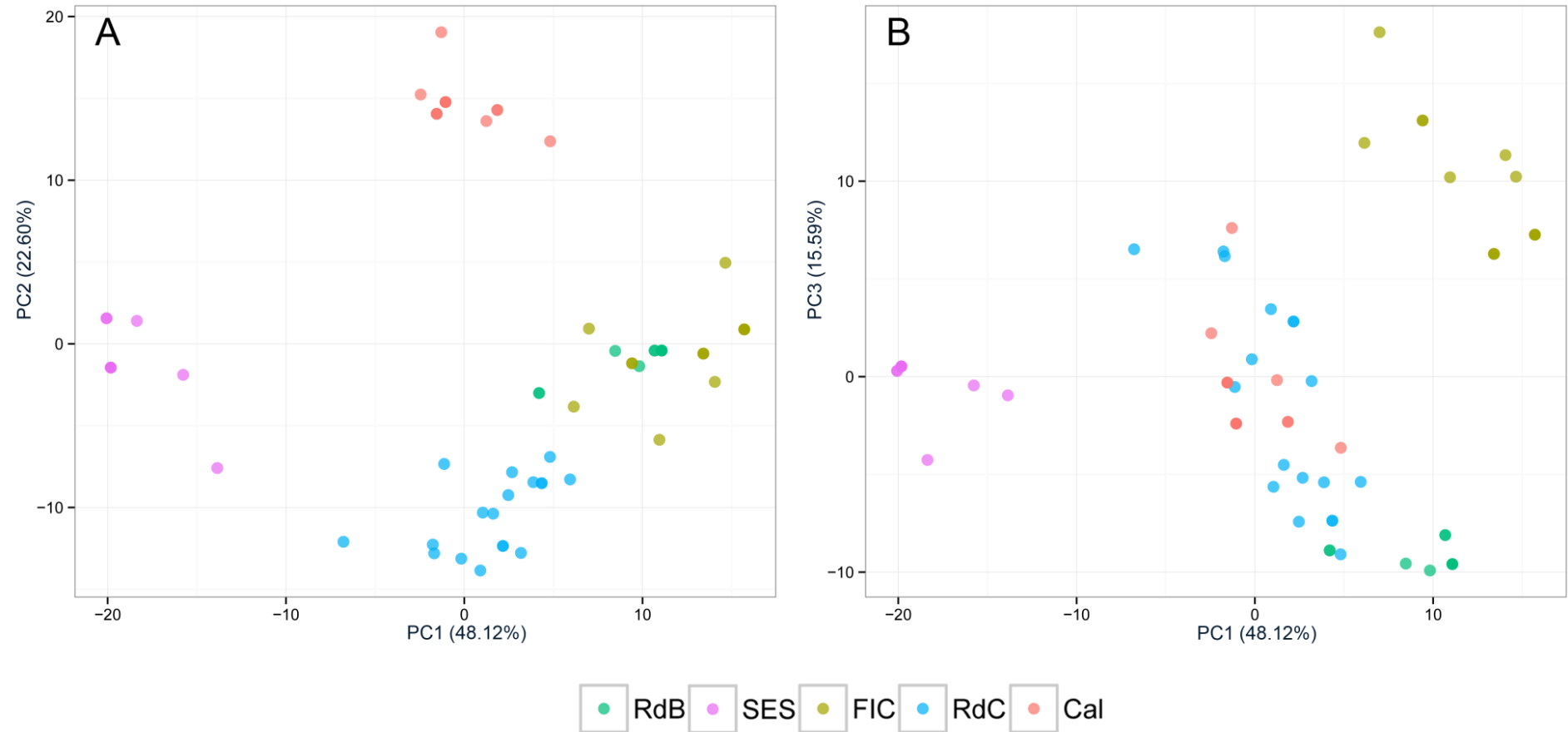
Molecular markers:

16S rRNA gene (bacteria); ITS (fungi)



Results: Genetic diversity of the cultivars

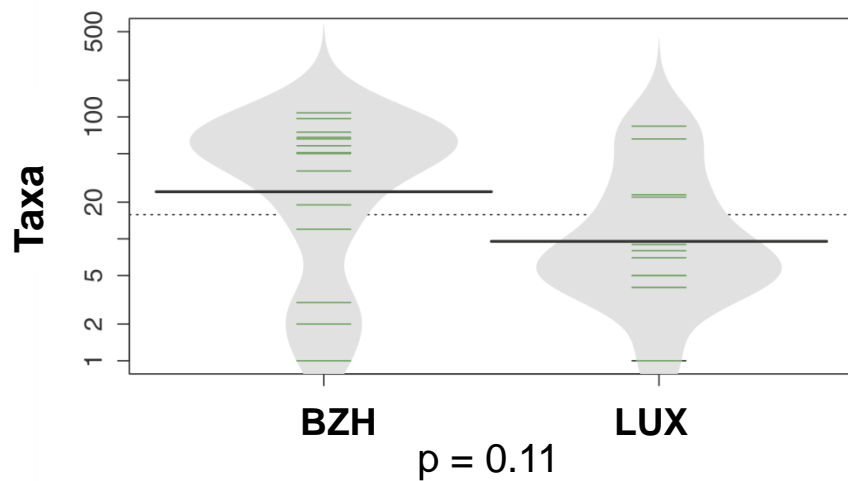
PCoA, 11 SSR markers



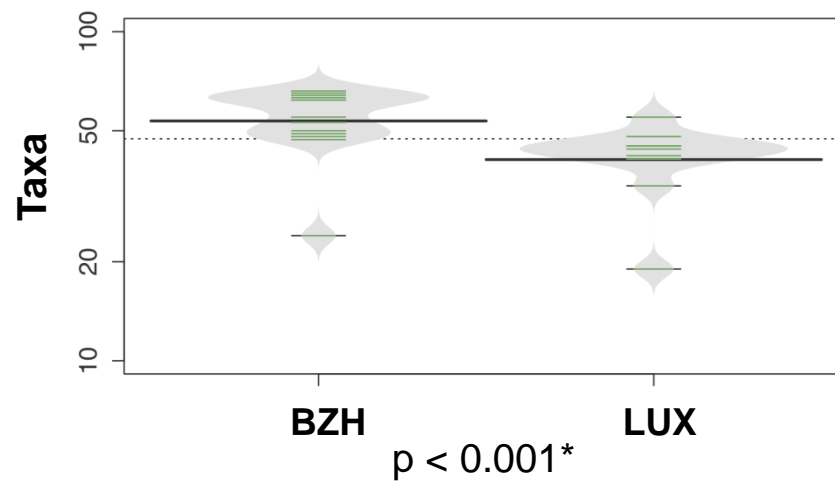
Results: Mean diversity within each site

Kruskal-Wallis test on diversity indices

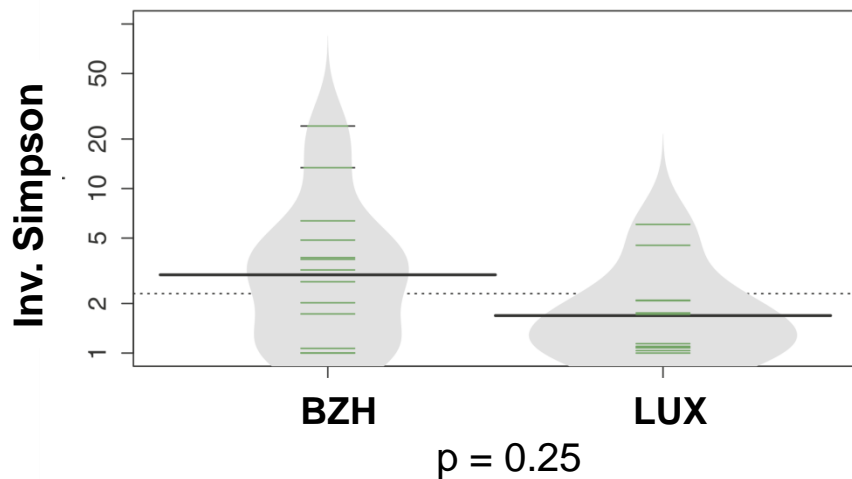
Bacterial richness (16S)



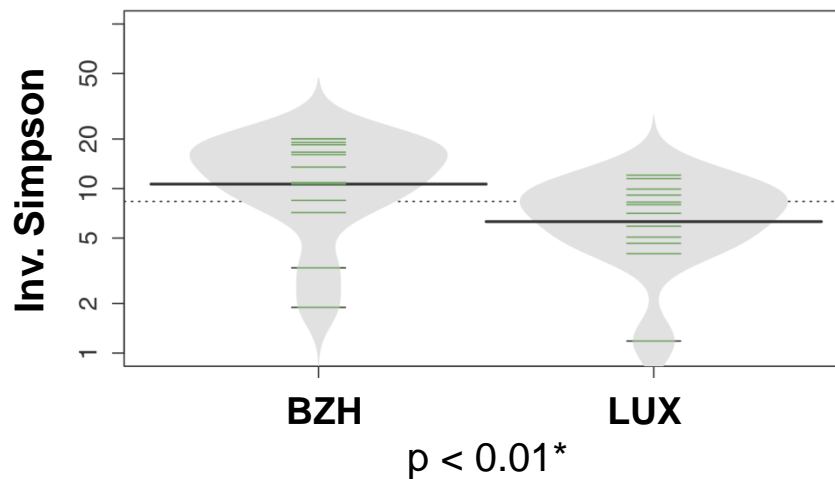
Fungal richness (ITS)



Bacterial diversity (16S)



Fungal diversity (ITS)



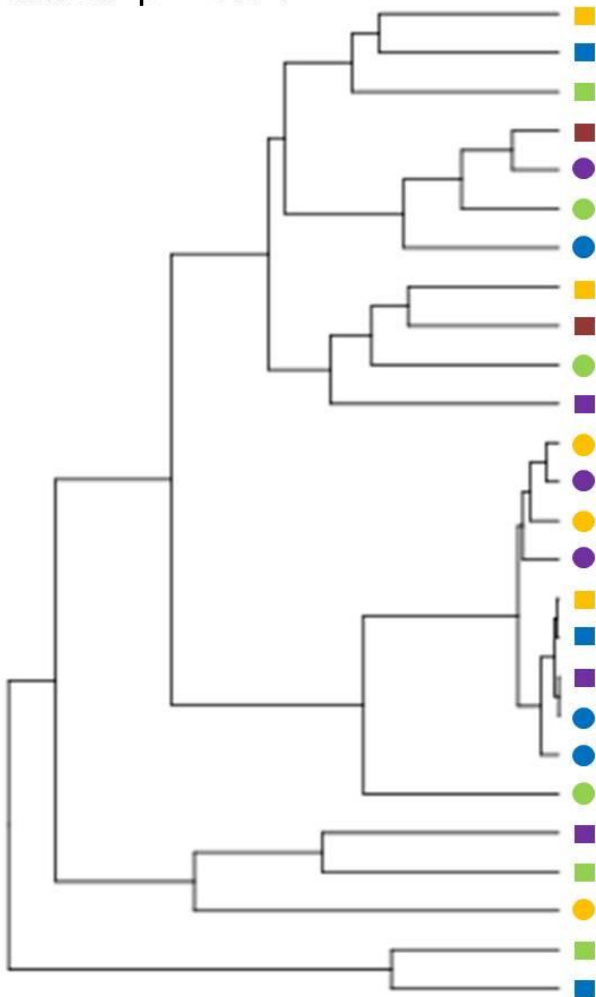
Results: Dissimilarities between microbial communities

AMOVA on Bray-Curtis distances

A) 16S (bacteria)

Site: $p = 0.032^*$

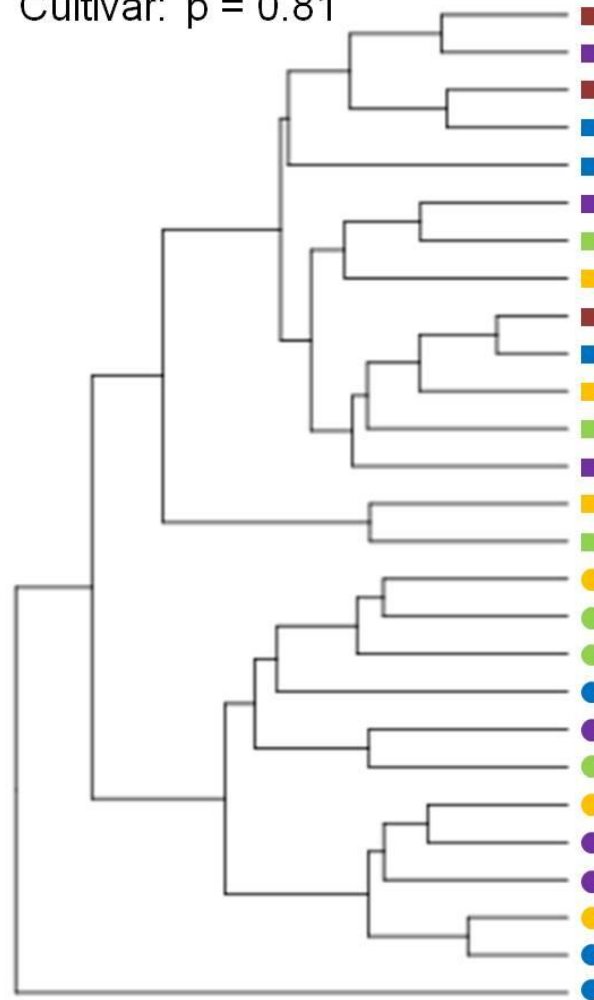
Cultivar: $p = 0.64$



B) ITS (fungi)

Site: $p < 0.001^*$

Cultivar: $p = 0.81$

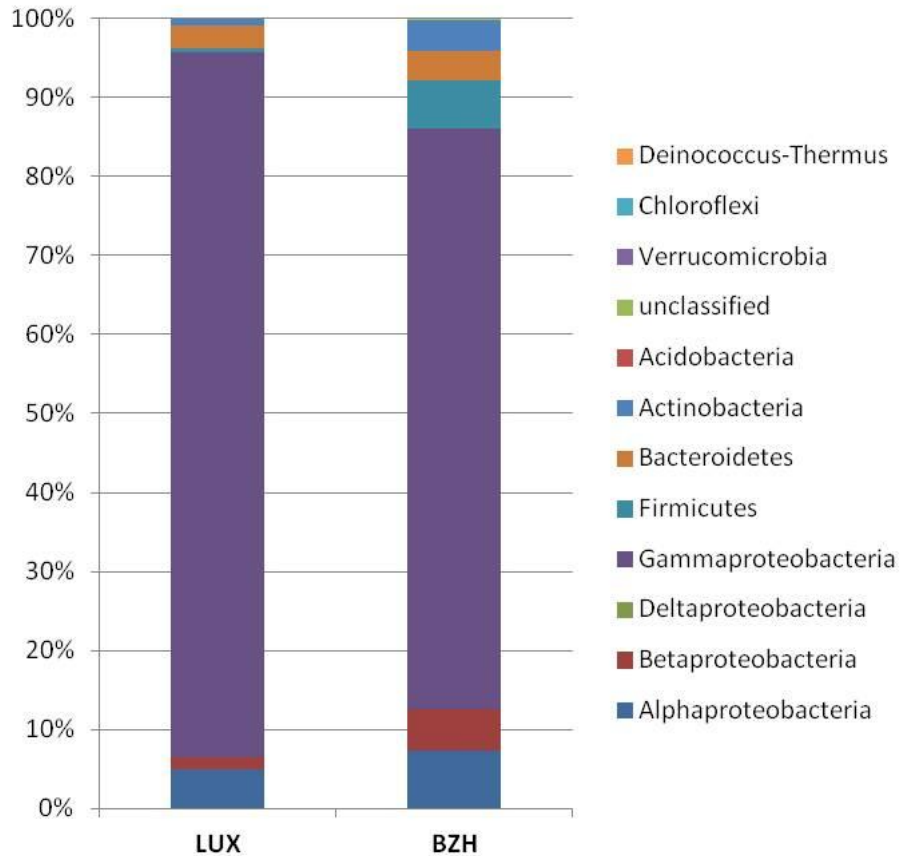


Legend:

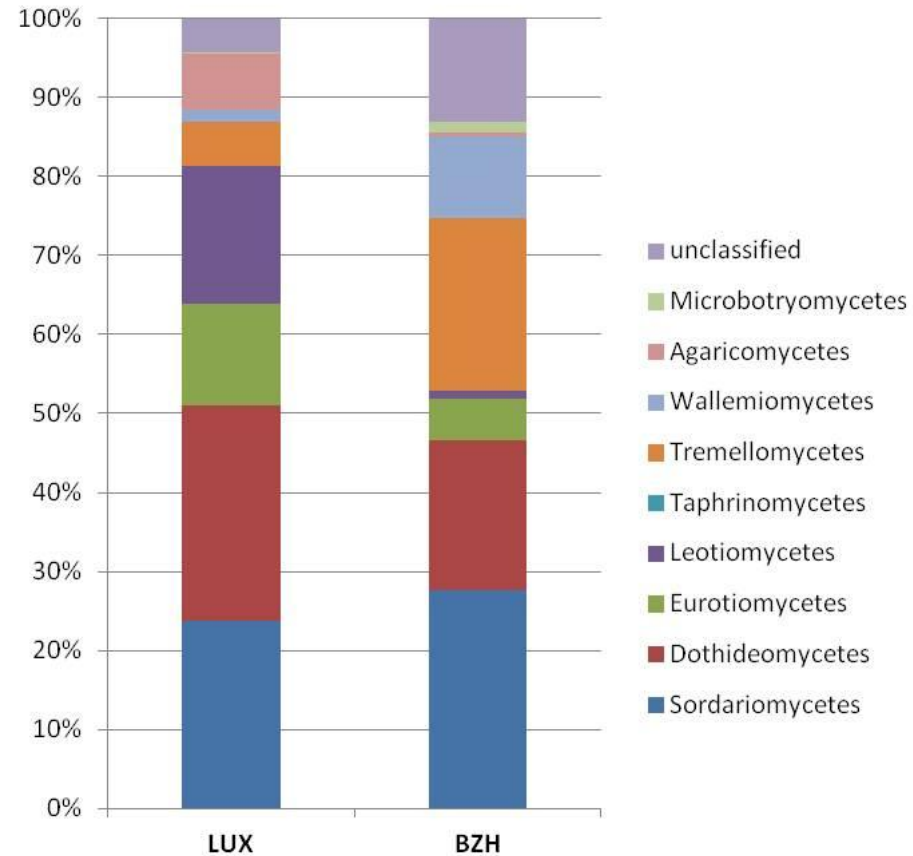
- Brittany (BZH)
- Luxembourg (LUX)
- 'Calima'
- 'Flageolet Chevrier'
- 'Roi des Belges'
- 'Rognon de Coq'
- 'St. Esprit à oeil rouge'

Results: Relative abundance per site

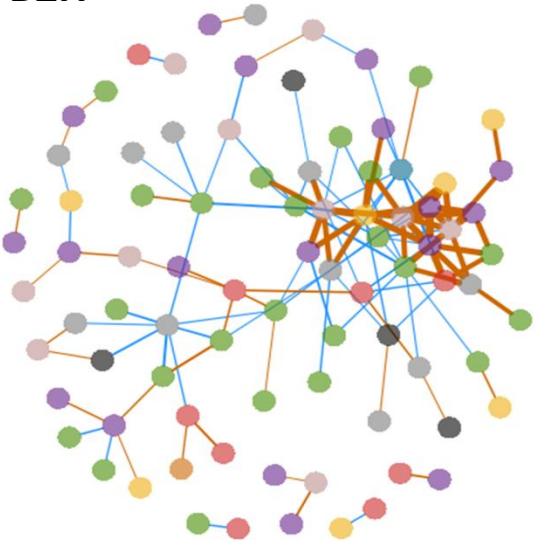
A) Bacterial phyla



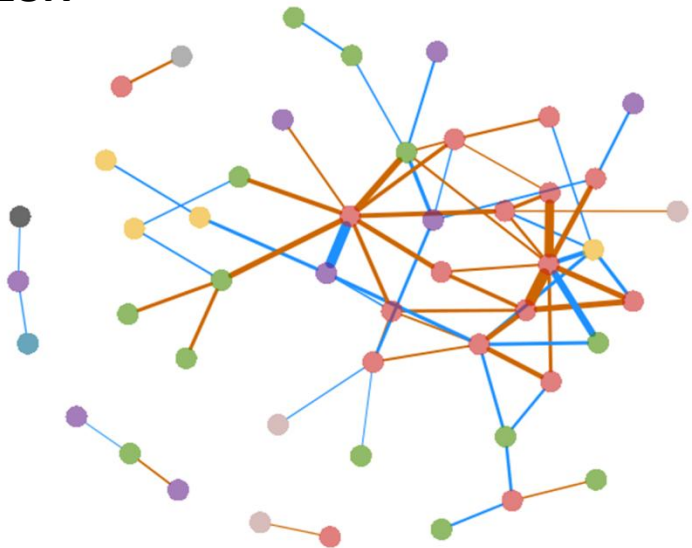
B) Fungal classes



**A. 16S
BZH**

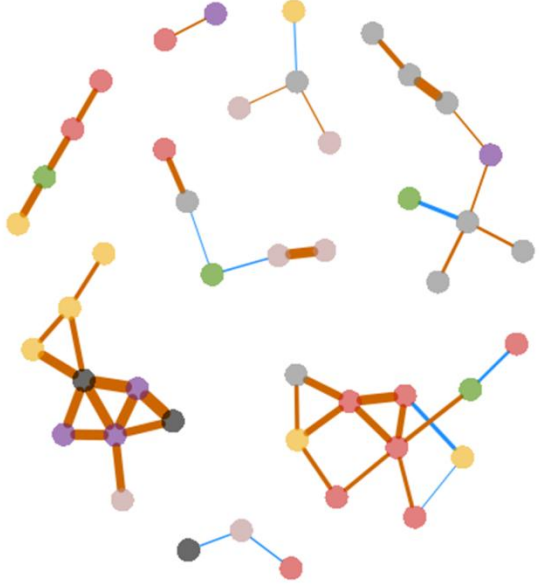


**B. 16S
LUX**

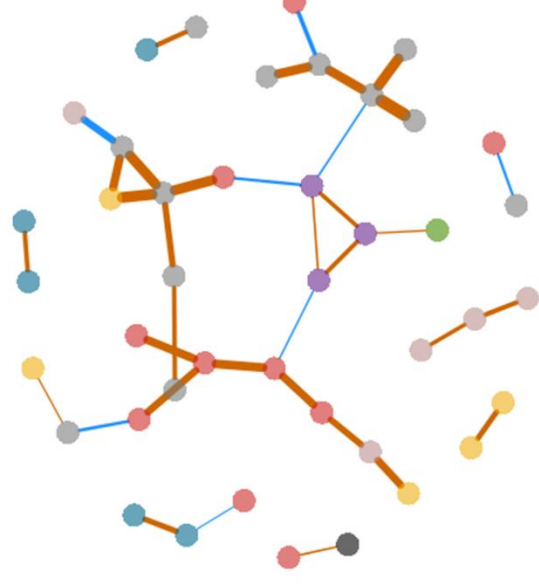


- Acidobacteria
- Actinobacteria
- Alphaproteobacteria
- Bacteroidetes
- Betaproteobacteria
- Firmicutes
- Gammaproteobacteria
- Other/unclassified
- Verrucomicrobia

**C. ITS
BZH**



**D. ITS
LUX**



- Agaricomycetes
- Dothideomycetes
- Eurotiomycetes
- Microbotryomycetes
- Other/unclassified
- Sordariomycetes
- Tremellomycetes
- Wallemiomycetes

**Correlation
networks**

Conclusions

- **Effect of terroir** on microbial communities after only 2 years of multiplication, especially for fungi.
- Insight into **functions** of the differing taxa limited due to taxonomic level of identification
- However, farmers engaged in small-scale, local seed production may also be **safeguarding seed-associated microbial assemblages** specific of their biogeographic location
- Indicates that microbial communities are driven by the terroir and that they are therefore **worth considering as a factor of plant adaptation** in further research.





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Approche : metabarcoding

