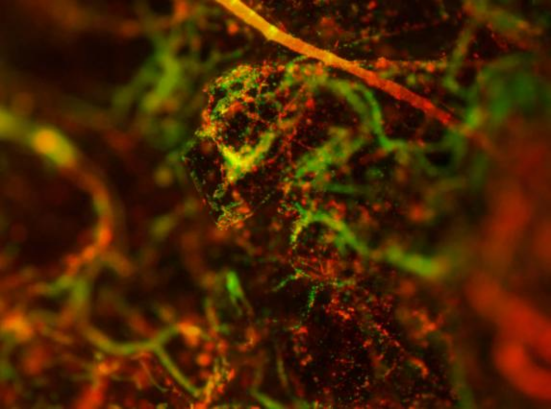
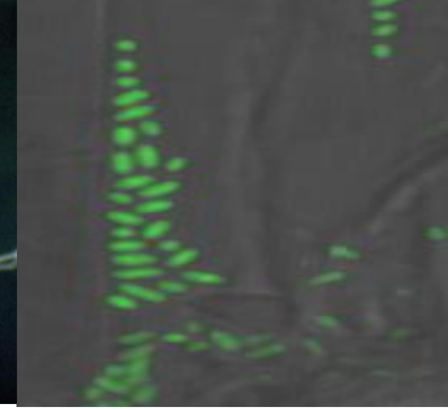


Ecosphère Continentale et Côtière

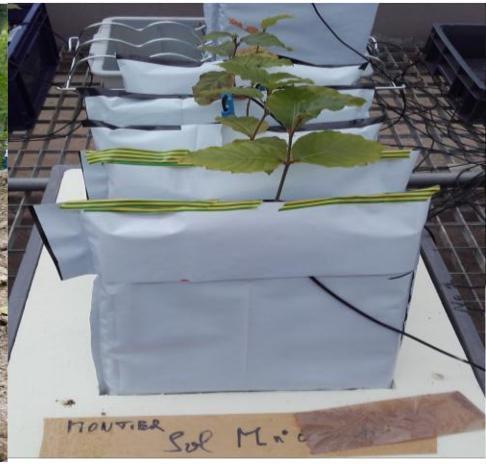


GENOMICS, ECOLOGY AND MOLECULAR MECHANISMS OF MINERAL WEATHERING BACTERIA

Stéphane UROZ and Co

Restitution : AAP 2019 (Microbien) Génomique et écologie du Microbiome de la Minéralosphère dans les sols forestiers (**GéMM**)

Collaborations: MP TURPAULT, P OGER, JH LEVEAU JH, L. PICARD, J. ARMEGAUD, …..



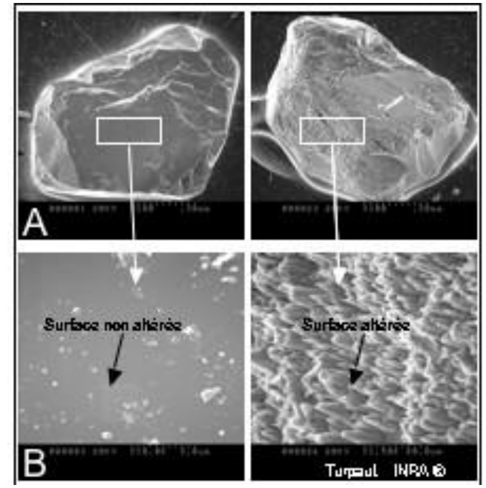
MINERAL WEATHERING

Architectural patrimony

Mineral weathering is everywhere

Physical integrity

Soil minerals



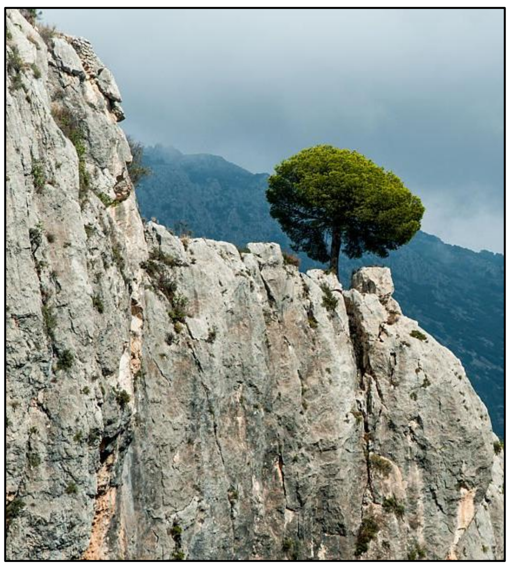
Mineral weathering has an important impact on Man and its environment, by shaping the landscape and determining soil fertility and water quality.

DISTRIBUTION OF FORESTS ON NUTRIENT-POOR SOILS

Forests are mainly located on **nutrient-poor** and/or **rocky** soils, hardly manageable and non amended (except the Landes region in France)

Forest ecosystems => **low-input ecosystems**

Recycling and nutrient access are key processes (especially in harvested forests)



=> Importance of organic matter decomposition and mineral weathering

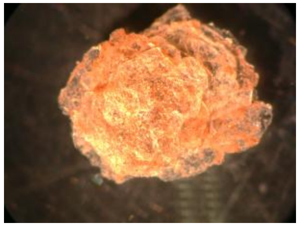
MINERAL WEATHERING : INTRINSIC PROPERTIES x ENVIRONMENTAL FACTORS

Soil : a mosaic of minerals/rocks….Different chemistry…. Different weatherability/reactivity

CHEMISTRY

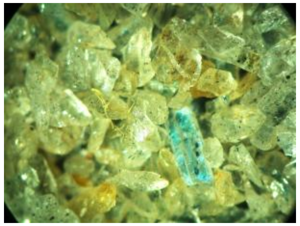
Phlogopite

Si, Al, K, Mg



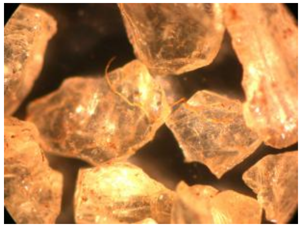
Plagioclase

Si, Al, Ca, Na



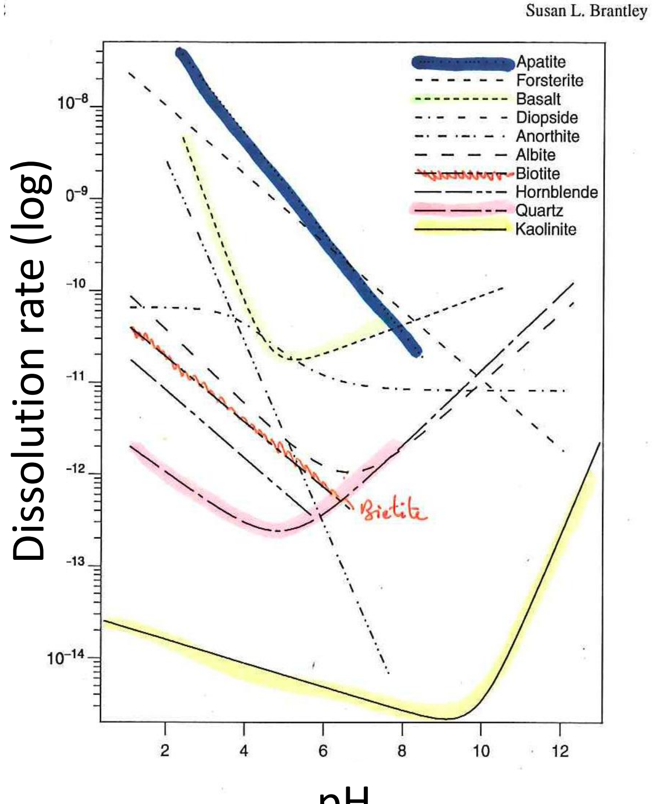
Apatite

Ca, P



WEATHERABILITY

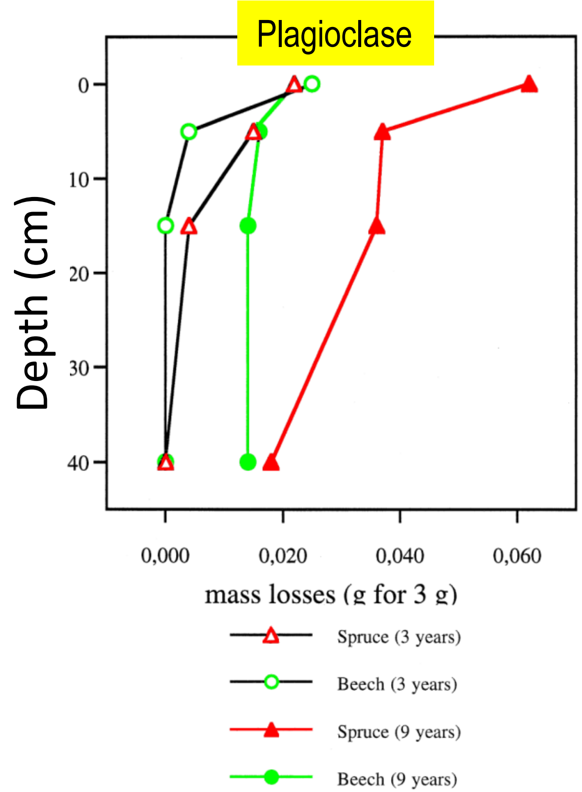
In labo



Brantley, 2003

WEATHERABILITY

In situ



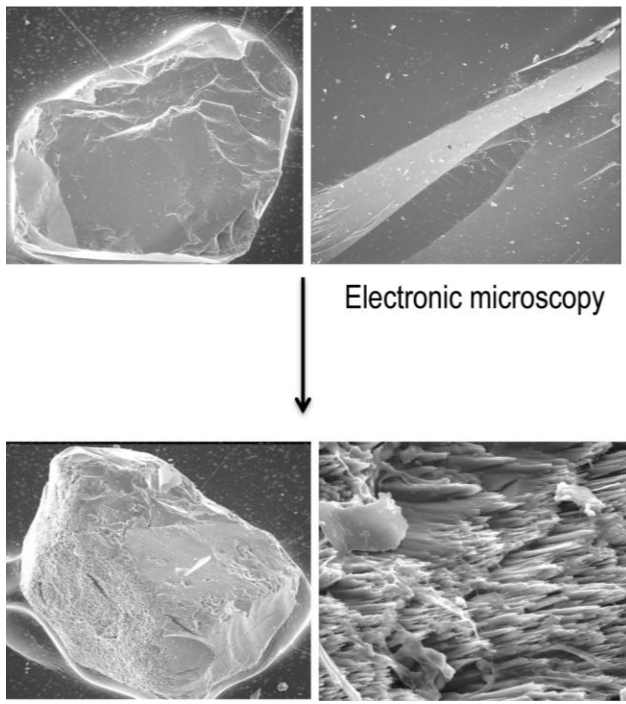
Augusto et al., 2000

MINERAL WEATHERING: SUM of ABIOTIC AND BIOTIC PROCESSES

Forest ecosystems = **low-input ecosystems**
Nutrient access and recycling are key processes
=> Importance of mineral weathering

Abiotic

- Water circulation
- Frost

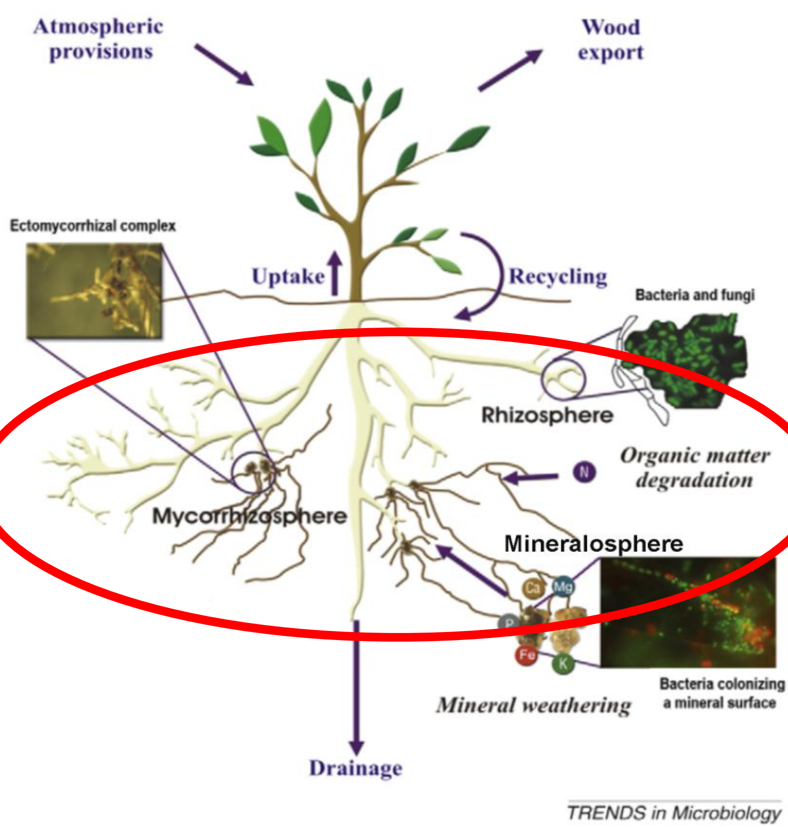


Biotic

Different actors

- Plant roots
- Ectomycorrhizal and saprophytic fungi
- Bacteria

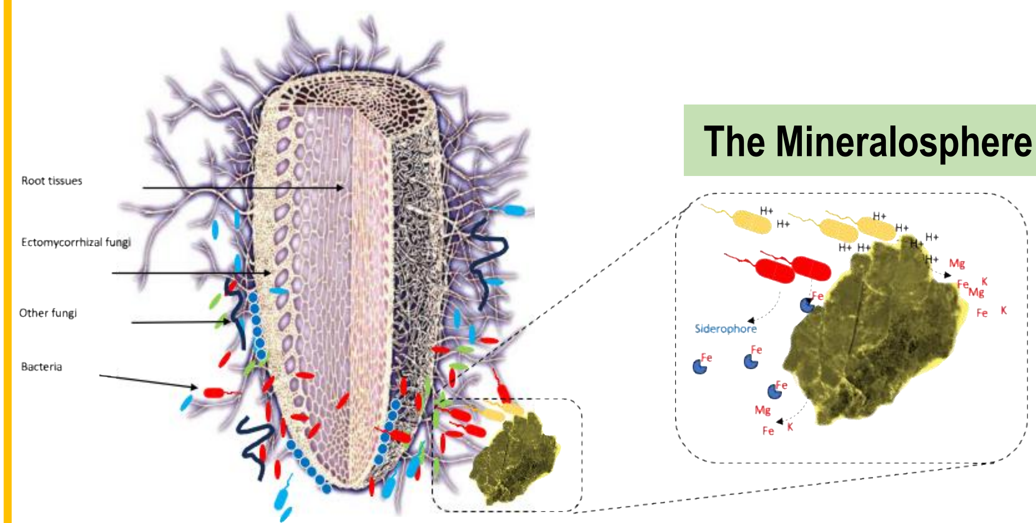
DIFFERENT REACTIVE INTERFACES : ROLE OF THE SOIL MICROBIOTA ?



Uroz et al., 2009

The Mycorrhizosphere

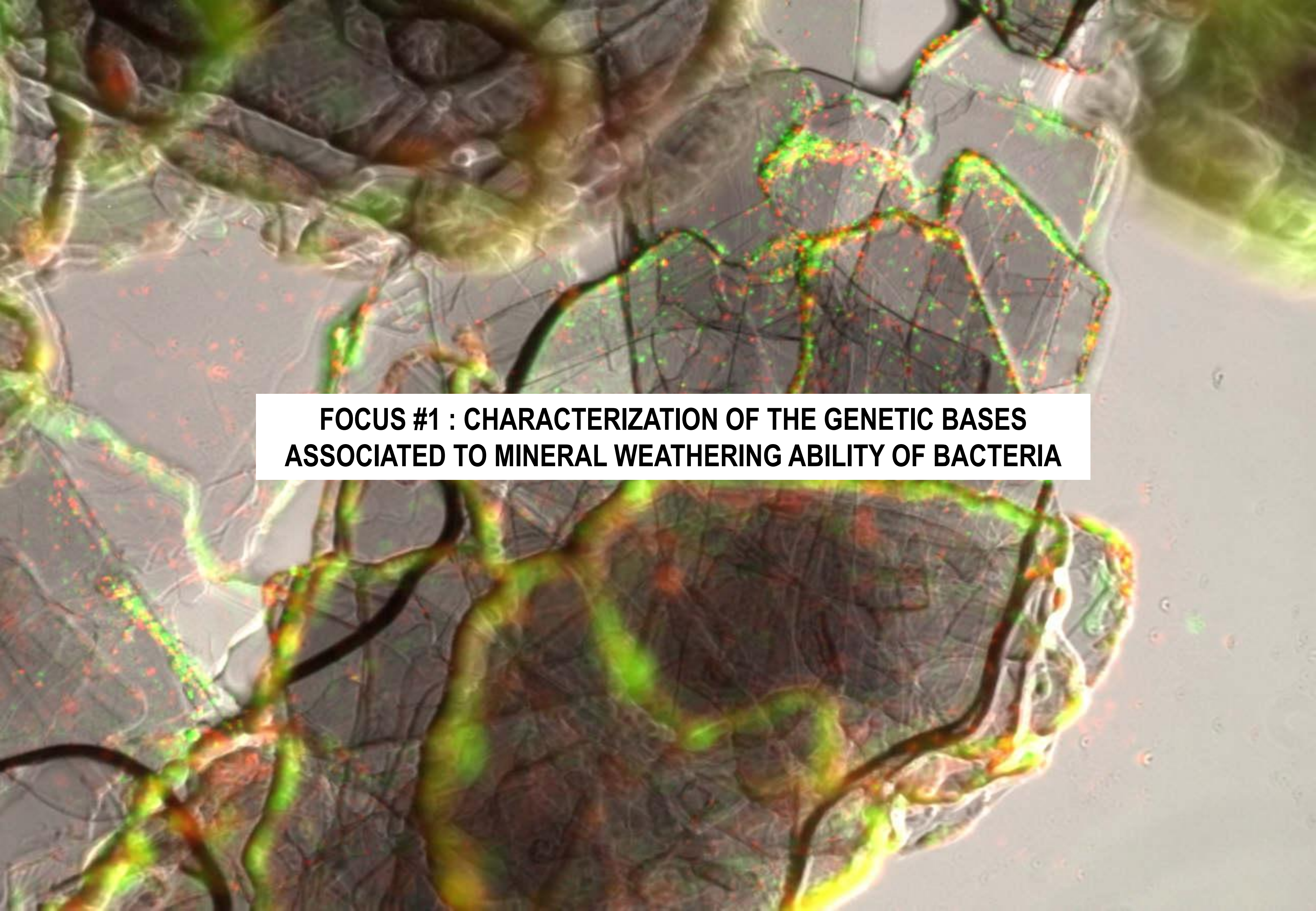
The Mineralosphere



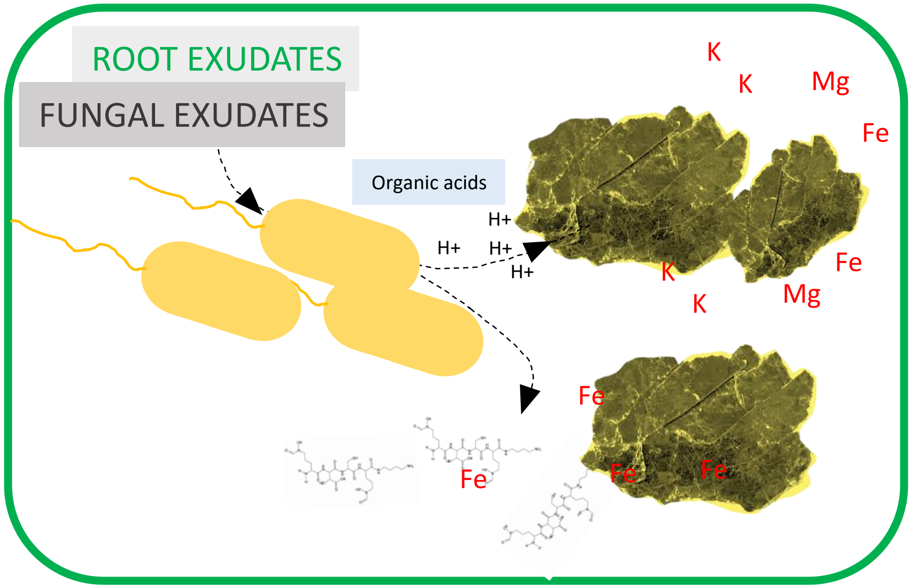
- Metagenomics (shotgun, amplicon-based…)
- Bacterial collections => screening for their mineral weathering ability (biotests)
- Mineralogy and geochemistry

Rhizosphere, mycorrhizosphere, mineralosphere…..all reactive interfaces at geochemical and microbiological levels…..enriched in effective MWe bacteria compared to the surrounding bulk soil

FOCUS #1 : CHARACTERIZATION OF THE GENETIC BASES ASSOCIATED TO MINERAL WEATHERING ABILITY OF BACTERIA



WHAT DO WE KNOW ABOUT THE MWe MECHANISMs ?

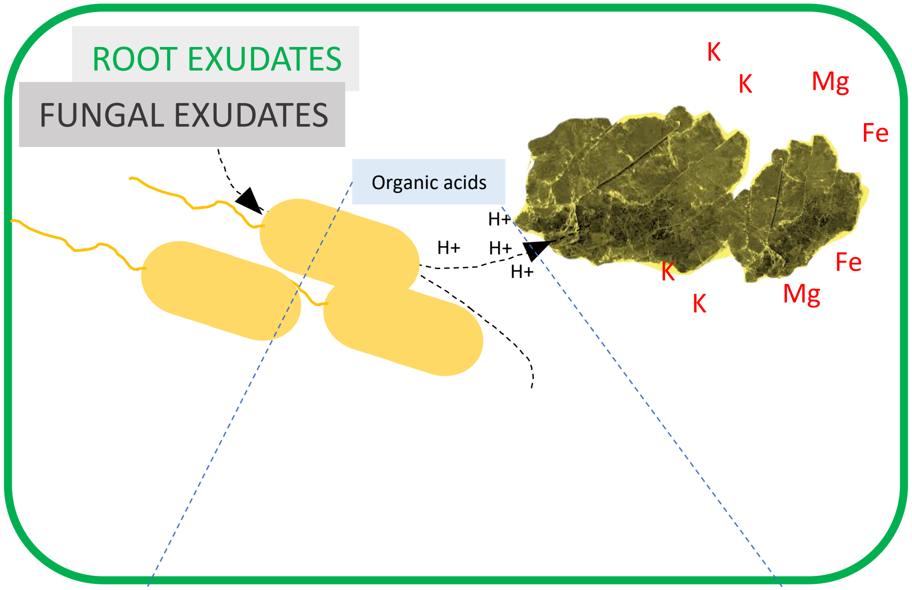


1. A link between the effectiveness at weathering of bacteria and the C substrates (root/fungal exudates) ?

2. A probable link with acidification processes => acidification-driven mineral weathering

3. A probable link with chelation processes => chelation - driven mineral weathering

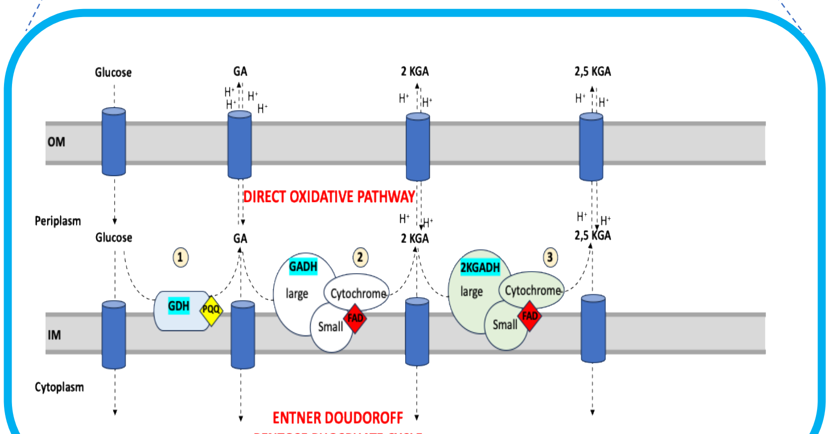
A FOCUS ON THE ACIDIFICATION-DRIVEN MINERAL WEATHERING MECHANISMs ?



1. A link between the effectiveness at weathering of bacteria and the C substrates (root/fungal exudates) ?

2. A probable link with acidification processes => **acidification-driven mineral weathering**

PQQ based glucose dehydrogenase



Goldstein, AH. 1986

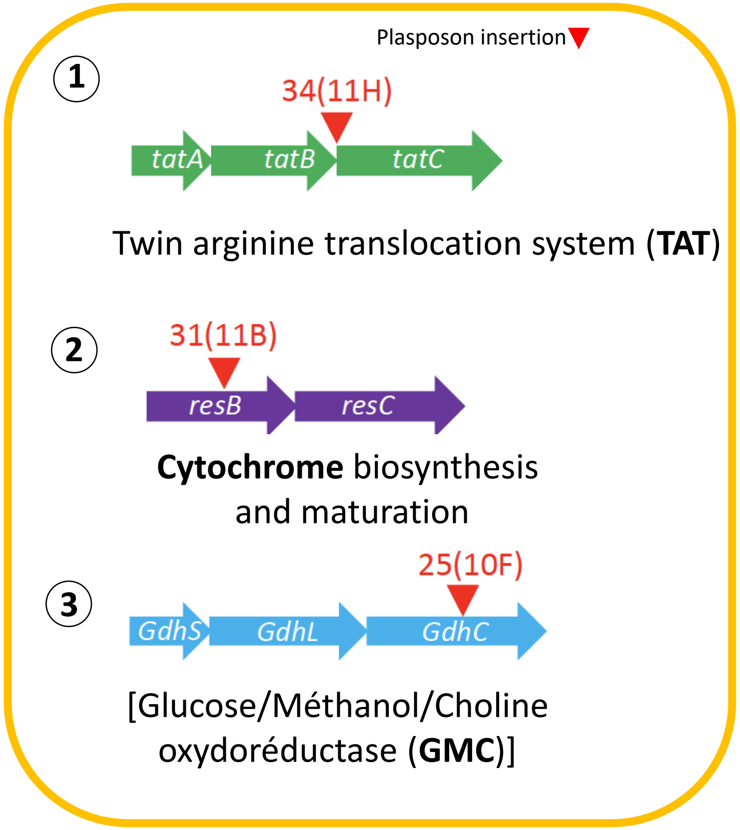
Several taxa (*Caballeronia*, *Collimonas*) from forest soil do not possess the expected genes ????

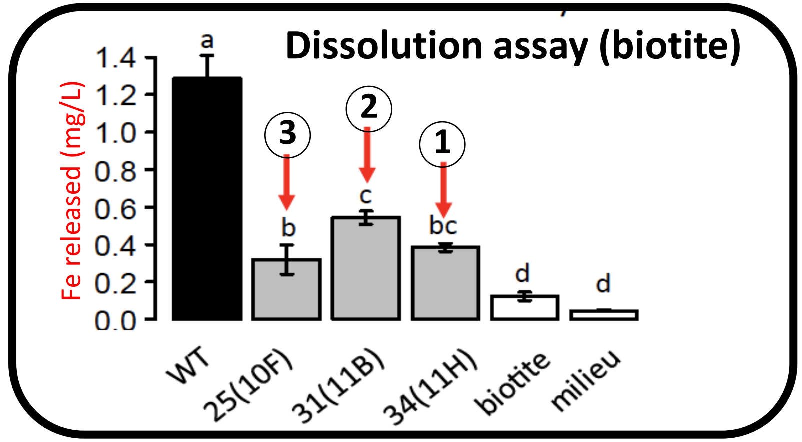
How to develop molecular markers to investigate this functional group ?

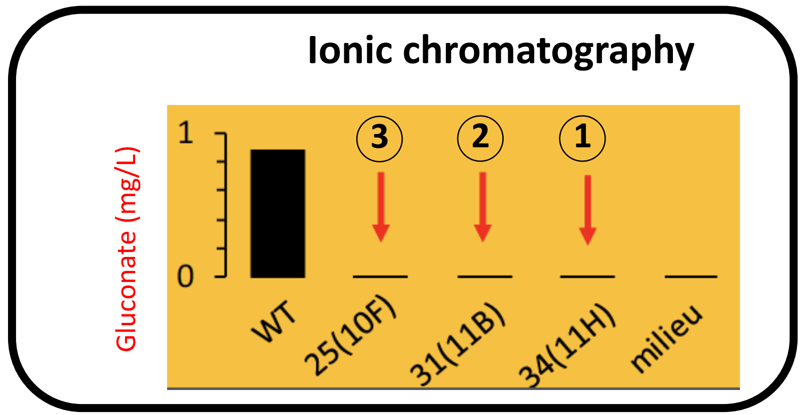
COLLIMONAS : AN INDICATOR GENUS OF NUTRIENT-POOR FOREST SOILs

Random mutagenesis

3 independent genomic regions in strain Collimonas pratensis PMB3(1)





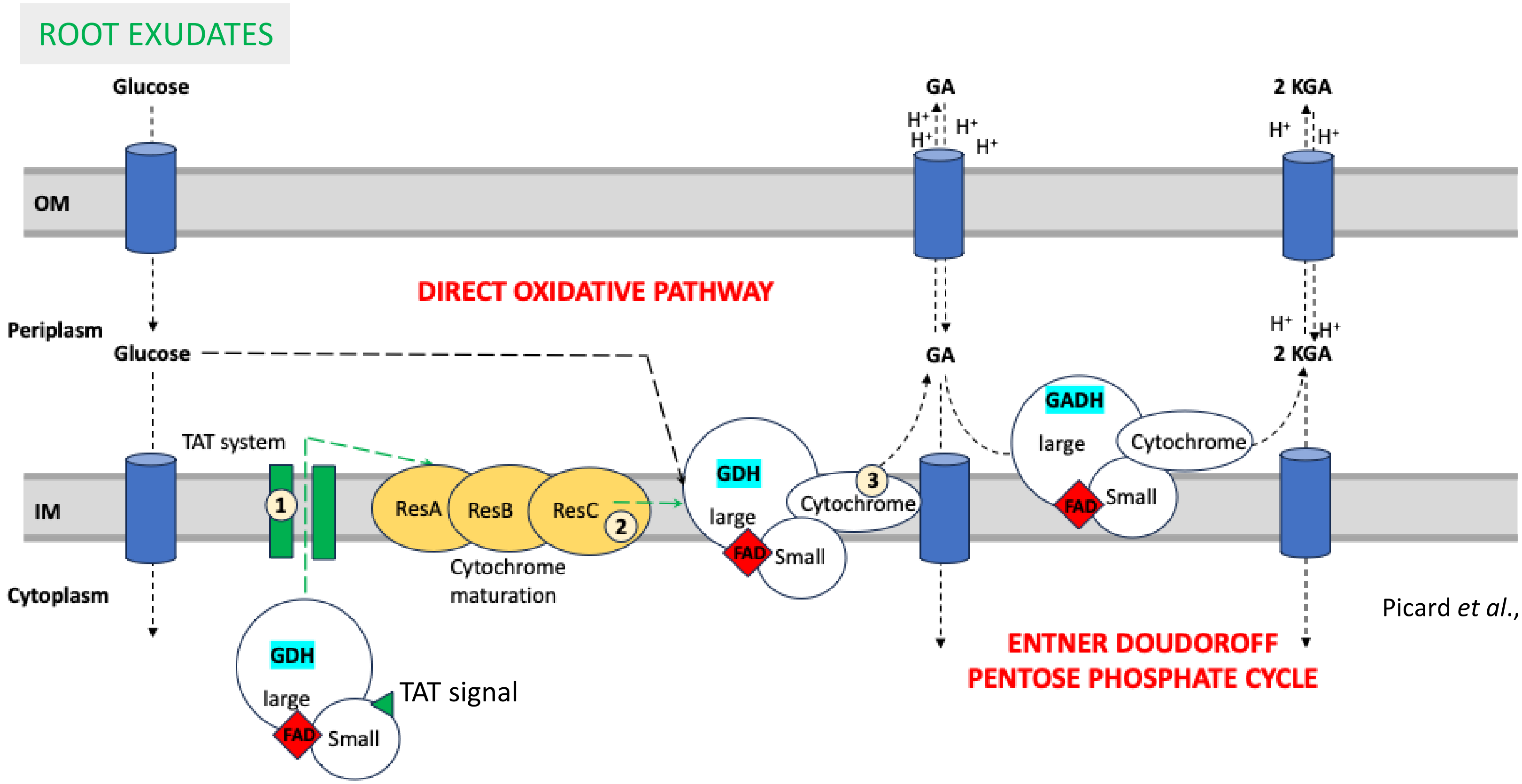


Identification of a new marker gene linked to a non described enzyme (GMC oxidoreductase => convergent evolution) conferring the MWe ability. What's about its conservation ?

Picard *et al.*, 2021

COLLIMONAS : AN INDICATOR GENUS OF NUTRIENT-POOR FOREST SOILs

Acidification-based MWe mechanism developed by Collimonas

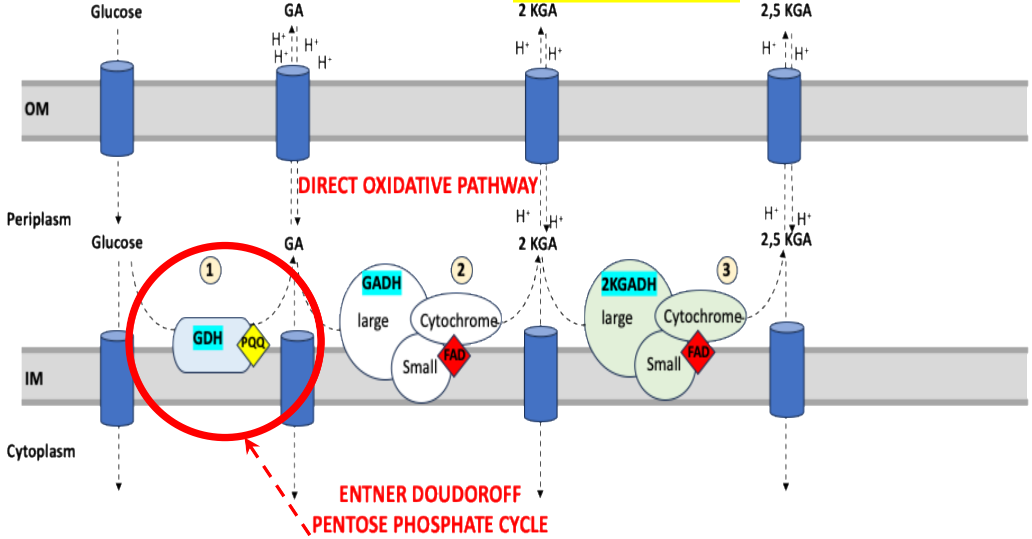


Picard *et al.*, 2021

Identification of a new marker gene linked to a non described enzyme (GMC oxidoreductase => convergent evolution?) conferring the MWe ability
What's about its conservation ?

A SAME PATHWAY : TWO MECHANISMS

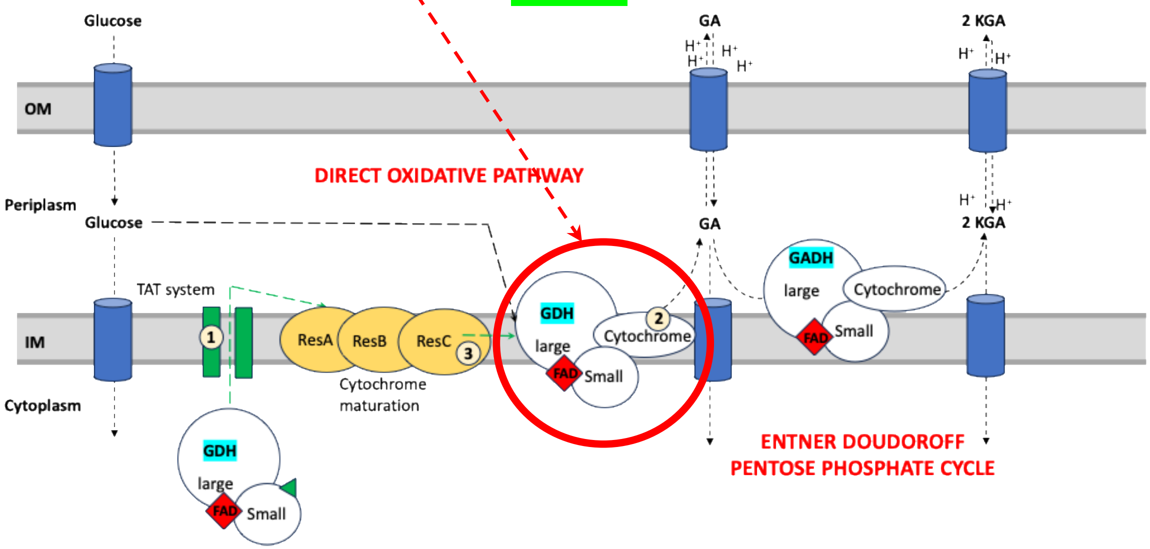
Since the 80's => bacteria from **agricultural** context



PQQ dependent GDH (monomeric)

Widely used in DNA based microbial ecology studies....

Since 2021 => bacteria from **forest** context



FAD dependent GDH (heterotrimeric)

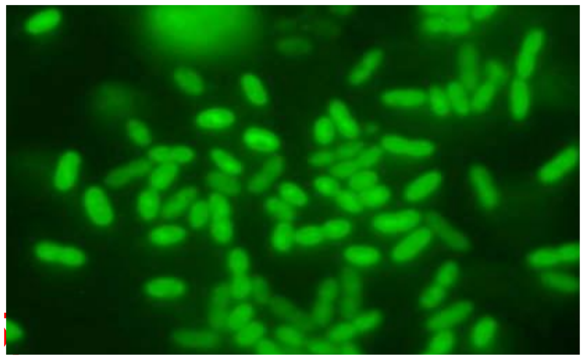
(GMC oxidorecductase)

FOCUS #2 : EVIDENCE OF REGUATIONS ASSOCIATED TO MINERAL WEATHERING AND MINERAL/BACTERIA INTERACTIONS

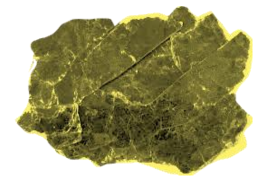
RESPONSE OF *CABALLERONIA MINERALIVORANS* TO NUTRIENT DEPLETION AND PRESENCE OF MINERAL IN ITS VICINITY

OMIC approach

Caballeronia mineralivorans PML1(12)



Short-term incubation with biotite



Biotite $[(Si_3Al_1)(Fe^{3+}_{0.12}Fe^{2+}_{0.61}Mg_{2.06}Mn_{0.02}Ti_{0.13})\ K_{0.88}Na_{0.06}O_{10}\ (OH_{0.98}F_{1.02})]$

Q1:Impact of nutrient deficiency ?

Q2: Impact of presence/absence of a mineral ?

Q3: Impact of the C substrate ?

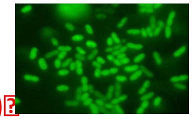
a) Solution analyses (chemistry)
b) proteome
c) transcriptome

RESPONSE OF *CABALLERONIA MINERALIVORANS* TO NUTRIENT DEPLETION AND PRESENCE OF MINERAL IN ITS VICINITY

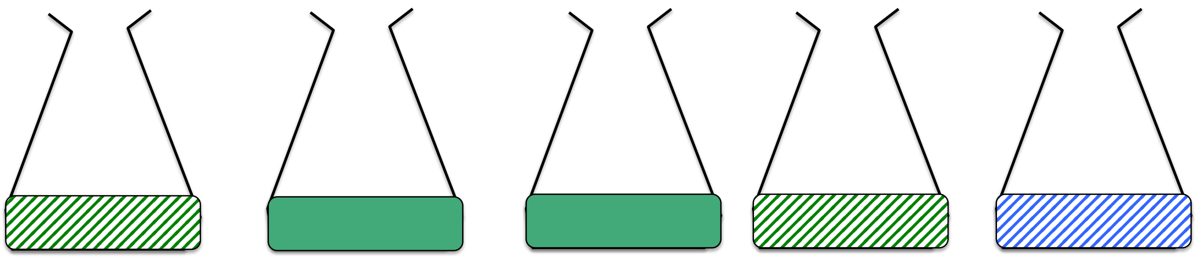
OMIC approach

Presence/absence of a mineral ?
Nutrient deficiency ?

Caballeronia mineralivorans PML1(12)



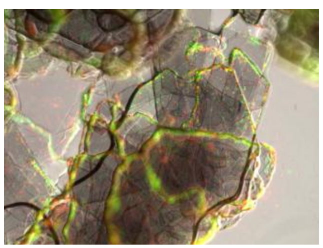
Abiotic | **+ PML1(12)**



n=4/treatment

Incubation 20h (25°C + shaking)

Abiotic	Abiotic	+ PML1(12)	+ PML1(12)	+ PML1(12)
BHm medium devoid of Mg/Fe + Glucose + biotite	BHm medium devoid of Mg/Fe + Glucose	BHm medium devoid of Mg/Fe +Glucose (GB)	BHm medium devoid of Mg/Fe +Glucose + biotite (GBwB)	BHm medium devoid of Mg/Fe + Mannitol + biotite (MBwB)



1 Deficiency effect

2 Mineral effect

3 C source effect

Biotite $[(Si_3Al_1)(Fe^{3+}_{0.12}Fe^{2+}_{0.61}Mg_{2.06}Mn_{0.02}Ti_{0.13})\ K_{0.88}Na_{0.06}O_{10}\ (OH_{0.98}F_{1.02})]$

a) Solution analyses (chemistry) ; b) proteome and c) transcriptome

CHEMICAL ANALYSES OF THE SOLUTION

a) Solution analyses (chemistry)

i) Inorganic nutrients released in solution

Conditions			Eléments chimiques mesurés dans la solution						
Substrat	*Biotite*	*Bactéries*	K	Na	P	Ca	Mg	Fe	Al
			Concentrations en mg/L						
-	-	-	45.53±1.60	36.55±0.13	32.14±0.12	0.81±0.01	0.01±0.00[a]	0.06±0.00[a]	0.01±0.00[a]
-	+	-	49.27±1.11	39.76±0.01	34.56±0.78	2.39±0.34	1.21±0.12[b]	0.34±0.09[b]	0.19±0.06[b]
Mannitol	+	+	47.82±1.03	35.47±0.01	29.57±0.97	2.49±0.39	1.13±0.12[b]	0.37±0.12[b]	0.40±0.07[c]
Glucose	+	+	48.99±0.34	35.70±0.00	30.71±0.28	2.81±0.02	1.47±0.00[c]	1.54±0.01[c]	0.75±0.00[d]
Glucose	-	+	45.52±1.27	36.54±0.01	32.17±1.20	0.81±0.33	0.01±0.01[a]	0.06±0.04[a]	0.01±0.01[a]

ii) Organic acids released in solution

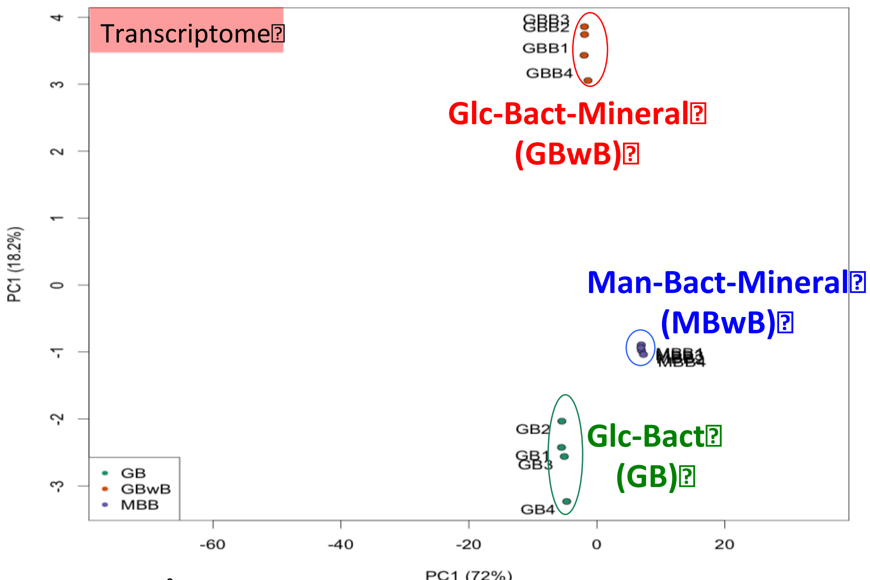
Conditions			pH	Acides organiques détectés					
Substrat	Biotite	Bactéries		Gluconate	Pyruvate	Acétate	Oxalate	Formate	Lactate
					Concentrations en mg/L				
-	-	-	6.49±0.00[a]	nd	nd	nd	nd	nd	nd
-	+	-	6.48±0.01[a]	nd	nd	nd	nd	nd	nd
Mannitol	+	+	4.03±0.03[b]	3.62 [a]	55.83	0.26	0.11	0.43	nd
Glucose	+	+	2.77±0.02[c]	586 [b]	69	1	2	2	1
Glucose	-	+	2.78±0.02[c]	874 [c]	59	2	1	1	1

- Significant increase of the dissolution (MWe measurable after only 20h incubation time)

- Gluconic acid as main acid measured / significant difference between treatments with and without mineral (? Particular regulation mechanism ? Metabolic switch ?)

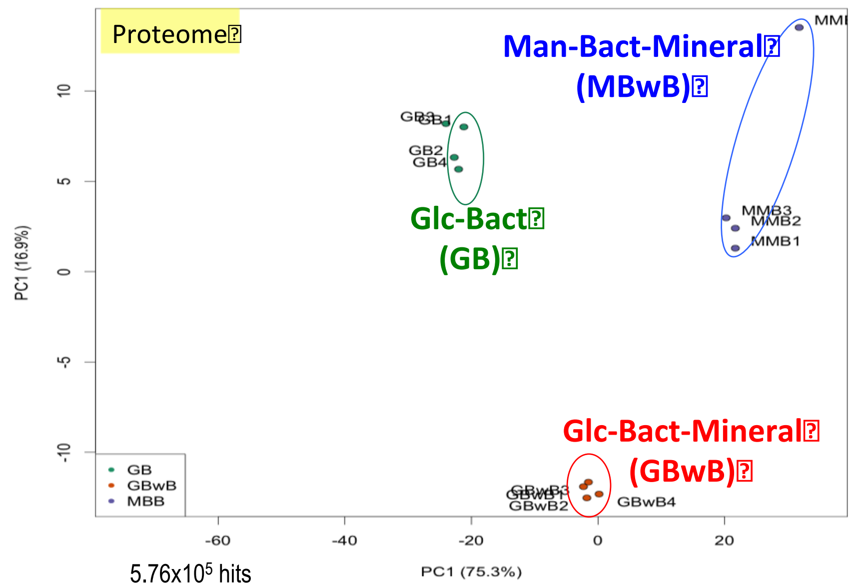
Multivariate analyses using all the genes/proteins after DEseq2 analyses

RNA seq (strand specific / Ribodepleted)



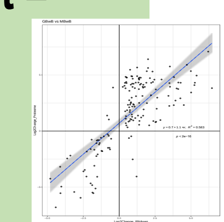
3.61×10^8 hits
Distributed on the 9195 CDS of the genome

Proteome (tryspin / NH4CO3)



5.76×10^5 hits
Distributed on 2408 proteins of the genome

- Difference in sequencing coverage linked to the method (proteomic lower)
- Differentiation of the transcriptome and proteome of PML1(12) according to the treatment = regulation ? (Details: GB vs GBwB: differentially expressed genes (DEG) = 1153 and differentially produced proteins (DPP) =41 ; GBwB vs MBwB: 1241 DEG vs 198 DPP)
- Validation of more than 200 genes (due to significant correlations between RNA/ protein)

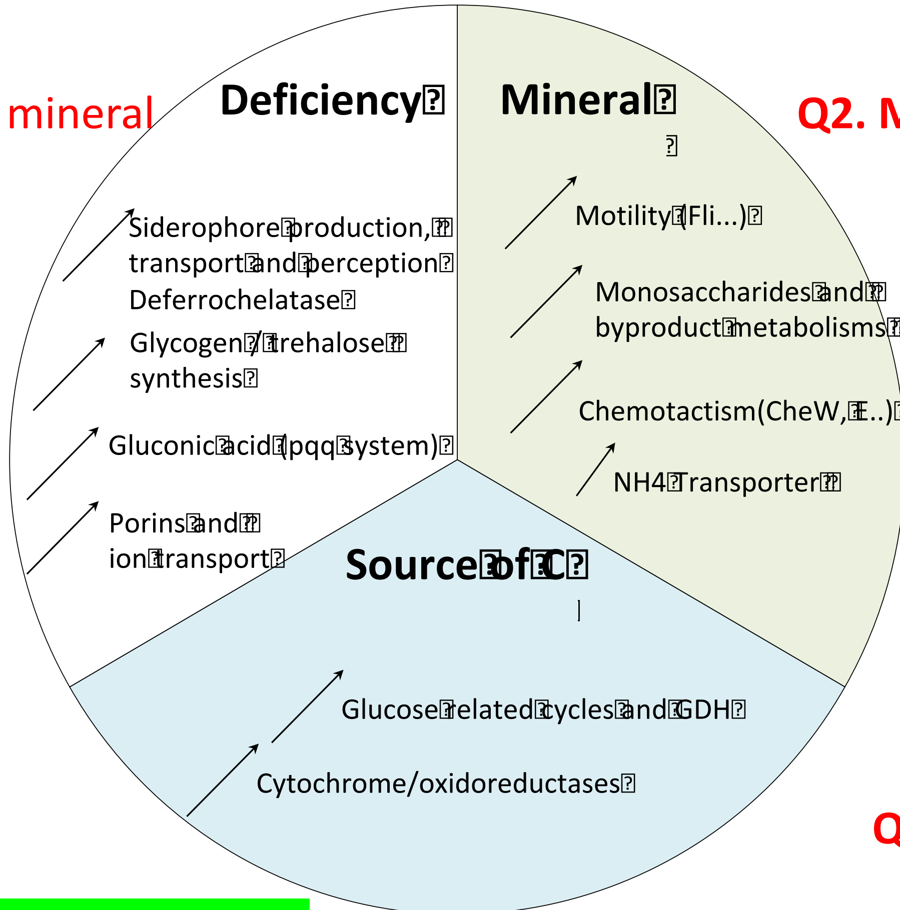


A DIFFERENTIAL RESPONSE ACCORDING TO MINERAL PRESENCE, NUTRIENT DEPLETION AND THE CARBON SUBSTRATE METABOLIZED

OMIC approach

Q1. Depleted > mineral

Q2. Mineral > Depleted



Q3. Glucose > Mannitol

Caballeronia mineralivorans PML1(12)

- **Bacteria adjust their physiology according to nutrient availability, presence of mineral and the type of C source**
- **Identification of candidate genes >> hypothetical or validated (ex: GMC oxidoreductases)**

(=> Importance of the mutagenesis step to identify the genes and assign them a function)

Uroz *et al.*, 2020
Picard et al. 2021

EVIDENCE OF SELECTIVE EFFECT OF MINERALS and NUTRITIVE CATIONS

CONCLUSIONS AND PERSPECTIVES

Ecosphère Continentale et Côtière
INRAE
UNIVERSITÉ DE LORRAINE
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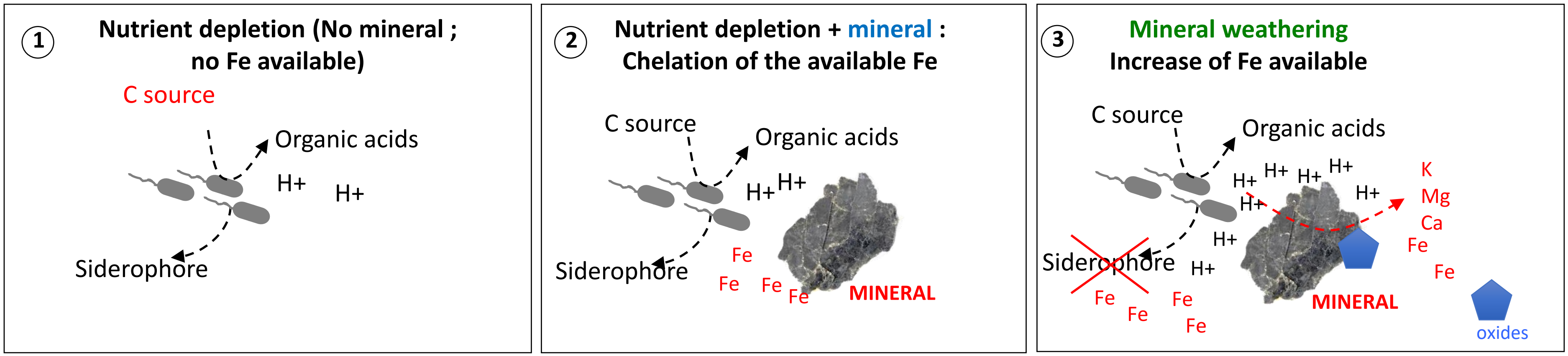
Regulation at the cellular level => genetic bases of MWe



- Identification of **new genes** associated to the MWe ability (GMC oxidoreductases and associated genes) => need a complete biochemical characterization and substrate specificity
- Evidence of **convergent evolution** (GMC vs PQQ-GDH) => need to investigate the distribution, and conservation of these enzymes
- Characterization of a **new siderophore** and its role in the MWe process => need to better understand the regulations and feedbacks effects occurring during the interactions b/m
- Evidence that **minerals are reactive** components to consider in environmental microbiology as stated using OMICs => need to develop more interdisciplinary research