



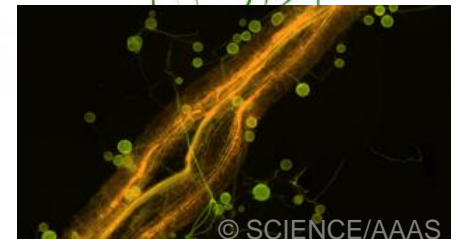
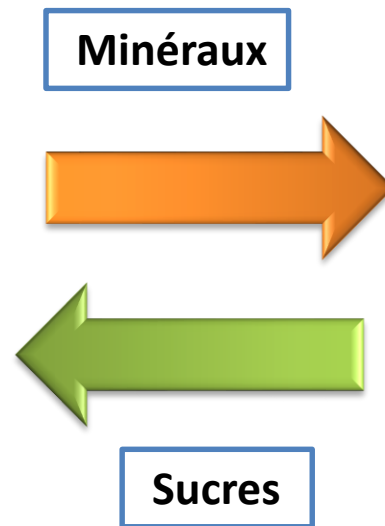
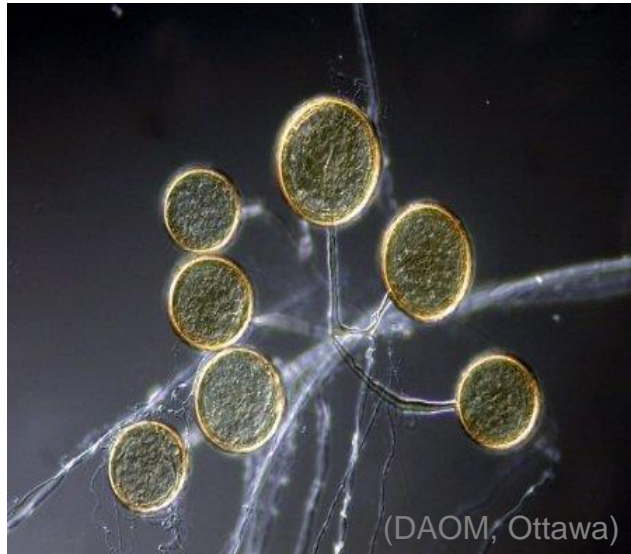
# **Étude de la biodiversité microbienne associée aux champignons mycorhiziens arbusculaires dans des sites hautement contaminés par des hydrocarbures pétroliers**

**Bachir IFFIS**

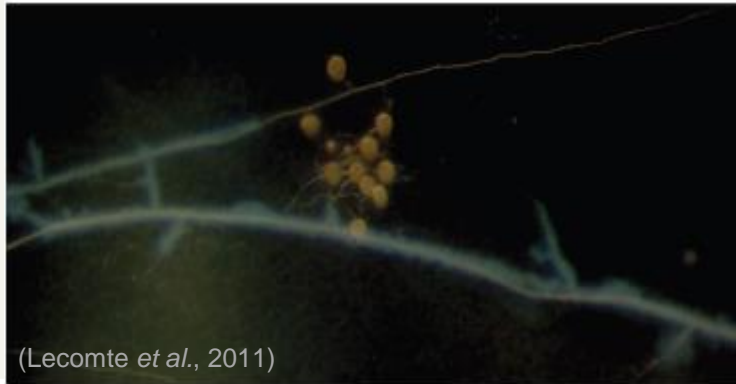
10 Mai 2017

# Les champignons mycorhiziens à arbuscules (CMA)

- Biotrophes obligatoires;
- Forment des associations symbiotiques avec 80 % des plantes vasculaires;
- Les CMA jouent aussi un rôle protecteur contre les pathogènes et les stress abiotiques.

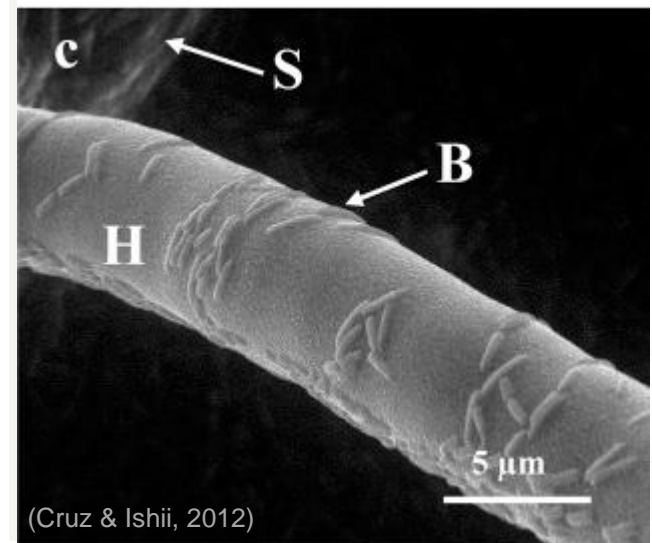
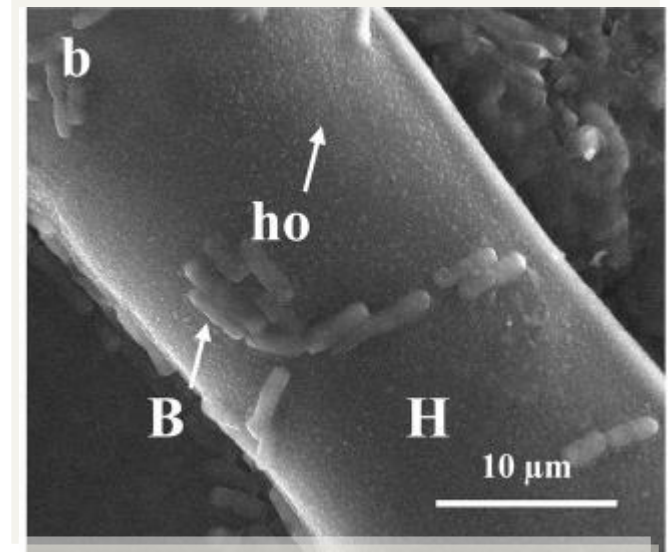


# Les microorganismes associées aux CMA



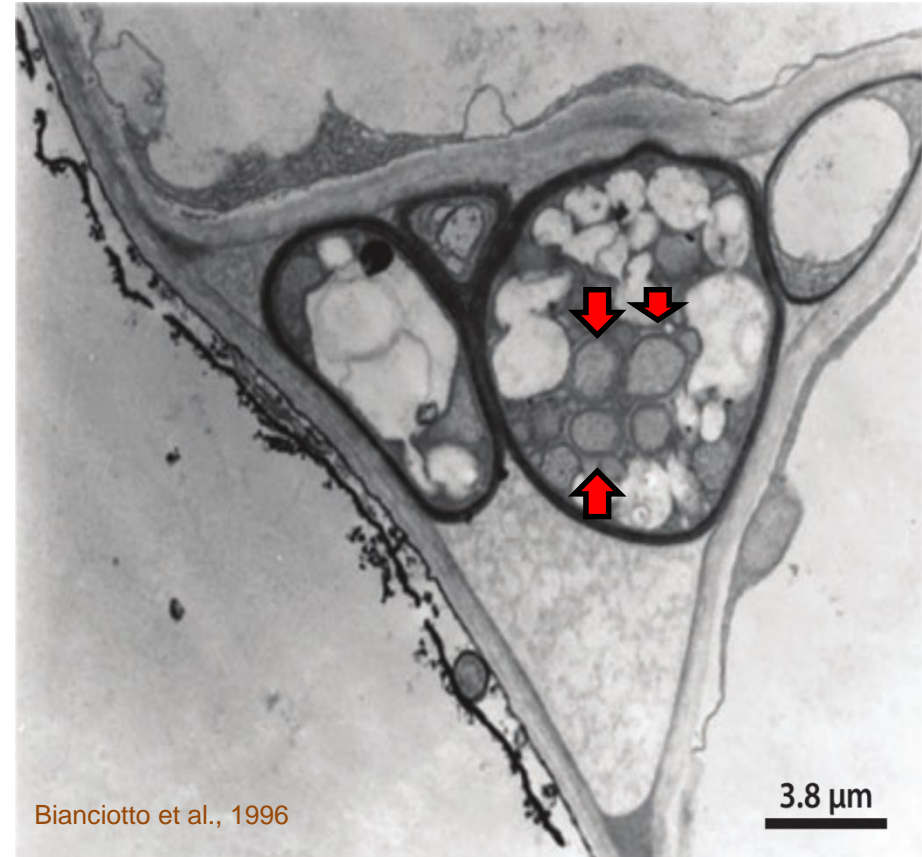
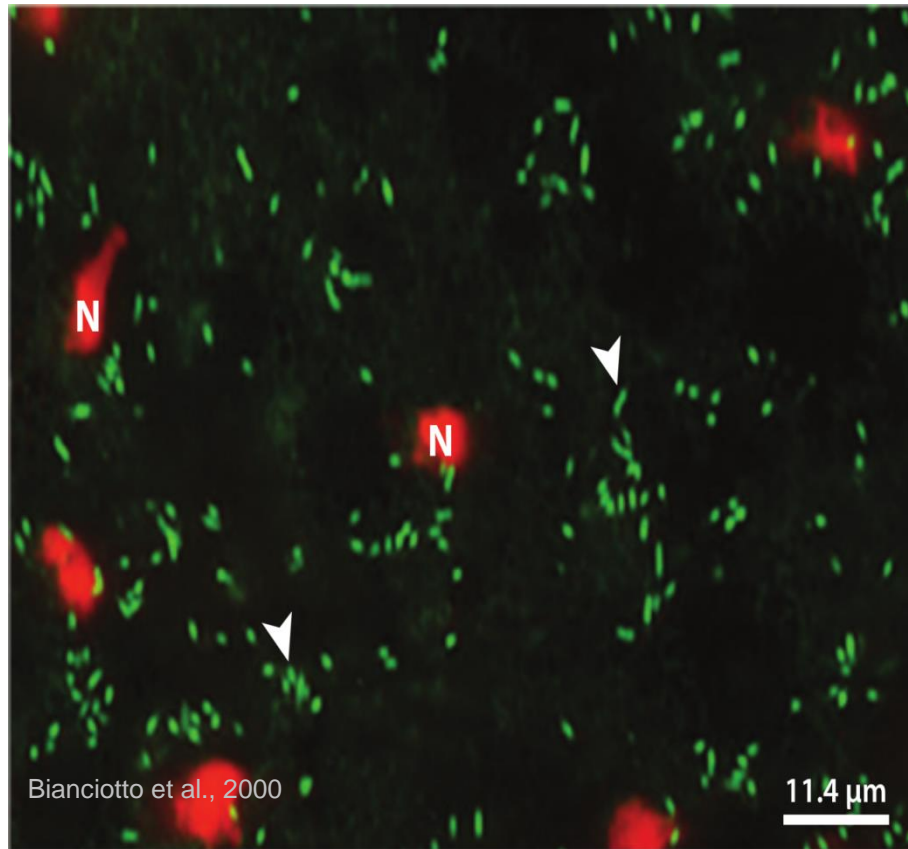
(Lecomte *et al.*, 2011)

- Les CMA peuvent contenir des bactéries à la surface des spores et du mycélium.



(Cruz & Ishii, 2012)

# Les microorganismes associées aux CMA

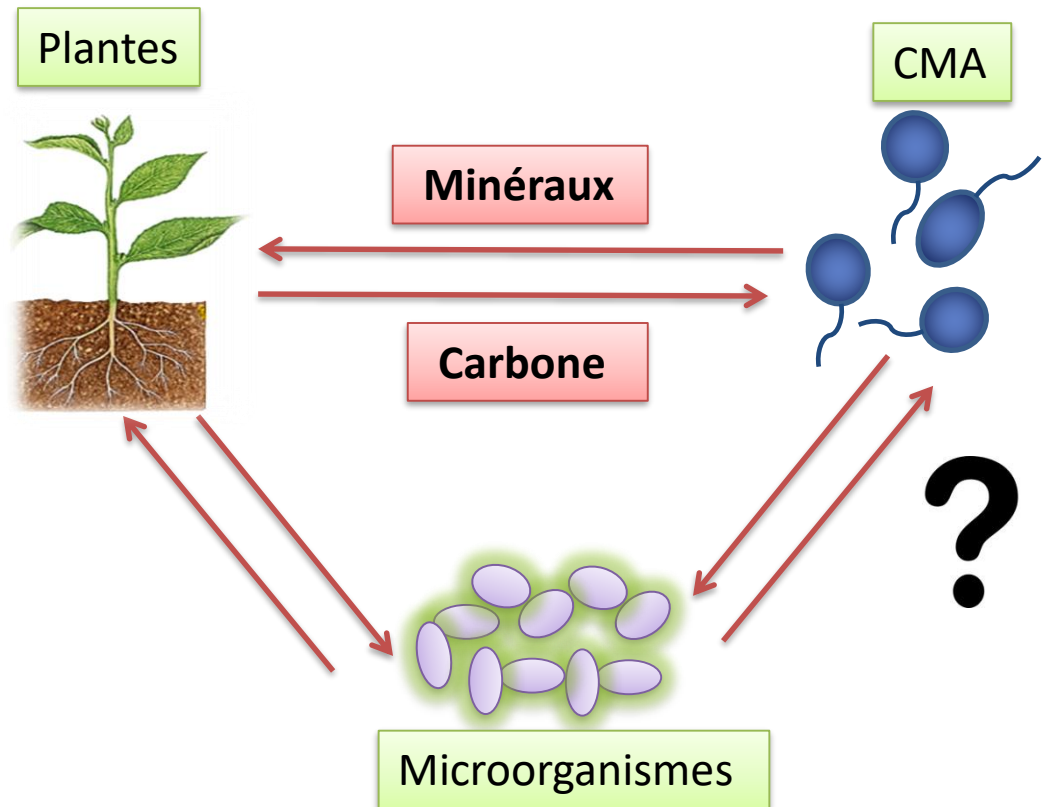


- Les CMA peuvent aussi héberger des bactéries à l'intérieur des spores.



# Les microorganismes associées aux CMA

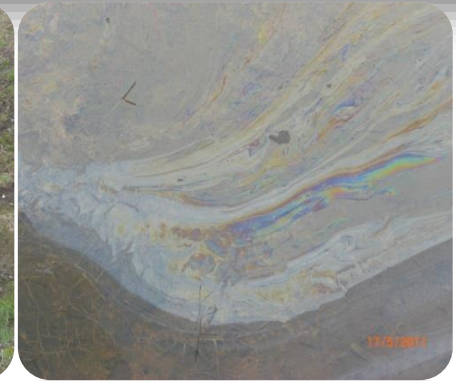
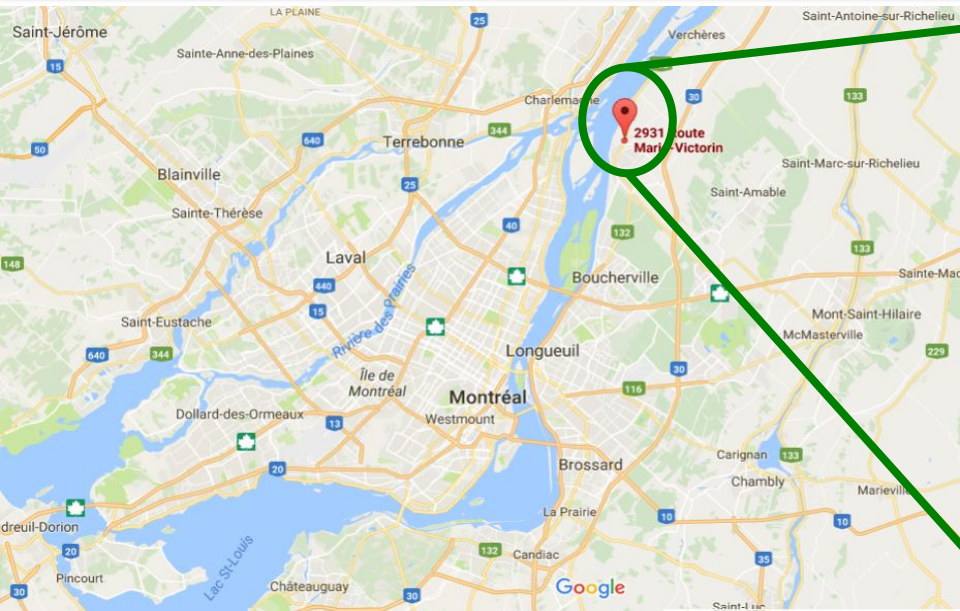
- Des associations tripartites;
- « Mycorrhiza helper bacteria »;
- La diversité et les rôles de ces microorganismes sont inconnus.



# Objectifs principaux

- Étudier la biodiversité microbienne associée aux CMA dans des sites contaminés.
- Étudier la **variation** de ces diversités par rapport aux **niveaux** de **contamination** et à l'**affiliation taxonomique** des plantes hôtes.
- Étudier les **relations** entre les **CMA** et les **microorganismes** qui sont associés à leurs spores.

# Description du site d'étude



	Phénanthrène	Fluorène	Alkanes
<b>Les concentrations mesurées (mg/kg de sol)</b>	<b>4300</b>	<b>710</b>	<b>41000</b>
<b>Les normes de MDDELCC (mg/kg de sol)</b>	<b>50</b>	<b>100</b>	<b>3500</b>



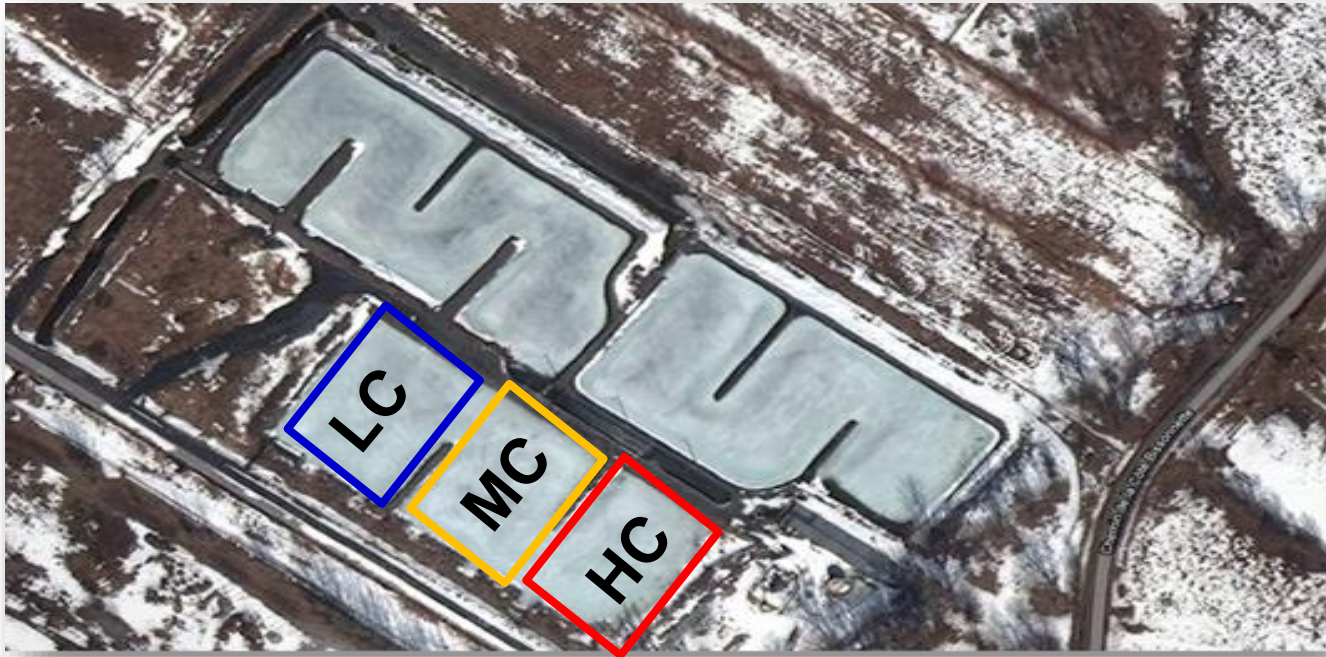
# Description du site d'étude

- Plusieurs espèces de plantes qui poussent **spontanément** dans ces **bassins** extrêmement **contaminés** par les hydrocarbures pétroliers.





# Description du site d'étude



*Solidago canadensis*



*Lycopodium europaeus*



*Populus balsamifera*



# Experience 1

## **Petroleum hydrocarbon contamination, plant identity and arbuscular mycorrhizal fungal community determine assemblages of the AMF spore-associated microbes**

Bachir Iffis, Marc St-Arnaud and Mohamed Hijri










Institut de Recherche en Biologie Végétale, Département de sciences biologiques, Université de Montréal, 4101 Sherbrooke est, Montréal (Québec) H1X 2B2, Canada

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environmental  
microbiology

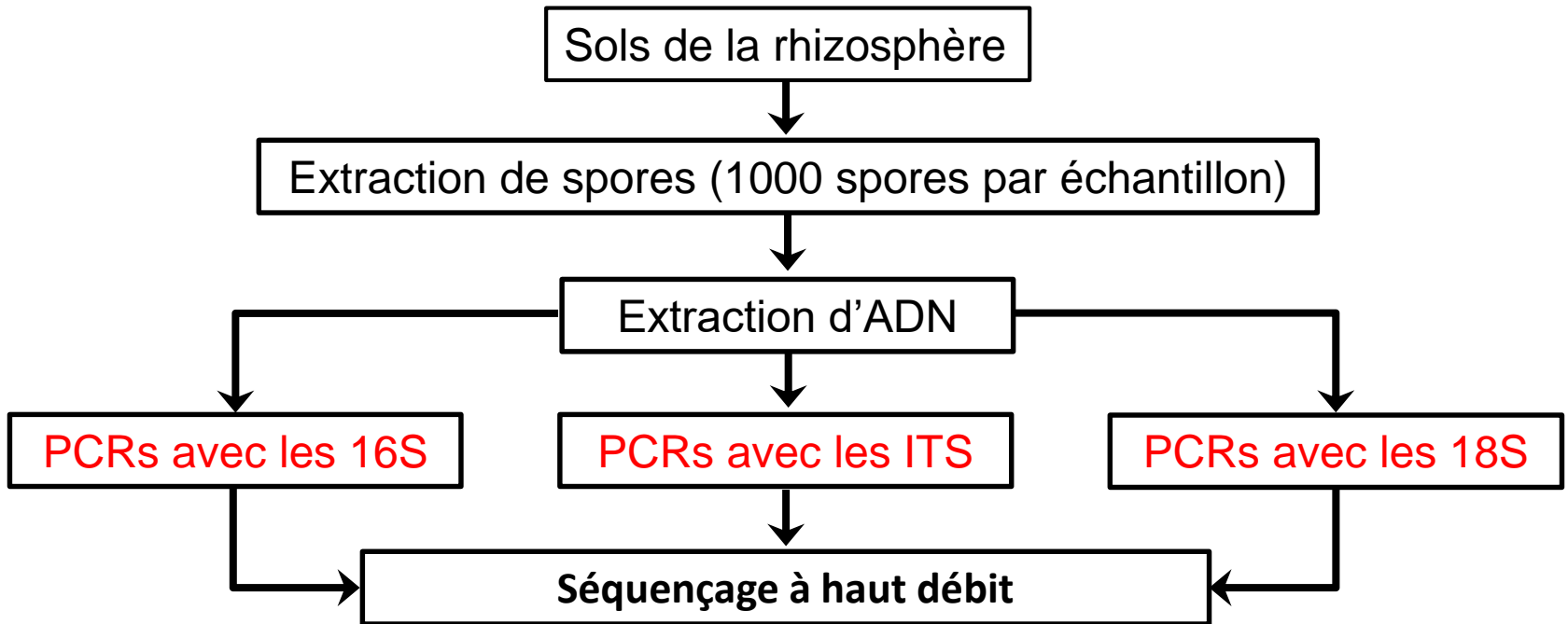


# Procédure expérimentale

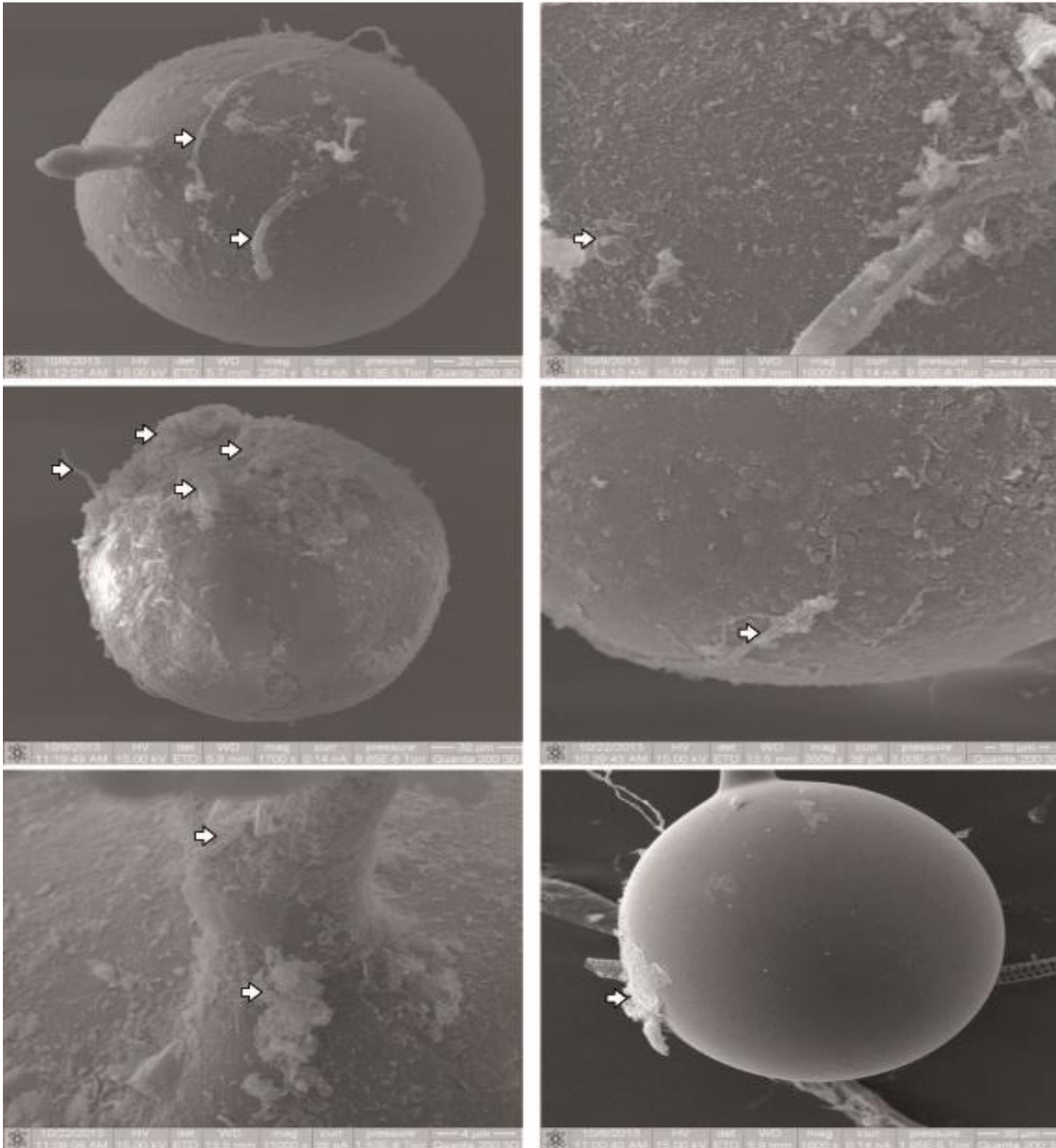
	(HC)	(MC)	(LC)
<i>Solidago canadensis</i>			
<i>Populus balsamifera</i>			
<i>Lycopus europaeus</i>			

Total = 27 échantillons

# Procédure expérimentale



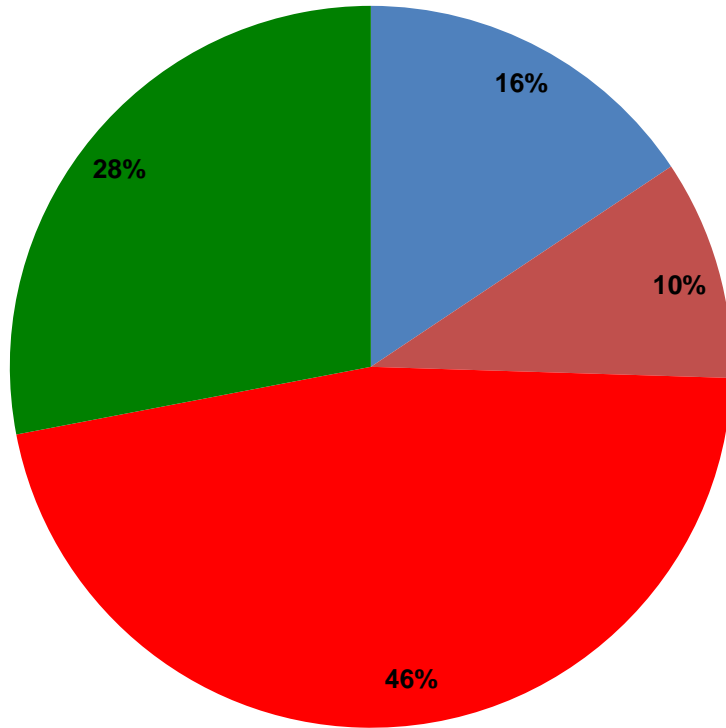
# Résultats



Des structures bactériennes et fongiques sur la surfaces des spores des CMA.

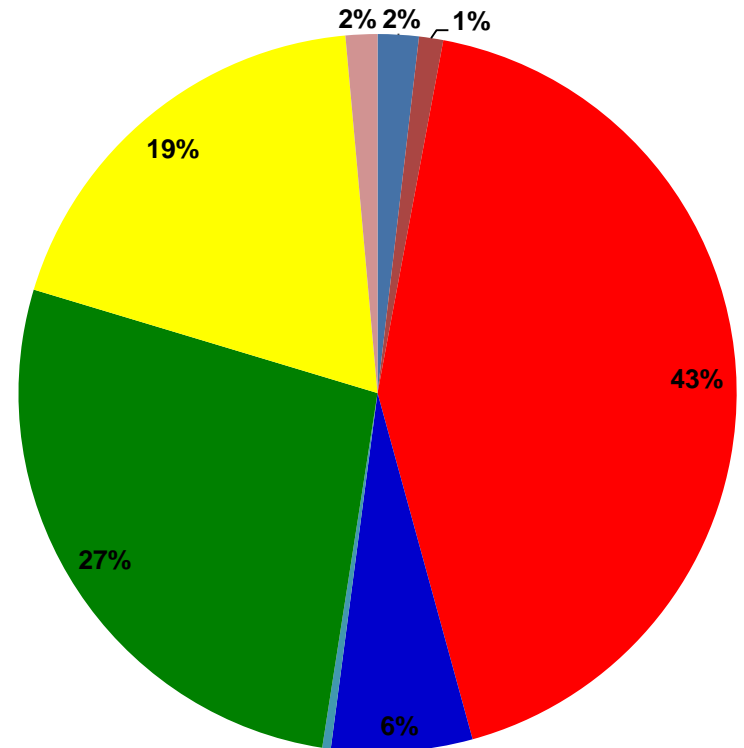
# La diversité des CMA

- Acaulospora sp
- Diversispora sp
- Claroideoglomus sp
- Glomus sp



**CMA\_18S**

- Acaulospora sp
- Diversispora sp
- Funneliformis sp
- Rhizophagus sp
- Claroideoglomus sp
- Entrophospora sp
- Glomus sp
- unclassified AMF

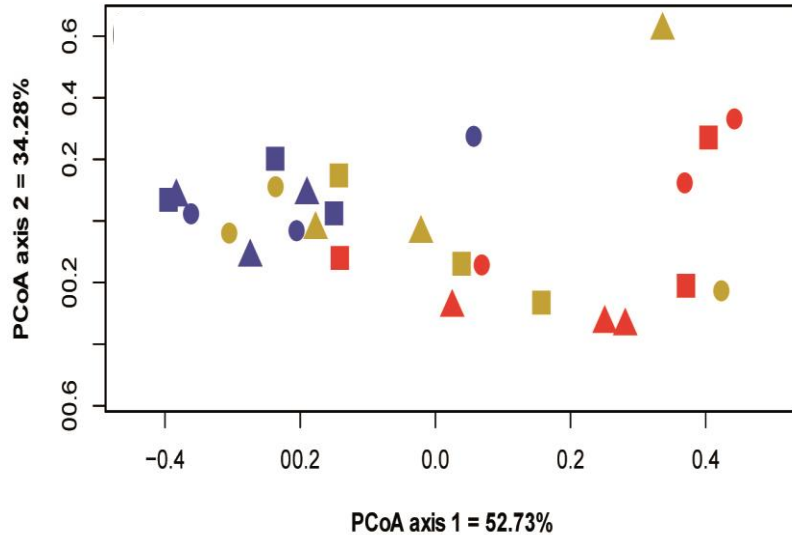


**CMA\_ITS**

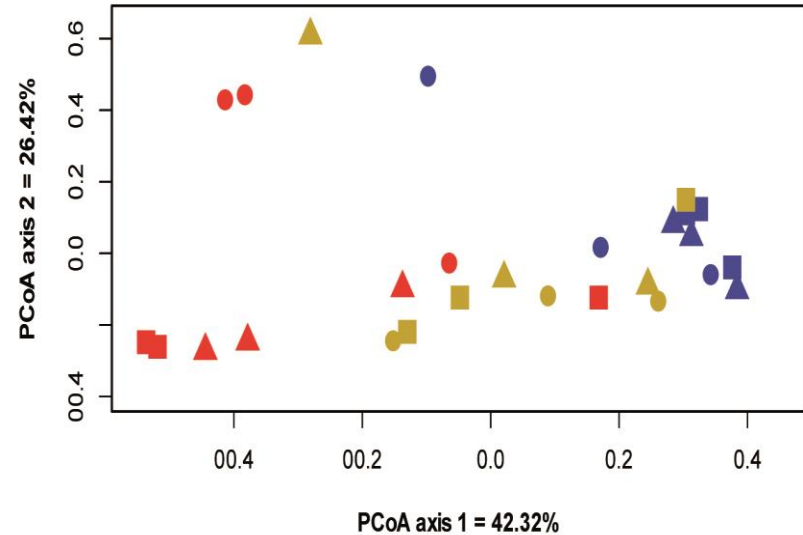


# La diversité des CMA

CMA avec les 18S



CMA avec les ITS



▲ Lycopodium-HC  
 ■ Populus-HC  
 ● Solidago-HC

▲ Lycopodium-LC  
 ■ Populus-LC  
 ● Solidago-LC

▲ Lycopodium-MC  
 ■ Populus-MC  
 ● Solidago-MC

Contamination level  
 PERMANOVA

Plant species  
 PERMANOVA

AMF 18S

0.004

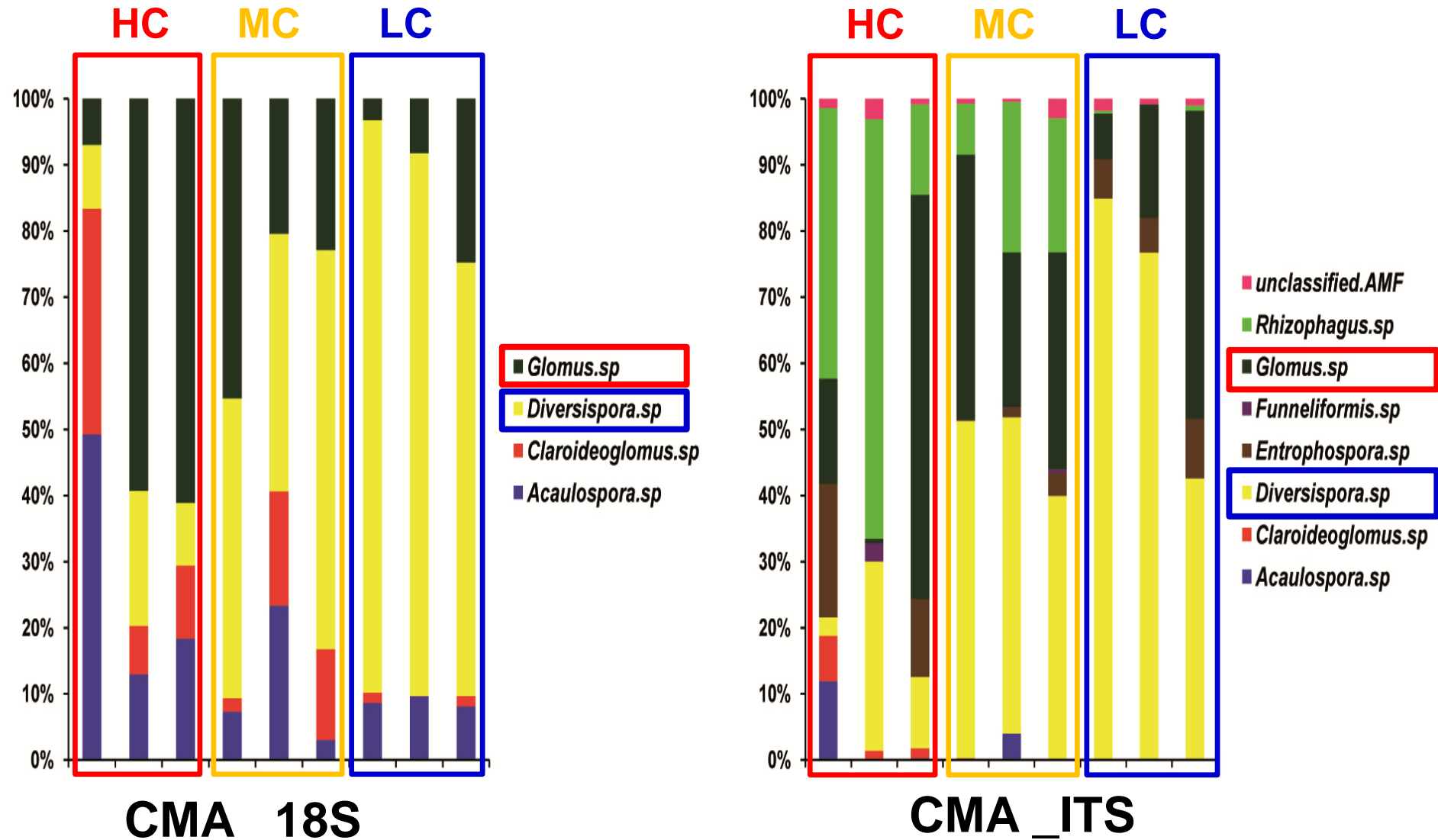
0.898

AMF ITS

0.016

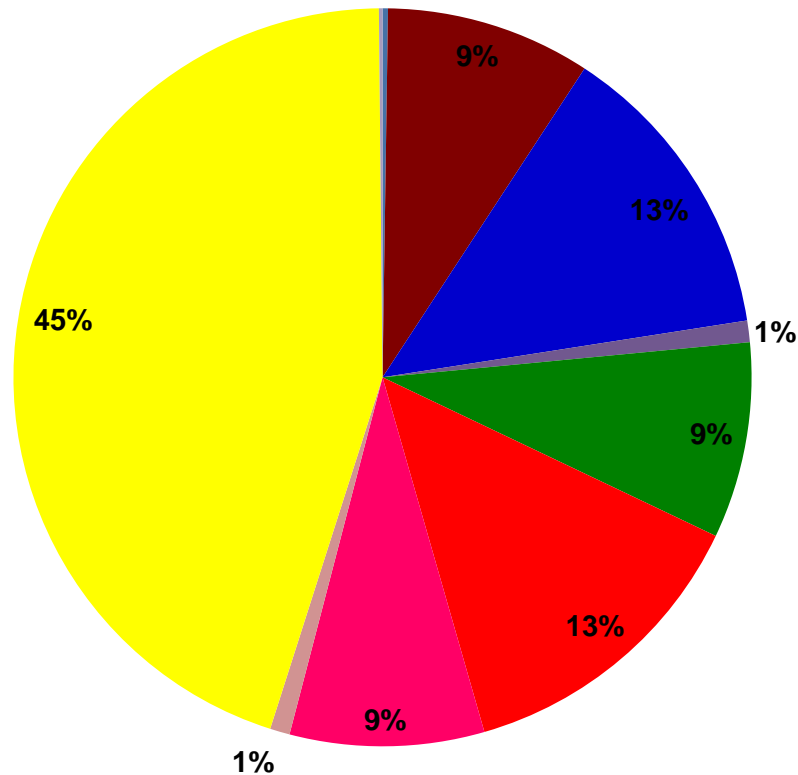
0.716

# La diversité des CMA



# La diversité des champignons

- Agaricomycetes
- Chytridiomycetes
- Dothideomycetes
- Leotiomycetes
- Microbotryomycetes
- Pezizomycetes
- Sordariomycetes
- Tremellomycetes
- Unclassified
- Ustilaginomycetes



**Champignons**

# La diversité des bactéries

■ Gammaproteobacteria

■ Alphaproteobacteria

■ Flavobacteriia

■ Acidobacteria\_Gp4

■ Bacilli

■ Opitutae

■ Clostridia

■ Thermomicrobia

■ Spartobacteria

■ Chloroflexia

■ Acidobacteria\_Gp3

■ Betaproteobacteria

■ unclassified

■ Bacteroidetes\_incertae\_sedis

■ Acidobacteria\_Gp16

■ Acidobacteria\_Gp17

■ Sphingobacteriia

■ Cytophagia

■ Subdivision3

■ Caldilineae

■ Acidobacteria\_Gp1

■ Candidatus\_Hydrogenedens

■ Actinobacteria

■ Deltaproteobacteria

■ Acidobacteria\_Gp6

■ Acidobacteria\_Gp10

■ Acidobacteria\_Gp7

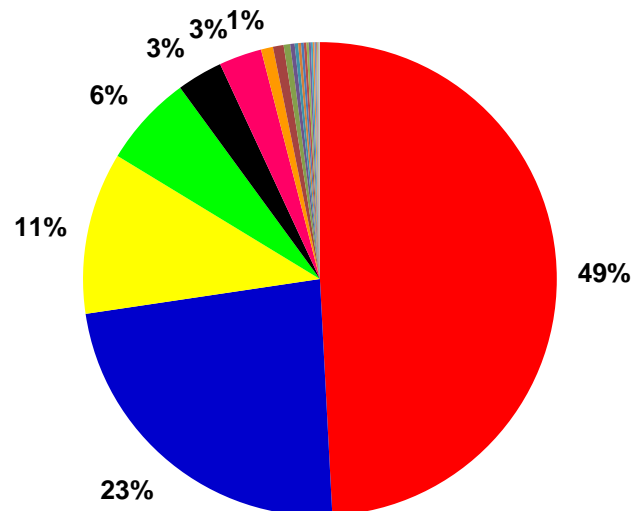
■ Gemmatimonadetes

■ Anaerolineae

■ Planctomycetia

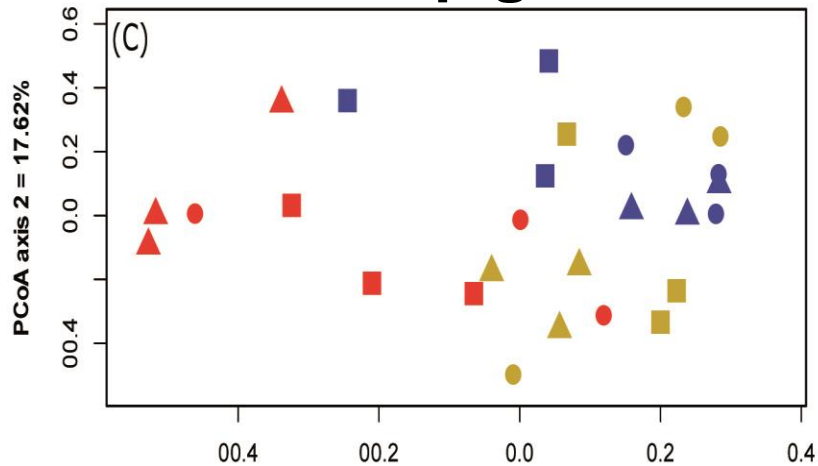
■ Negativicutes

■ Bacteroidia



# La structure des communautés microbiennes associées aux CMA

## Champignons



PCoA axis 1 = 18.24%

▲ **Lycopus-HC**

■ **Populus-HC**

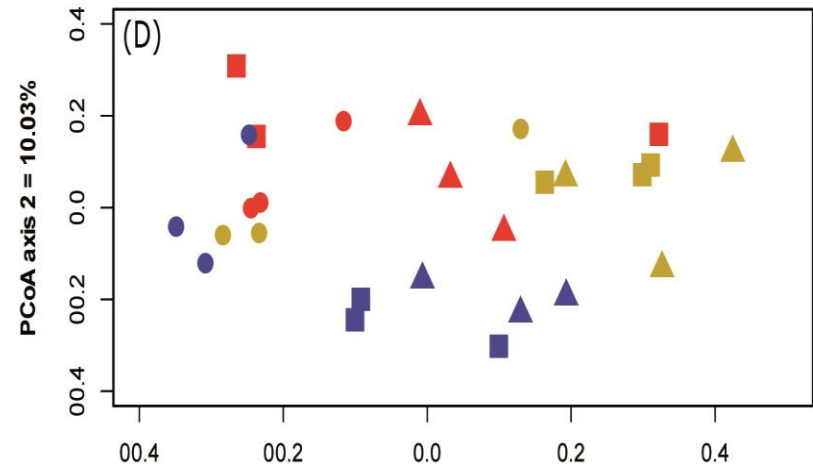
● **Solidago-HC**

▲ **Lycopus-LC**

■ **Populus-LC**

● **Solidago-LC**

## Bactéries



PCoA axis 1 = 21.53%

▲ **Lycopus-MC**

■ **Populus-MC**

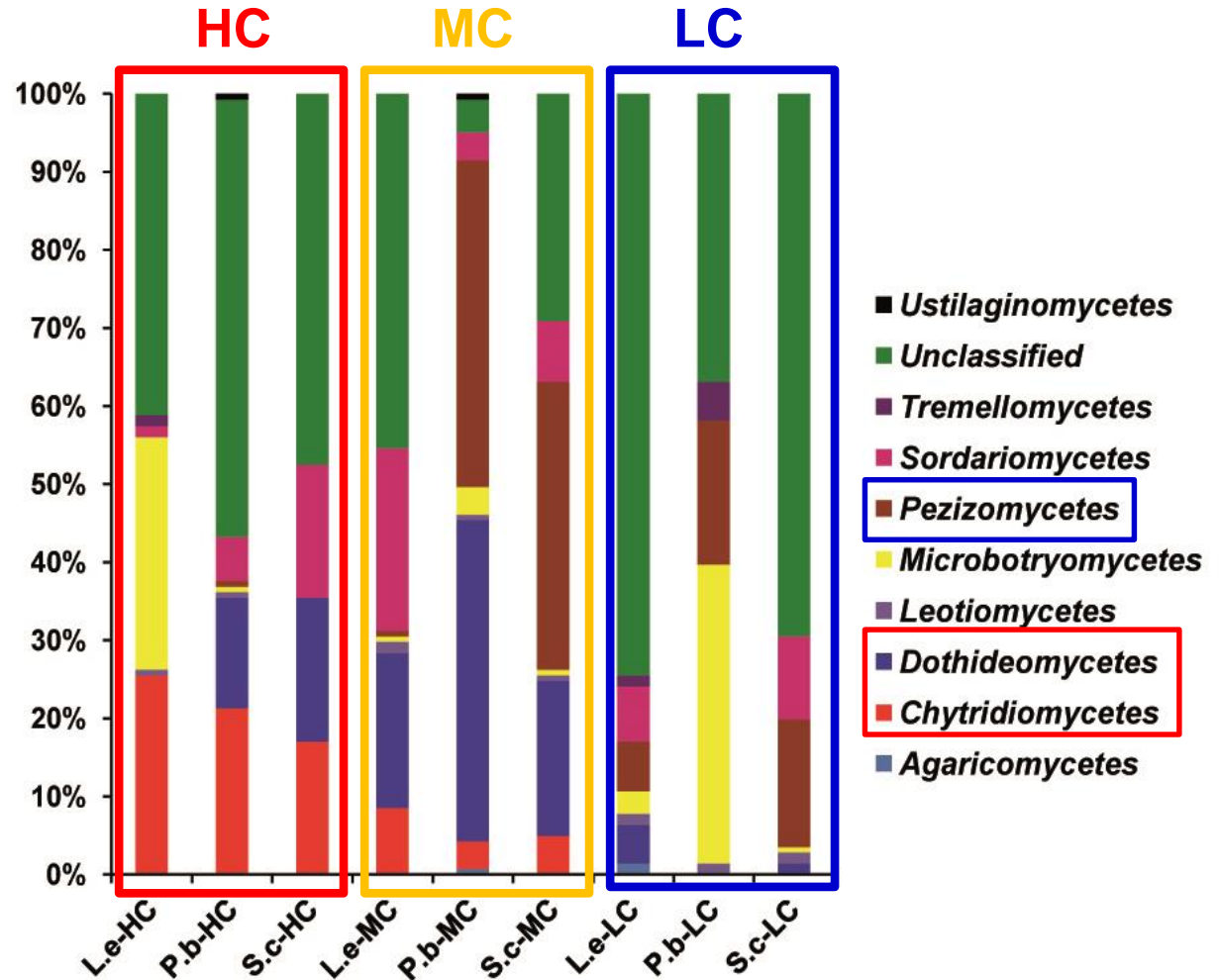
● **Solidago-MC**

	Contamination level	Plant species
	PERMANOVA	PERMANOVA
Fungal ITS	0.002	0.010
Bacterial 16S	0.010	0.001



# La diversité des champignons

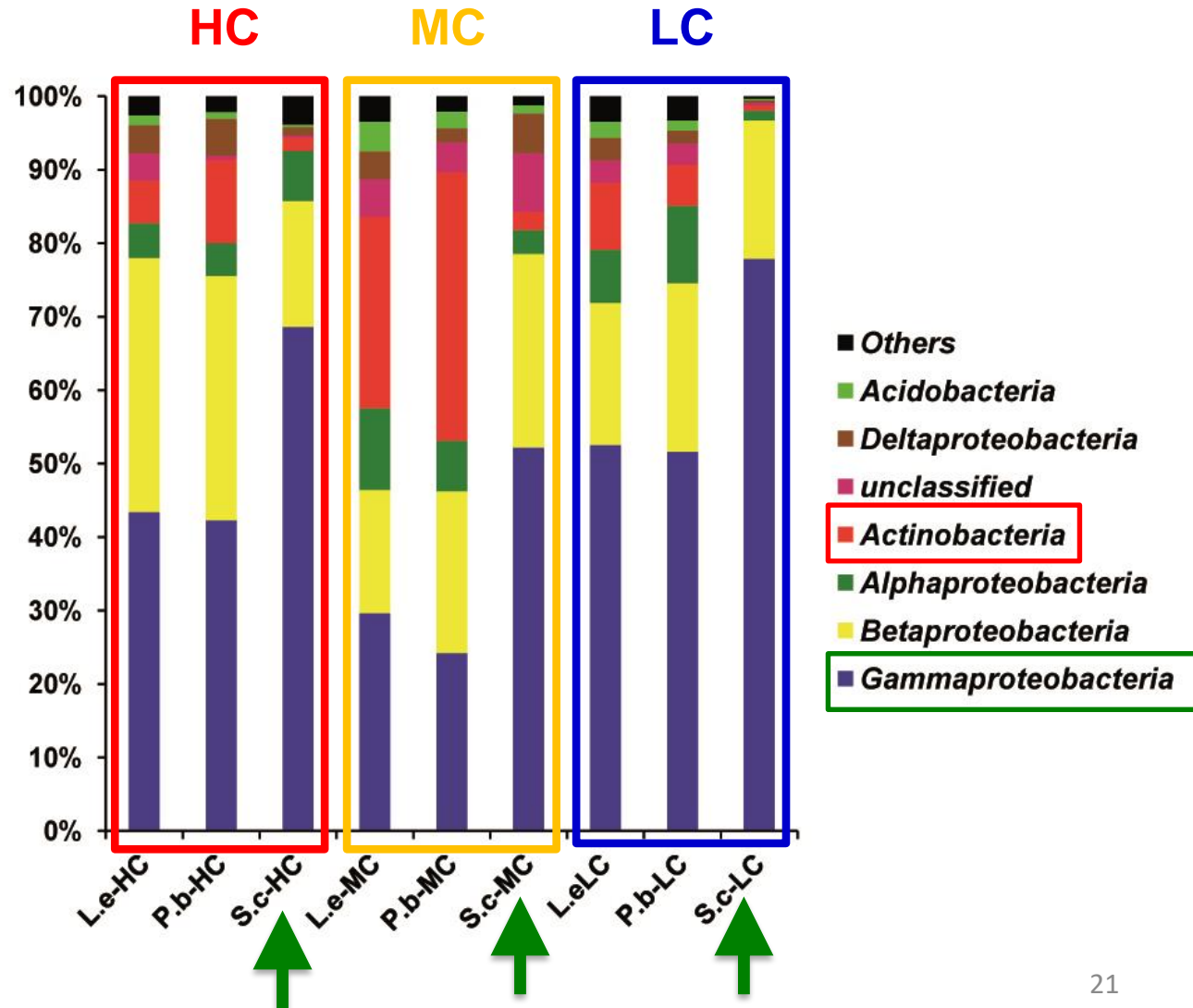
## Champignons



■ Les abondances des champignons varient en fonction des niveaux de contamination et les plantes hôtes.

# La diversité des bactéries

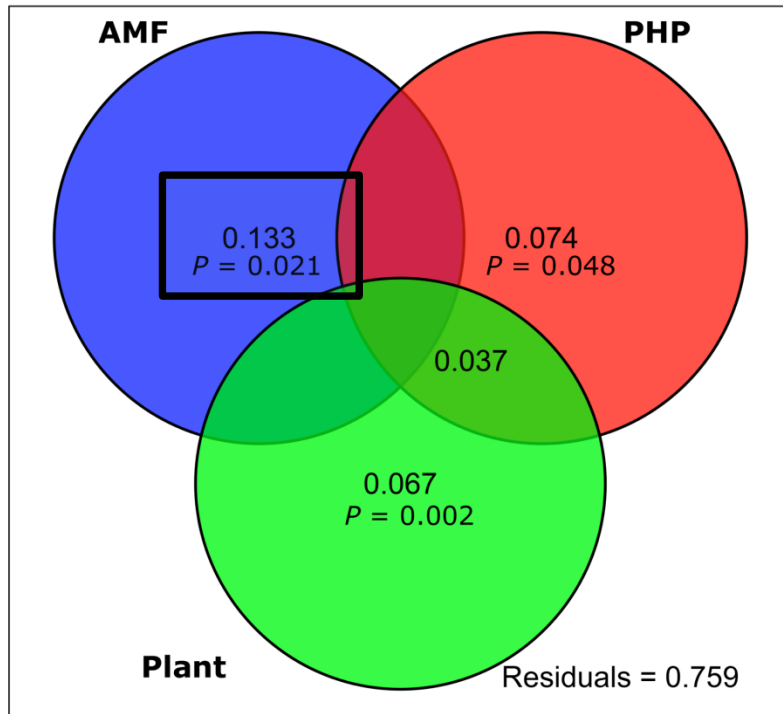
## Bactéries



Les abondances des bactéries varient en fonction des niveaux de contamination et les plantes hôtes.

# Analyse de partition de variance

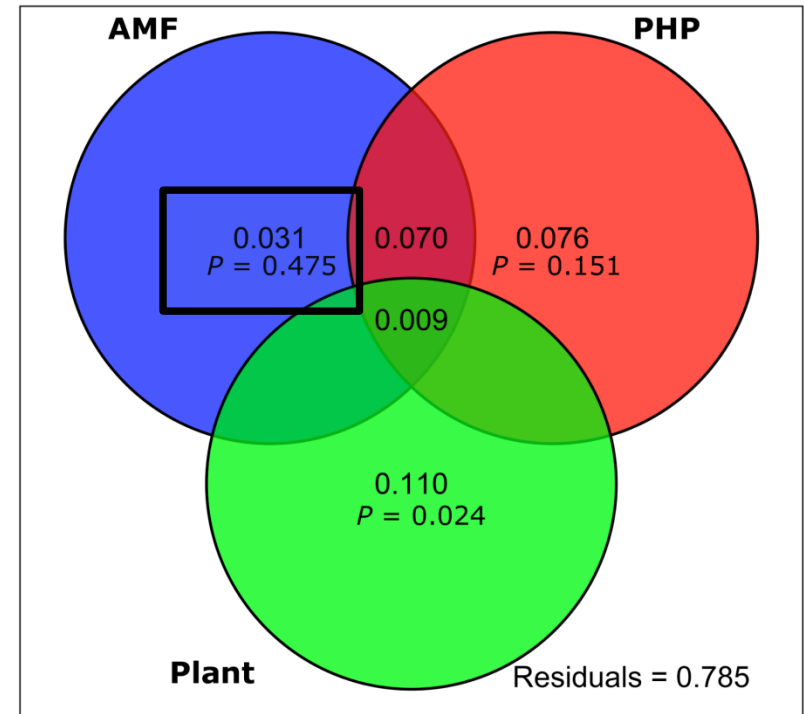
## Bacteria



Values <0 not shown

sum of negative fractions = -0.07014

## Fungi



Values <0 not shown

sum of negative fractions = -0.08008

# Experience 2

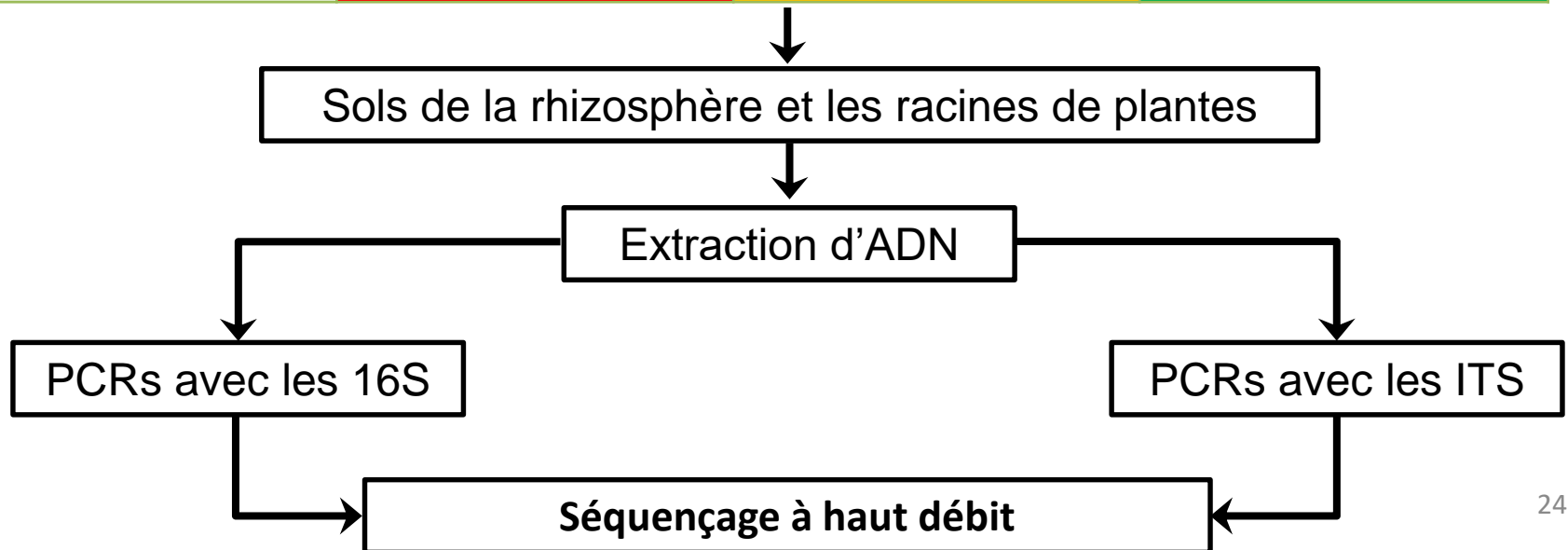
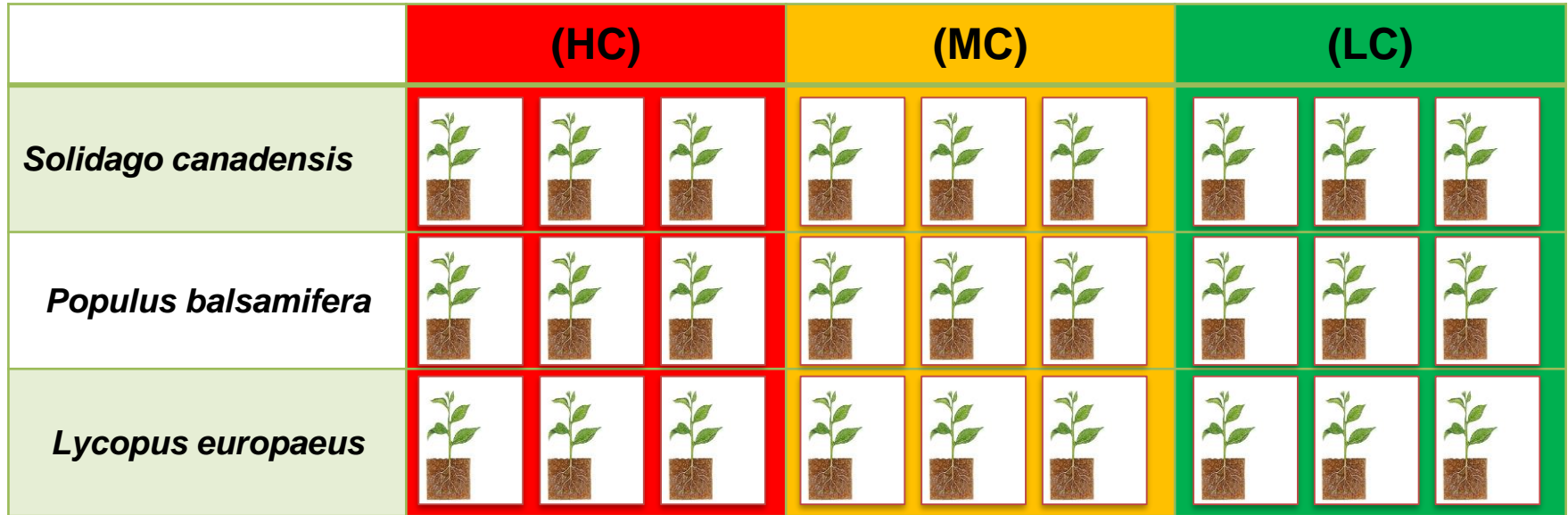
**Petroleum contamination and plant identity influence soil and root microbial communities while AMF spores retrieved from the same plants possess markedly different communities**

Bachir Iffis, Marc St-Arnaud and Mohamed Hijri

Institut de Recherche en Biologie Végétale, Département de sciences biologiques,  
Université de Montréal, 4101 Sherbrooke est, Montréal (Québec) H1X 2B2, Canada

article soumis pour publication dans le journal *Frontiers*

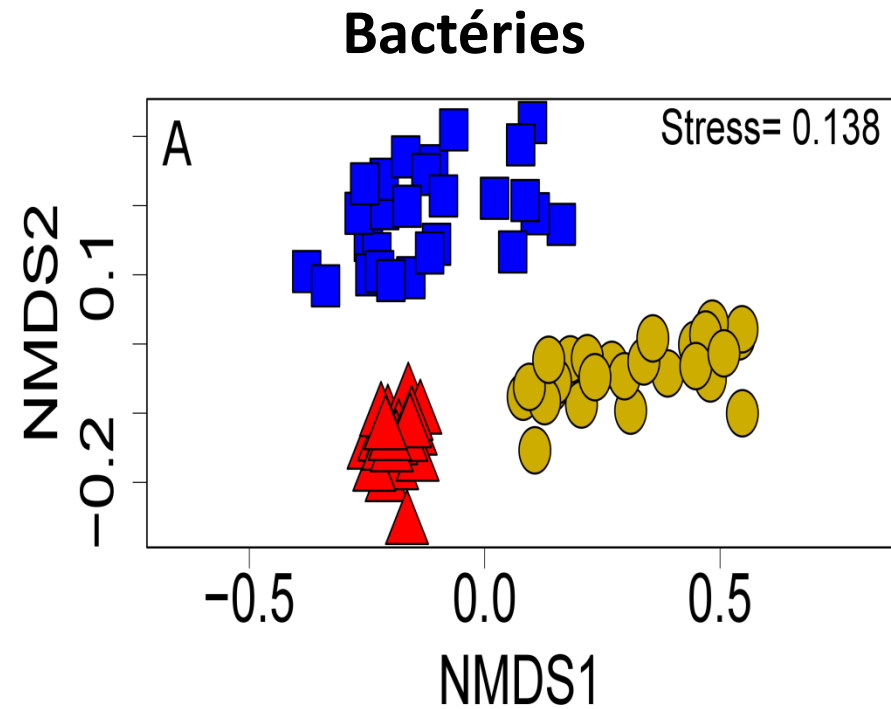
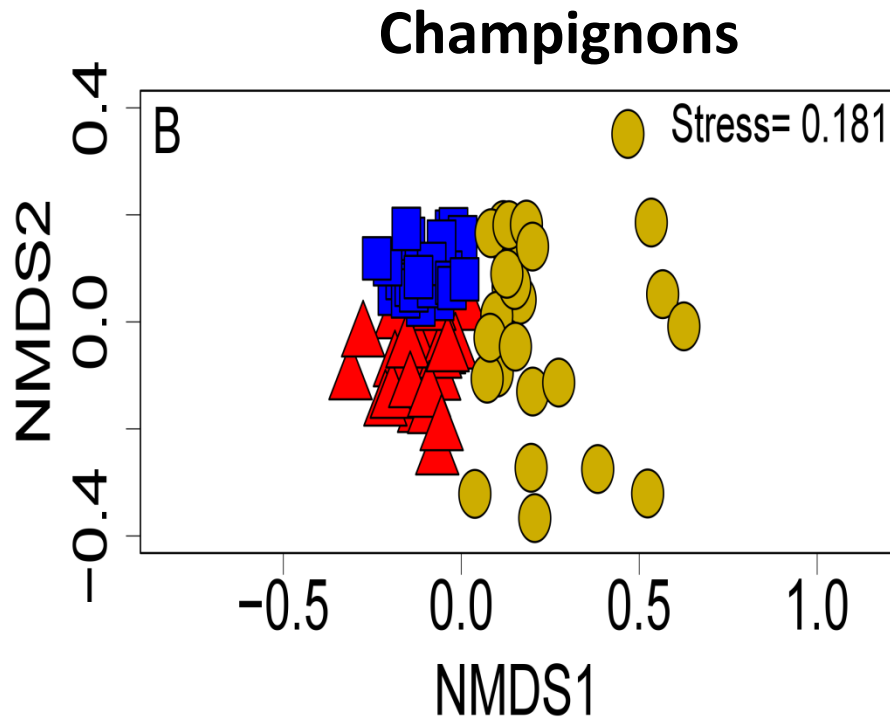
# Procédure expérimentale





# Résultats

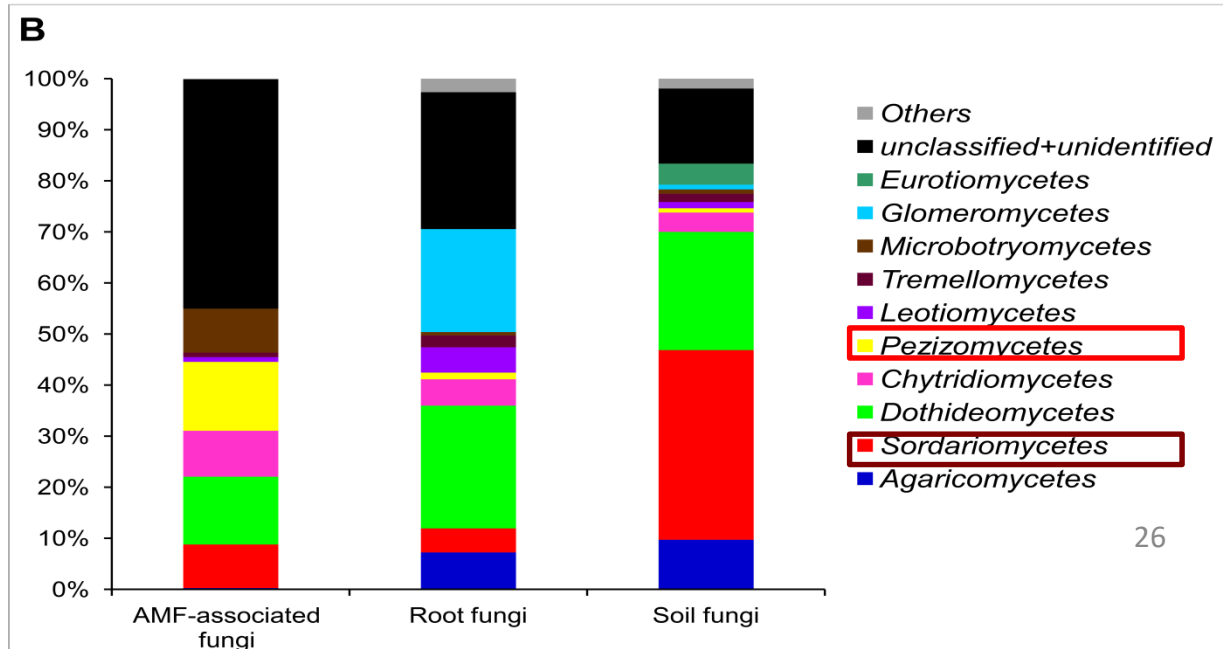
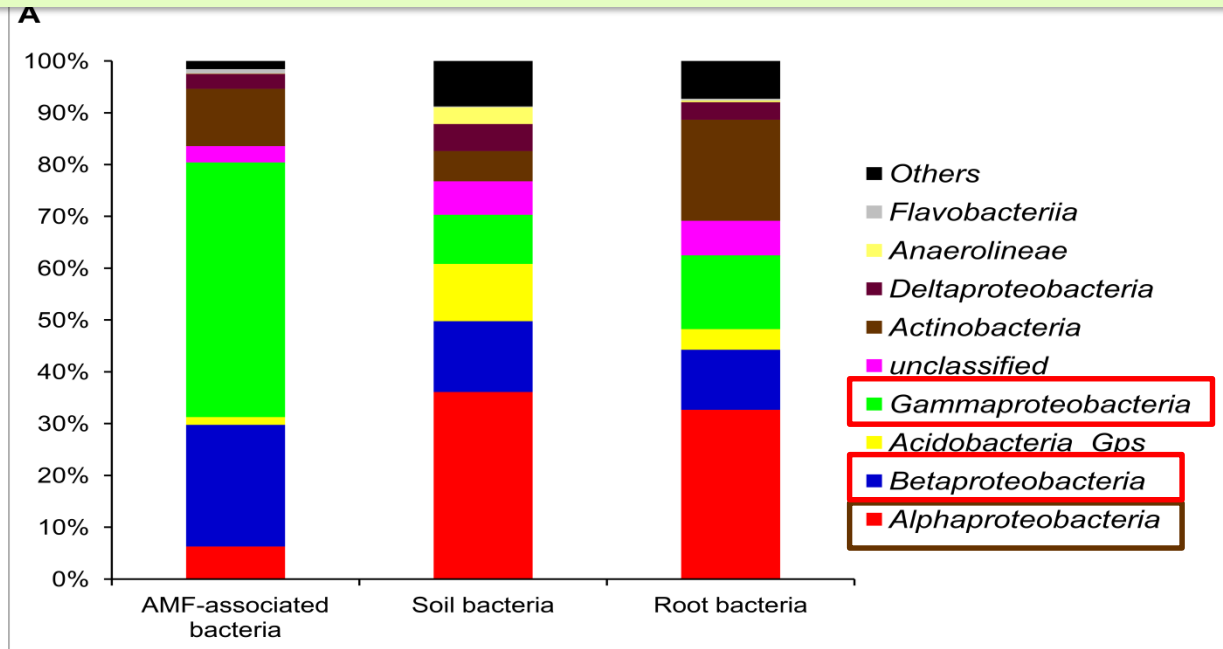
- Les communautés **microbiennes** associées aux CMA sont **significativement différentes** de celles des **racines** de plantes et des **sols** ( $P < 0.05$ ).



▲ Soil      ■ Roots      ● AMF spores

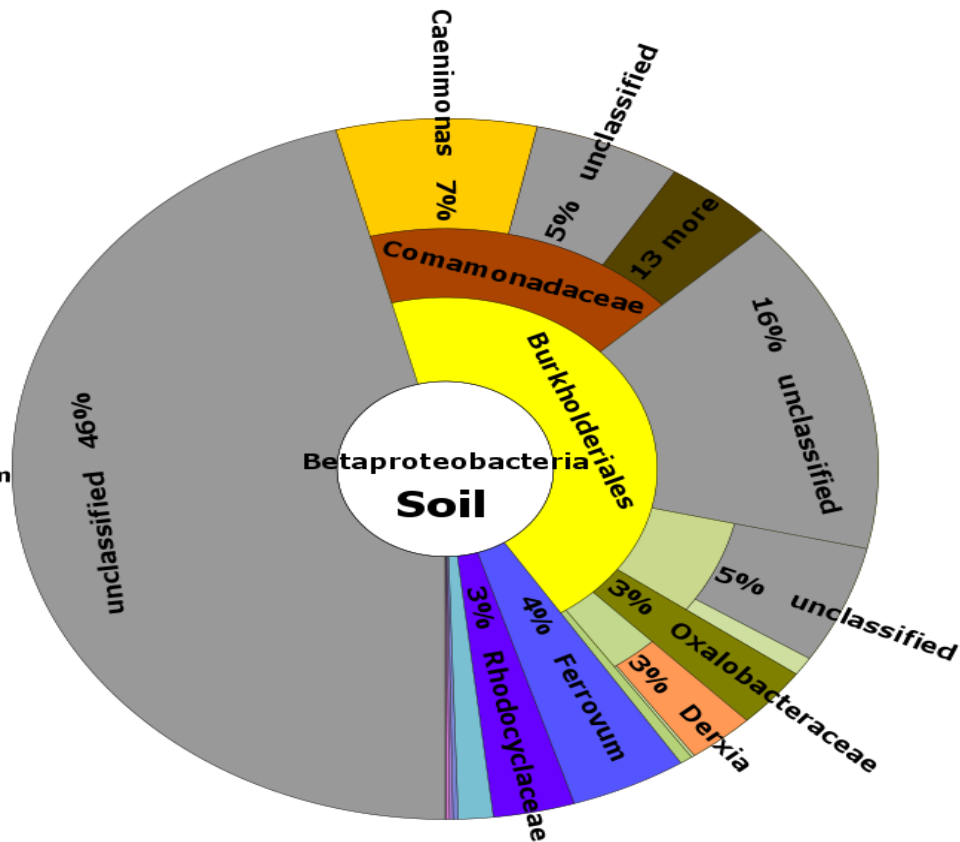
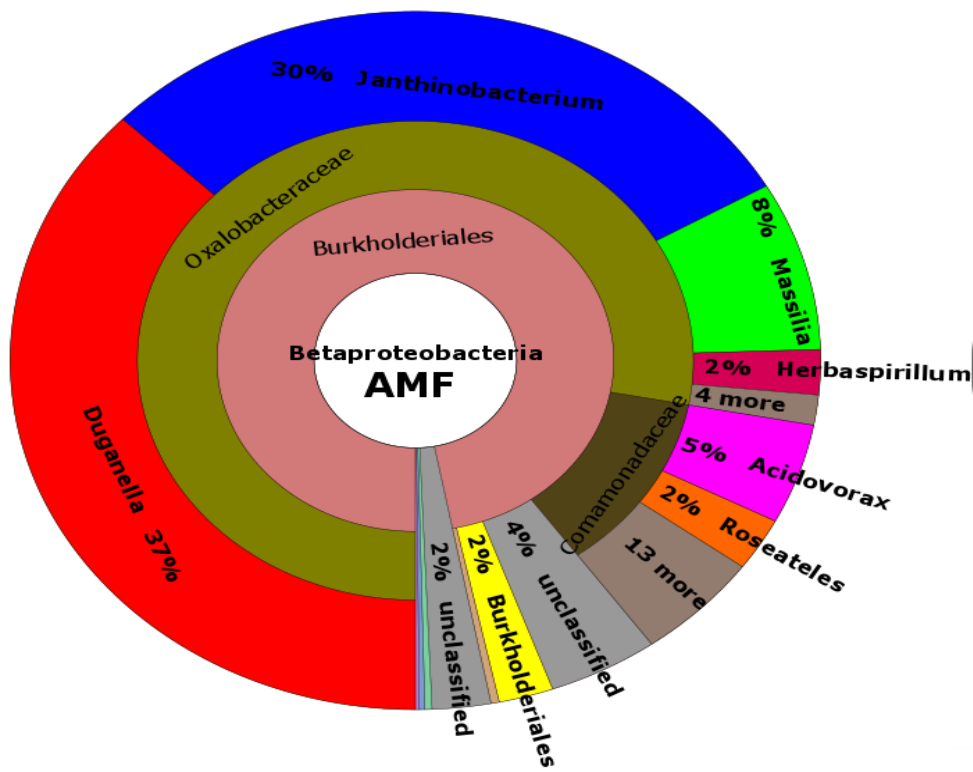
# Résultats

■ Les abondances des bactéries et des champignons varient en fonction des trois biotopes.



# Résultats

- Le profil des bactéries associées aux CMA est **différent** de celui des racines et des sols.

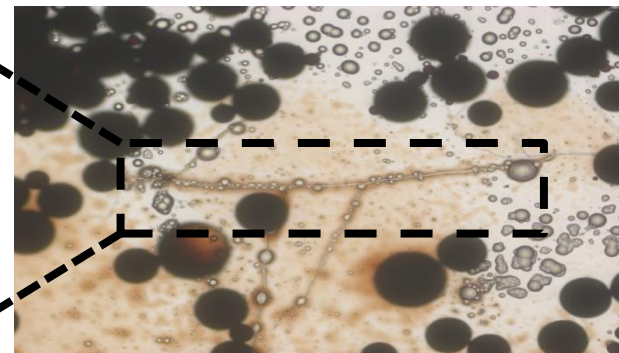
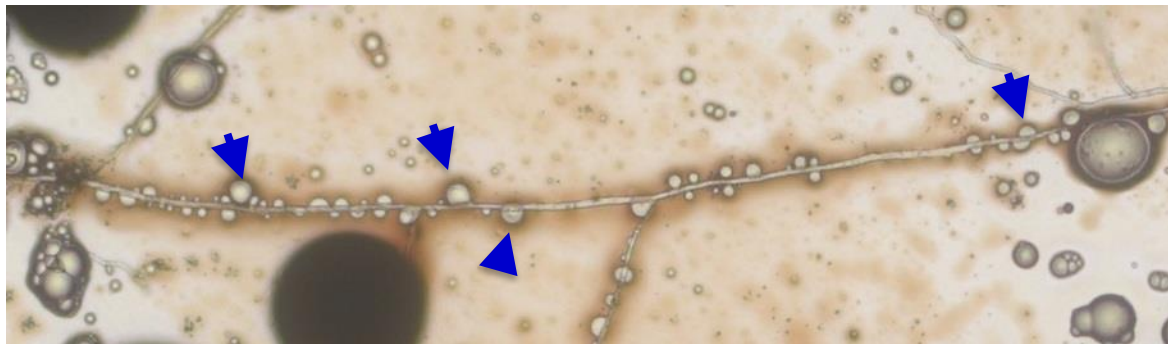
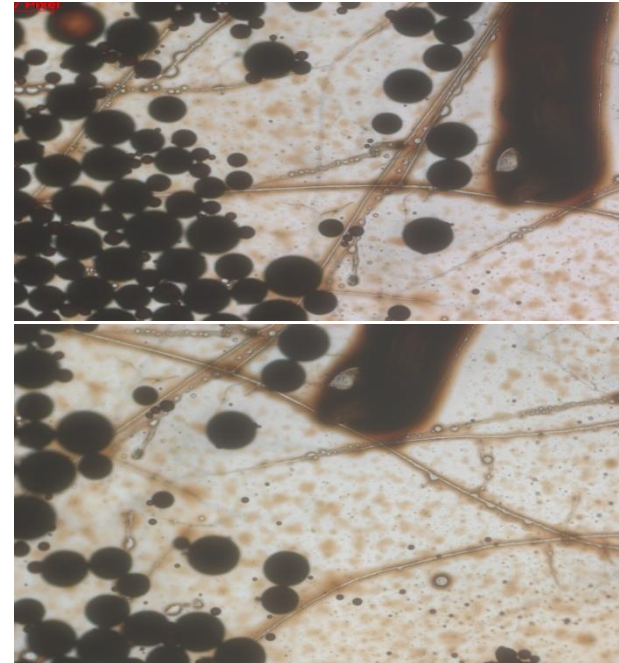


# Conclusion

- Malgré les concentrations extrêmes en HP mesurées dans ce site, le **niveau de diversité** des CMA et des bactéries associées reste **élevé**.
- En plus des HP et les plantes hôtes, les CMA peuvent jouer aussi un **rôle clé** dans la **structure** des communautés microbiennes associées à leurs spores.
- Même si les spores des CMA, les racines de plantes et les sols de la rhizosphère **partagent** le même microenvironnement, les **communautés microbiennes** présentent dans ces trois biotopes sont complètement **différentes**.

# Perspectives

- Étudier le(s) rôle(s) des microorganismes associées aux CMA.
- Tester l'effet de la **co-inoculation** des microbes avec les CMA sur la **phytoremédiation**.
- Étudier les **mécanismes** de bioremédiation des HP par les CMA.
- **Déterminer** la composition des **exsudats** mycéliens des CMA .



# Remerciements

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Dimitri Dagher  
MengXuan Kong (Cathy)  
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Tous les étudiants de l'IRBV



Université   
de Montréal

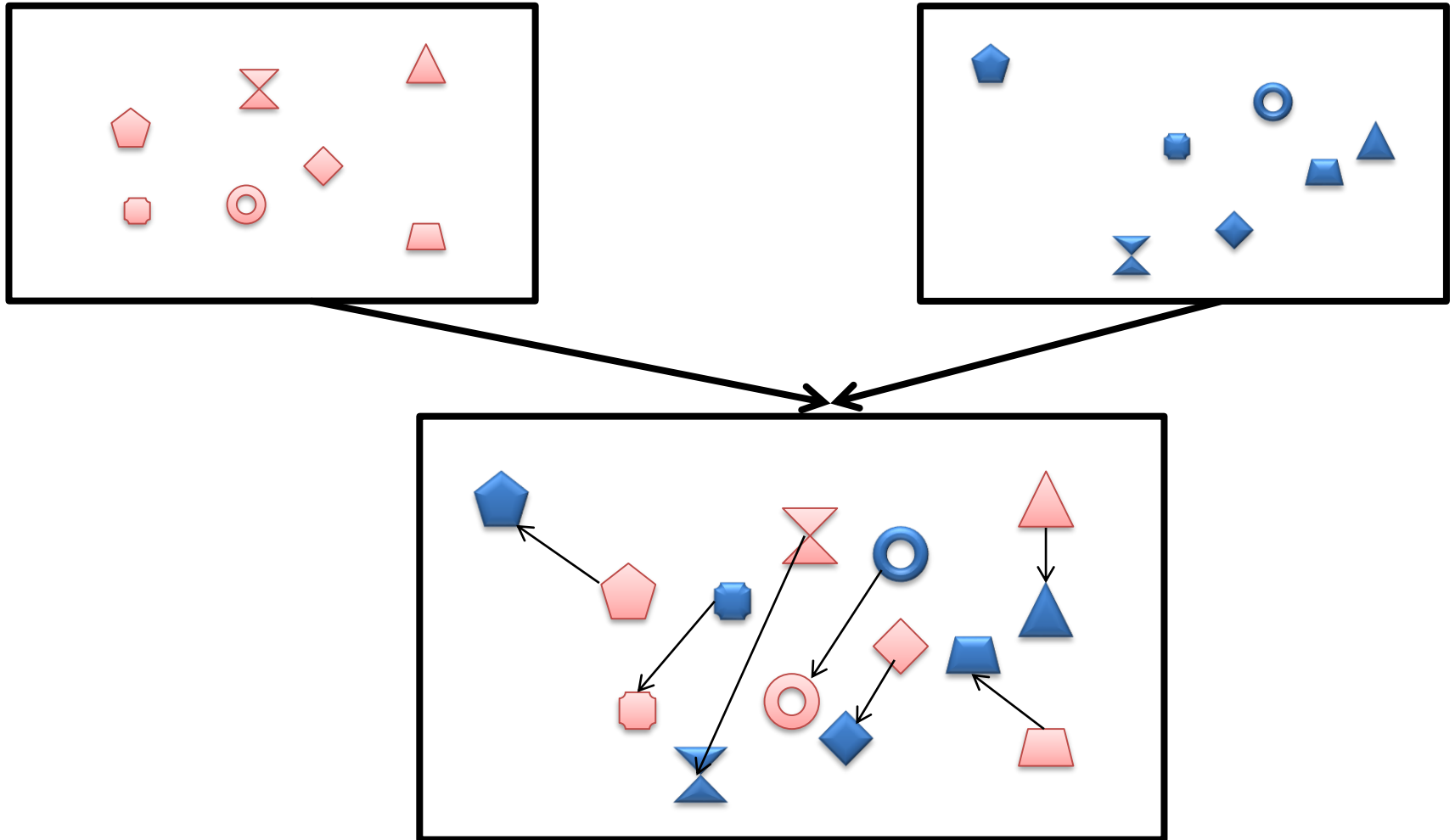
Faculté des études supérieures et postdoctorales



CENTRE DE LA SCIENCE DE LA BIODIVERSITÉ DU QUÉBEC  
QUEBEC CENTRE FOR BIODIVERSITY SCIENCE

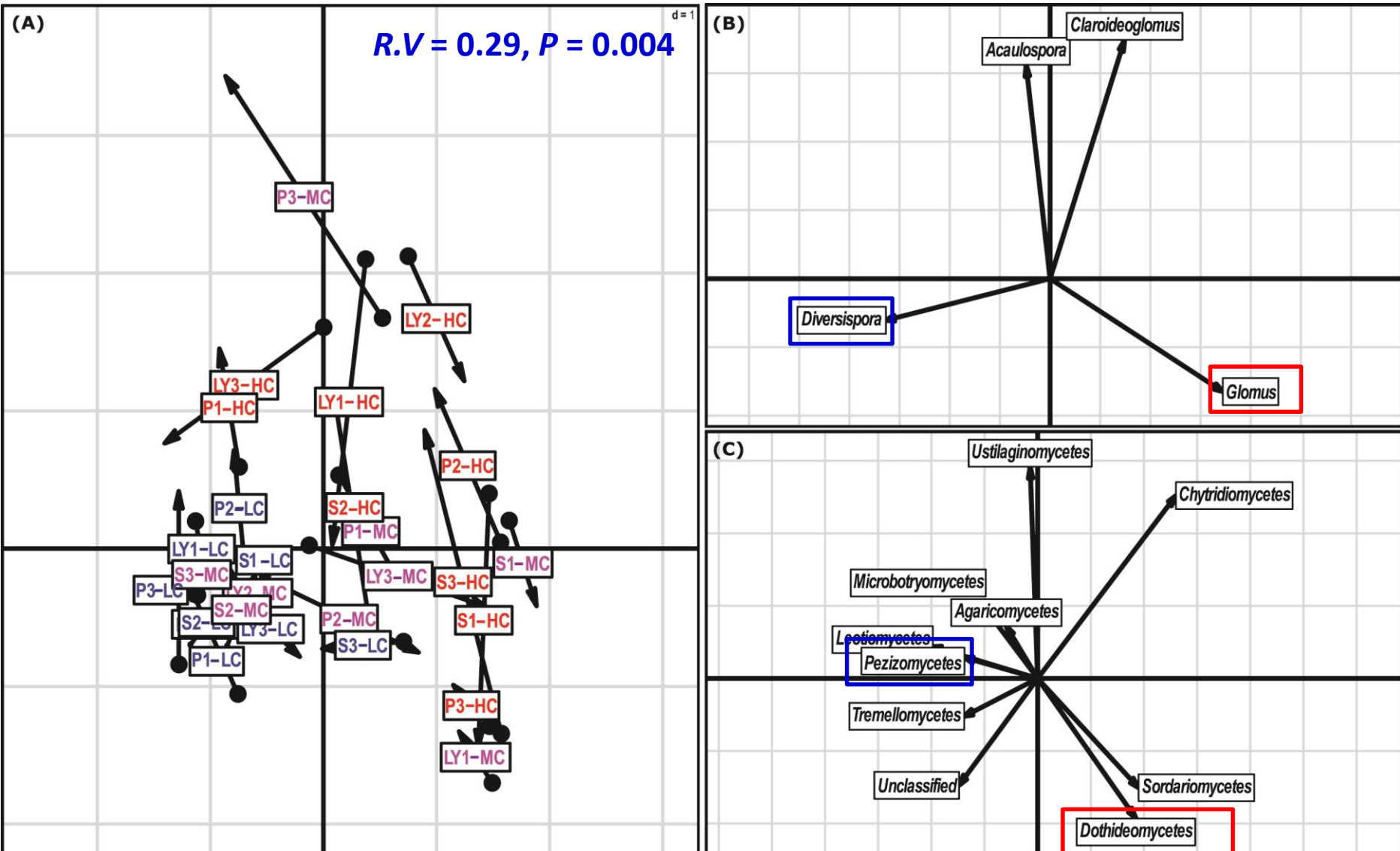
# Les relations entre CMA et les autres microorganismes

## Les analyses de co-inertie



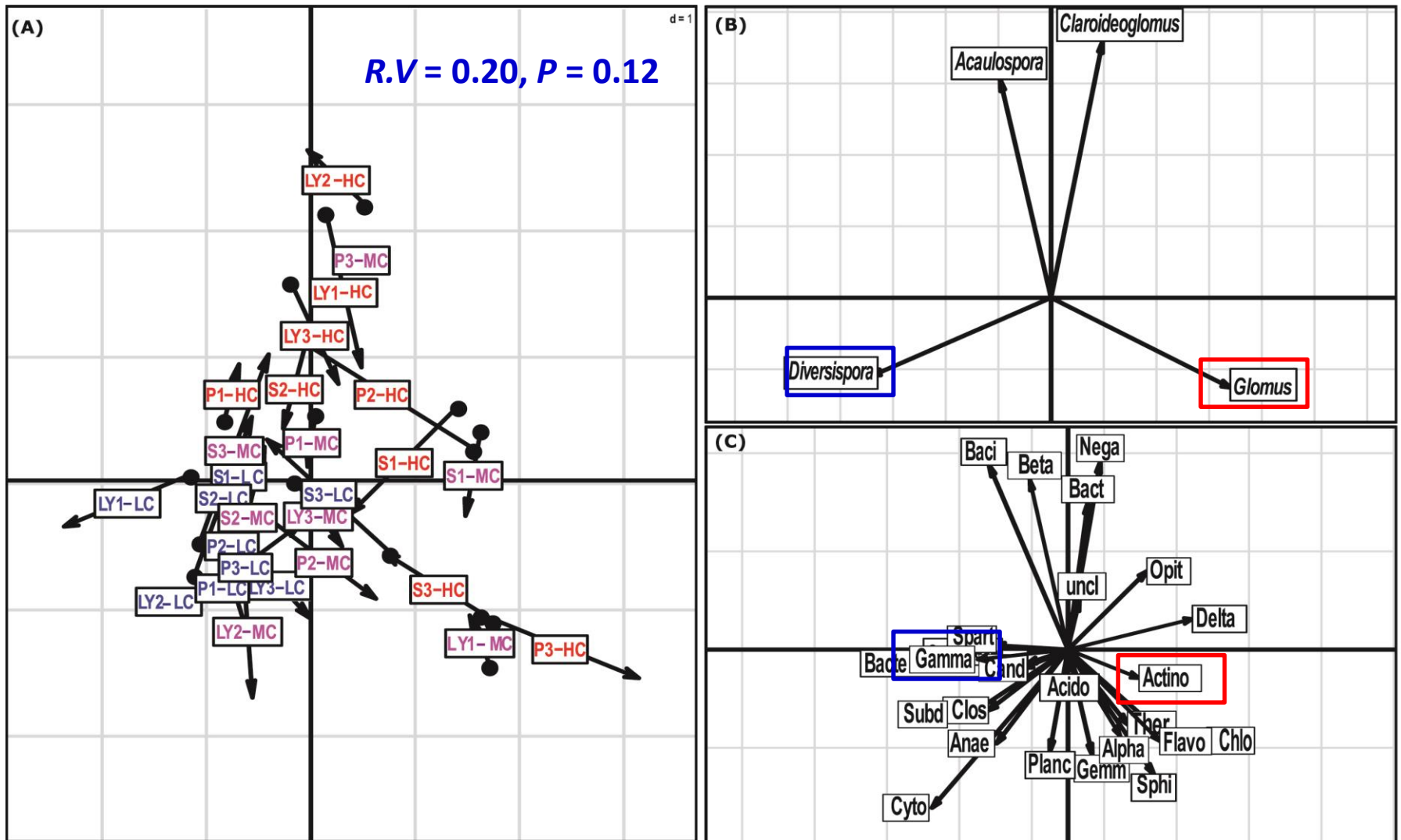


# Les relations entre CMA et champignons

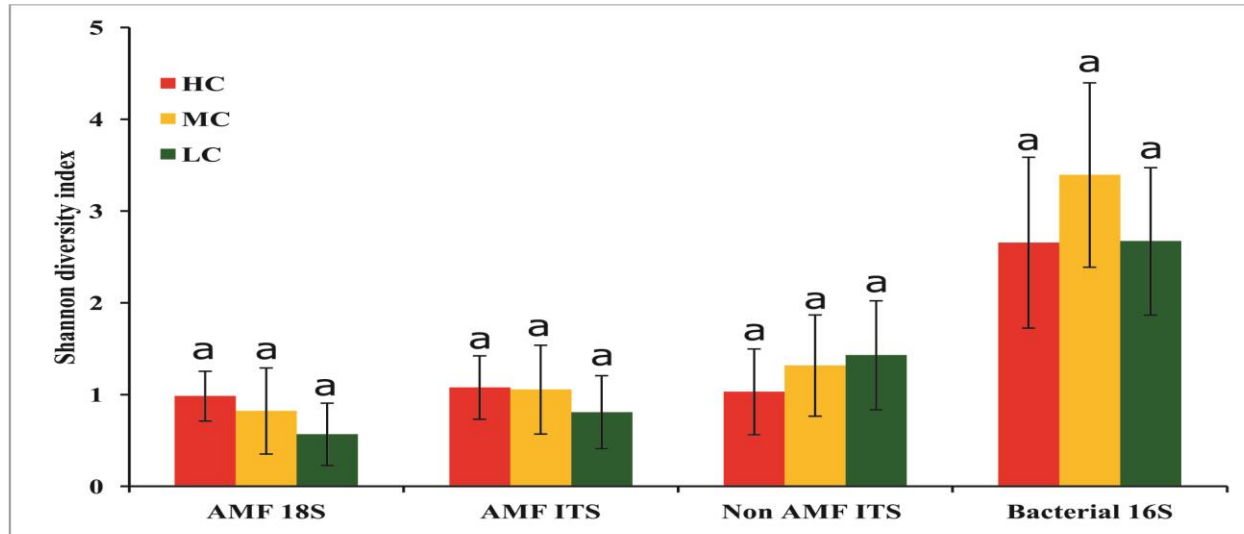




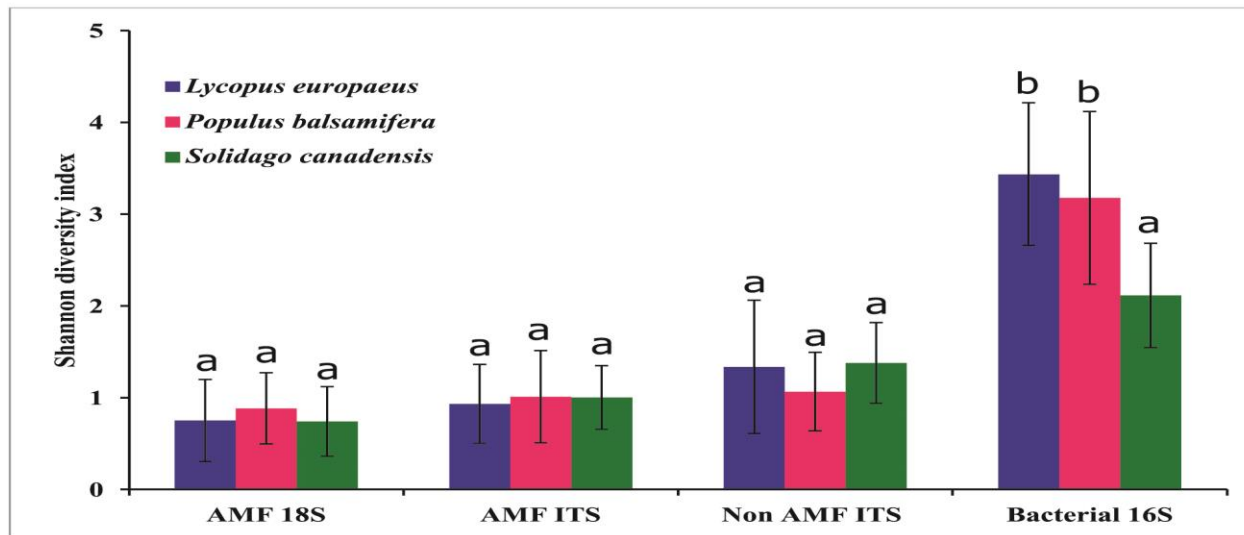
# Les relations entre CMA et bactéries

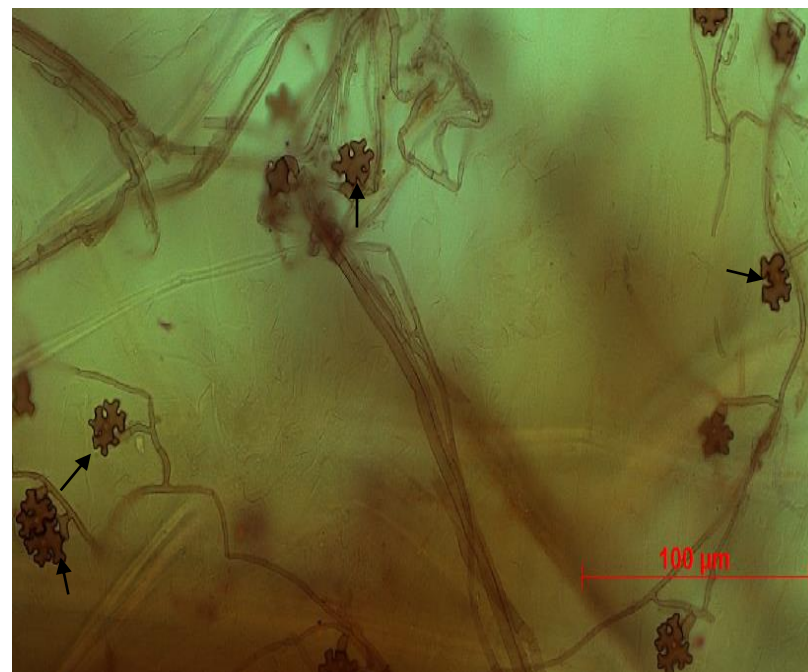


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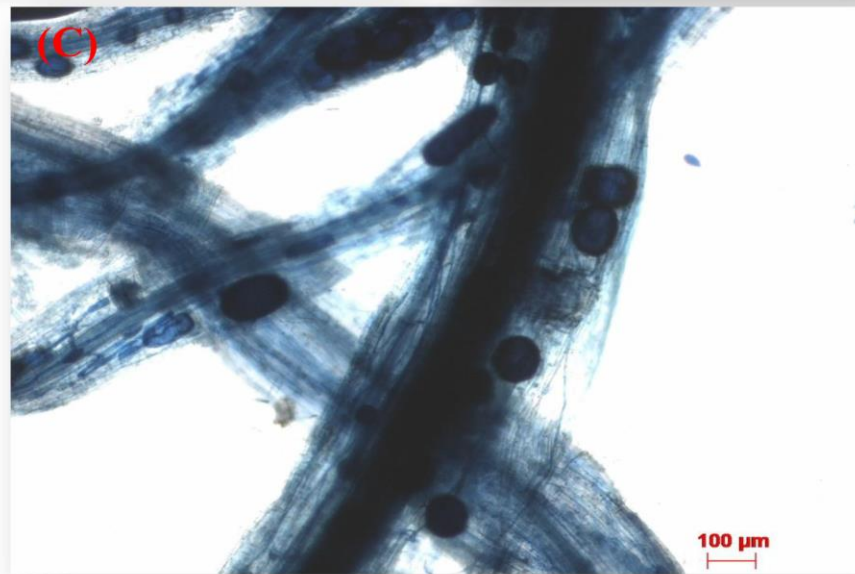
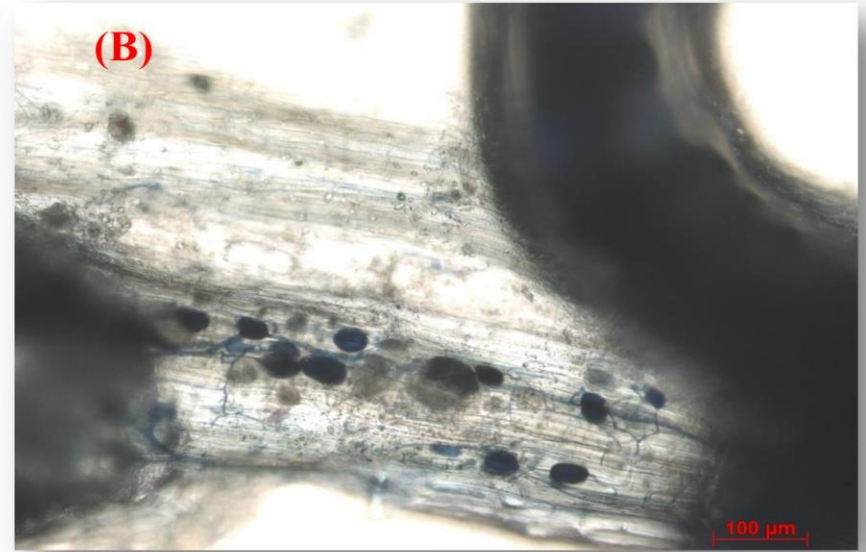
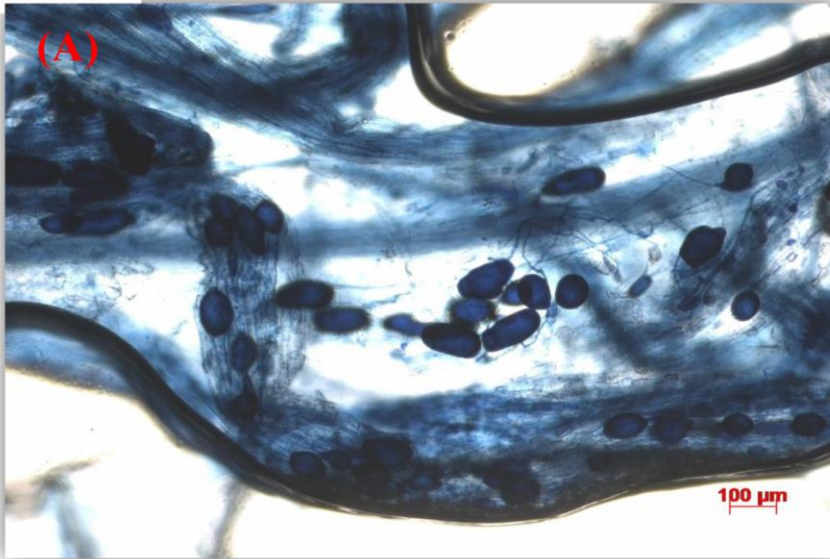


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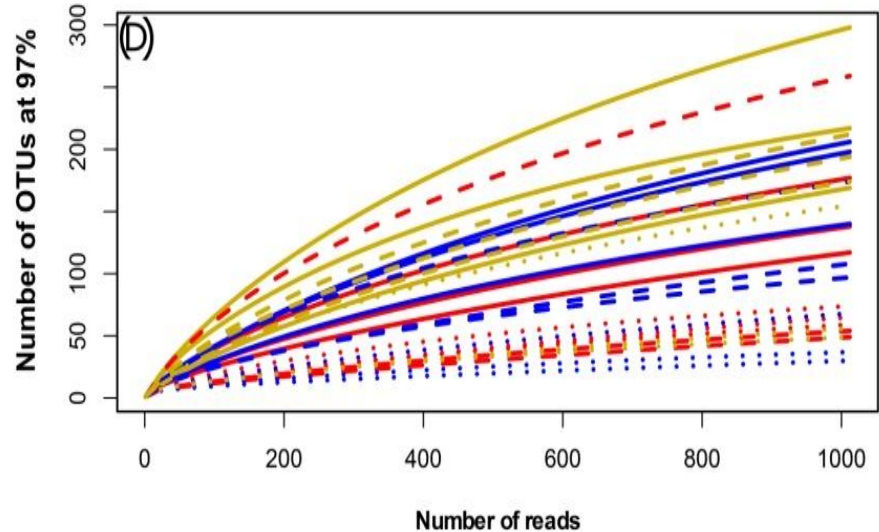
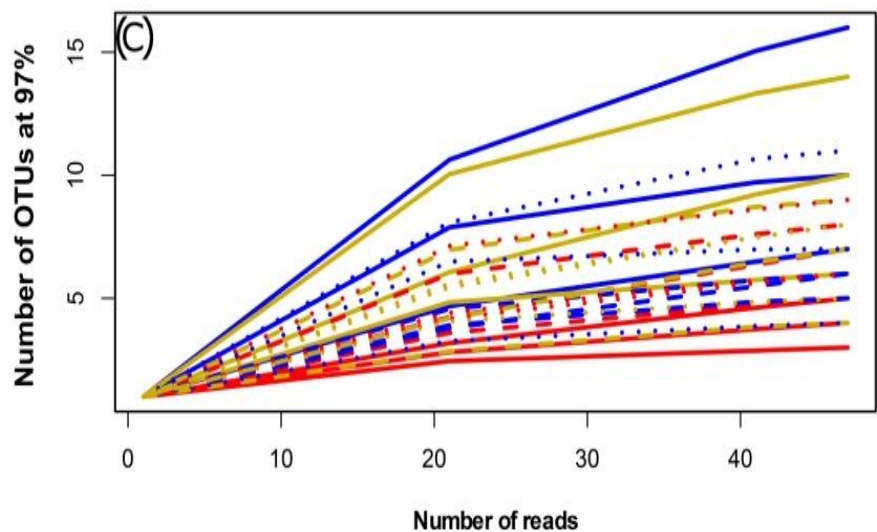
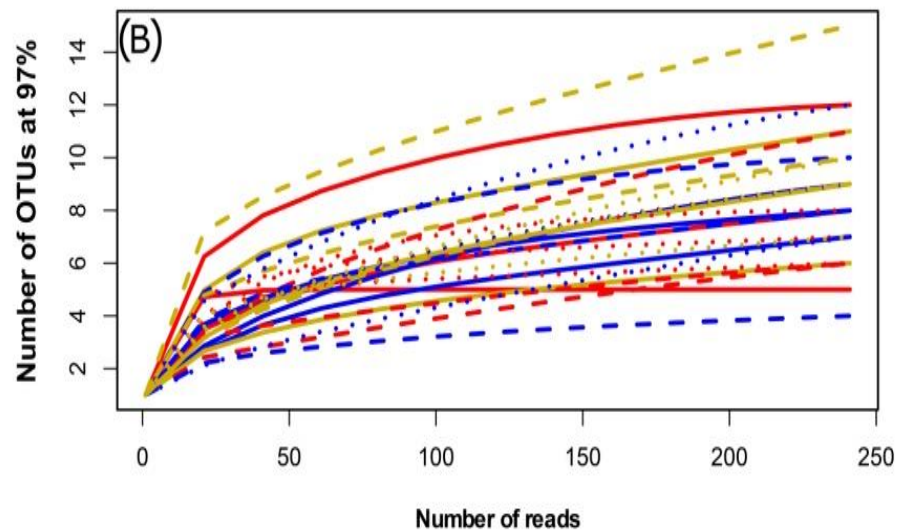
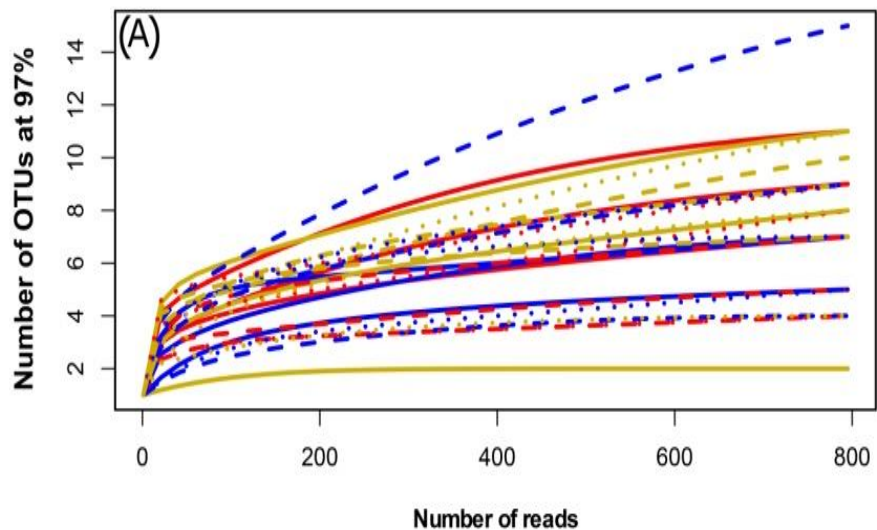




Images de microscope photonique des formes fongiques observées à l'intérieur des racines de *Eleocharis sp* collectée sur le site contaminé de Varennes.





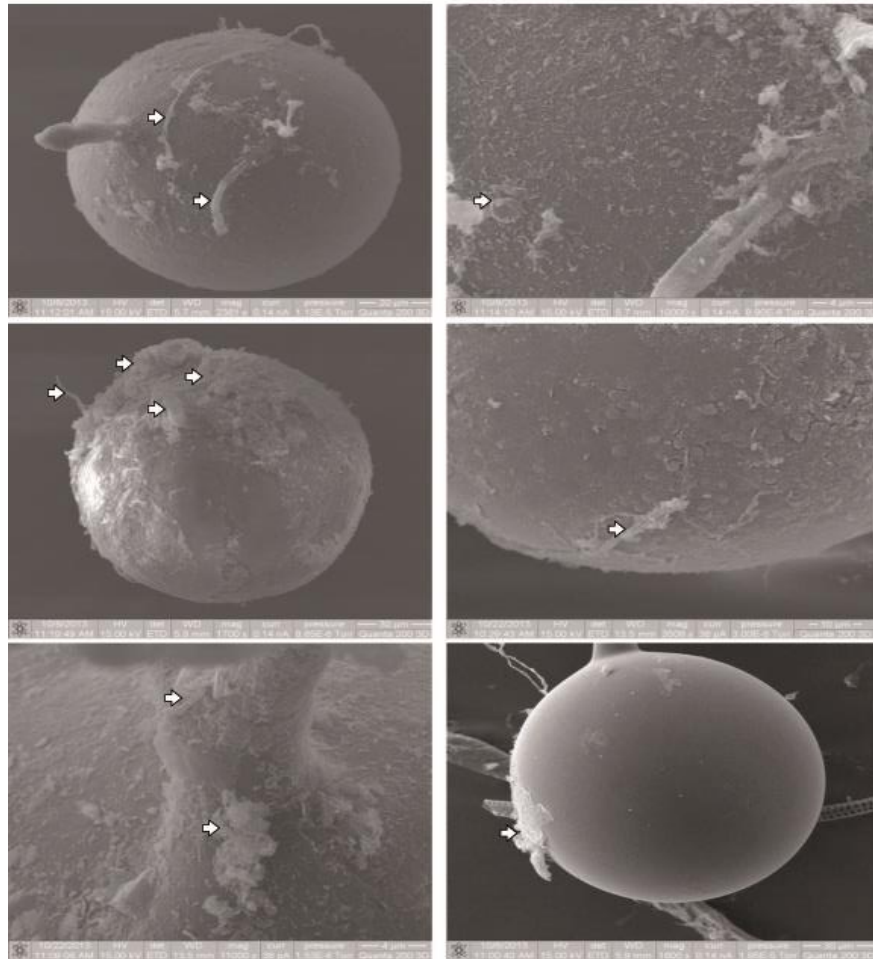


— Lycopus-HC  
 — Lycopus-LC  
 — Lycopus-MC

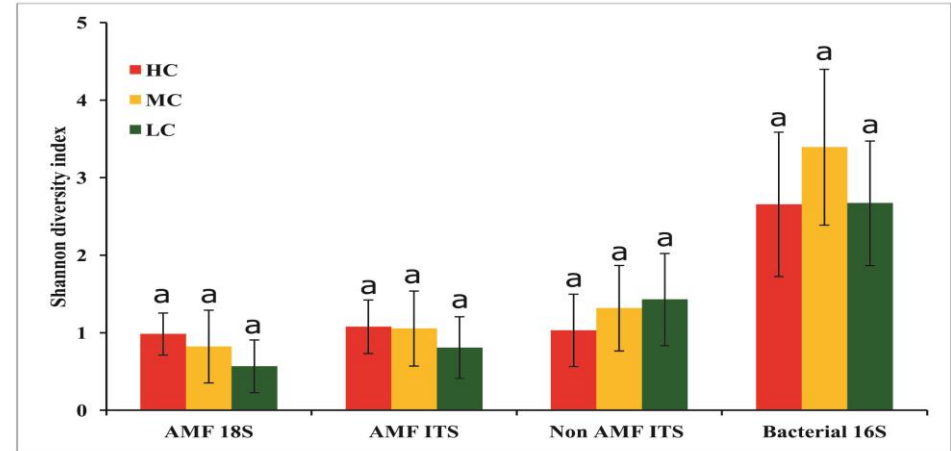
- - - Populus-HC  
 - - - Populus-LC  
 - - - Populus-MC

... Solidago-HC  
 ... Solidago-LC  
 ... Solidago-MC

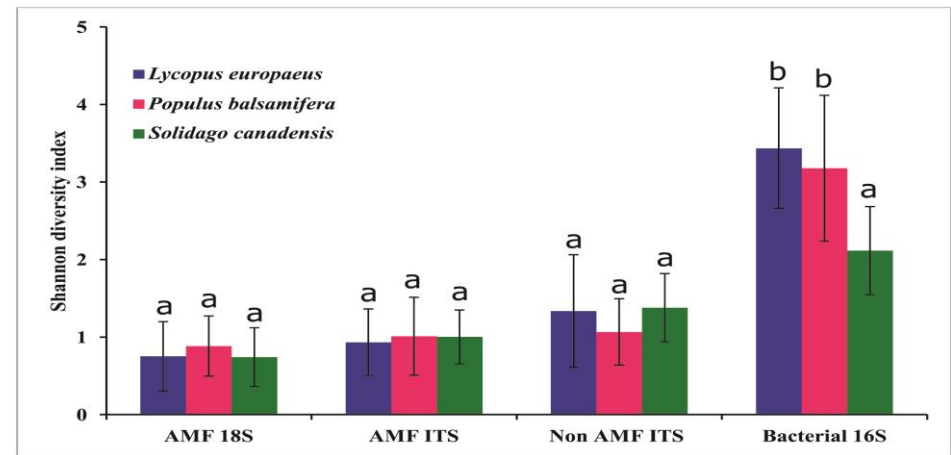
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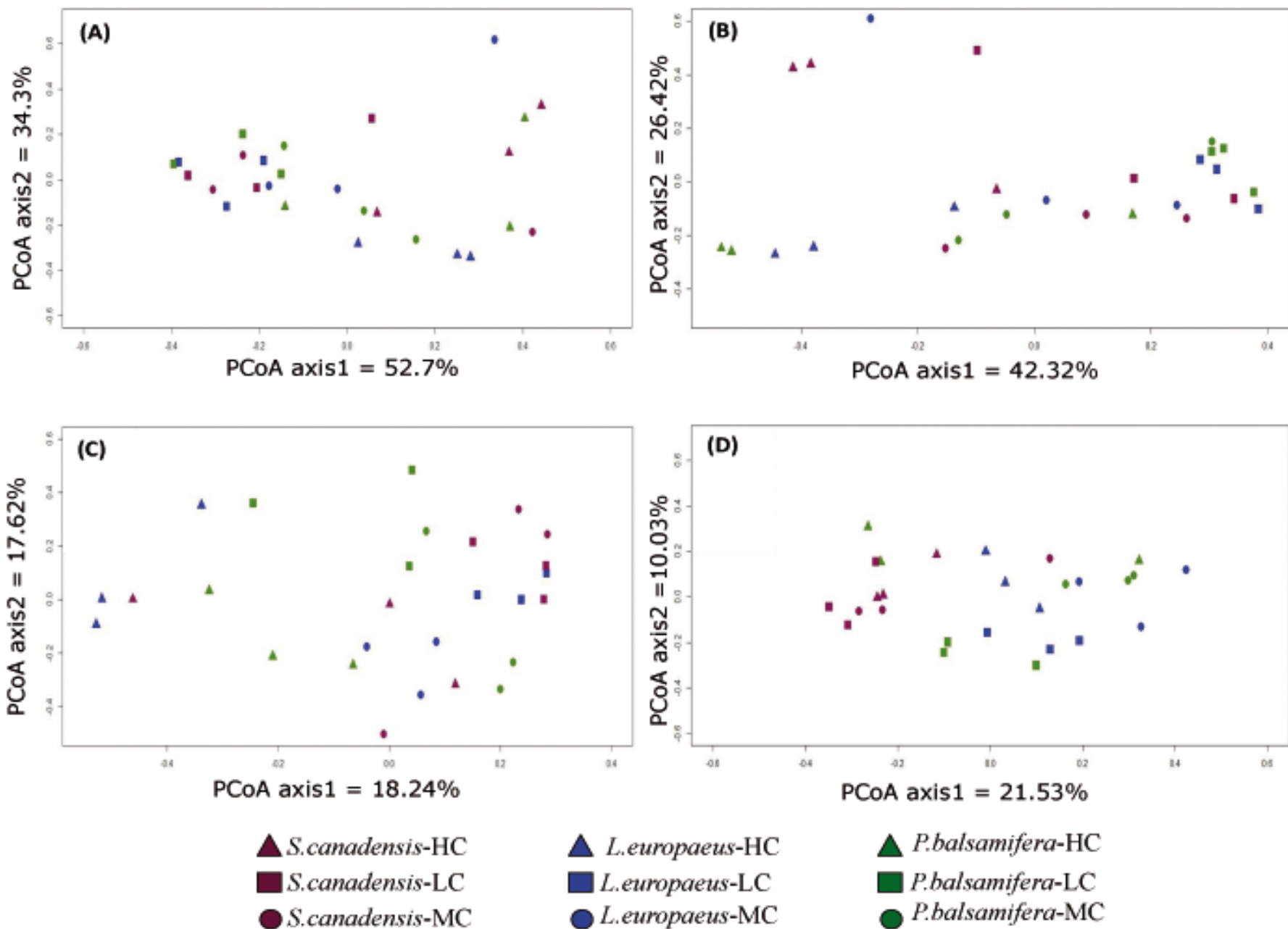
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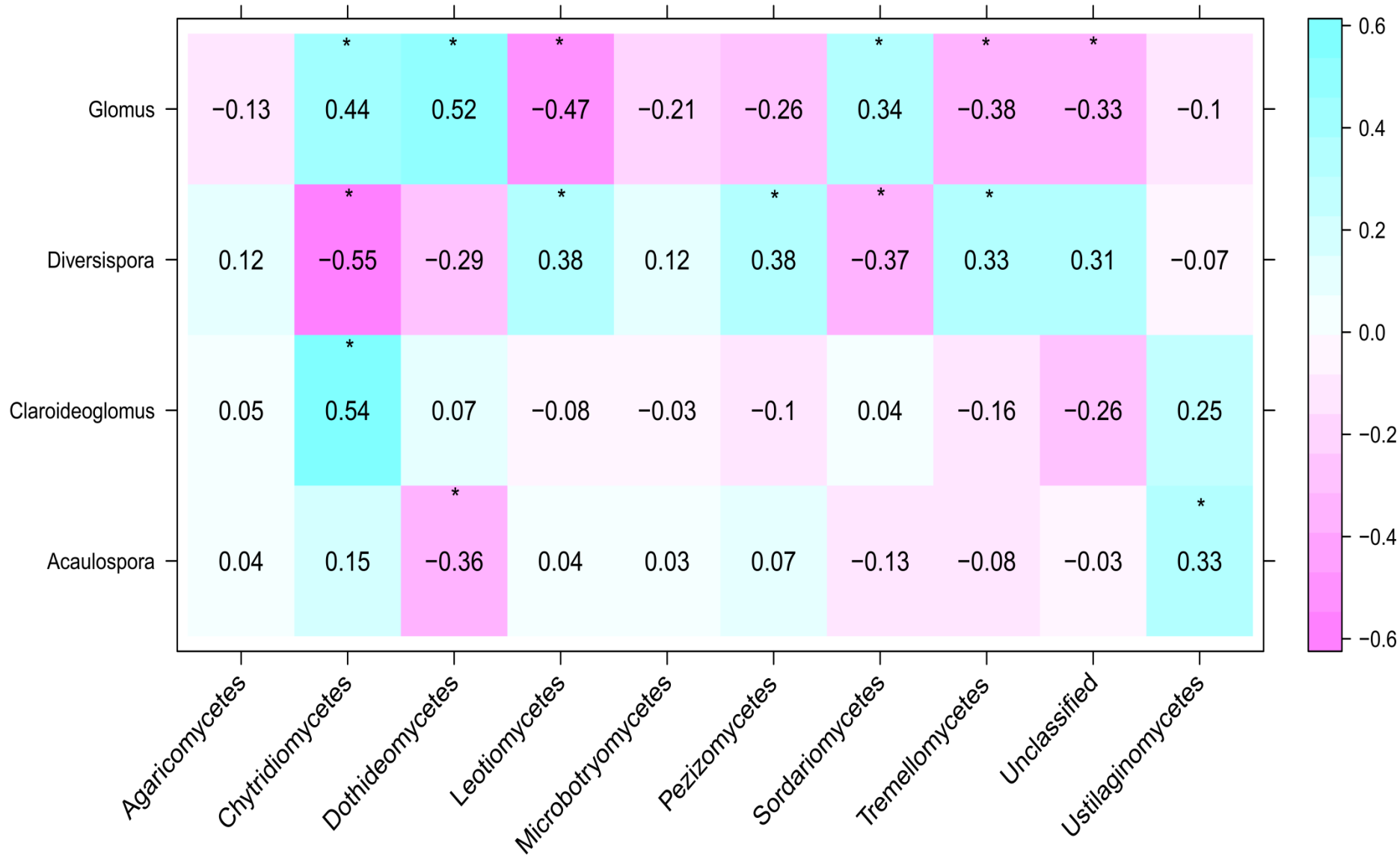


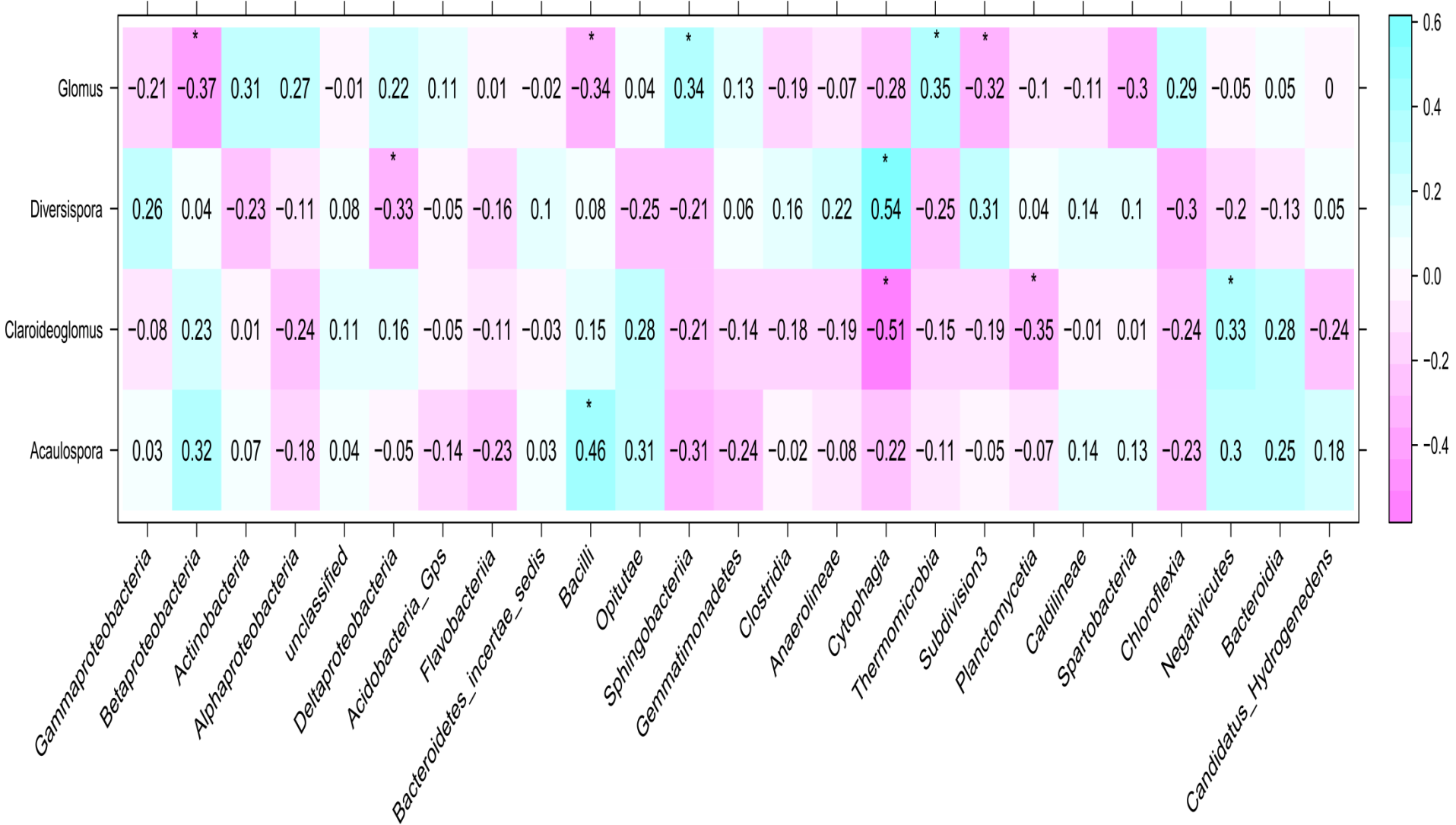
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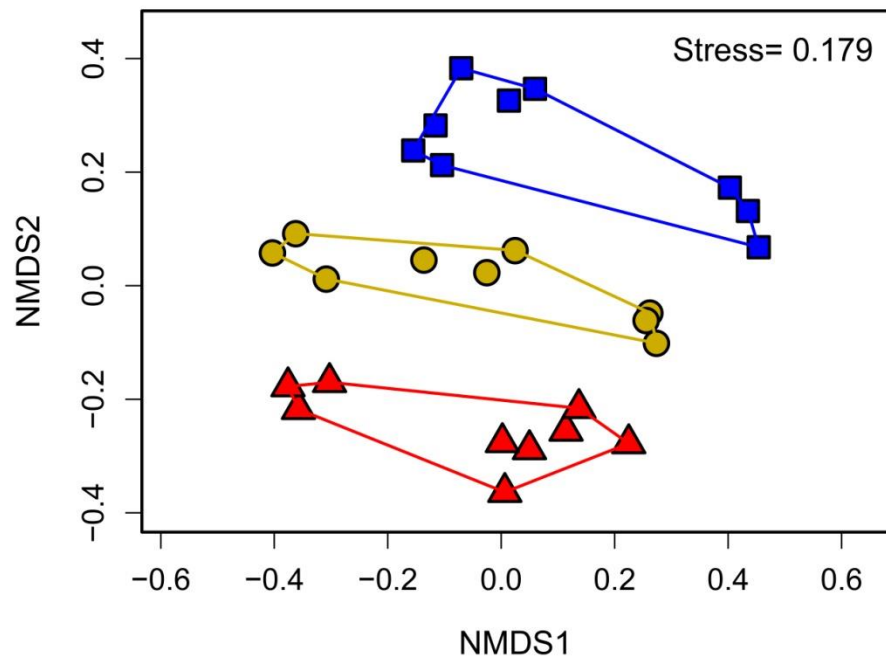
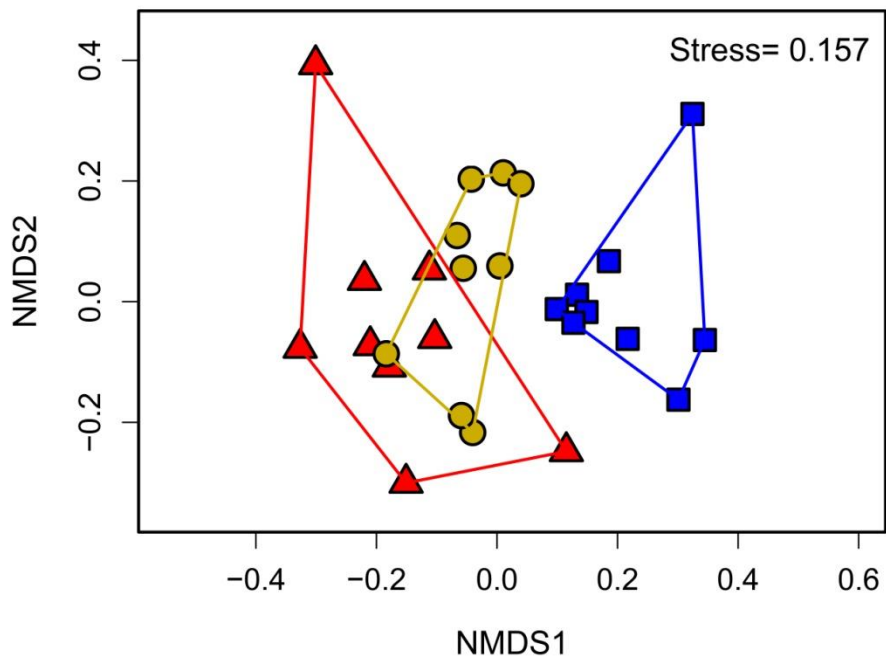
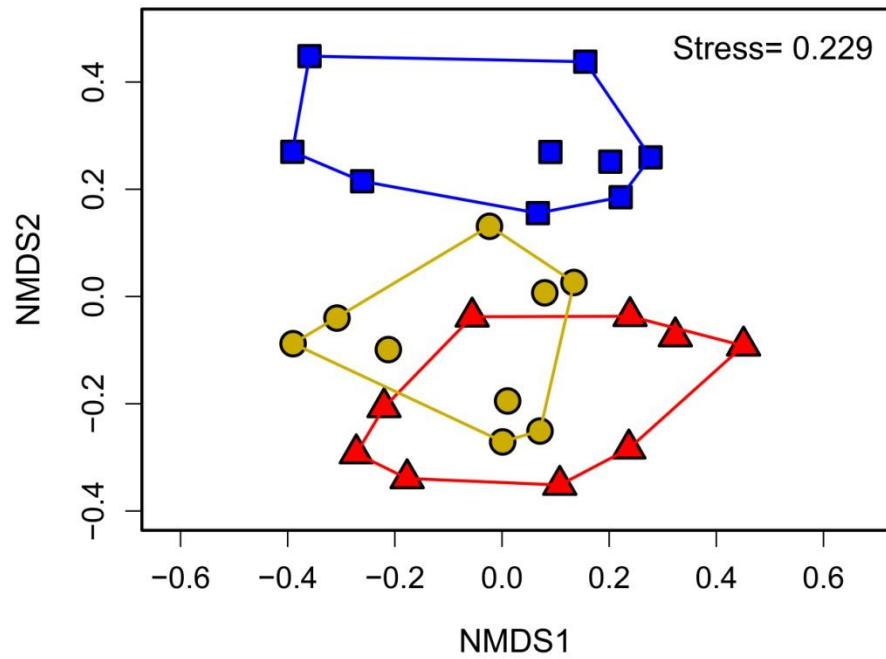
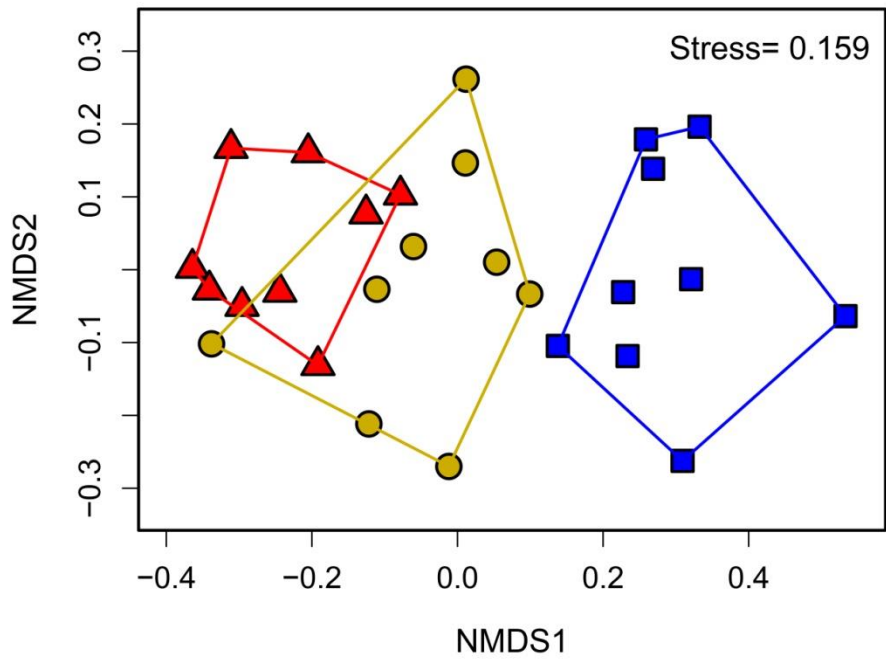


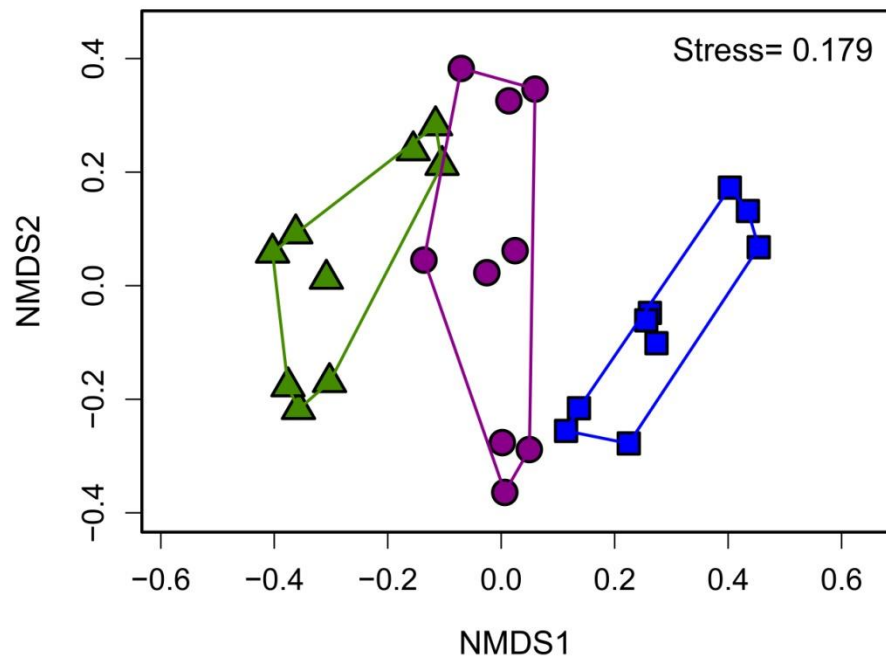
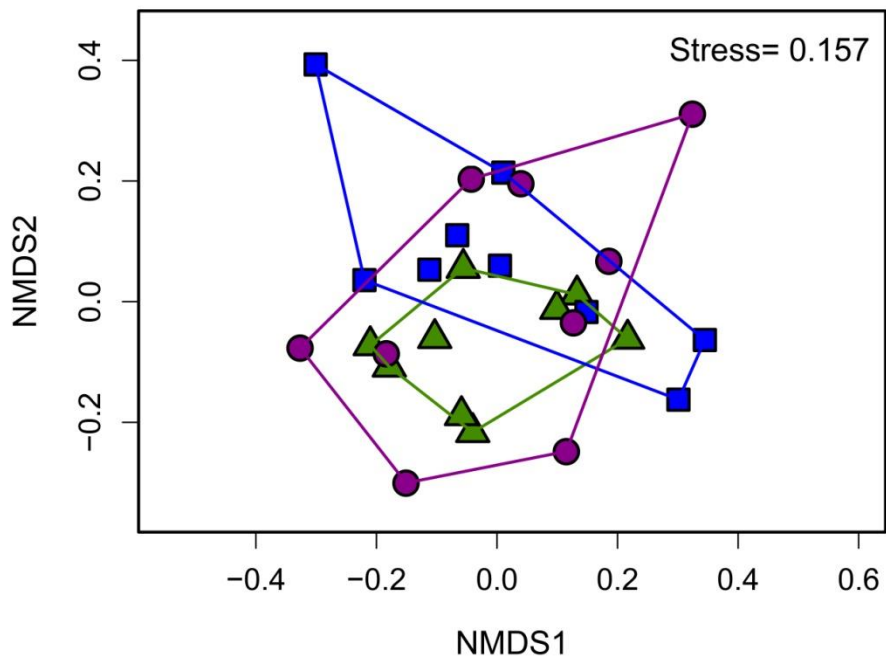
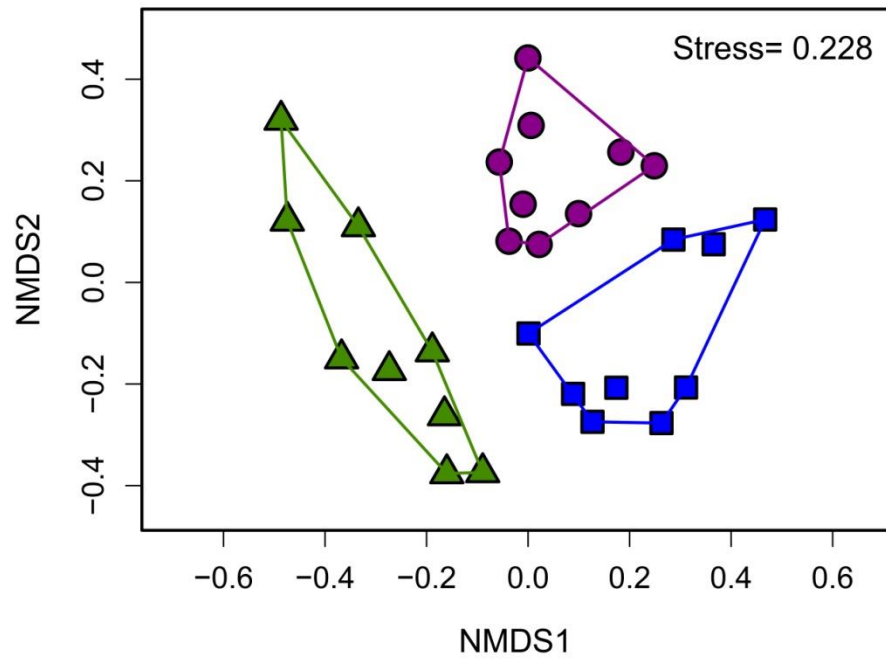
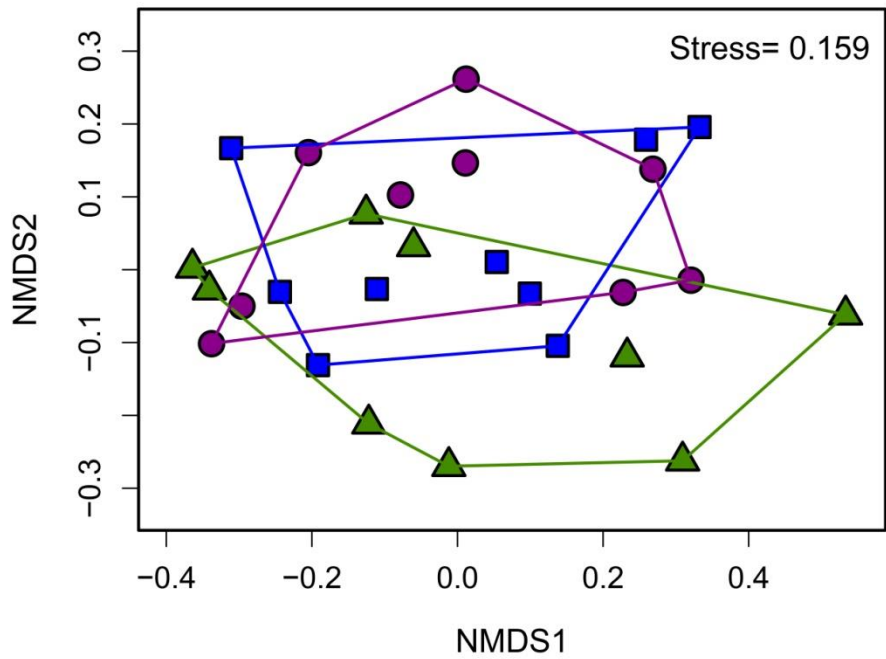


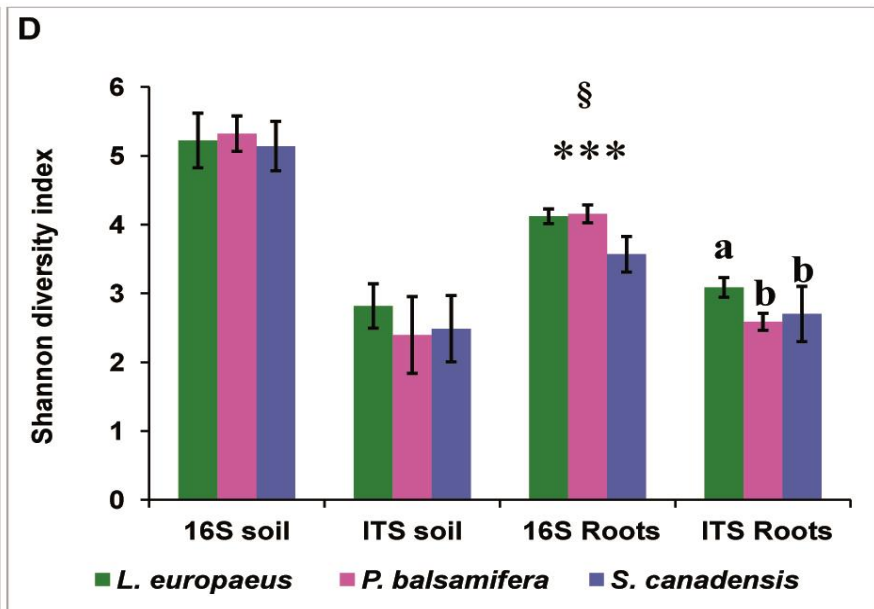
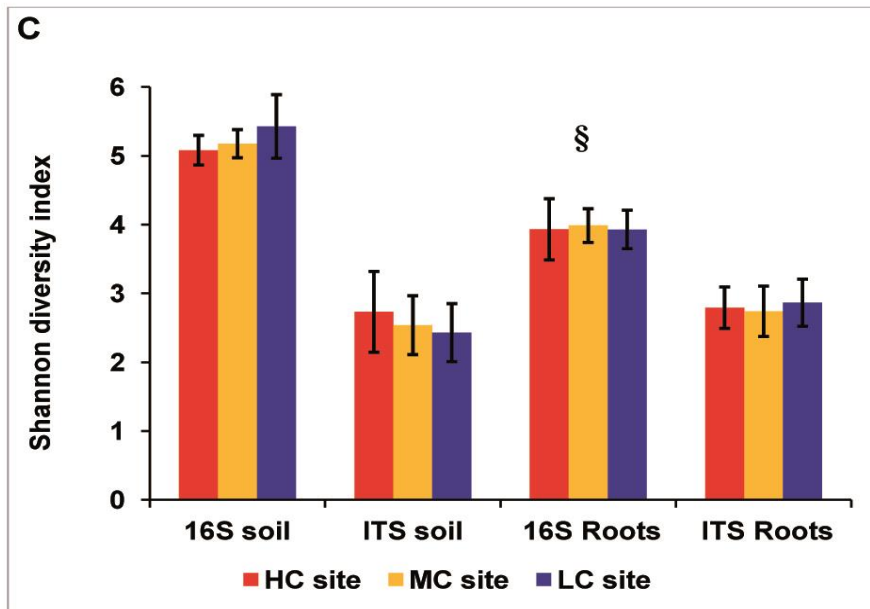
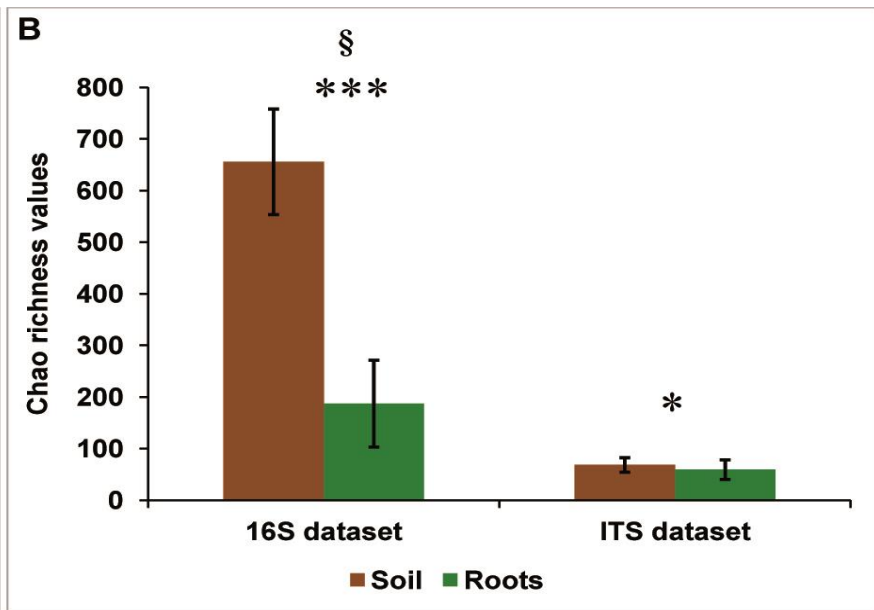
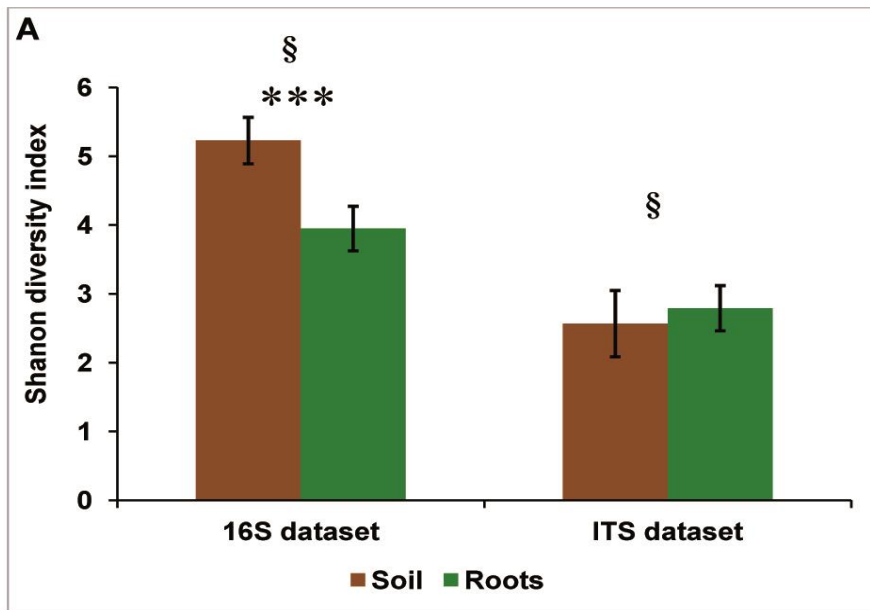




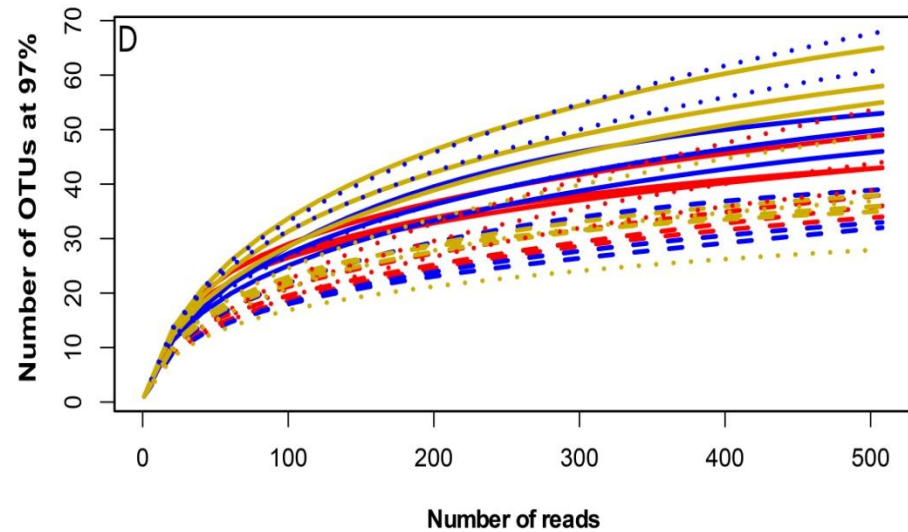
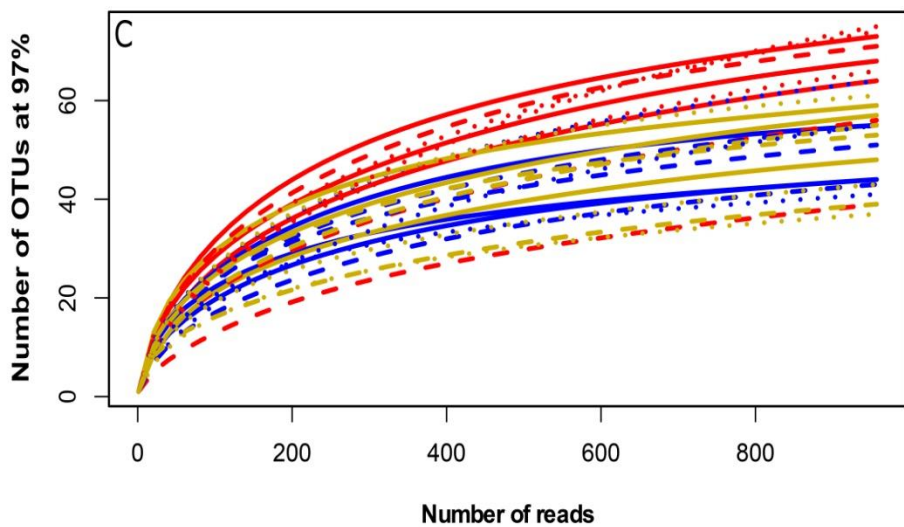
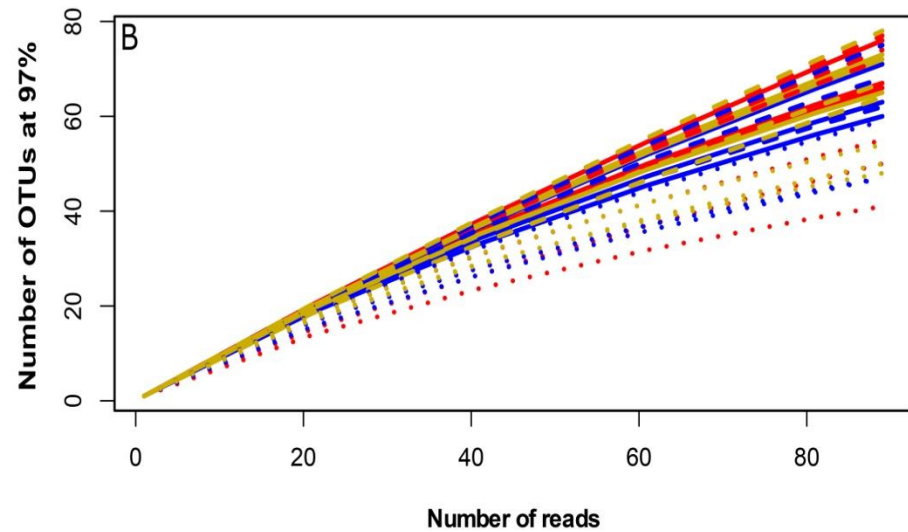
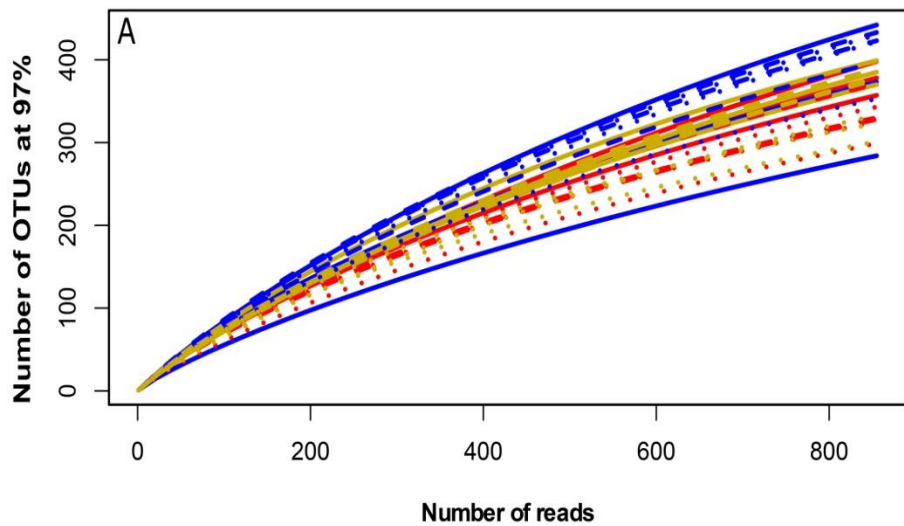




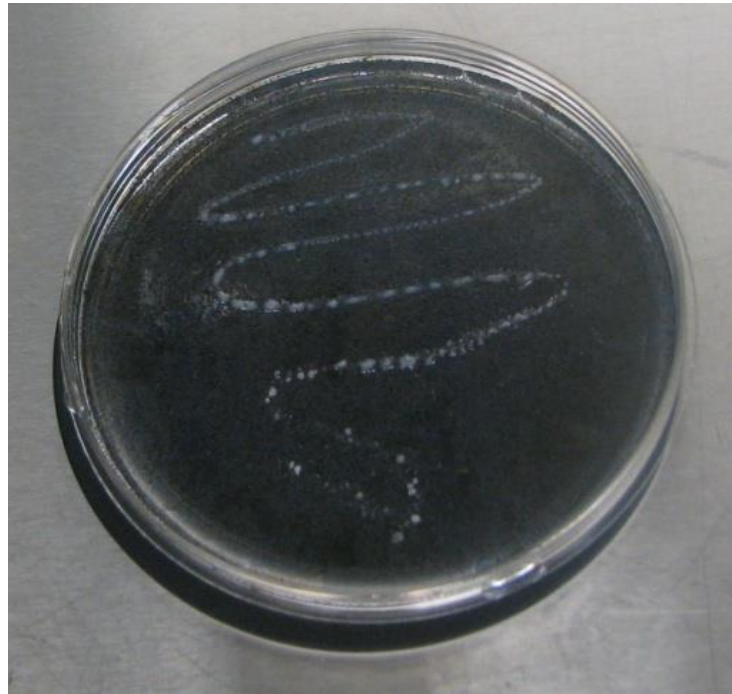








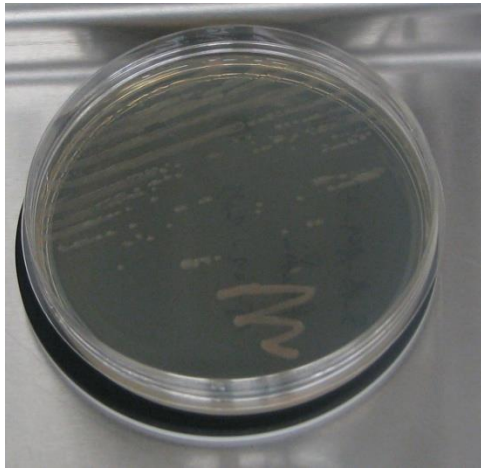
# Perspectives



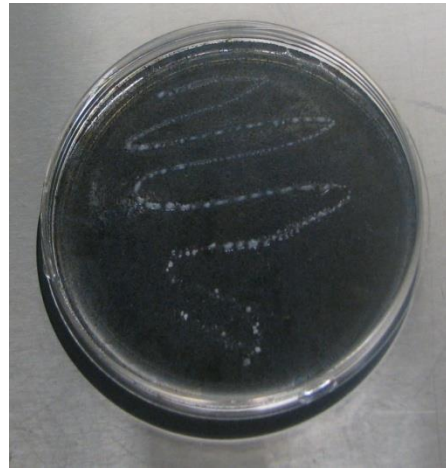
***Paenibacillus glycanilyticus***

# Isolement des bactéries associées aux CMA

**82 isolats**



57 isolates from TSA



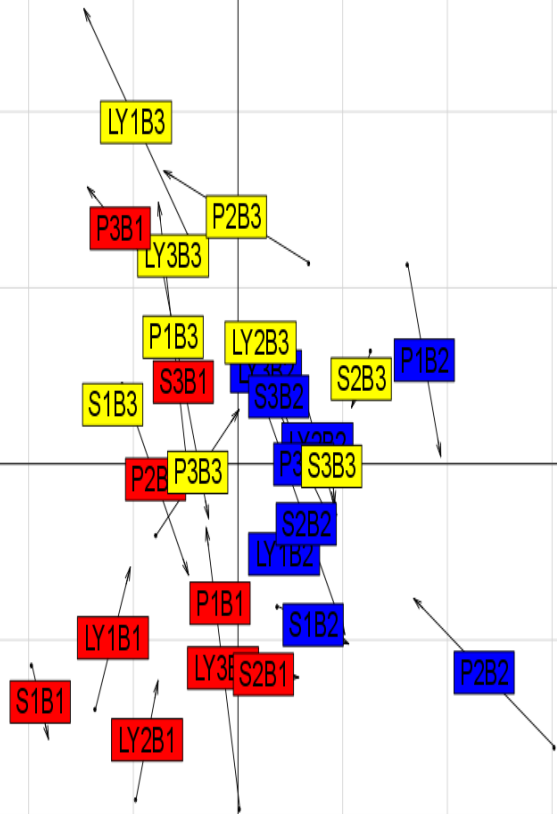
25 isolates from COM

PCR avec les amorces 16S bactériennes

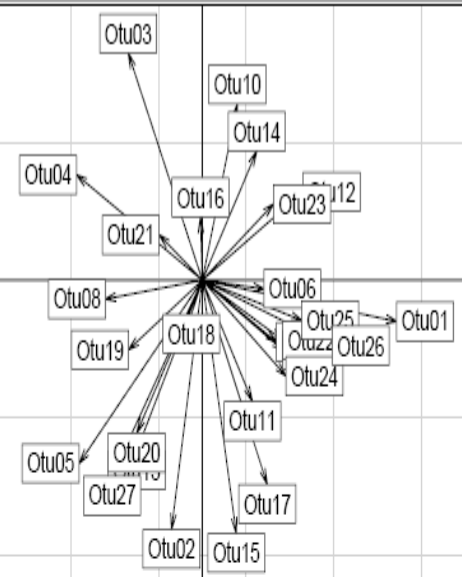


*Paenibacillus glycanilyticus*

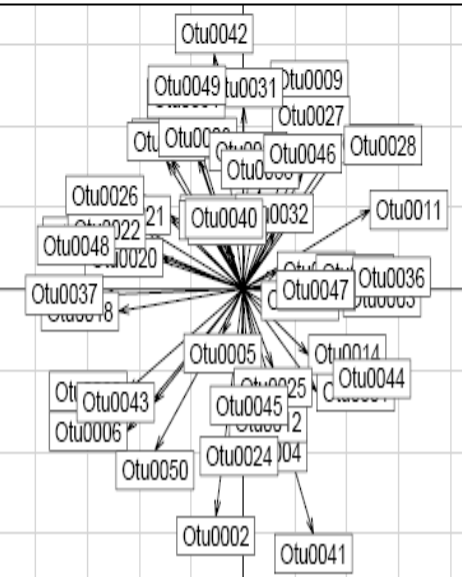
R.V = 0.42, P = 0.09



d = 0.2

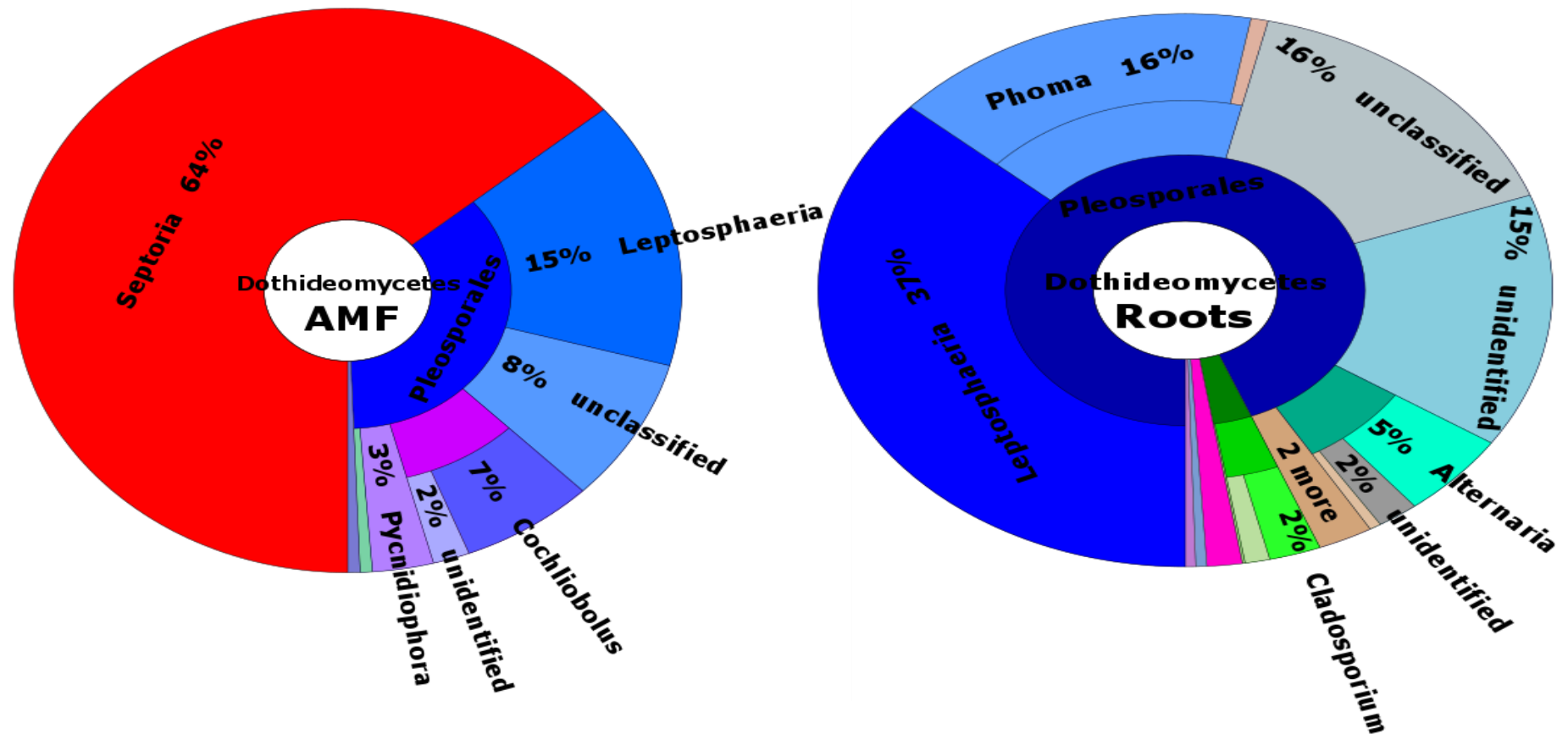


d = 0.1

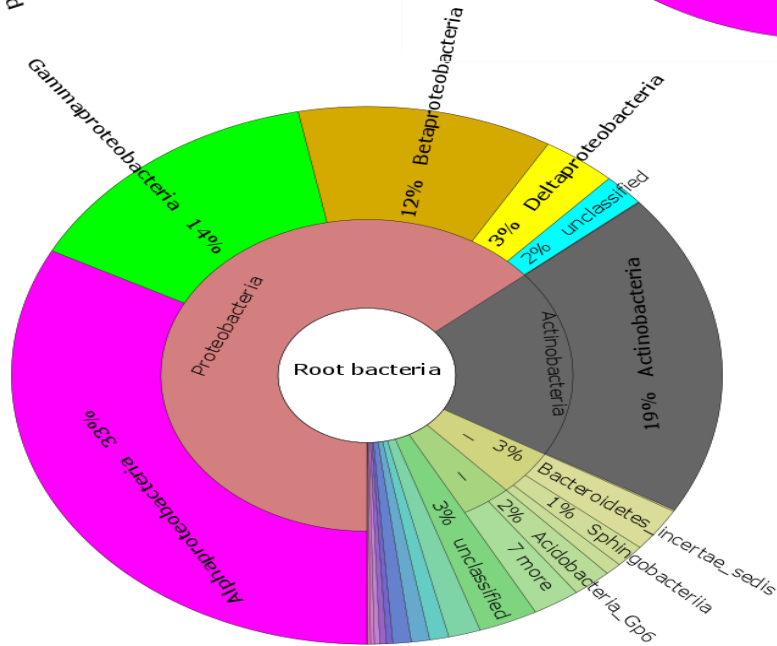
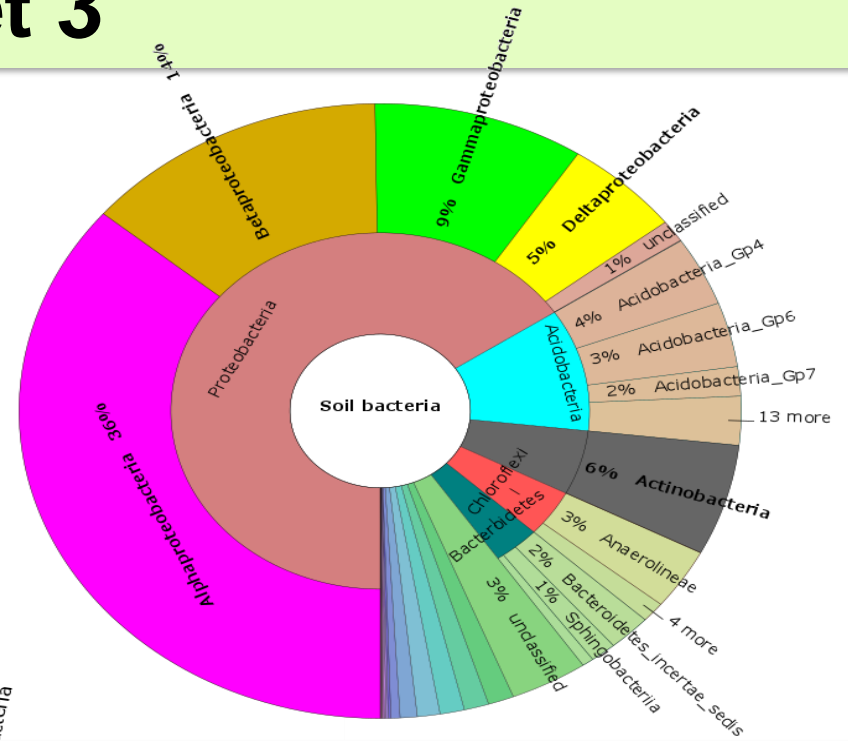
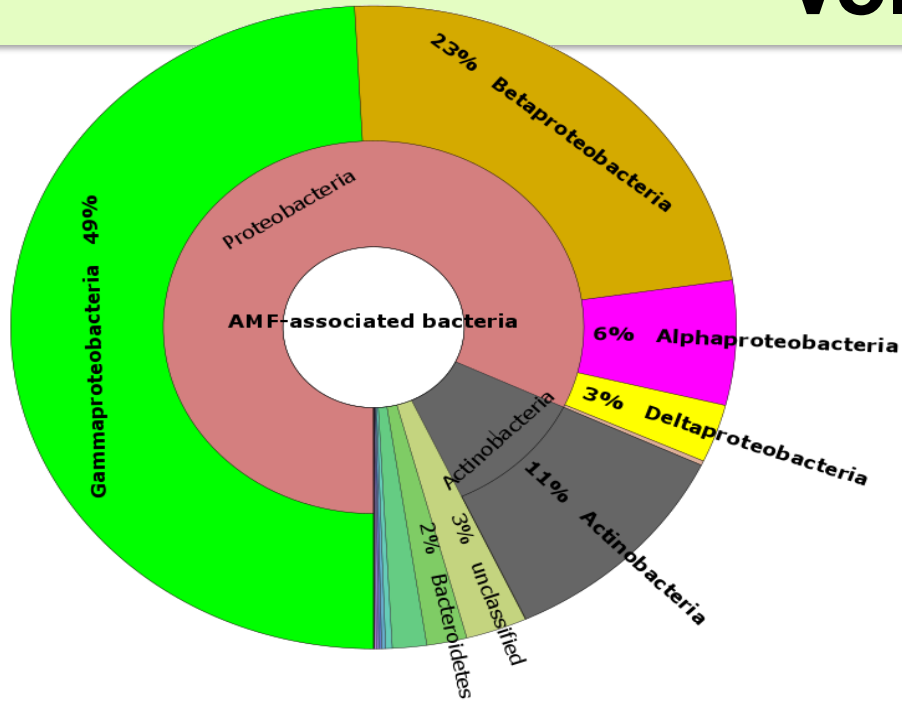


# Résultats

- Le profil des champignons associés aux CMA est **différent** de celui des racines et des sols.

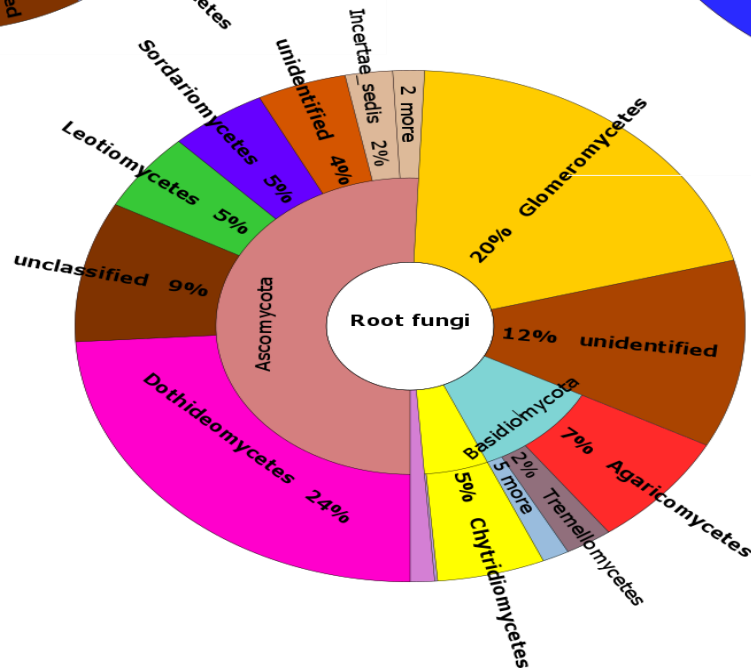
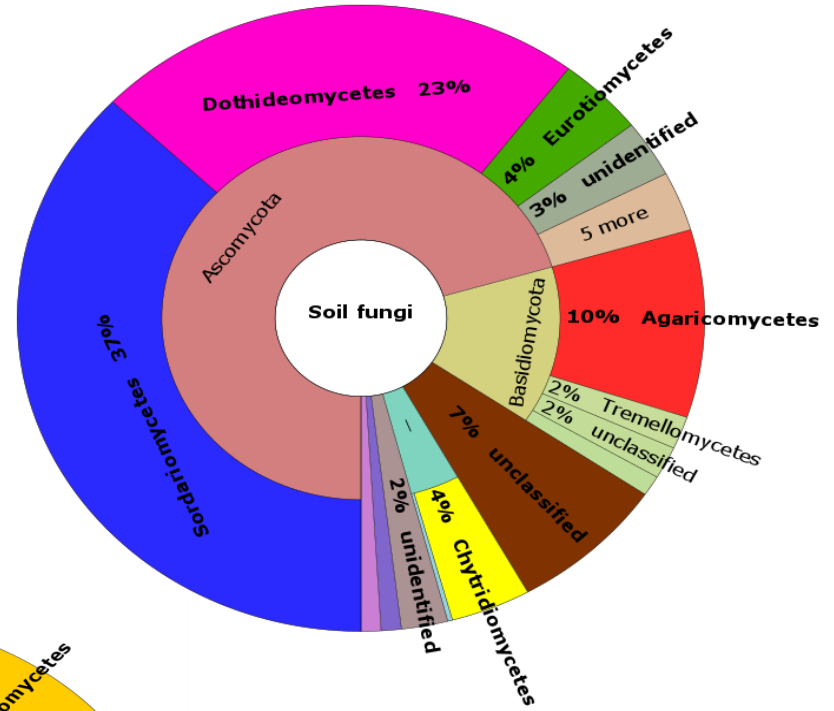
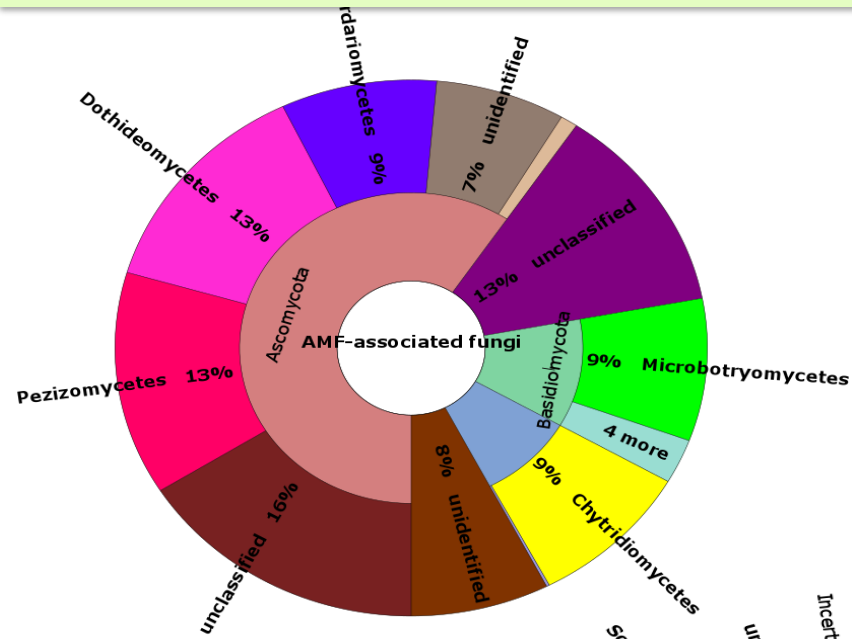


# Volet 3

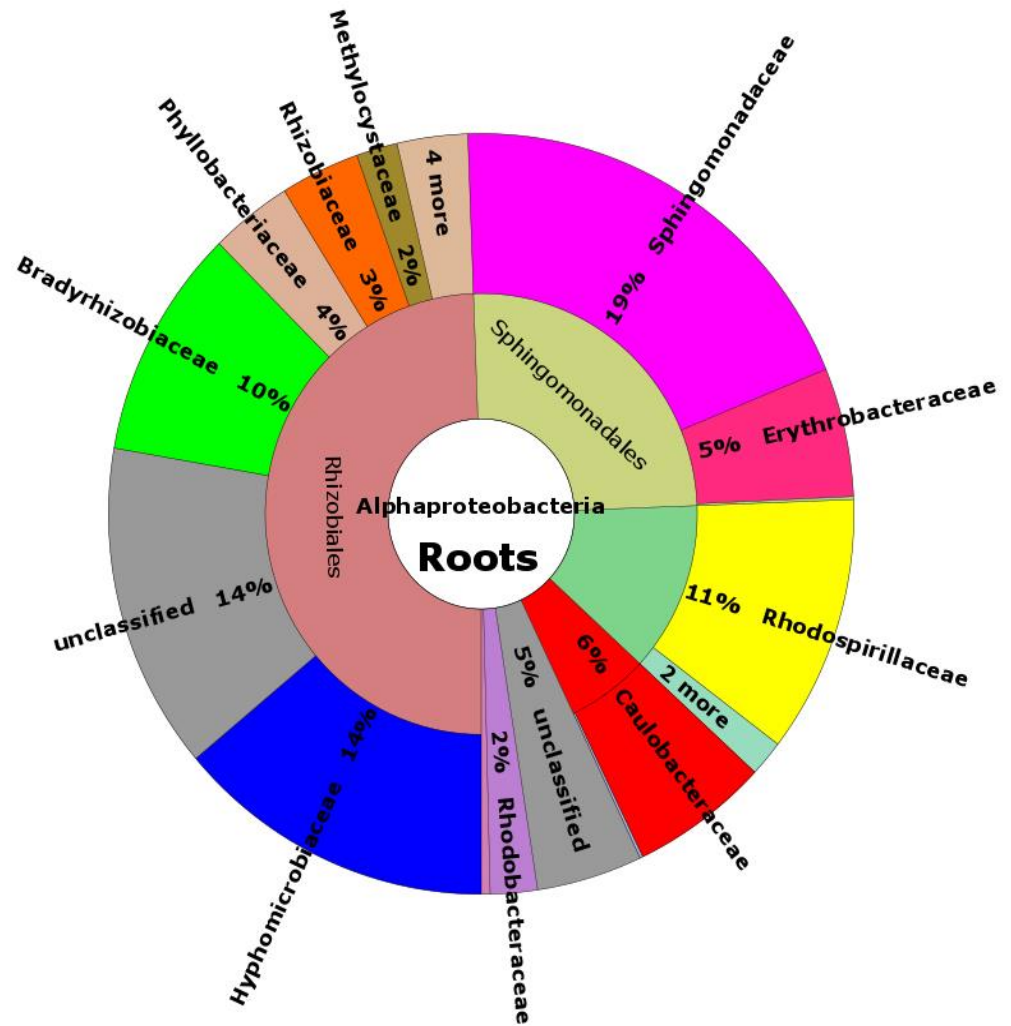
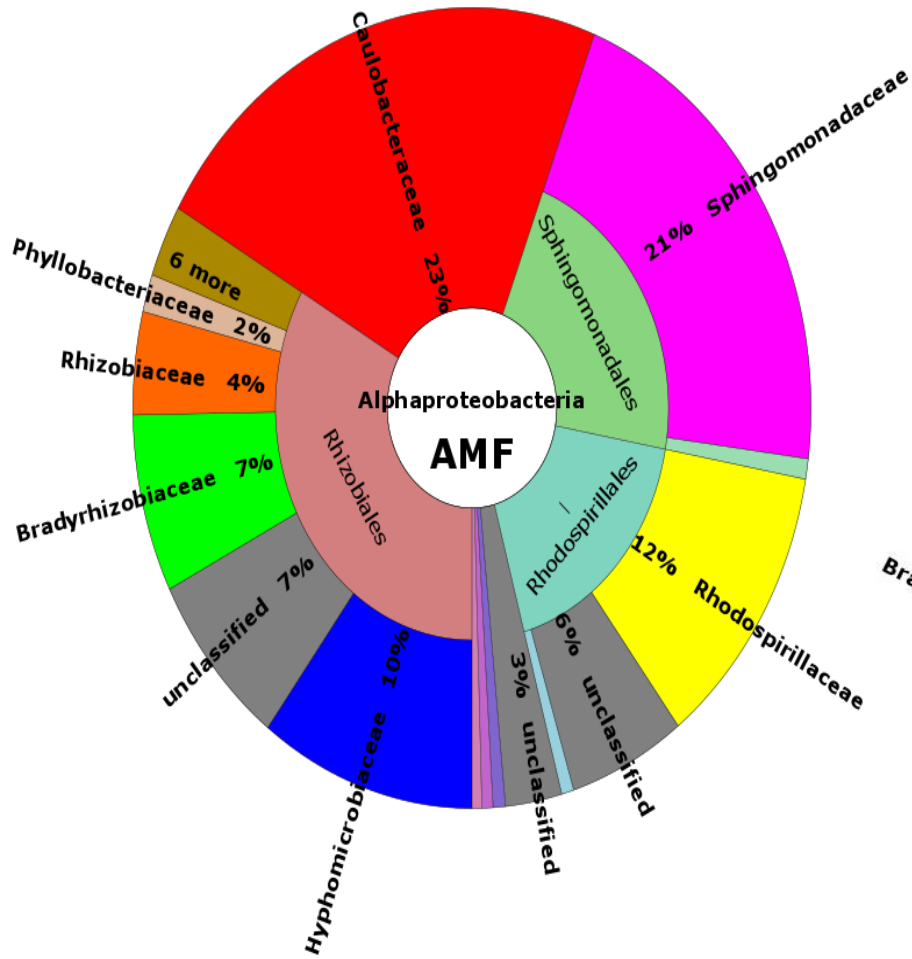


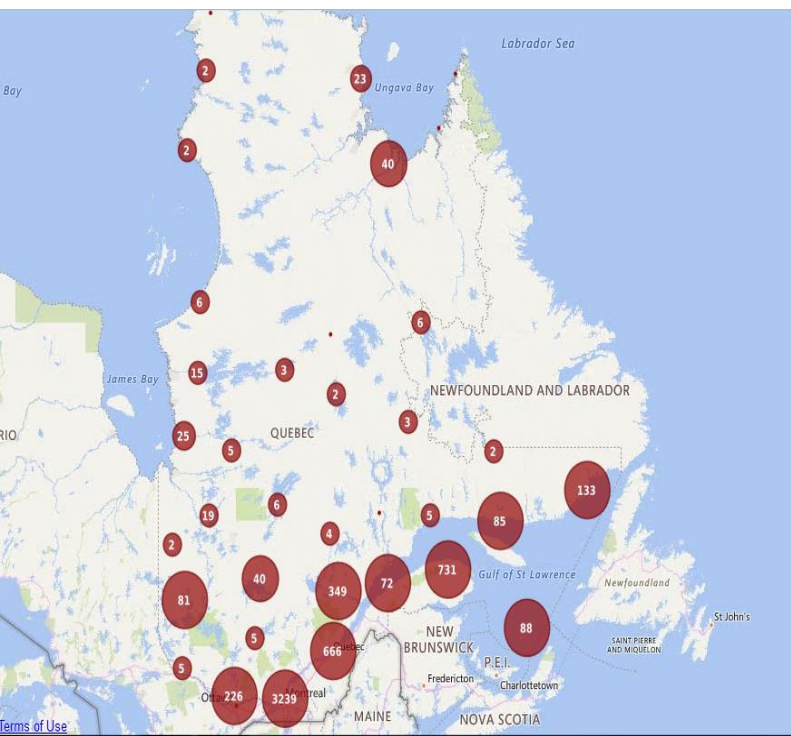


# Volet 3



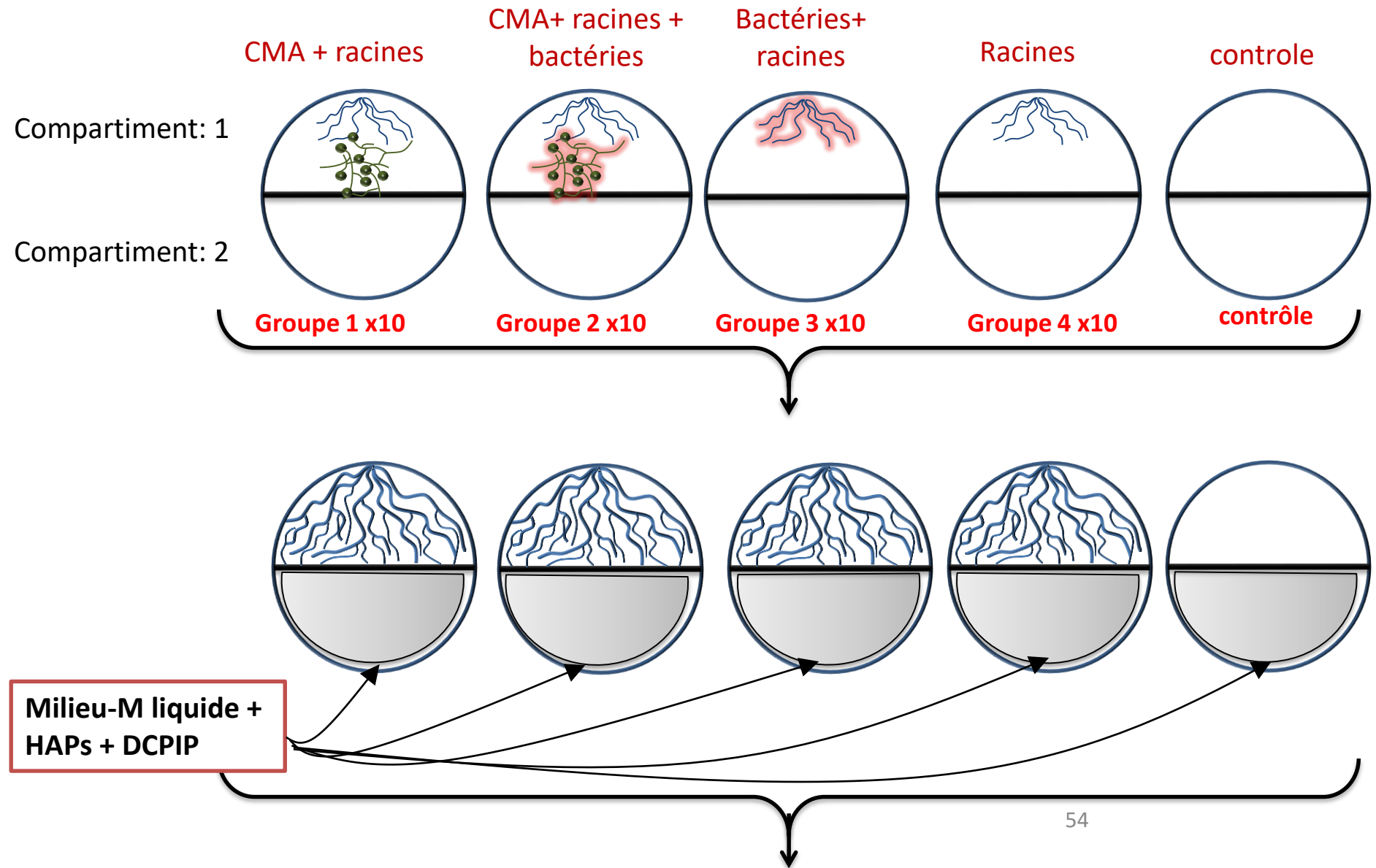
# Chapitre 4



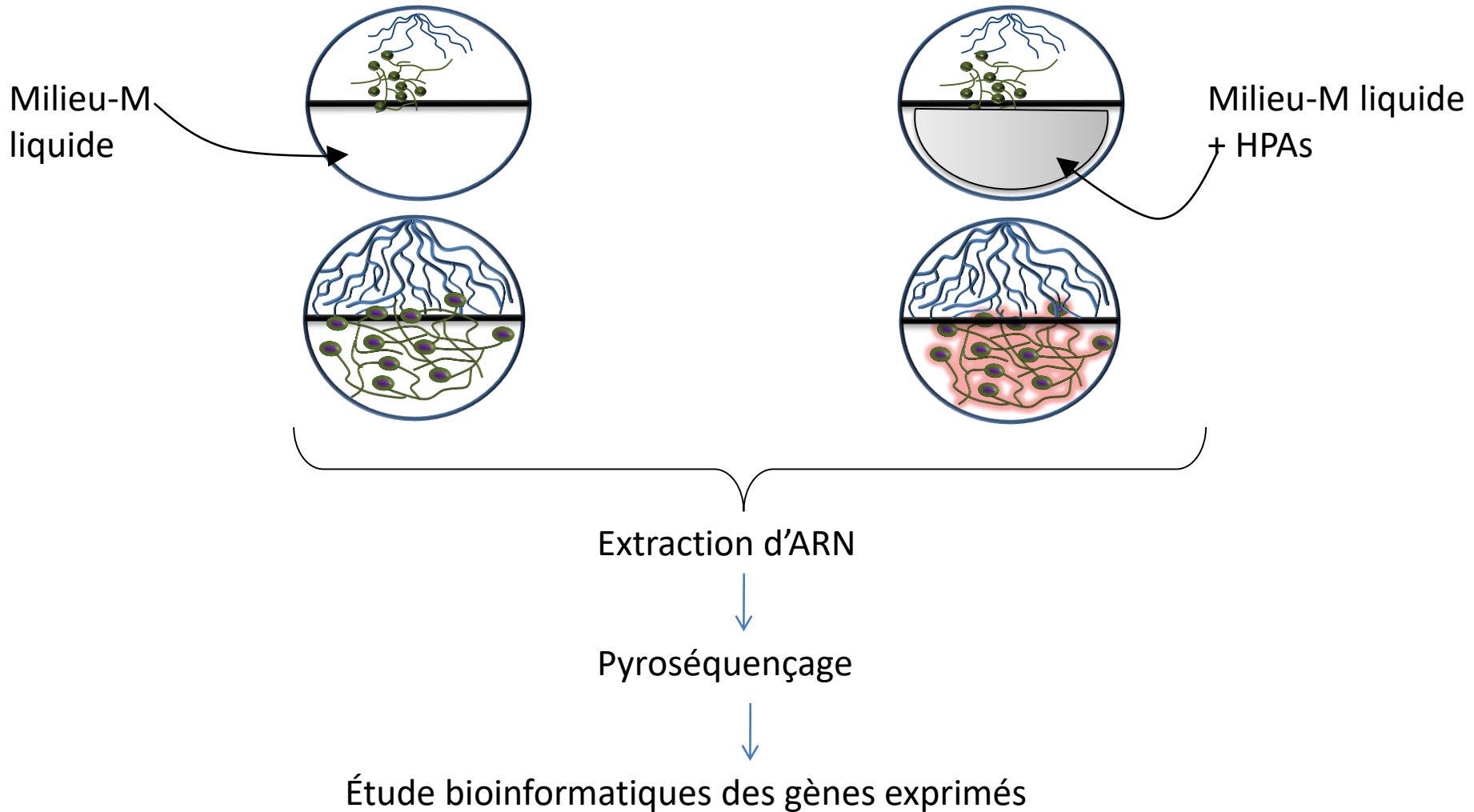


(1 M\$/hec);

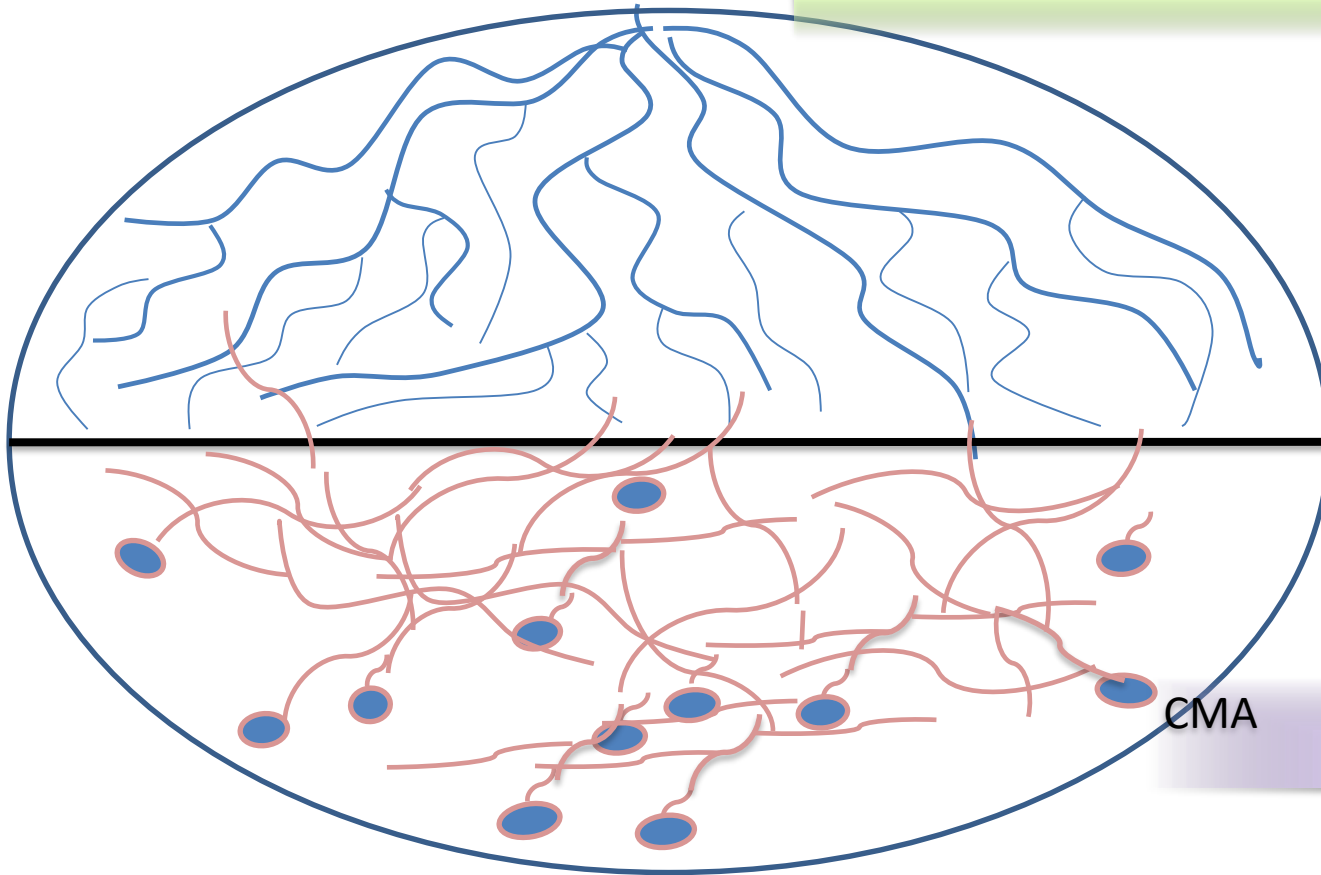
## A- Capacité de bioremédiation dans des conditions *in vitro*



## Étude de l'expression des gènes de CMA en présence des HAPs



Racines de carotte



CMA



NMDS with 16S rRNA

