

Metagenomic insights into the microbial diversity of bauxite residues in the mining basin of Provence and the Marseille agglomeration

Julie Foulon, Mathieu Luglia, Héloïse Verdier, Camille Fourier, Catherine Keller, Jean-Paul Ambrosi, Virgile Calvert, Daniel Pavon, Bernard Angeletti, Stéven Criquet



MARS Project

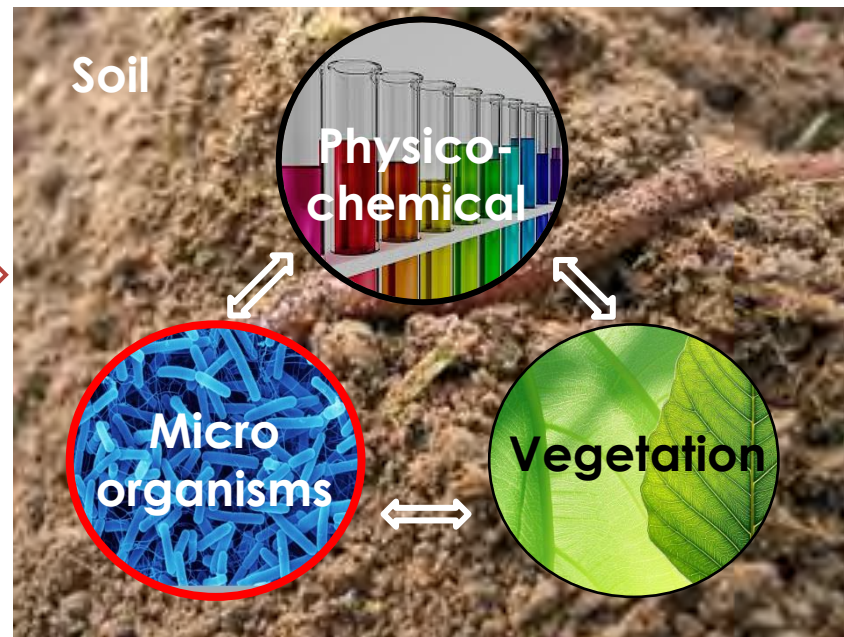
- MARS : Microbial diversity Assessment in Redmud Soils
- Study of microbial communities by **metabarcoding**
- in the continuity of the DORIS, REDMUD I & II Projects





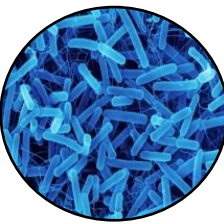
Bauxite
residues

transfer ⁽¹⁾

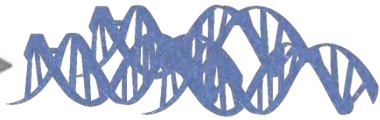


impacts ?

(1) : Jones et Haynes, 2011



Extraction
Zymobiomics™ DNA Miniprep
(Zymo Research)



ITS 2

Amplification



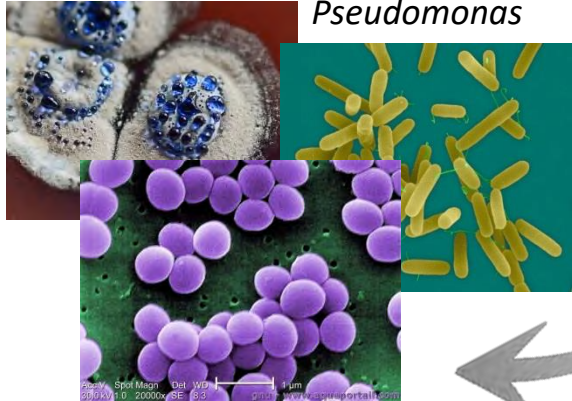
16s
(V34)



MiSeq sequencing
(LECA Grenoble)

Streptomyces

Pseudomonas



Staphylococcus

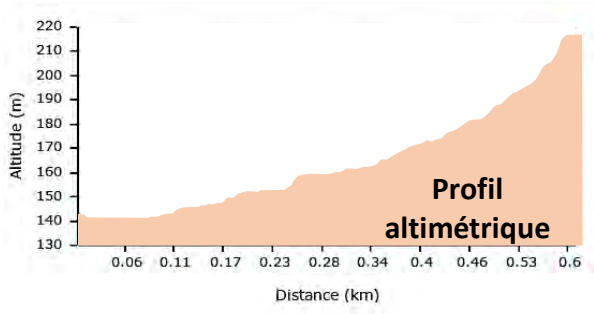
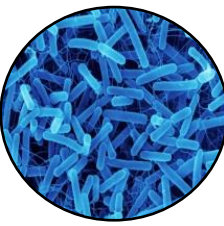


- 5,264,665 bacterial sequences
- 8,604,198 fungal sequences

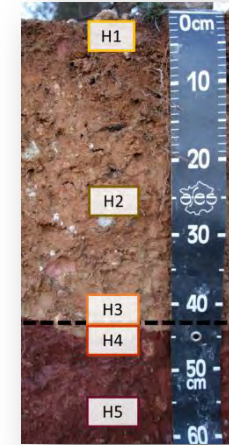


Journées Scientifiques :
"Bauxite Résidues"

Aix-en-Provence, du 08 au 09 juin 2021



Properties of vegetated uncapped and capped bauxite residues



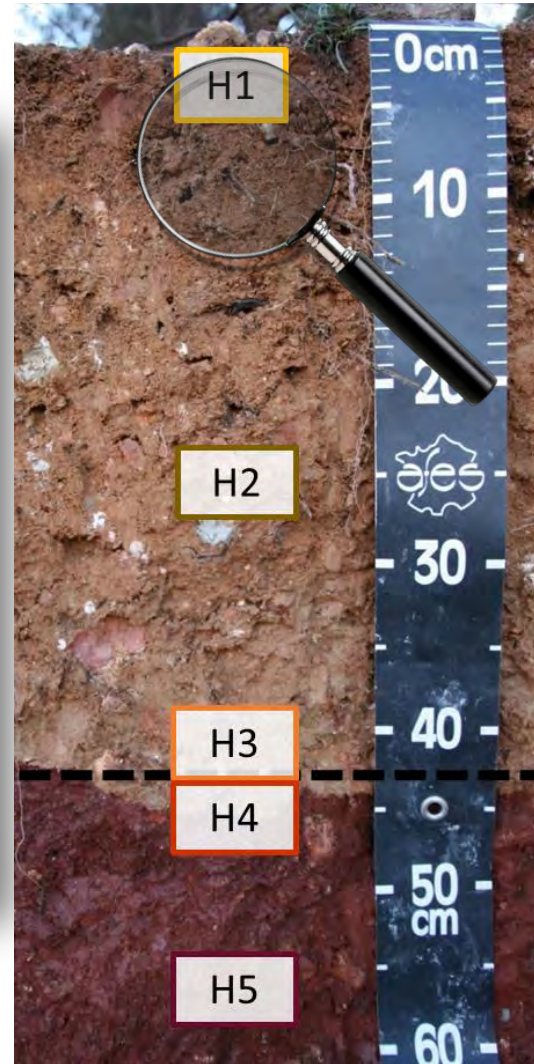
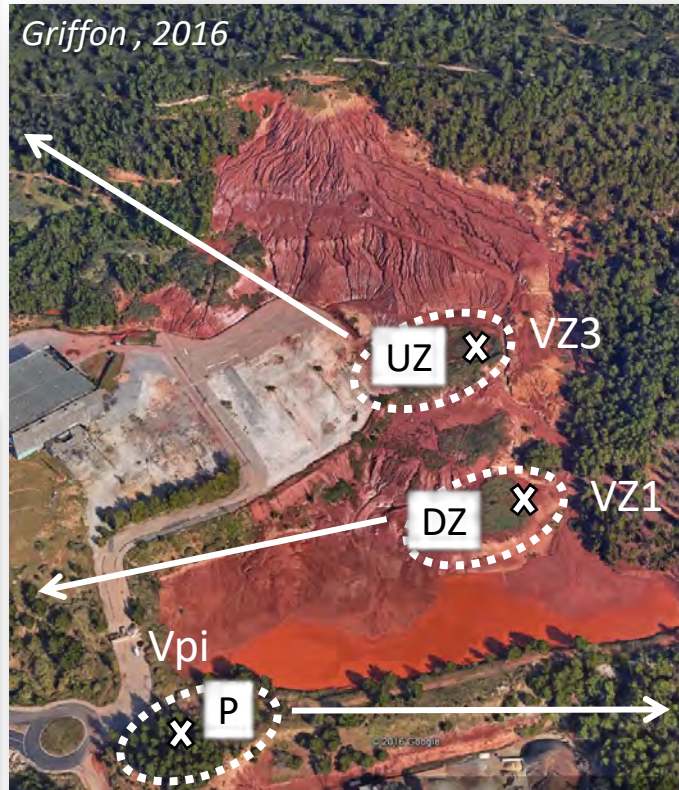
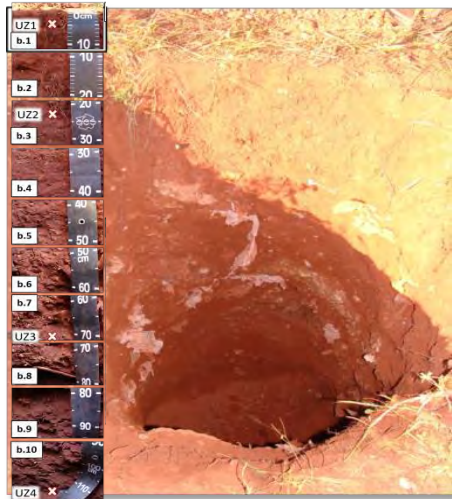
Environmental metabarcoding uncovers the microbial diversity in red mud deposits

How does *Suaeda vera* affect microbial diversity and nutrient status of soils from the extreme environment: the case of salt marsh and red mud soils?





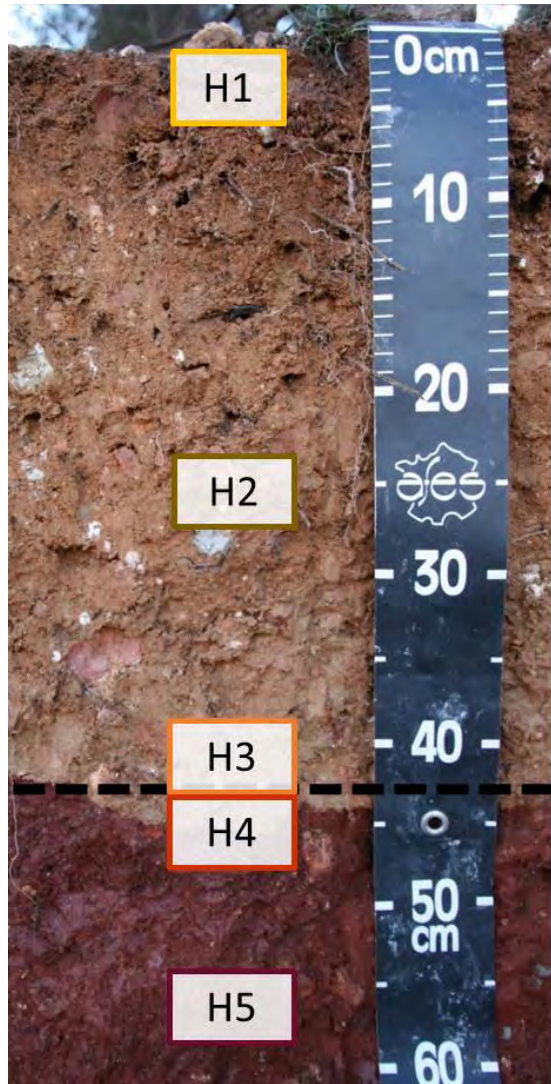
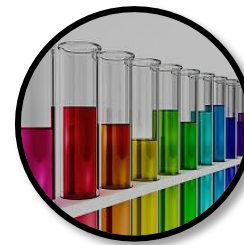
Sites and sampling



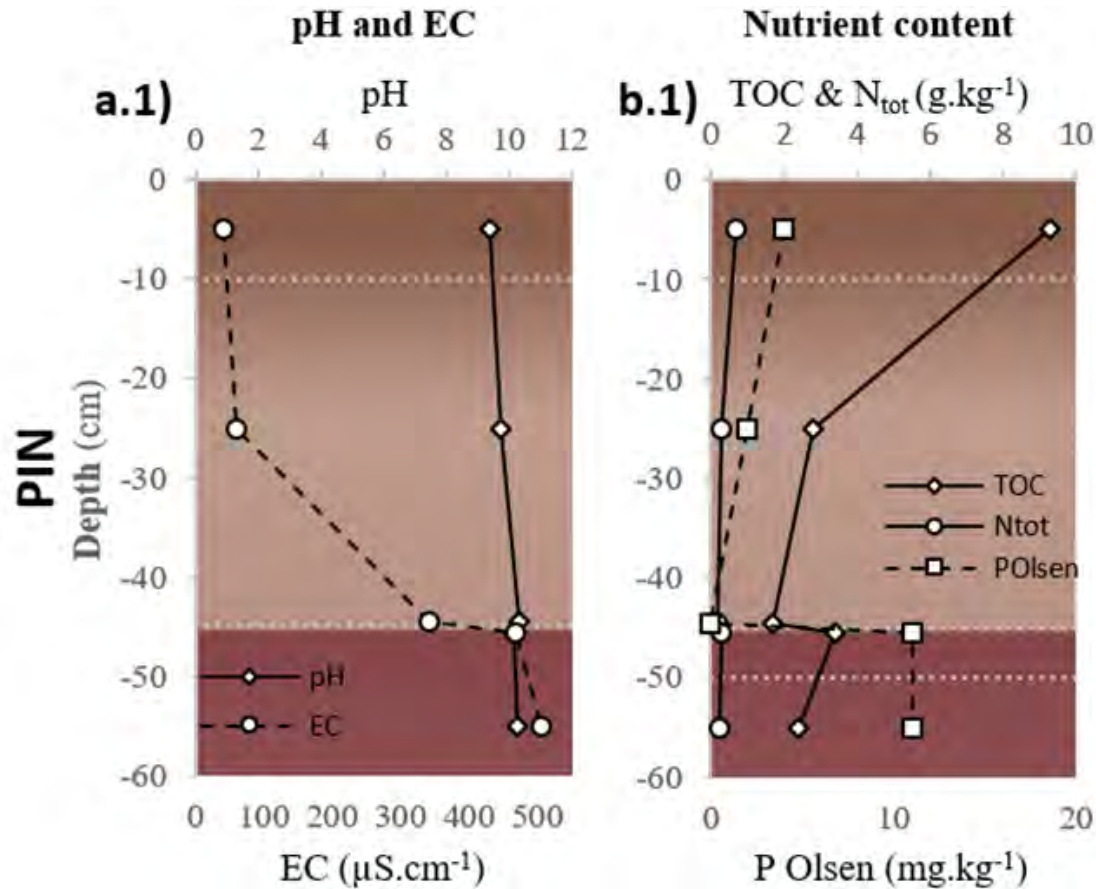
3 plots :

- 2 soil pits on herbaceous plots :
 - UZ (upzone)
 - DZ (downzone)
- 1 pinewood (P) plot
= natural outcrop

Fourrier *et al.*, In prep.



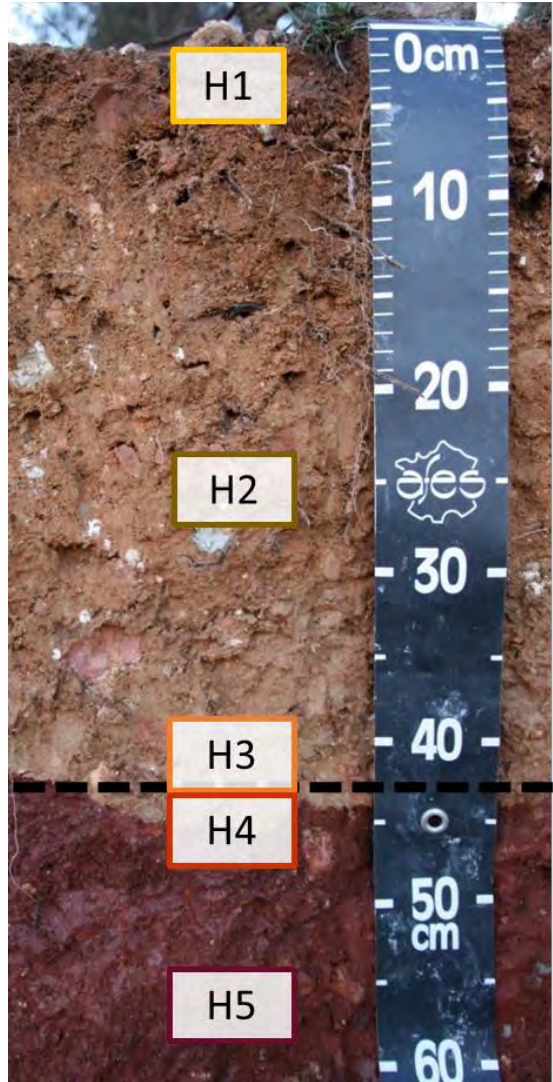
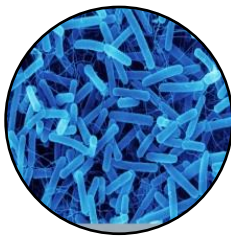
Physico-chemical characteristics



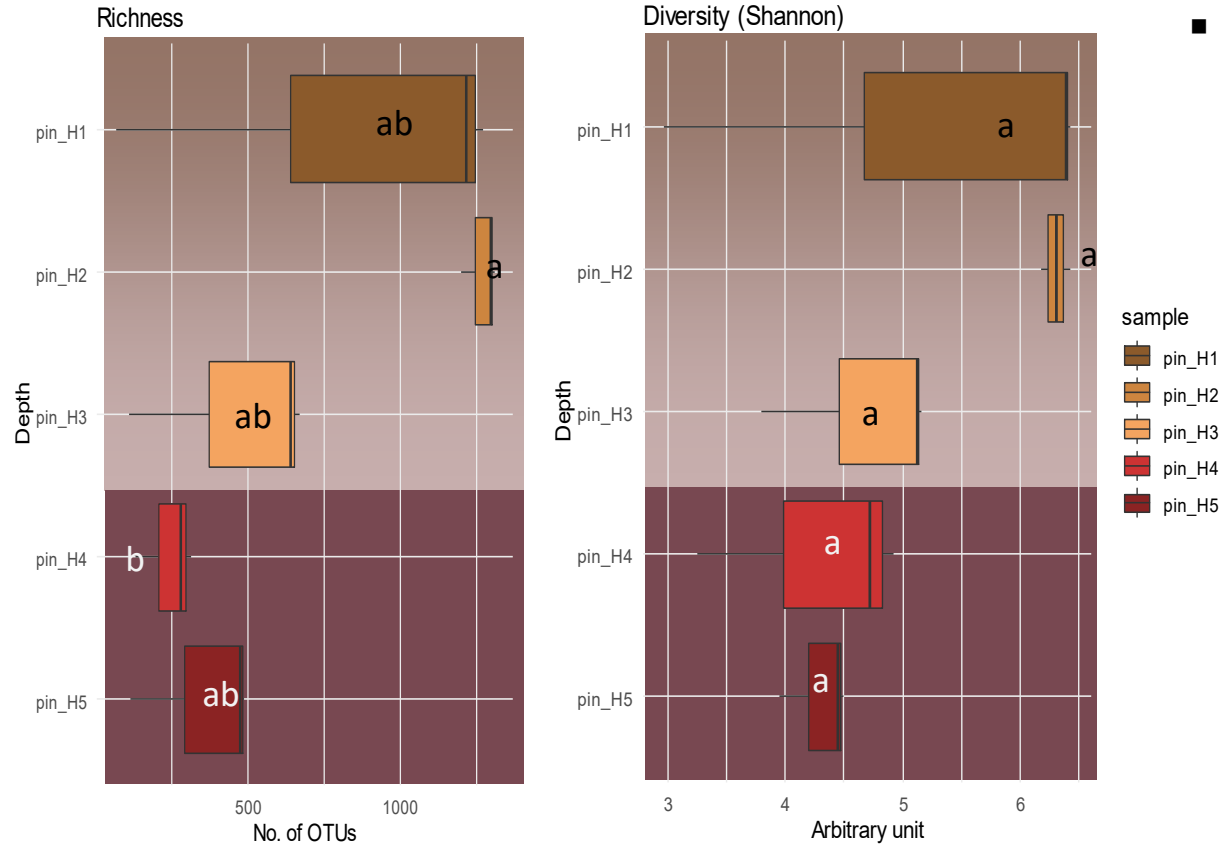
- Increase in pH and salinity with depth
- Decrease in nutrient content with depth (C, N, and P)

Fourrier *et al.*, In prep.





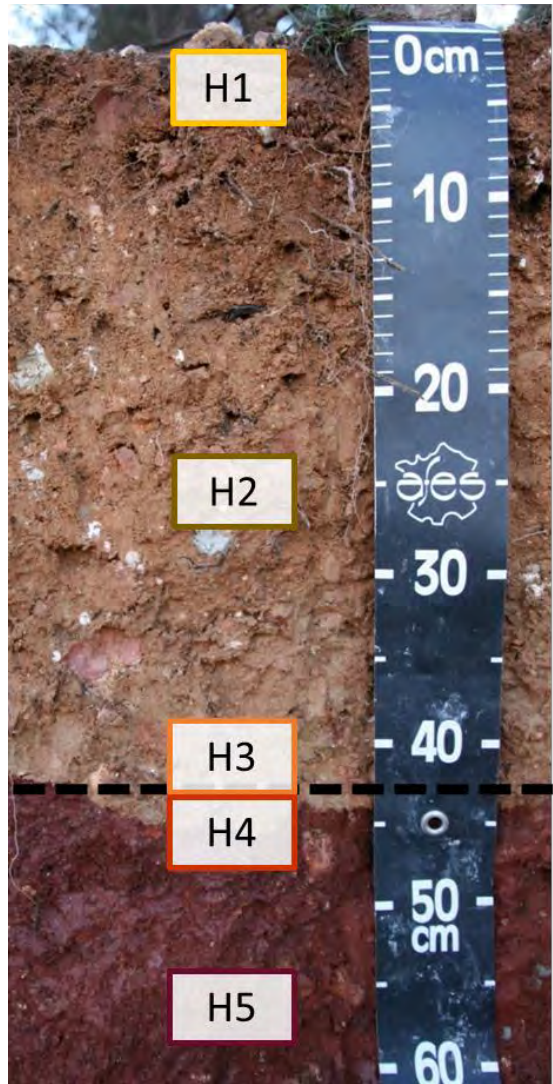
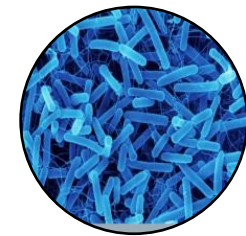
Bacterial richness and diversity



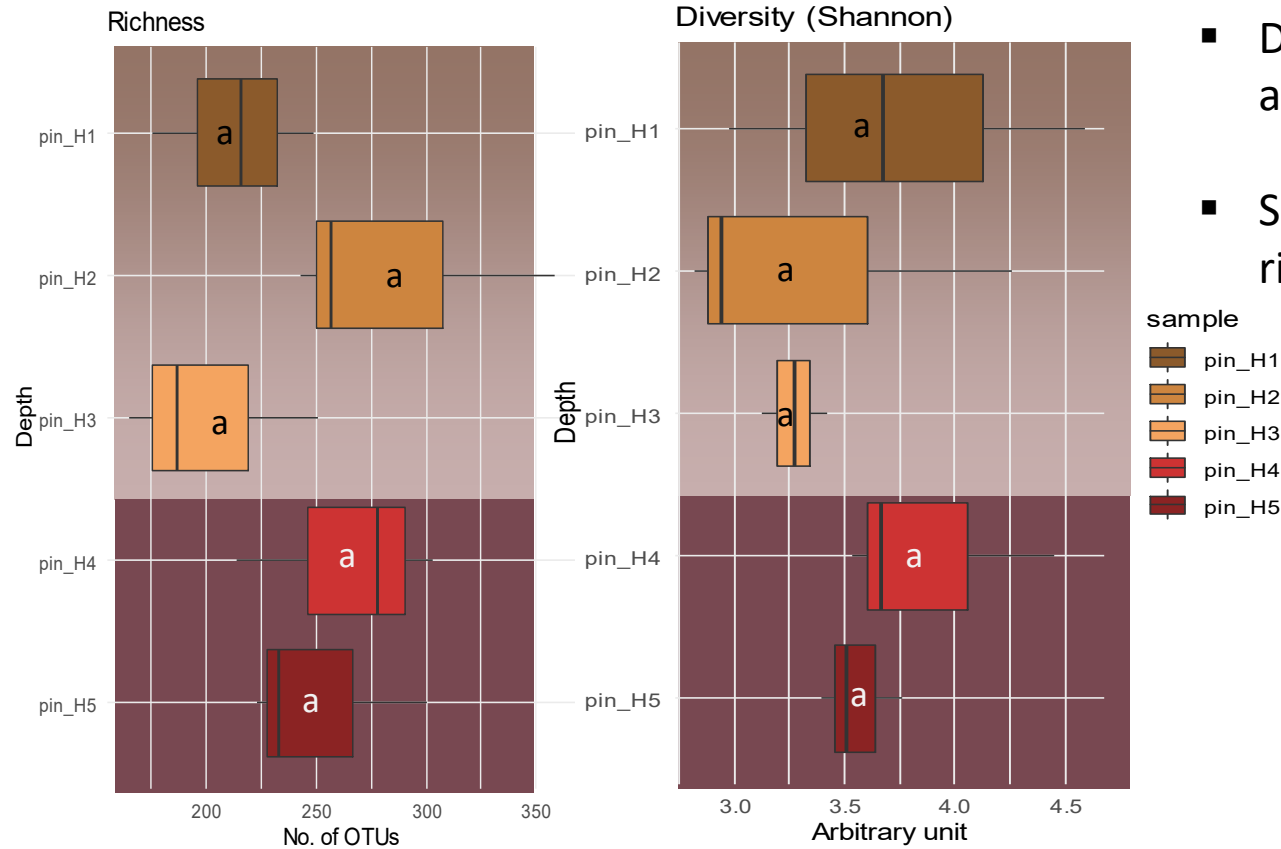
- Decrease in bacterial richness and diversity



Fourrier *et al.*, In prep.



Fungal richness and diversity

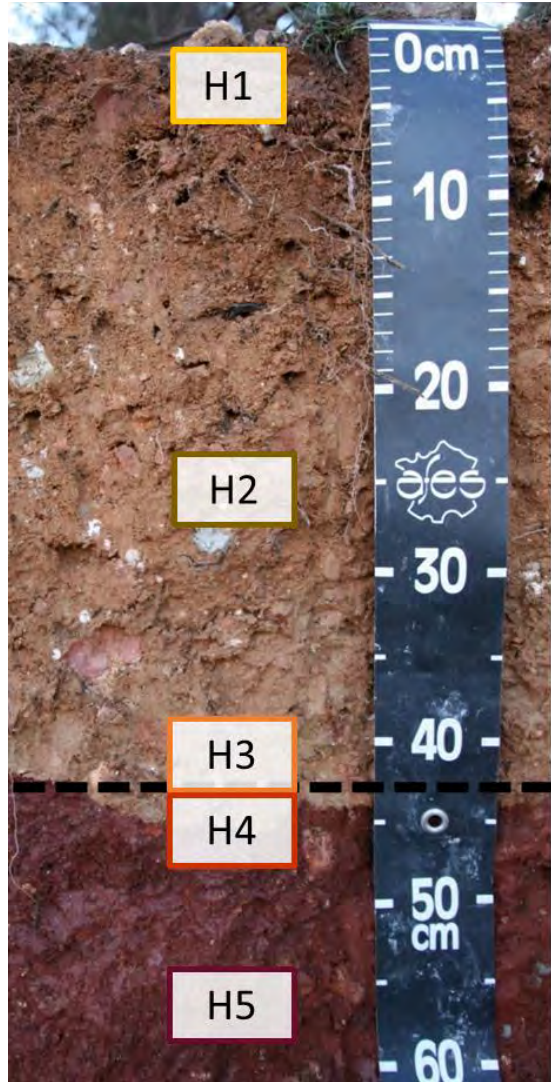
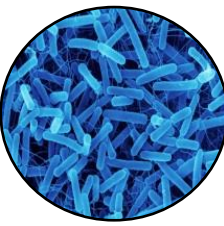


- Decrease in bacterial richness and diversity
- Slight decrease in fungal richness and diversity

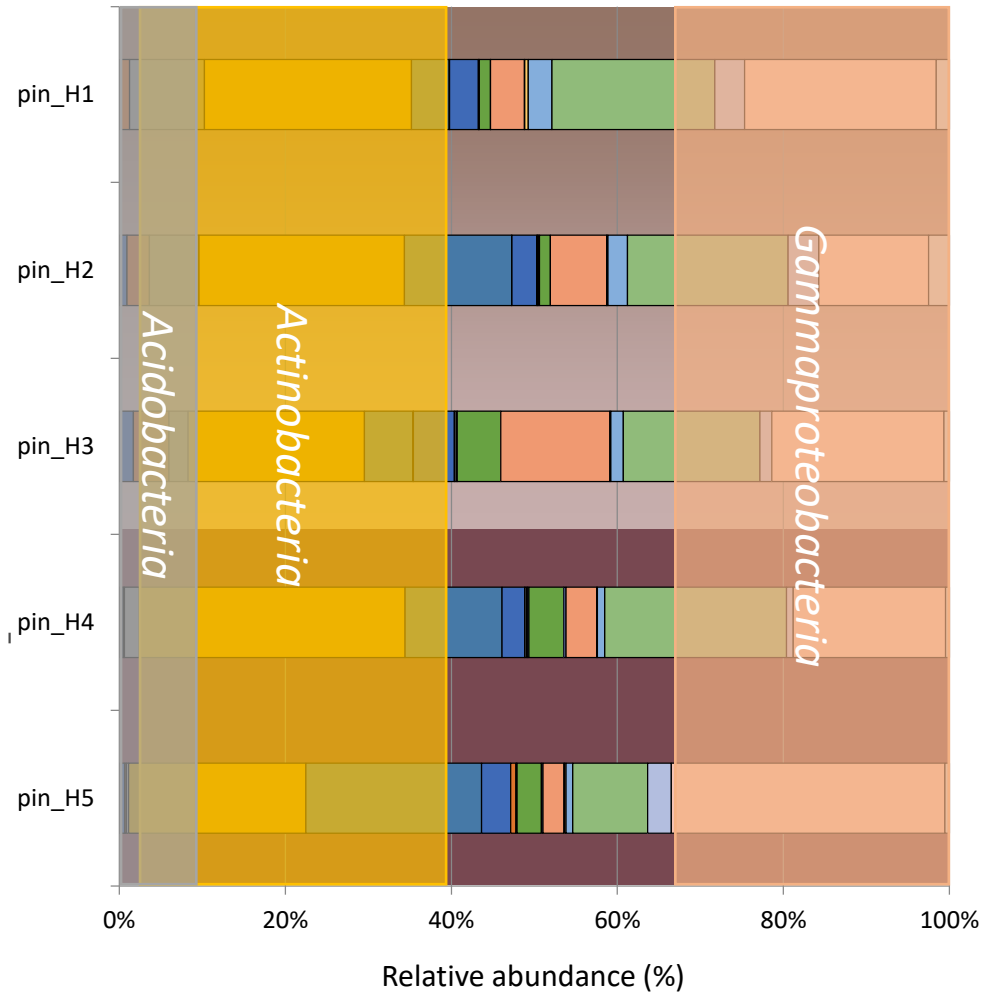


Fourrier *et al.*, In prep.





Bacterial phyla

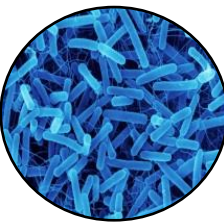


- Euryarchaeota
- Thaumarchaeota
- Acidobacteria
- Actinobacteria
- Bacteroidetes
- Chlamydiae
- Chloroflexi
- Deinococcus-Thermus
- Dependuntiae
- Elusimicrobia
- Fibrobacteres
- Firmicutes
- Fusobacteria
- Gemmatimonadetes
- Nitrospirae
- Patescibacteria
- Planctomycetes
- Alphaproteobacteria
- Deltaproteobacteria
- Gammaproteobacteria
- Verrucomicrobia

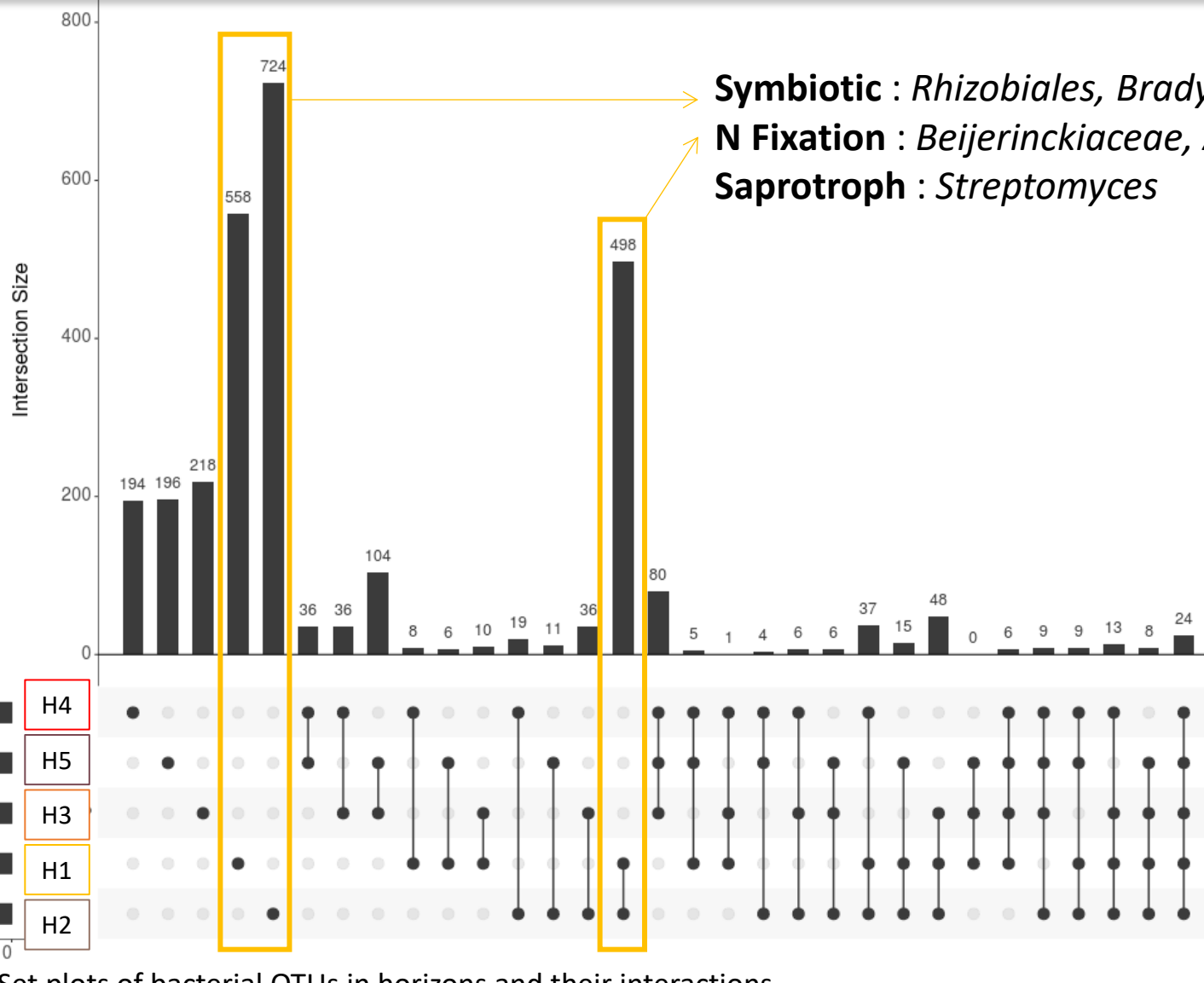
- Decrease in *Acidobacteria* with depth
- Increase in *Actinobacteria* and *Archaea*
- the deepest horizon dominated by *Gammaproteobacteria*
- *Actinobacteria* + *Gammaproteobacteria* = unweathered residue (Wu *et al.*, 2020)

Fourrier *et al.*, In prep.

Properties of vegetated uncapped and capped bauxite residues



Bacterial species

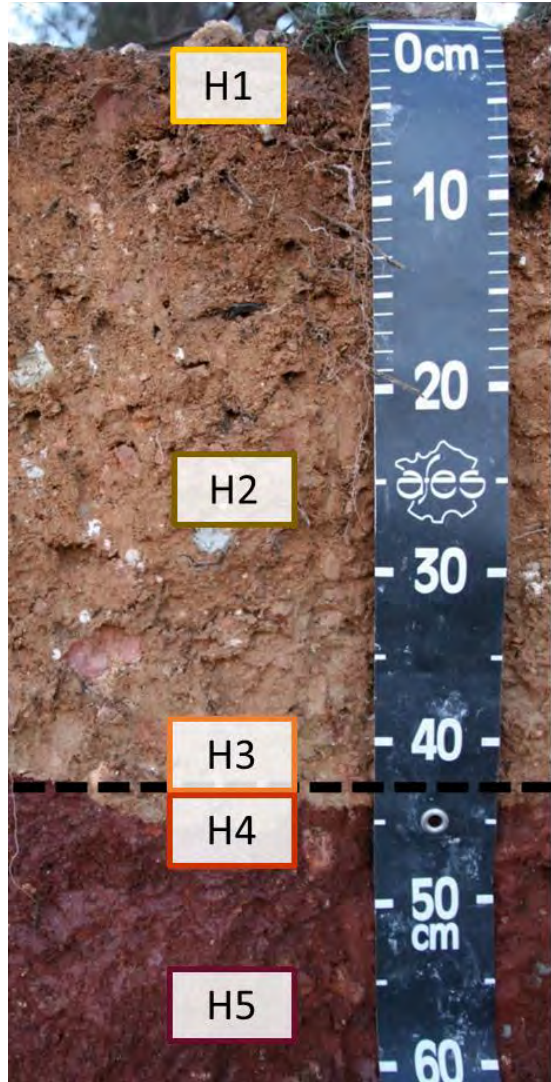
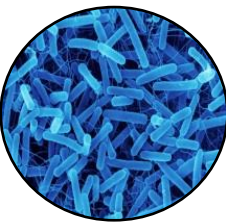


Symbiotic : *Rhizobiales*, *Bradyrhizobium japonicum*
N Fixation : *Beijerinckiaceae*, *Azospirillales*, *Nitrospira*
Saprotroph : *Streptomyces*

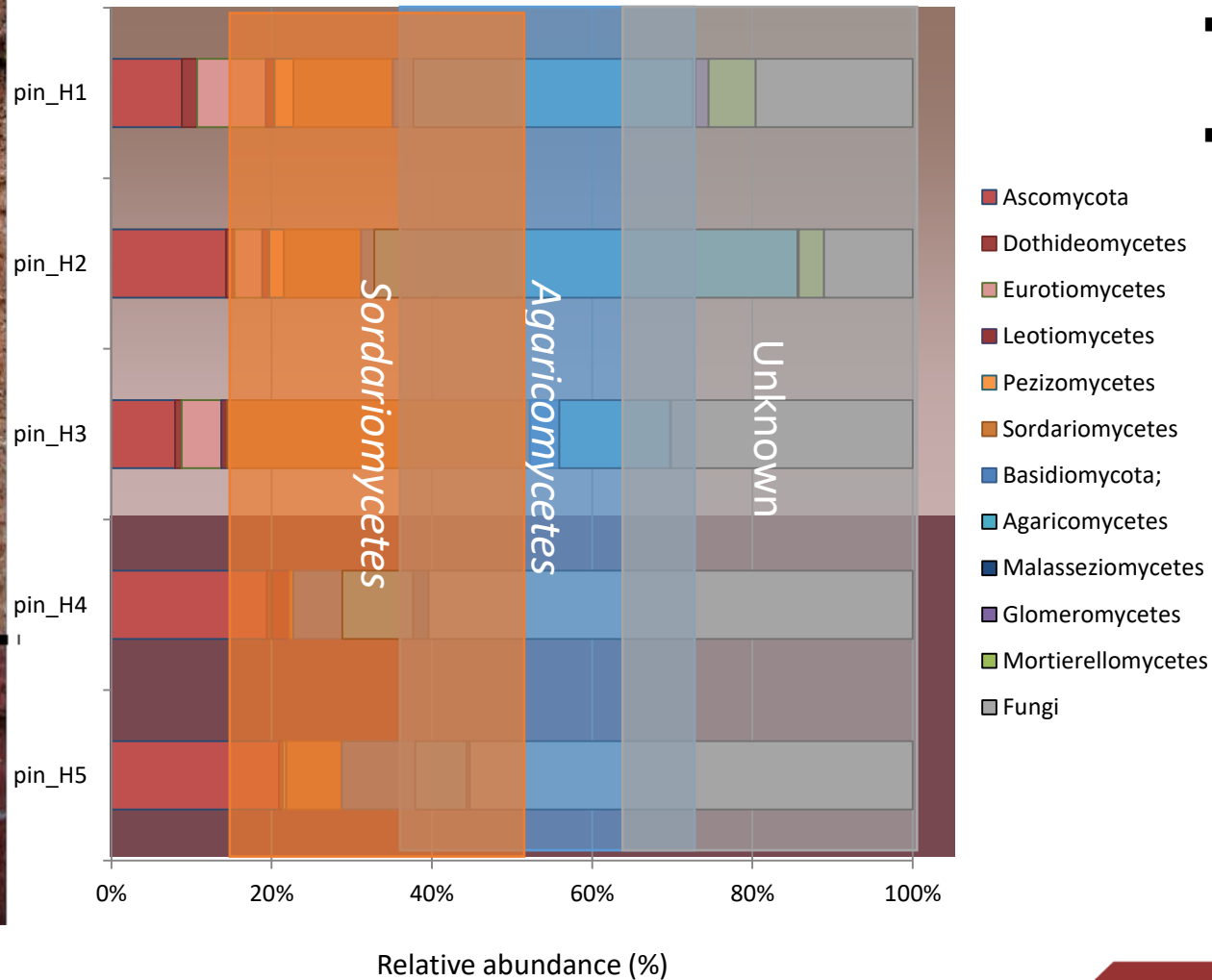
UpSet plots of bacterial OTUs in horizons and their interactions

Fourrier *et al.*, In prep.



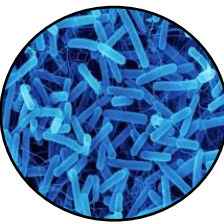


Fungal class

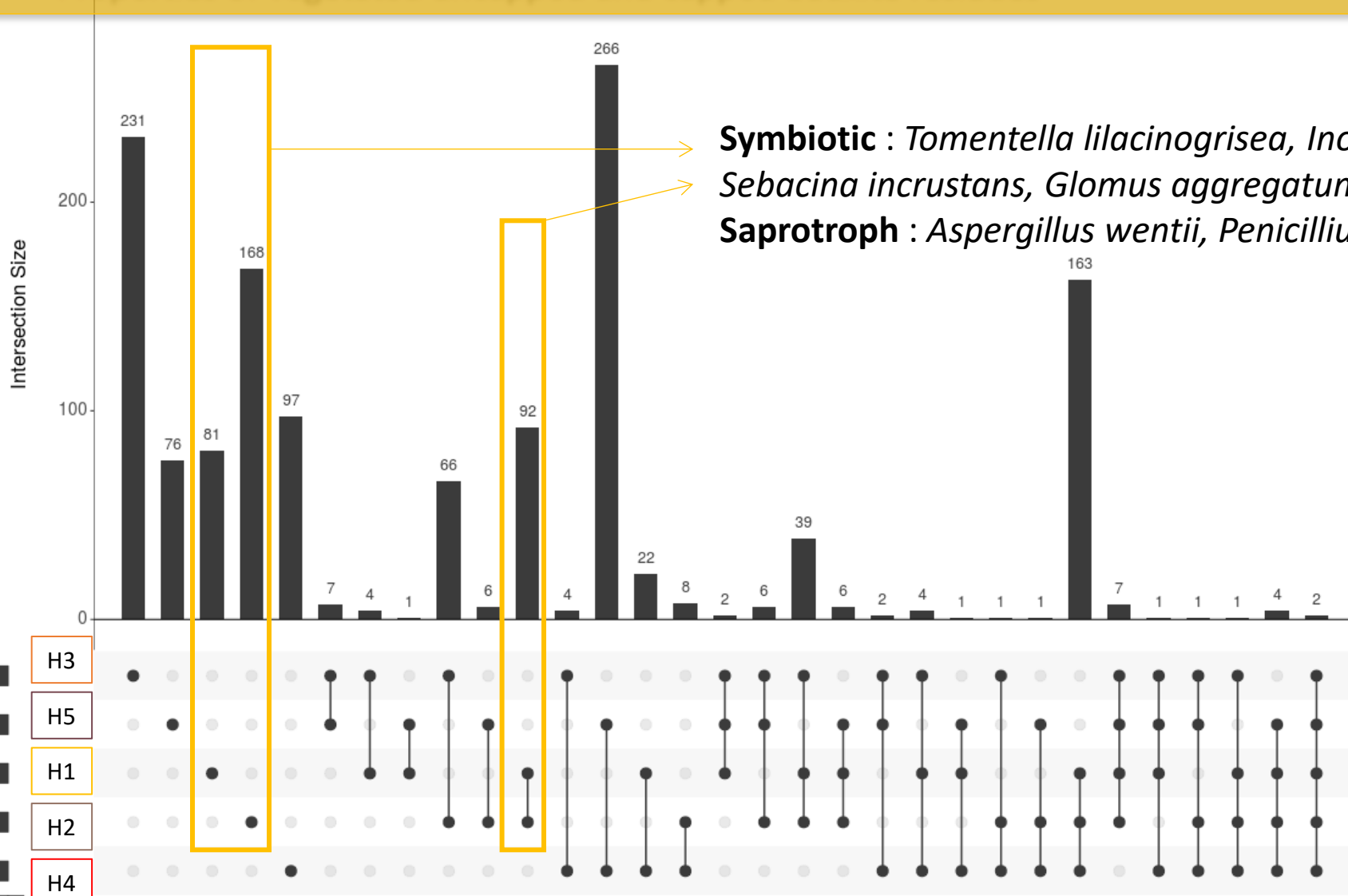


- Decrease in *Agaricomycetes*
- Increase in *Sordariomycetes* and Unknown fungi

Fourrier *et al.*, In prep.



Fungal species

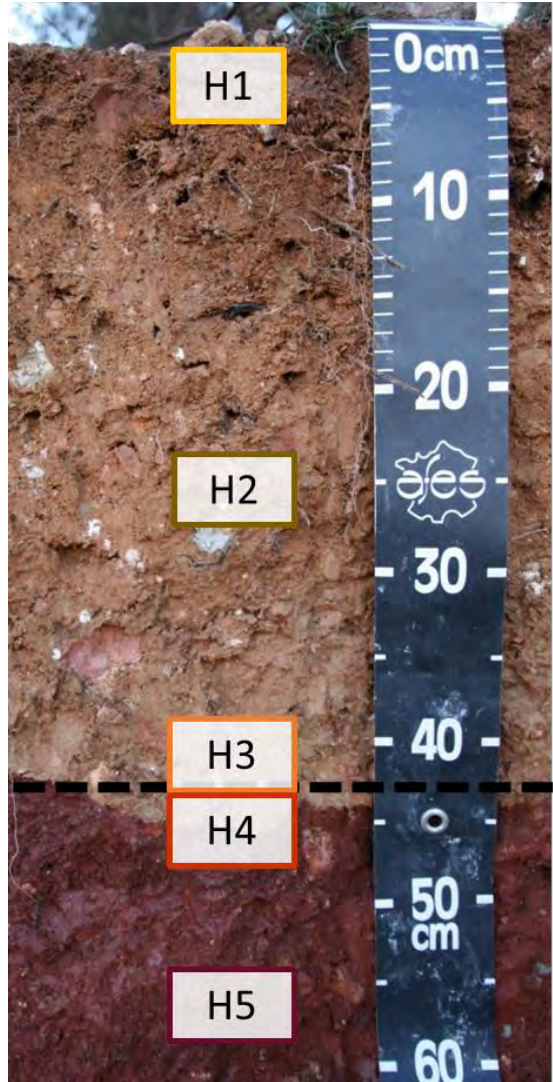


Symbiotic : *Tomentella lilacinogrisea*, *Inocybe arenicola*, *Sebacina incrustans*, *Glomus aggregatum*
Saprotroph : *Aspergillus wentii*, *Penicillium nodositatum*

UpSet plots of fungal OTUs in horizons and their interactions

Fourrier *et al.*, In prep.





Conclusion

Red muds capped with fill soil with pinewood roots

- Presence of rhizosphere microorganisms
- Restoration of soil biological function

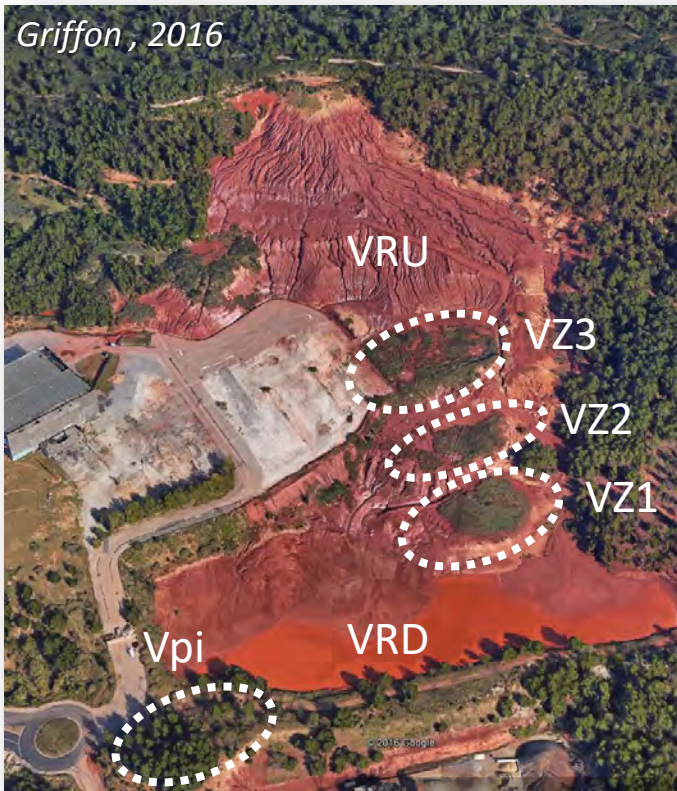
Enzymatic activities of profile samples.

Profile	Horizon	mU.g DW ⁻¹							
		Arylamidase		β-glucosidase		Alkaline phosphatase		Arylsulfatase	
PIN	P1	3.60 ± 0.47	a	2.50 ± 0.13	a	9.47 ± 0.63	a	1.03 ± 0.01	a
	P2	0.59 ± 0.06	d	0.58 ± 0.03	b	4.44 ± 0.09	b	0.21 ± 0.01	b
	PSUP	1.85 ± 0.23	b	0.05 ± 0.01	c	1.04 ± 0.28	c	0	d
	PINF	1.47 ± 0.12	bc	0.06 ± 0.02	c	0.49 ± 0.02	c	0	d
	P3	0.95 ± 0.03	cd	0	c	0.24 ± 0.05	c	0.05 ± 0.04	c

Fourrier *et al.*, In prep.



Sites and sampling

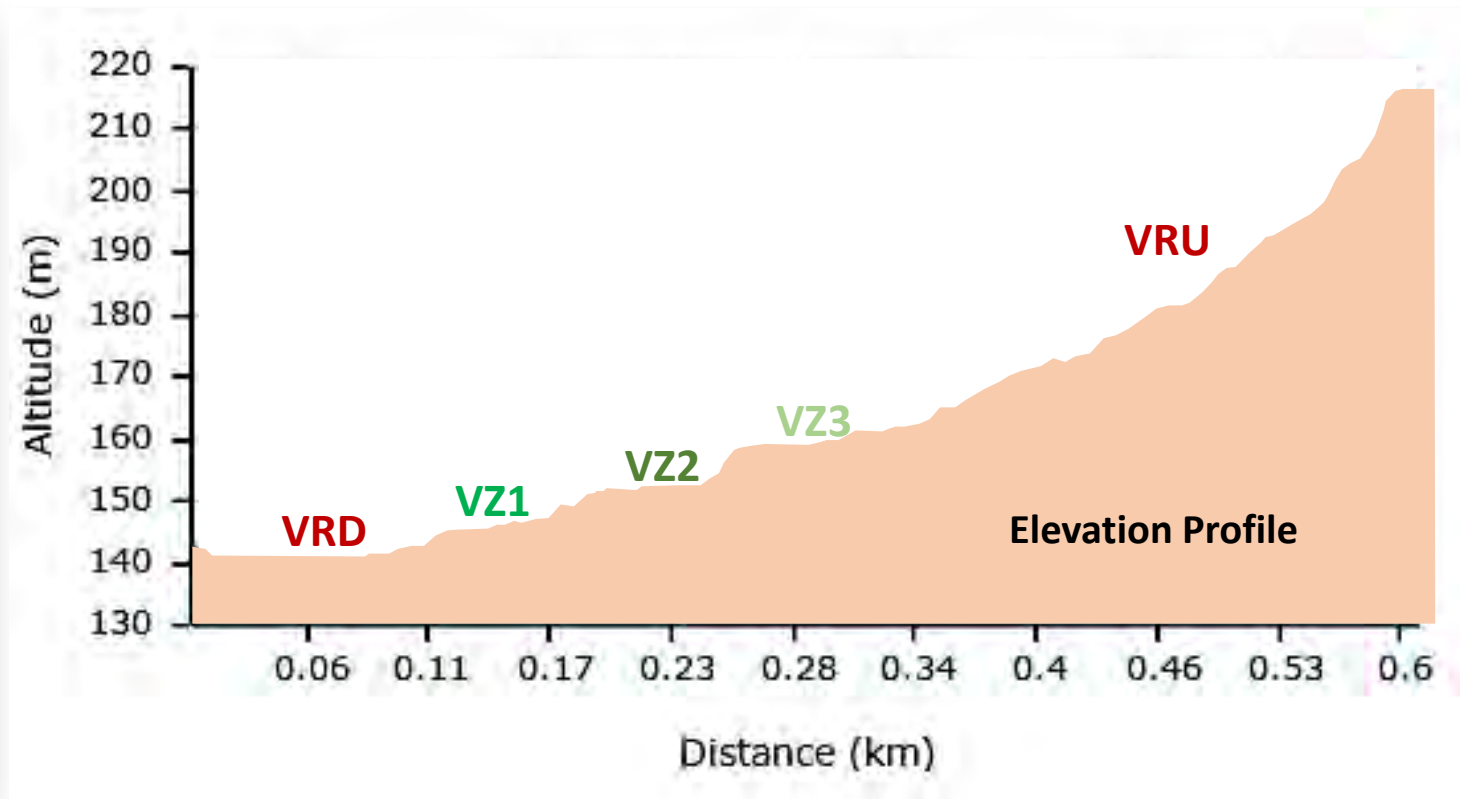
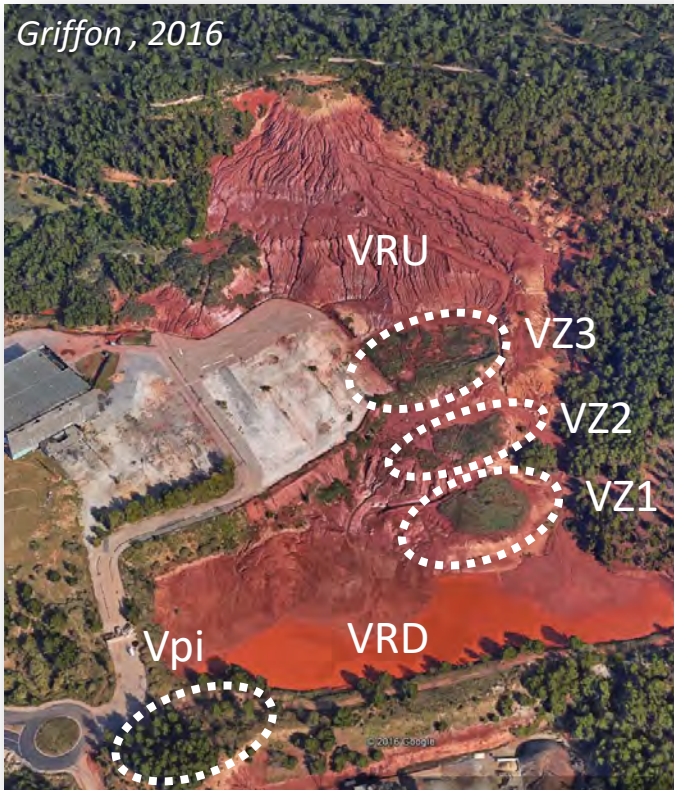


- 2 sites
- 5 vegetalized plots (VZ1, VZ2, VZ3, MG1, MG2)
- 2 unvegetalized plots (top of the fields (VRU) and the bottom of the fields (VRD))
- 1 pinewood soil (Vpi)

Foulon *et al.*, In prep.

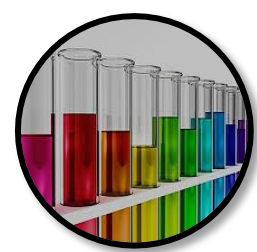


Sites and sampling

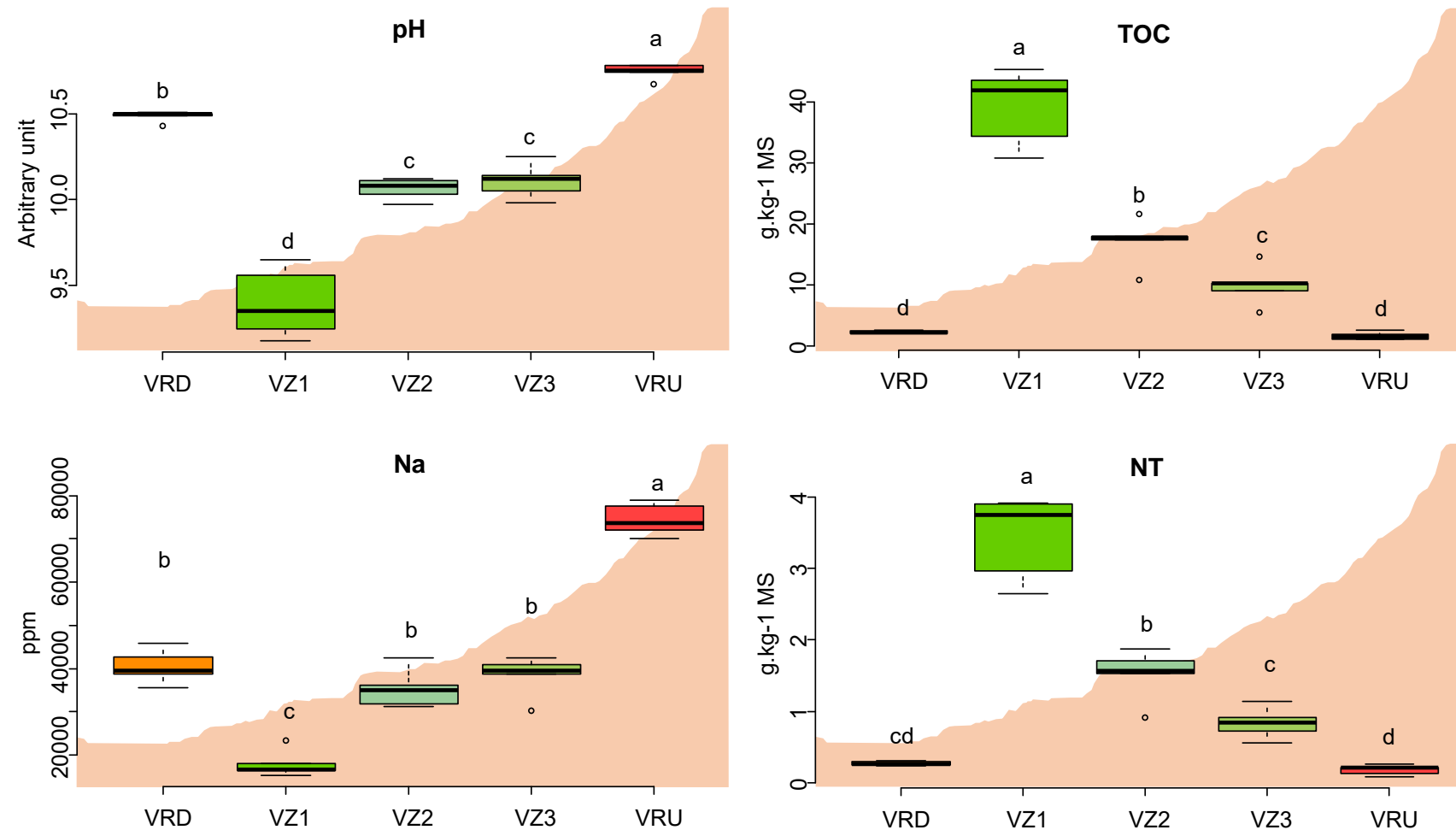


- Elevation profile along the sampling transects
- three terraced ground areas with spontaneous vegetation

Foulon *et al.*, In prep.



Physico-chemical characteristics

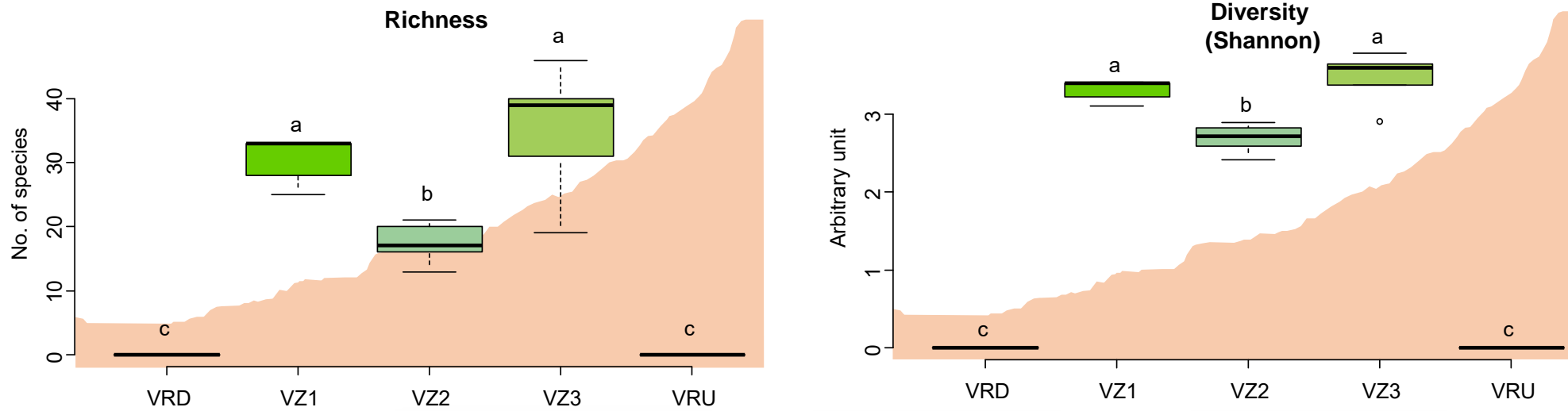


Foulon *et al.*, In prep.





Plant richness and diversity

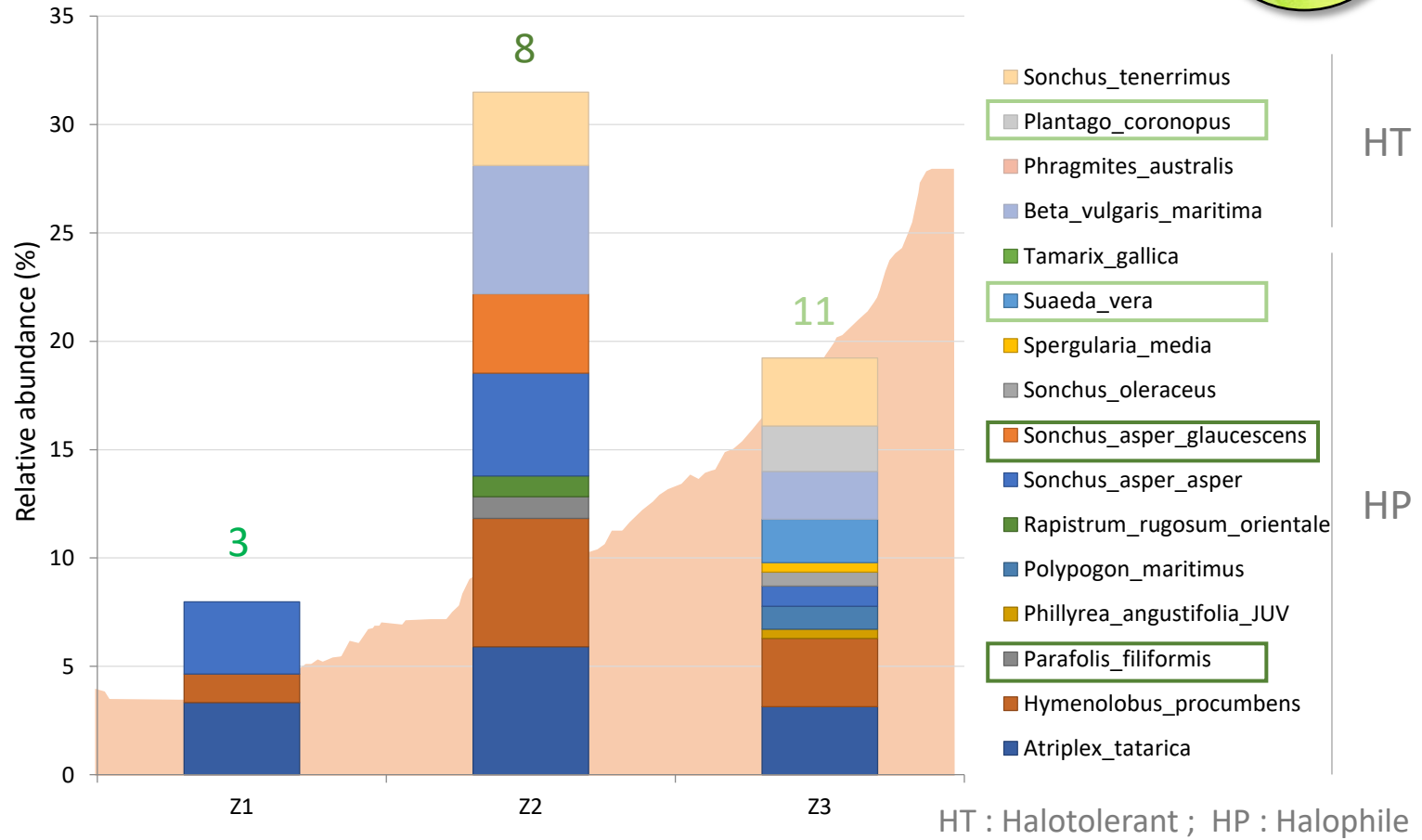
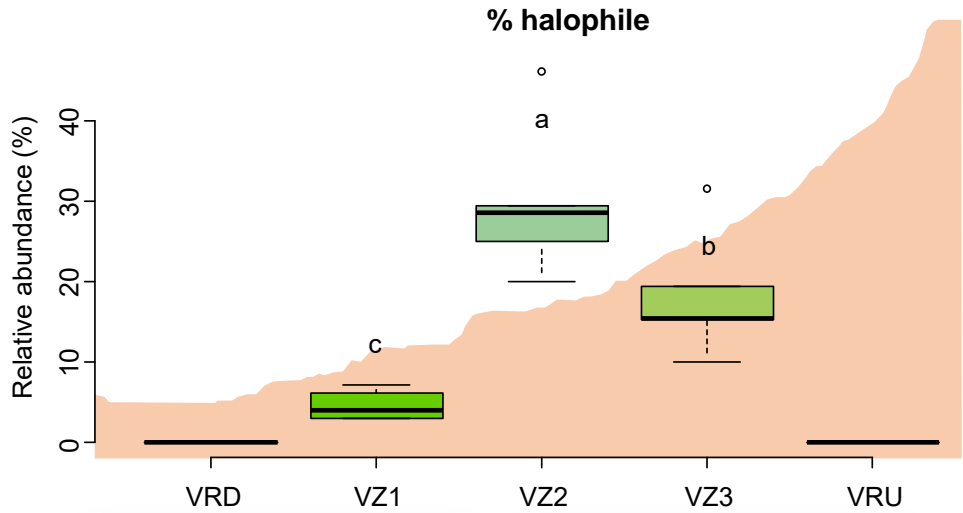


- Vegetation richness and diversity is higher in vegetated plot 1 and 3

Foulon *et al.*, In prep.



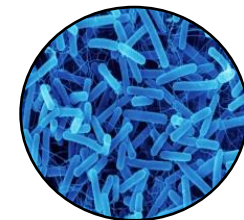
Halophilic vegetation



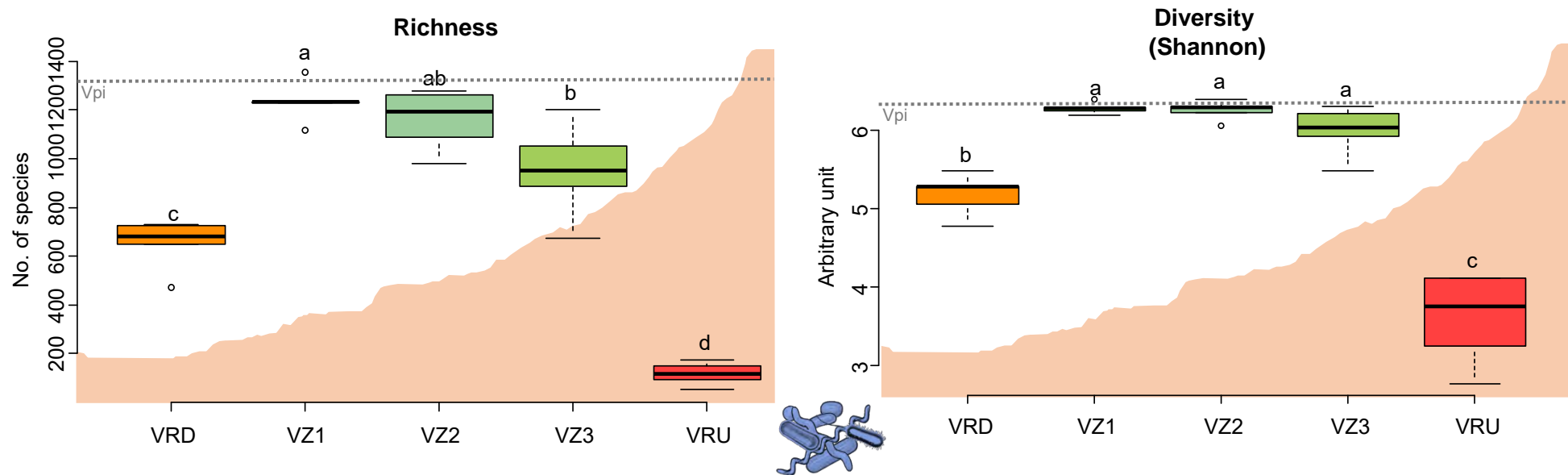
- Proportion of halophilic vegetation is higher in upper plots

Foulon *et al.*, In prep.



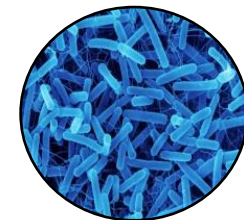


Bacterial richness and diversity

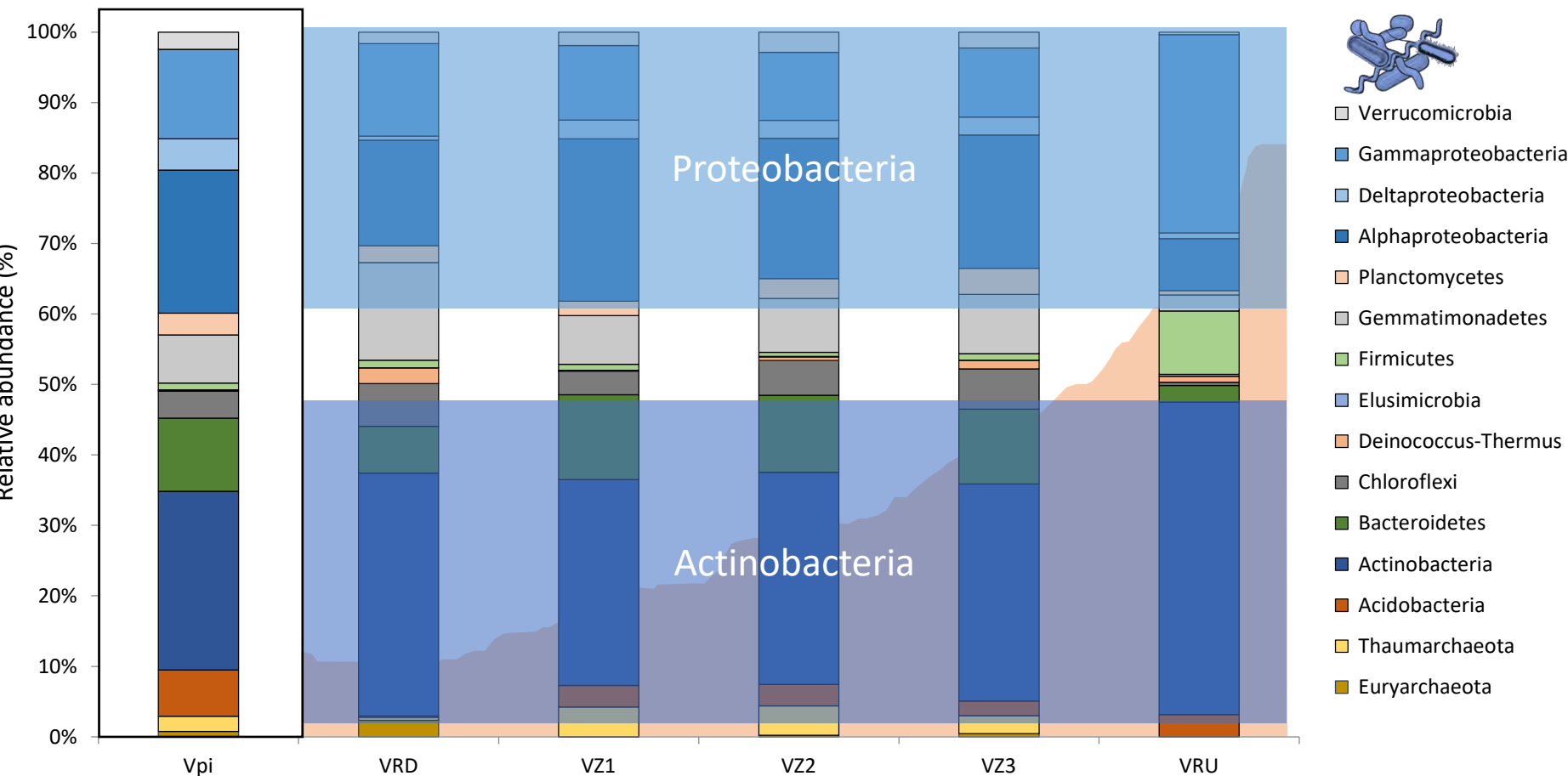


- Bacterial richness and diversity is higher in vegetalized plots relative to unvegetalized red mud
- Increased in bacterial richness with decreased elevation
- Bacterial richness and diversity in vegetalized plots close to the pinewood soil

Foulon *et al.*, In prep.



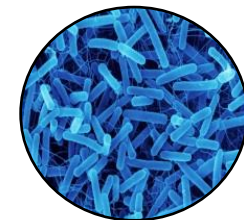
Bacterial phyla



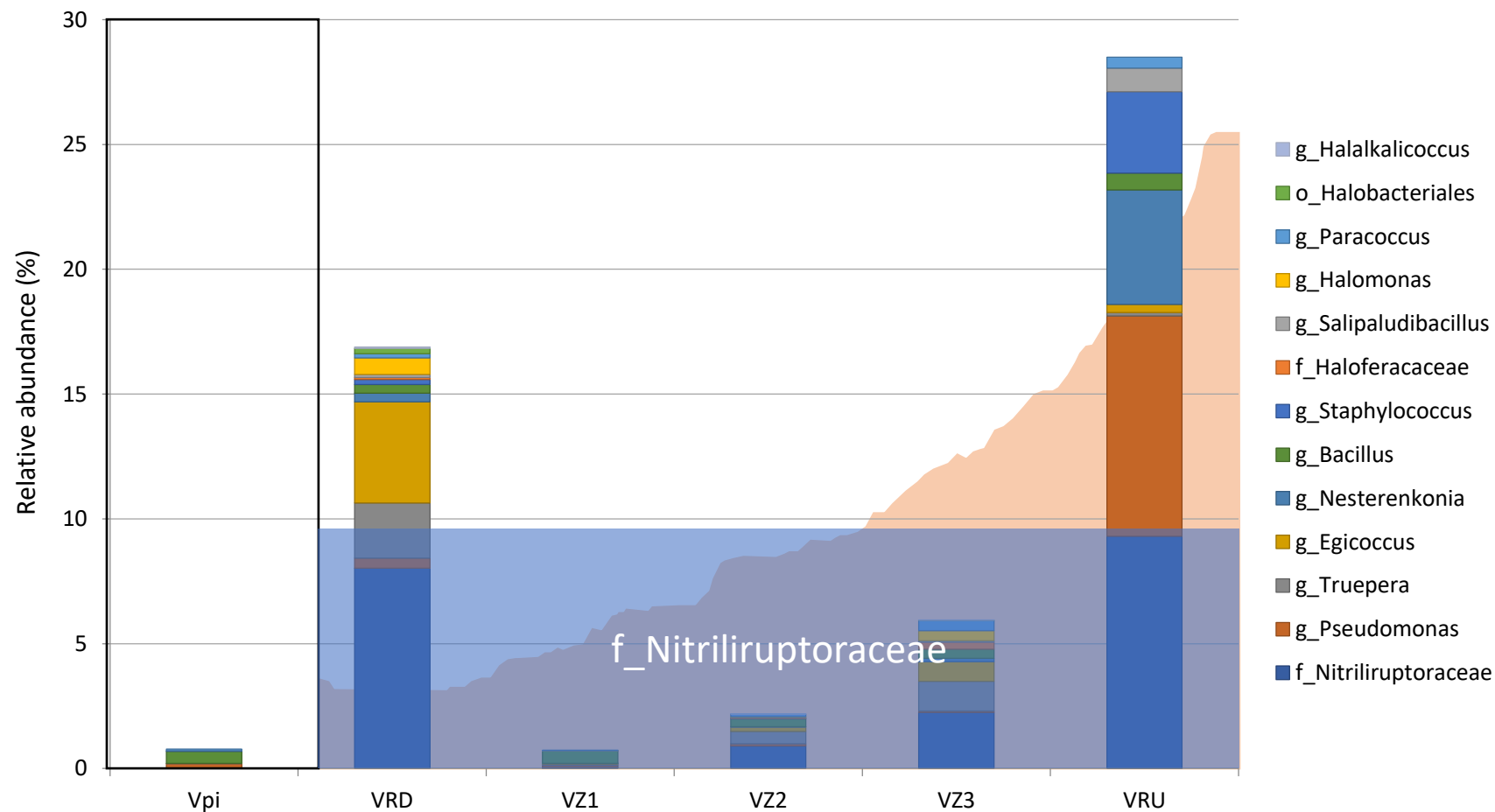
- All red mud soil dominated by *Actinobacteria*
- *Proteobacteria*, especially *Gammaproteobacteria* more present in red mud soil
- *Acidobacteria* + *Alphaproteobacteria* = old weathered residue (Wu *et al.*, 2020)
- *Firmicute* + *Actinobacteria* = high alkaline and saline conditions (Krishna *et al.*, 2014)

Foulon *et al.*, In prep.



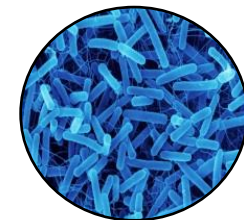


Halophilic and alkaliphilic bacteria

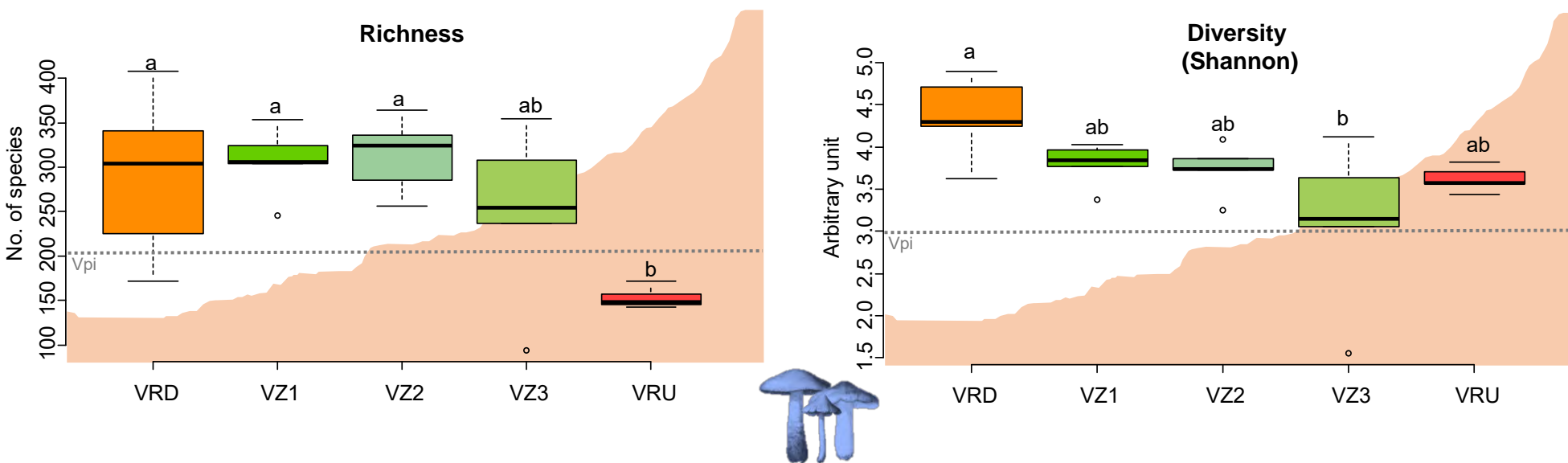


- Halophilic and alkaliphilic bacteria more abundant in red mud soil : ex. *Egicoccus*, *Truepera*, *Halomonas*, *Nesterenkonia*
- Alkaliphilic family *Nitriliruptoraceae* increase in red mud soil, specifically *nitriliruptor* genus

Foulon *et al.*, In prep.

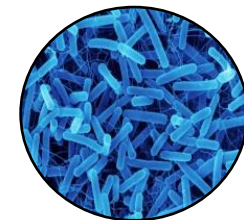


Fungal richness and diversity

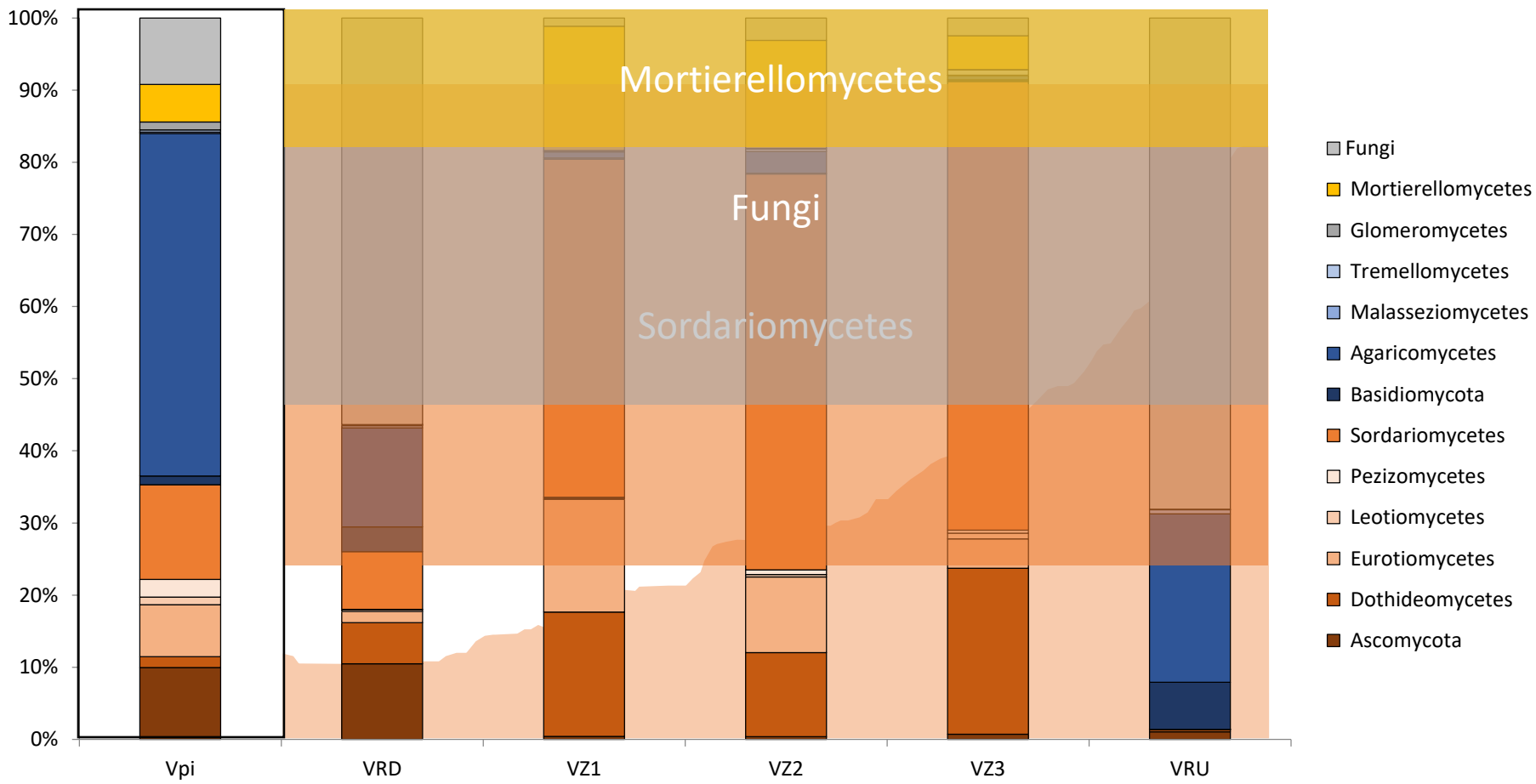


- Fungal richness and diversity is higher in vegetalized soil relative to unvegetalized red mud at the top of the fields (VRU)
- Increased in fungal richness with decreased elevation
- Fungal richness and diversity in vegetalized plots is greater than pinewood soil

Foulon *et al.*, In prep.



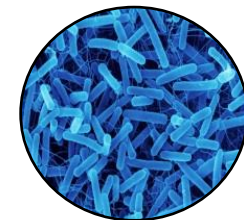
Fungal class



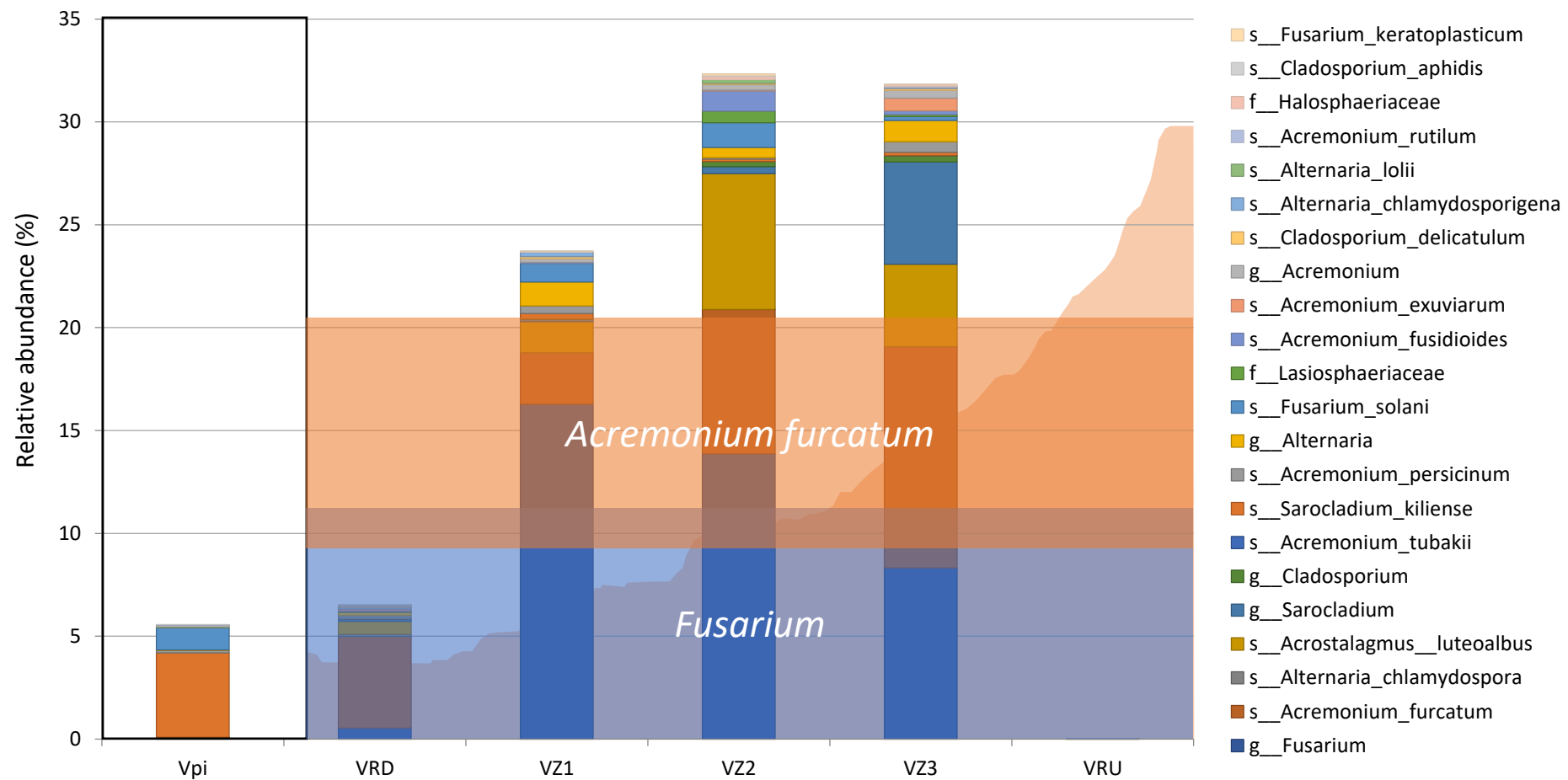
- *Sordariomycetes* and *Mortierellomycetes* increase in vegetalized red mud
- All red mud soil dominated by unknown Fungi
- *Sordariomycetes* + unknown Fungi = like hypersaline environments (Santini *et al.*, 2015)

Foulon *et al.*, In prep.





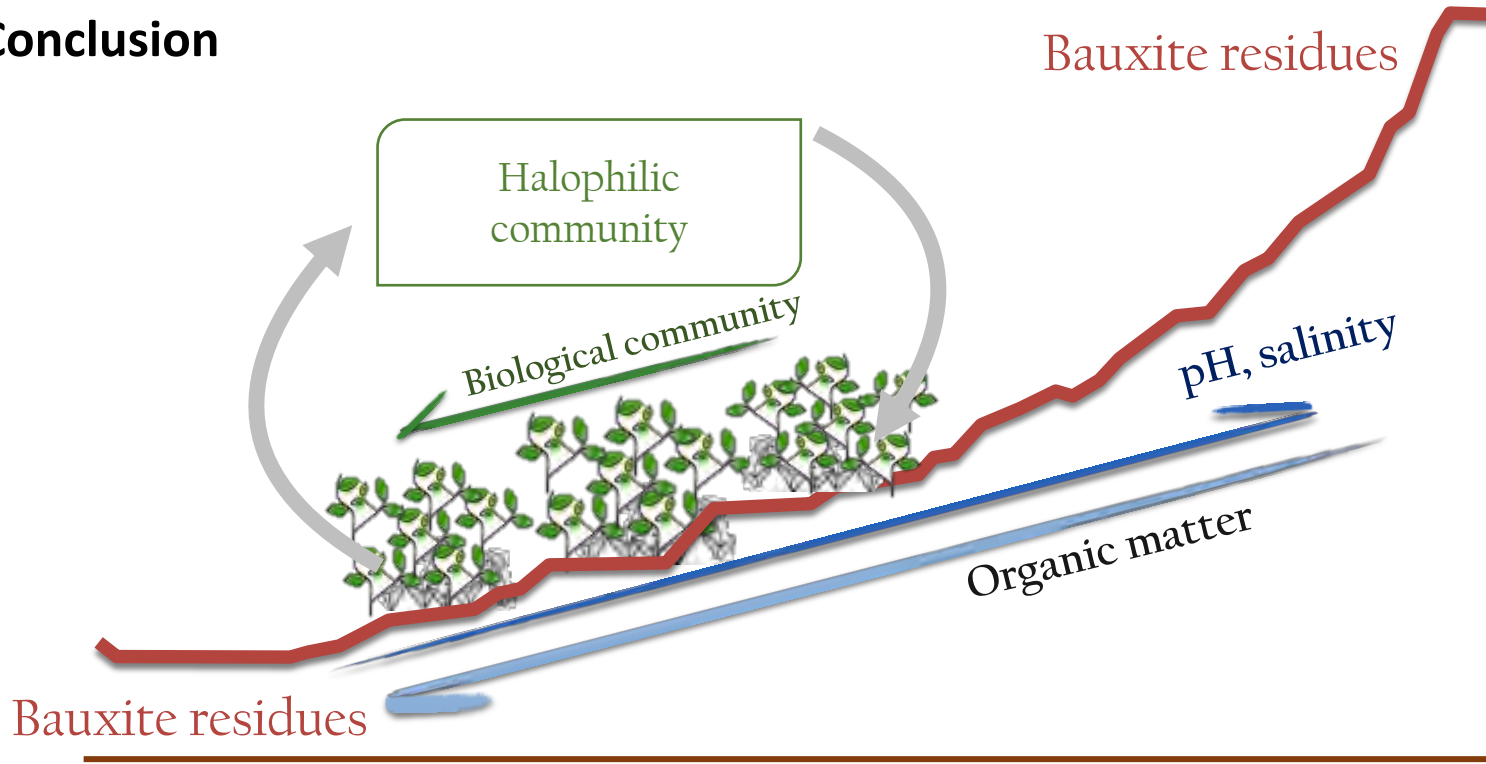
Halophilic and alkaliphilic fungal



- Halophilic and alkaliphilic fungi more abundant in red mud soil
- Genus *Fusarium* and species *Acremonium furcatum* dominated vegetalized soils

Foulon *et al.*, In prep.

Conclusion



Sansouires biotope



Mediterranean salt marsh

Foulon et al., In prep.



Atriplex tatarica



Beta vulgaris subsp. *maritima*

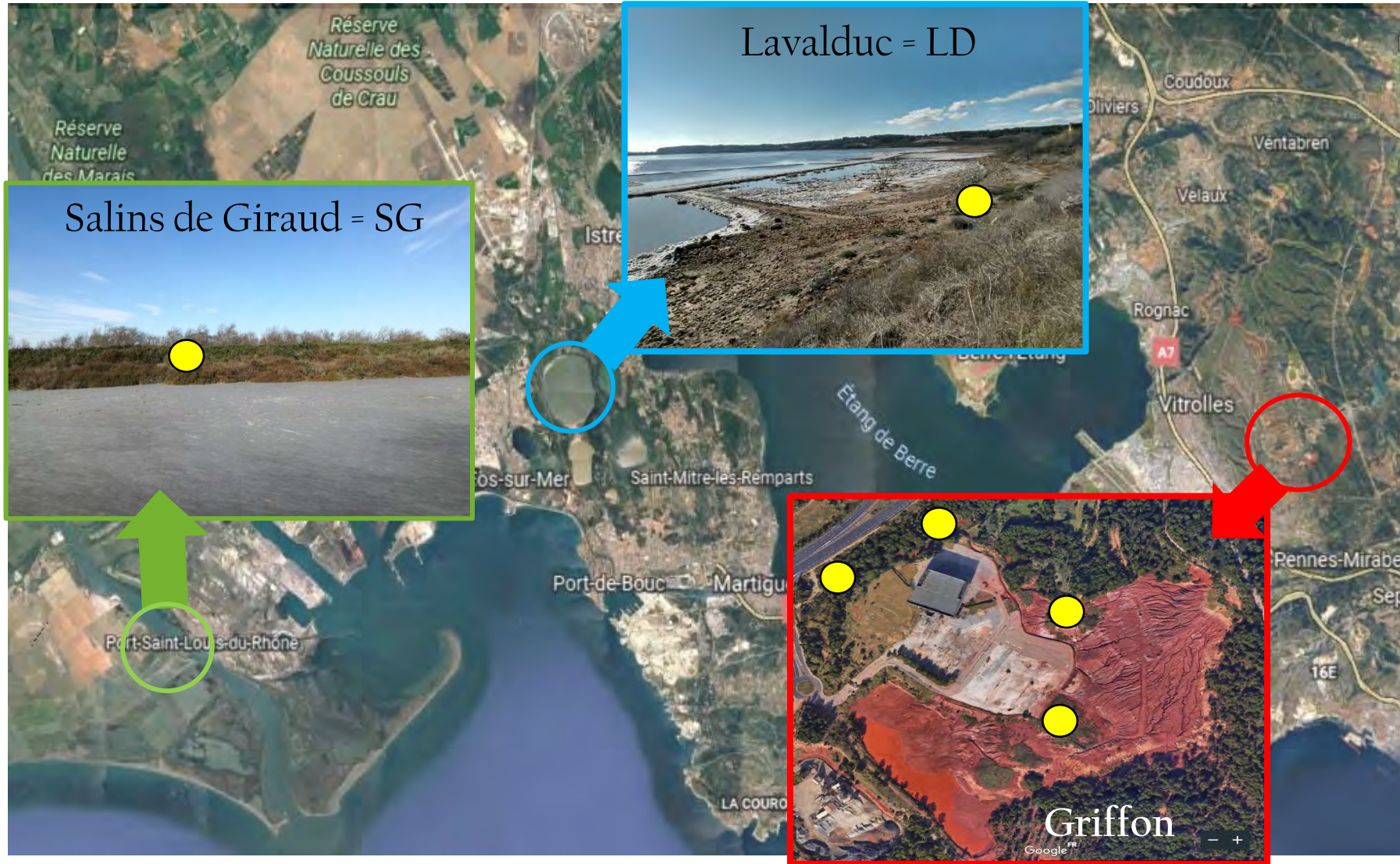


Plantago coronopus



Suaeda vera



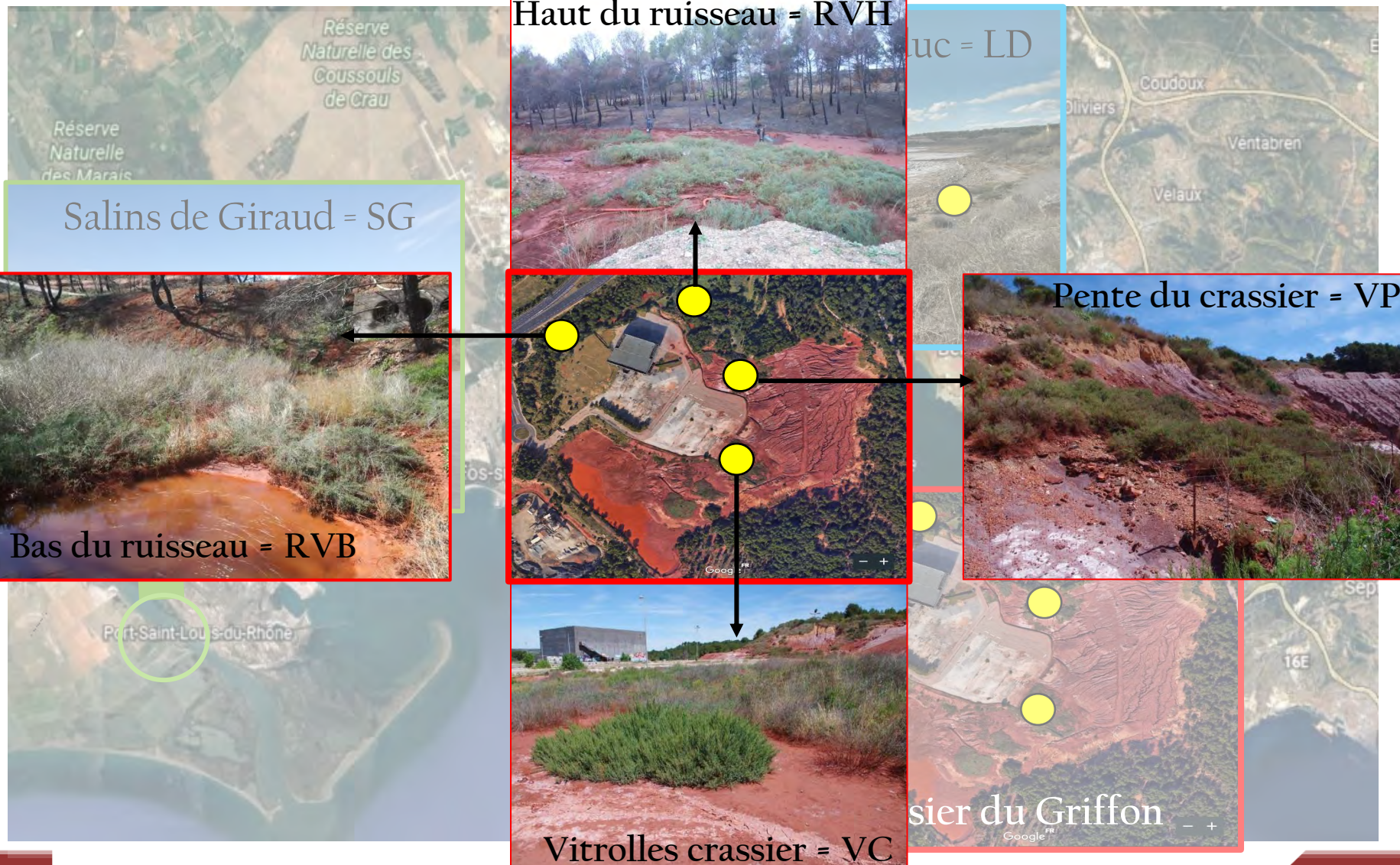


Sites and sampling

- 3 sites -> 6 plots
- *Suaeda vera* plants = 1 plot

Verdier *et al.*, In prep.

How does *Suaeda vera* affect microbial diversity and nutrient status of soils from extreme environment



Sites and sampling

- 3 sites -> 6 plots
- *Suaeda vera* plants = 1 plot

Verdier *et al.*, In prep.

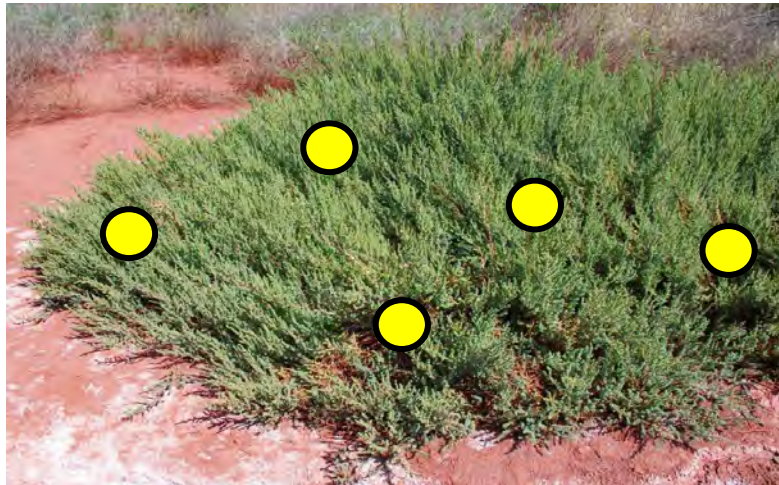


Journées Scientifiques :
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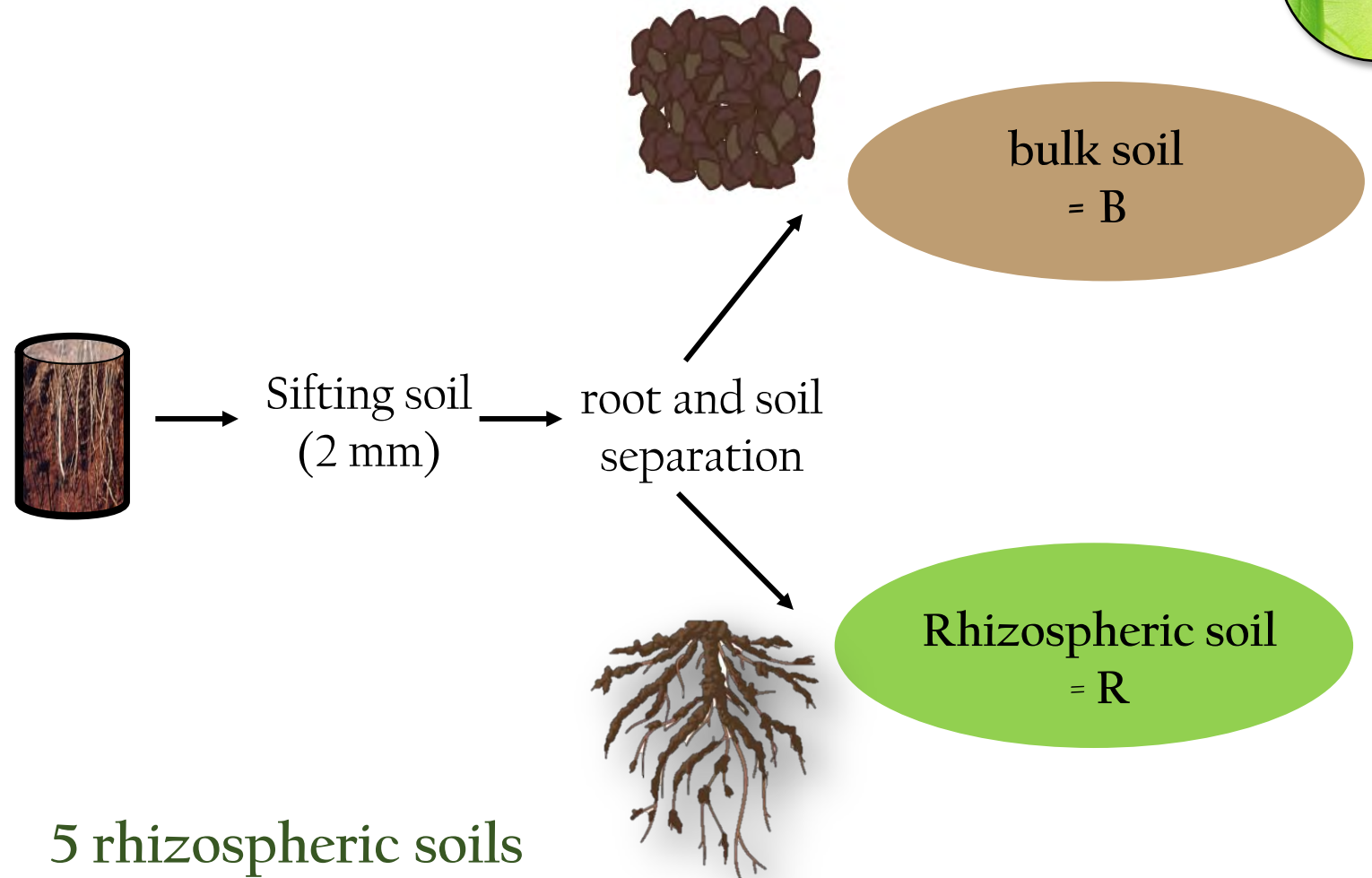
Aix-en-Provence, du 08 au 09 juin 2021



Material and Methods



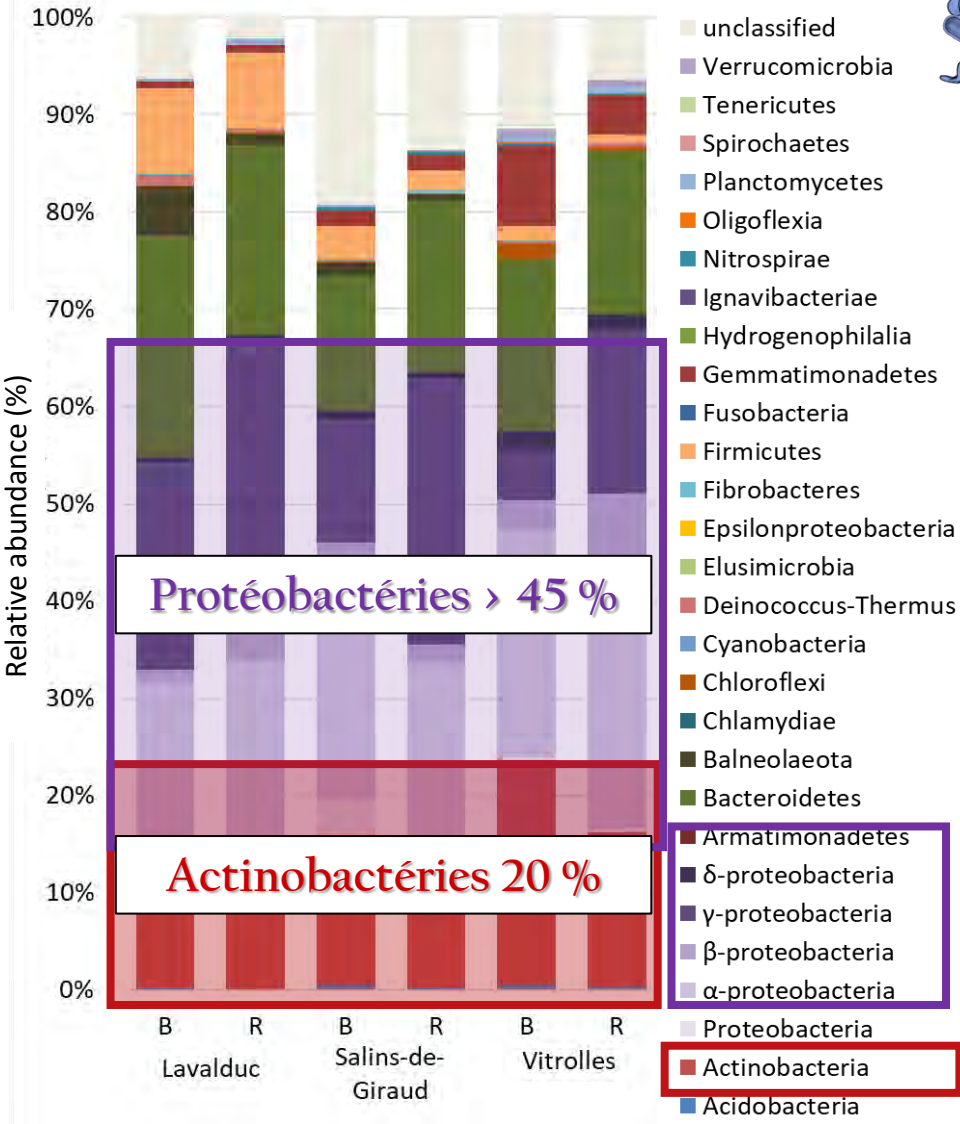
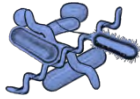
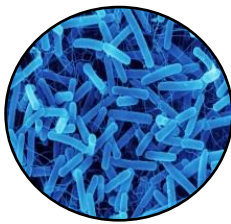
5 samples by plants



5 rhizospheric soils
5 bulk soil

6 Plots

Verdier *et al.*, In prep.

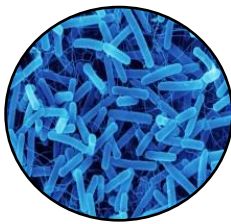


Bacterial phyla

- Similar bacterial phyla
- More *Actinobacteria* at Vitrolles soil
- Bulk and rhizospheric soil dominated by *Proteobacteria*

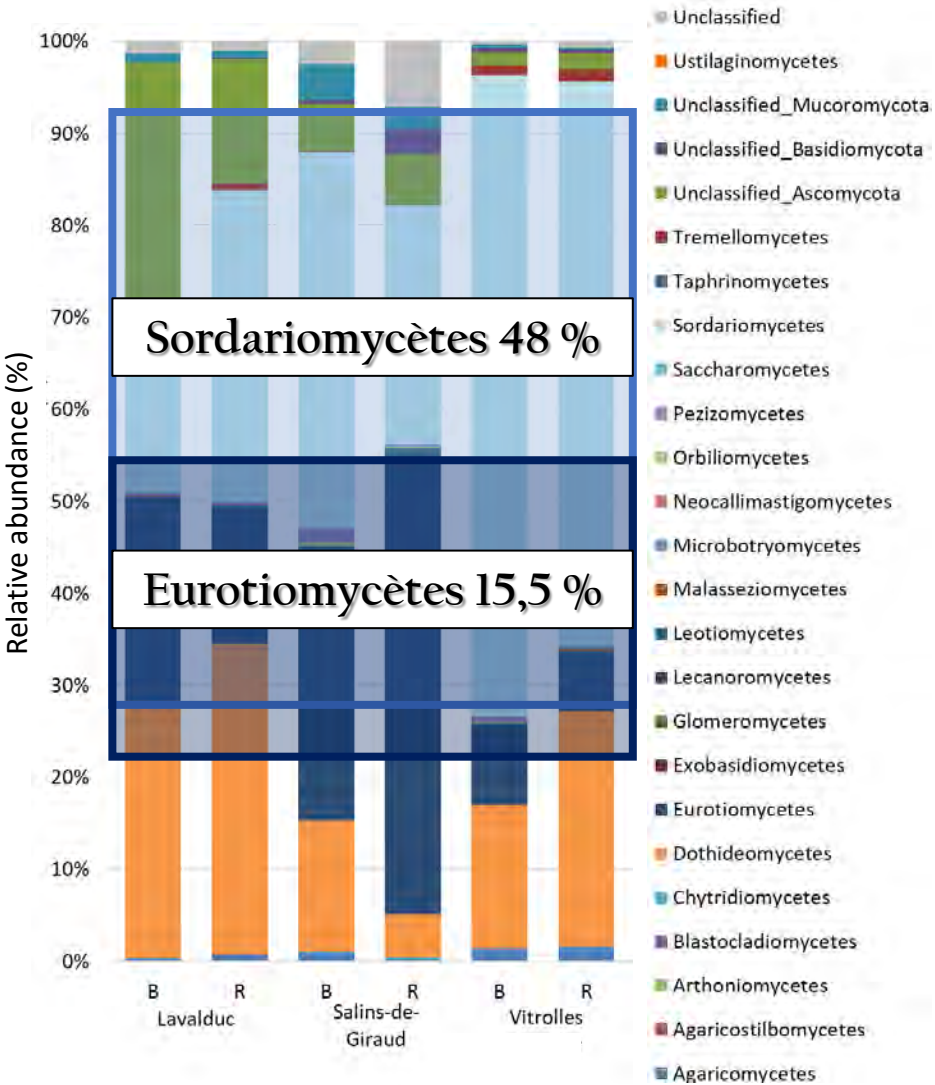
Verdier *et al.*, In prep.





Fungal class

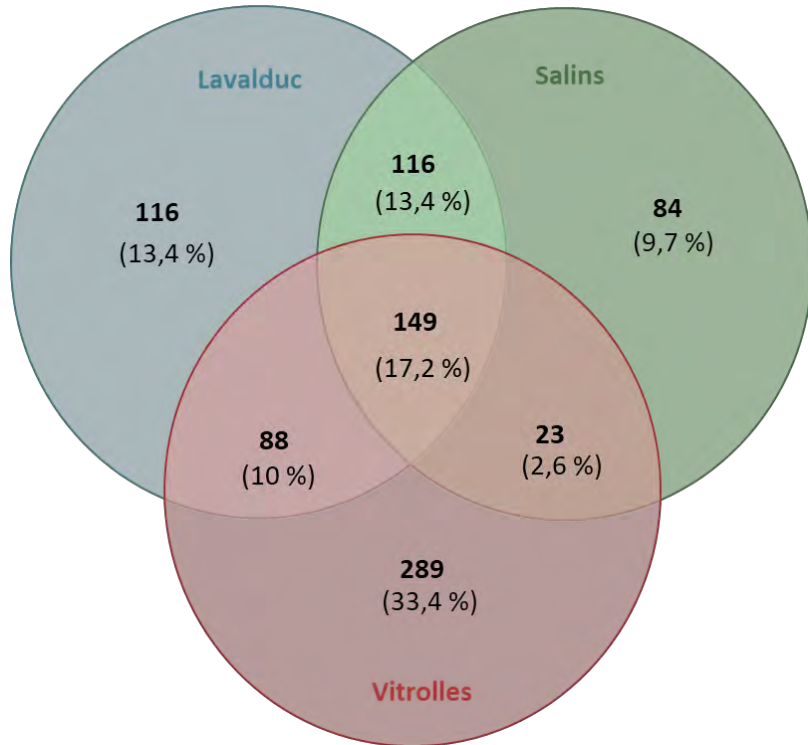
- Fungal phyla dominated by *Ascomycota* in all soils
- *Sordariomycetes* more abundante at Vitrolles soils
- *Eurotiomycetes* dominated in SG and LD soils



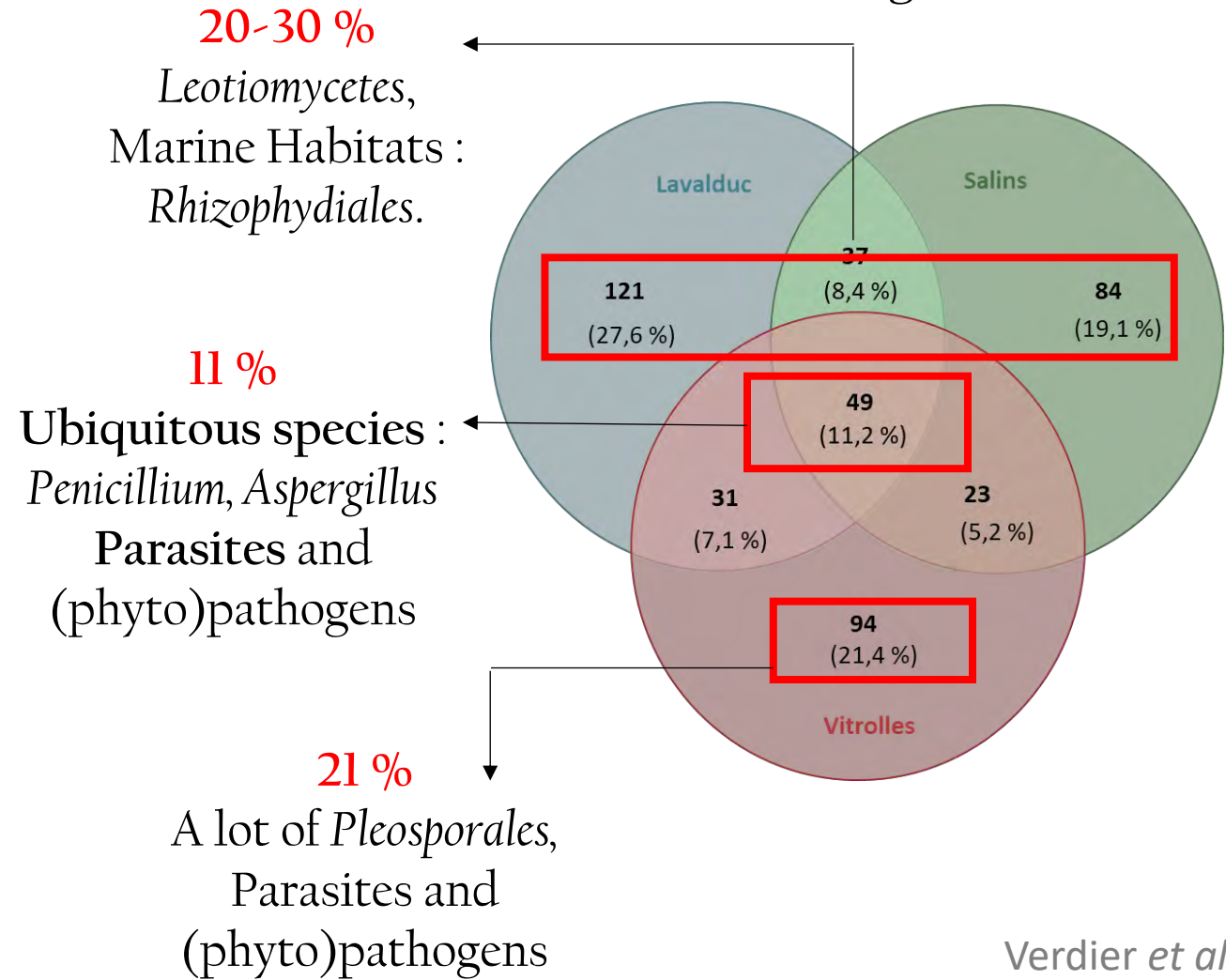
Verdier *et al.*, In prep.



Bacteria

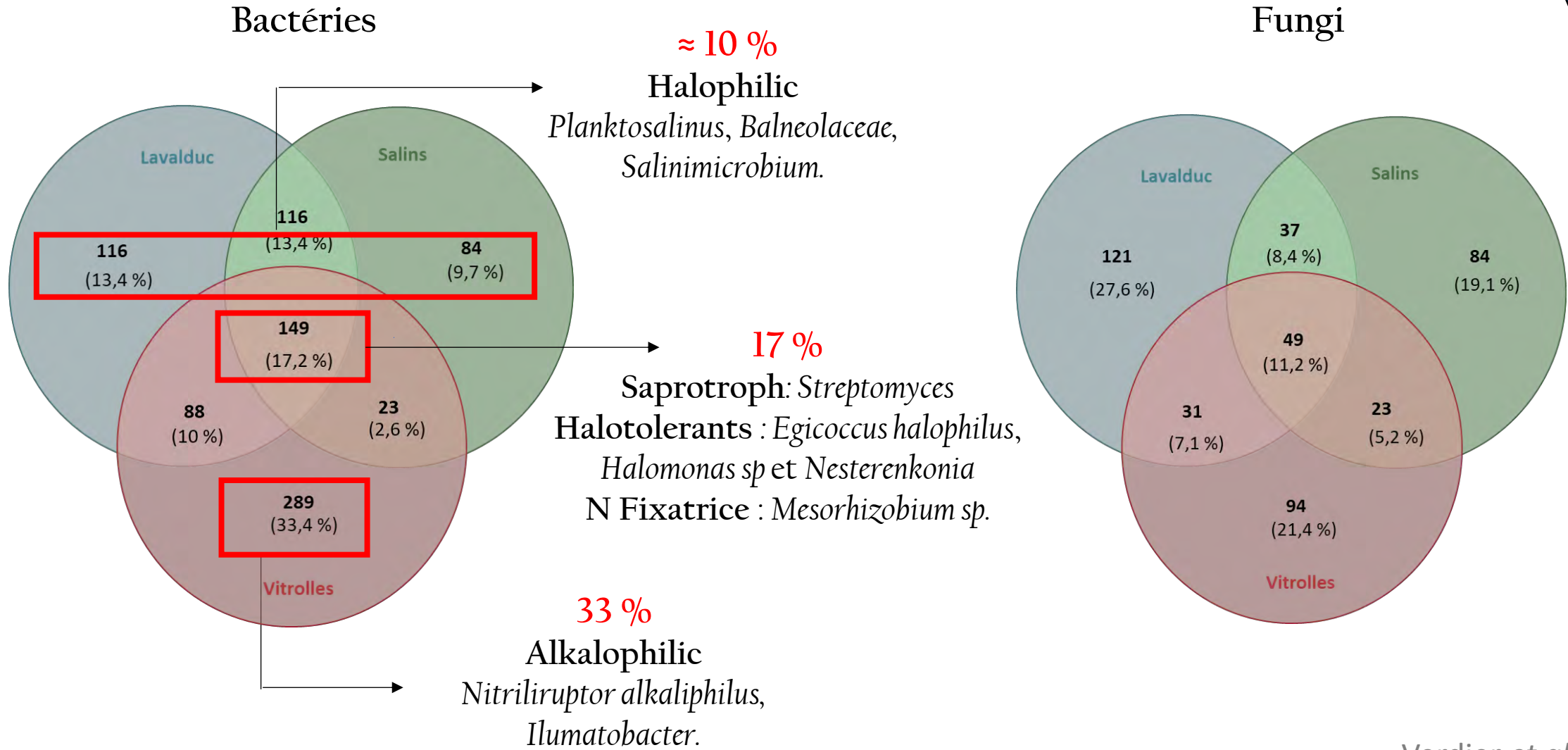


Fungi



Verdier *et al.*, In prep.

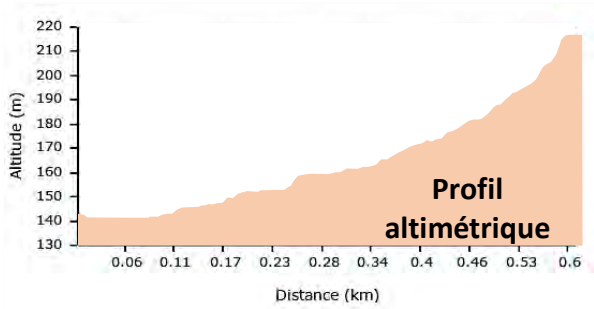




Verdier *et al.*, In prep.



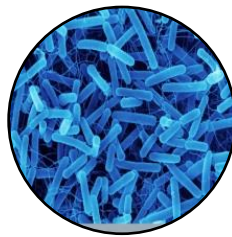
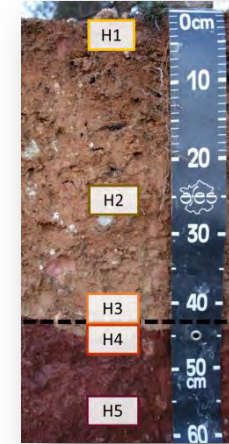
Conclusion



Fill soil with roots show restoration of soil biological functions

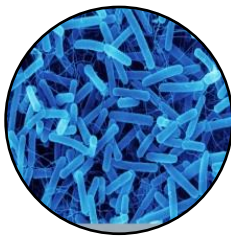
Red mud show more plants and microbial communities adapted to Halo/ alkali soils

Red mud show similar microbial communities to salty soil of salt marsh





- Addition of fill soil to restore biological functions
- And choice of plant species suitable for restoration of bauxite residues



Thanks for your attention

