Microbial diversity in hydrogen-rich and high-pH waters of hyperalkaline springs (New Caledonia)

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Colloque annuel du GDR CNRS HydroGEMM - 2023 15-17 nov. 2023, Université de Pau et des Pays de l'Adour

Serpentinization and associated reactions

H_2O

Serpentinization

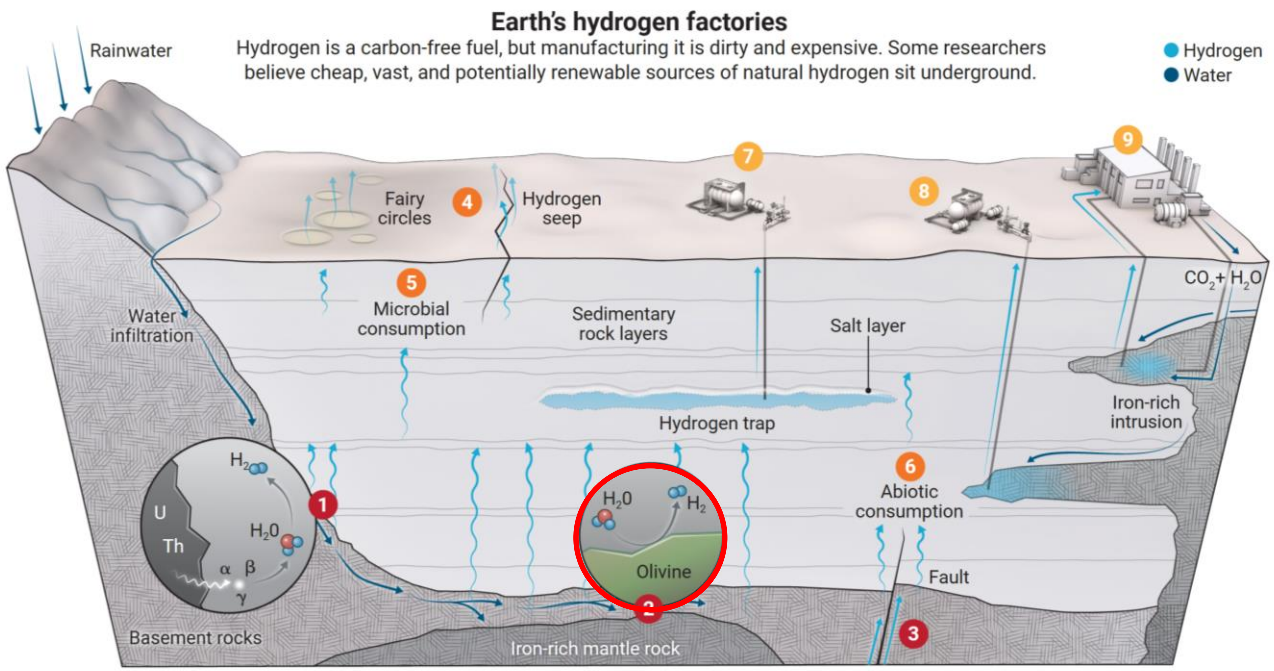
+ Brucite + Magnetite + H_2

Mantle rocks (peridotite)

CO_2 (mantle)

Fischer-Tropsch-Type/Sabatier /formation of abiotic formate

CH_4 + C_nH_{2n+2} + $HCOO^-$



(GRAPHIC) C. BICKEL/*SCIENCE*; (DATA) GEOFFREY ELLIS/USGS

Serpentinization

Serpentinization and associated reactions

Subsurface microbial communities sustained by serpentinization

Serpentinization

H_2O

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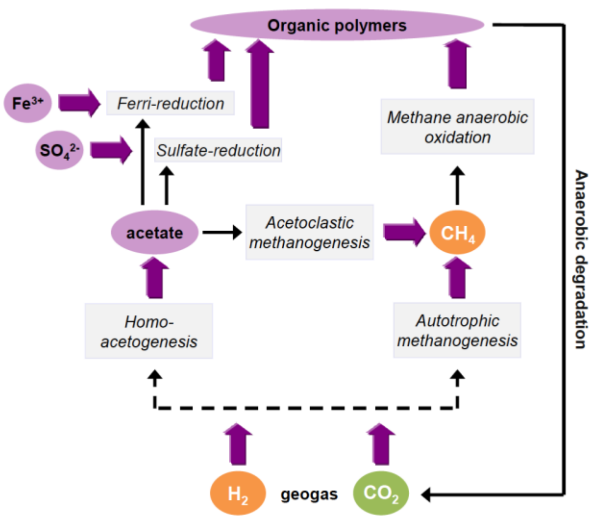
$CH_4 + C_nH_{2n+2} + HCOO^-$

Trends in Microbiology

Hydrogen-driven subsurface lithoautotrophic microbial ecosystems (SLiMEs): do they exist and why should we care?

TRENDS in Microbiology Vol.13 No.9 September 2005

Kenneth H. Nealson[1,2], Fumio Inagaki[2] and Ken Takai[2]



Sources of energy and carbon sources for microorganisms living in subsurface environments (in the absence of light)

Autotrophic methanogenesis:

$HCO_3^- + 4H_2 + H^+ \rightarrow CH_4 + 3H_2O$

methane

Homoacetogenesis:

$2HCO_3^- + 4H_2 + H^+ \rightarrow CH_3COO^- + 4H_2O$

acetate

Serpentinization and associated reactions

Subsurface microbial communities sustained by serpentinization

H_2O **Serpentinization**

+ Brucite + Magnetite + H_2

Mantle rocks (peridotite)

CO_2 (mantle)

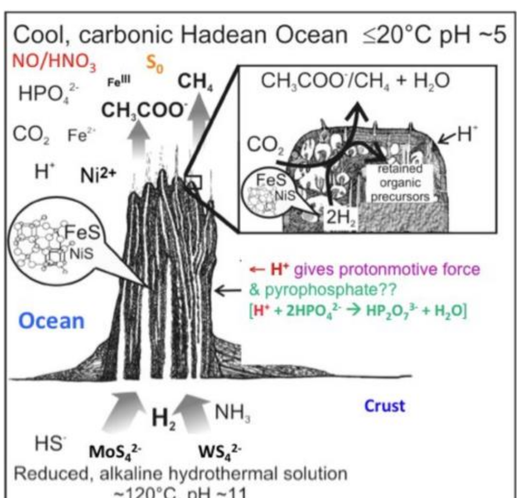
Fischer-Tropsch-Type/Sabatier /formation of abiotic formate

CH_4 + C_nH_{2n+2} + HCOO-

Geobiology
Geobiology (2010), 8, 355–371 DOI: 10.1111/j.1472-4669.2010.00249.x

Serpentinization as a source of energy at the origin of life

M. J. RUSSELL,[1] A. J. HALL[2] AND W. MARTIN[3]

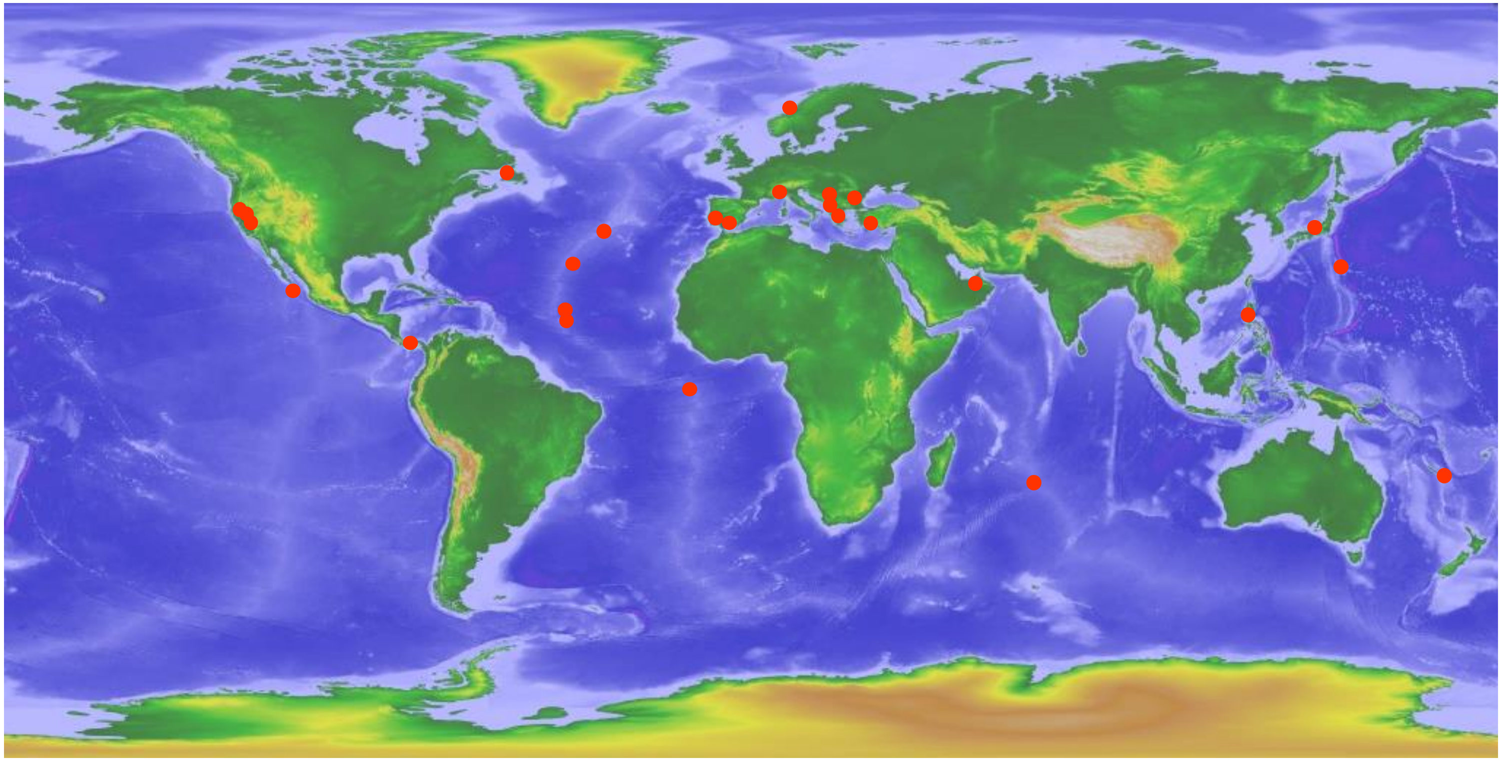


Research interests in microbiology:

- Origin and limits of life on Earth or other planets?
- Biotechnologies of extremophiles (pH>10)
- CO_2 Storage
- Natural H_2 production

Microbial ecosystems associated with serpentinization

Hydrothermal systems: windows into the subsurface



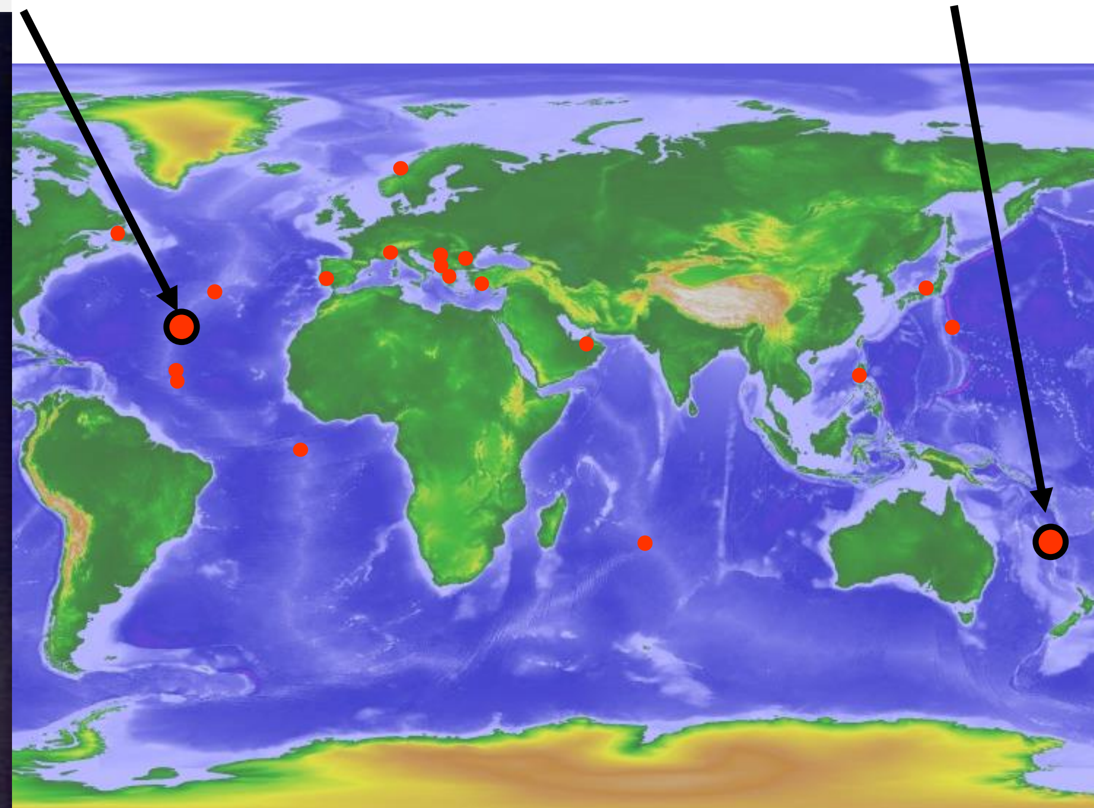
Microbial ecosystems associated with serpentinization

Hydrothermal systems: windows into the subsurface

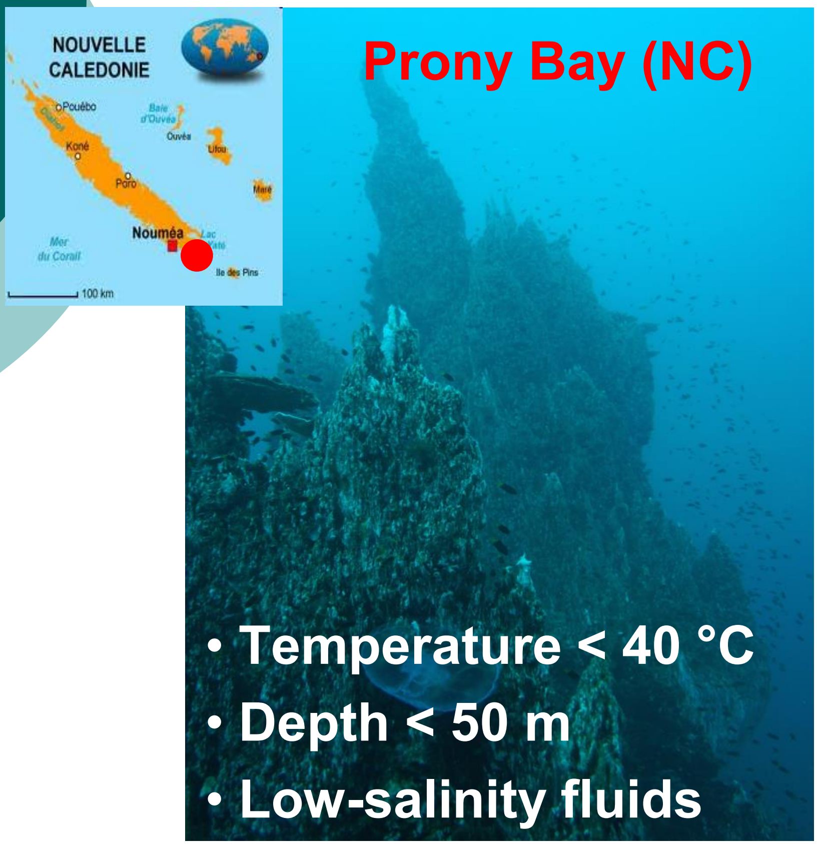
Lost City (MAR)

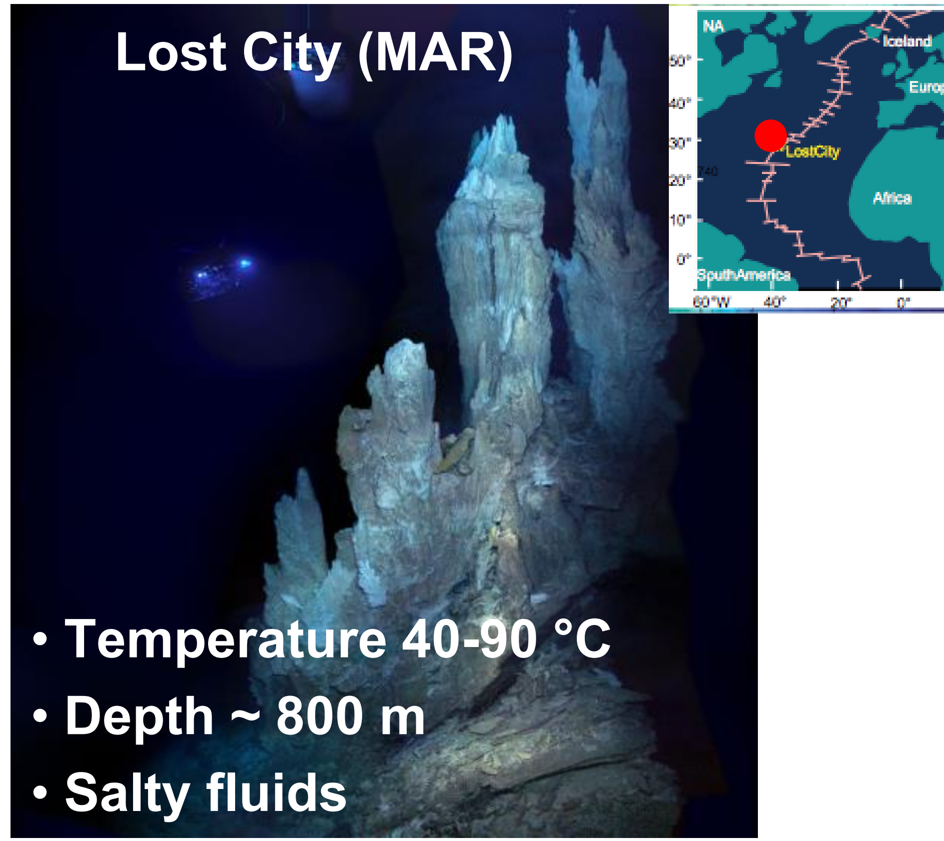
Kelley et al., 2001

Prony bay (New Caledonia)



Submarine hydrothermal systems associated with serpentinization : Prony (New Caledonia) *vs* Lost City





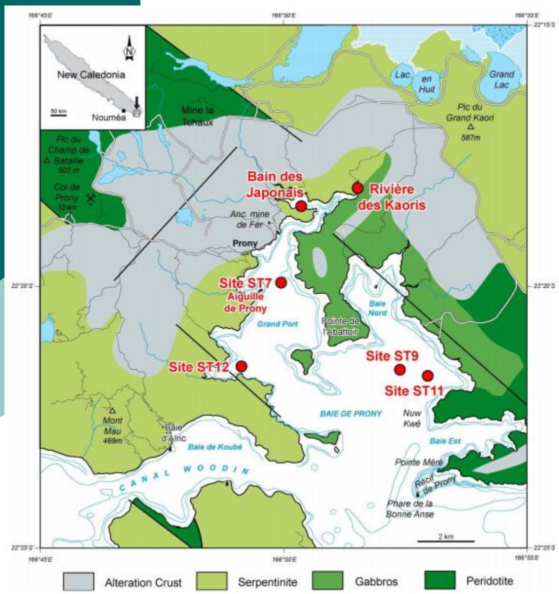
Active chimneys composed of calcite ($CaCO_3$) and brucite ($Mg(OH)_2$)

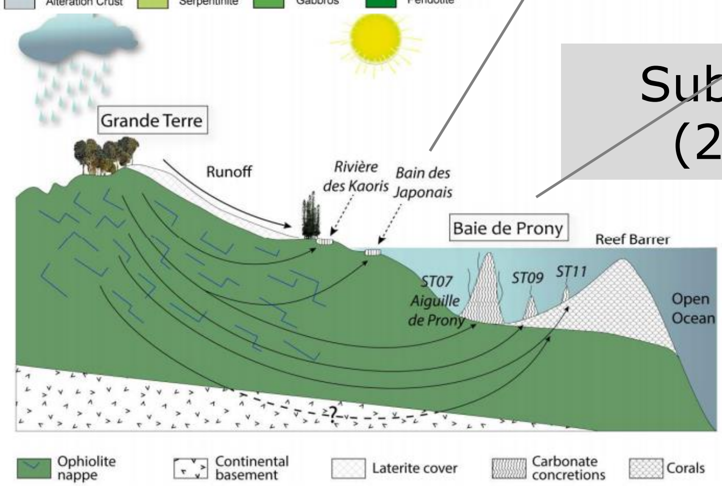
Anoxic and alkaline fluids (pH 9-11) rich in H_2, CH_4 et others C_nH_{2n+2}

HYDROPRONY Campaign 2011

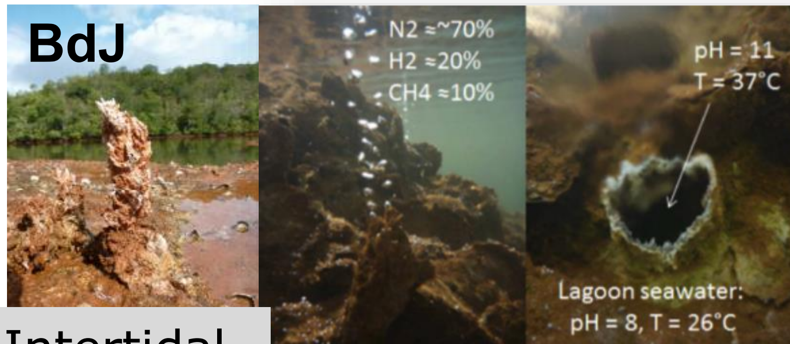
(Head of mission : Bernard Pelletier, IRD Nouméa)

Coastal Hydrothermal Field of the Prony Bay (NC)



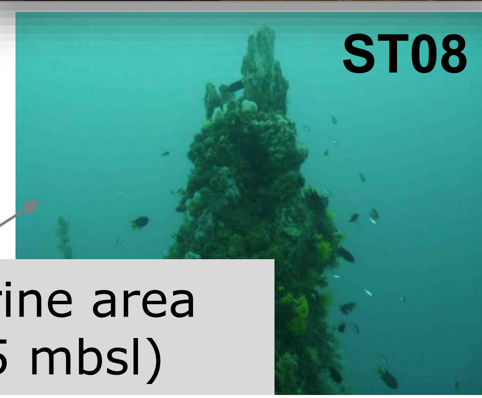


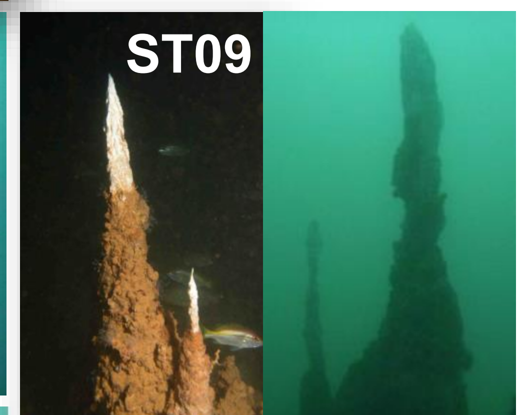
Monnin et al., Biogeosciences, 2014



Intertidal area

Submarine area (20-45 mbsl)





Fluid composition of New Caledonian springs

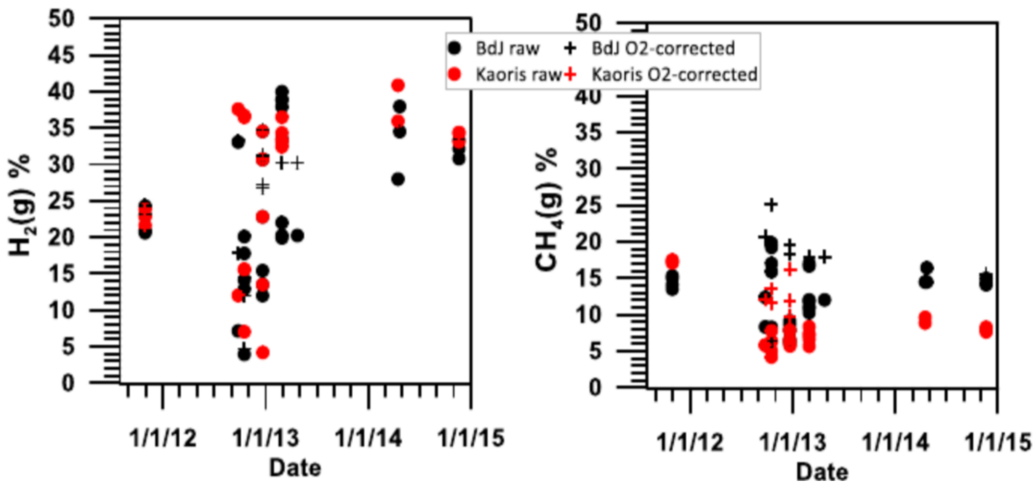
Monnin et al., Biogeosciences, 2014

Site	Sample name	pH	T	Si mmol L^{-1}	DIC mmol L^{-1}	Mg mmol L^{-1}	Ca mmol L^{-1}	Na mmol L^{-1}	K mmol L^{-1}	F^{-} mmol L^{-1}	Cl^{-} mmol L^{-1}	SO$_4$ mmol L^{-1}
Bain des Japonais	HP11-BdJ-Ilot1-W1C	11.08		0.0009	0.3921	0.006	0.518	2.38	0.32	0.0071	2.22	0.10
	HP11-BdJ-Ilot1-W2	10.48		0.0028	0.3573	0.198	0.525	7.29	0.42	0.0055	5.47	0.28
	HP11-BdJ-Ilot1-W3	10.01		0.0102	0.4737	2.344	0.957	26.14	0.81	0.0059	21.61	1.24
	HP11-BdJ-Ilot1-W5	11.07		0.0025	0.4601	b.d.l.	0.444	1.28	0.30	0.0055	0.41	0.01
	HP11-BdJ-Ilot1-W6	10.68		0.0059	0.3616	0.092	0.667	5.68	0.41	0.0061	4.02	0.19
	HP11-BdJ-Ilot2-W1	10.87		b.d.l.	0.4096	0.029	0.594	2.98	0.36	0.0069	1.84	0.07
	HP11-BdJ-Dil1	10.05	36.0	0.0073	0.4599	3.98	1.29	22.63	1.54	0.0053	52.67	3.36
	HP11-BdJ-Dil2	9.13	31.1	0.0432	1.2946	23.83	5.00	197.98	4.75	0.0064	179.27	11.85
	HP11-BdJ-Dil3	8.66	29.8	0.0498	1.6152	33.58	6.87	284.15	6.36	0.0204	241.80	16.09
	HP11-BdJ-Dil4	10.20	36.8	0.0052	0.4774	1.34	0.97	1.91	1.15	0.0055	34.52	2.16
	HP11-BdJ-Dil5	8.30	28.2	0.0316	1.7884	45.16	9.16	392.81	8.52	0.0257	322.05	21.45
	HP11-BdJ-Dil6	11.00	37.1	0.0039	b.d.l.	0.028	0.21	2.55	0.43	0.0269	1.44	0.06
	HP11-BdJ-Dil7	10.92	37.6	0.0029	0.3097	0.024	0.22	1.65	0.39	0.0109	0.66	0.02
	HP11-BdJ-Dil8	10.88	36.9	0.0025	0.3996	0.036	0.41	1.75	0.40	0.0064	0.76	0.02
Rivière des Kaoris	HP11-CarKao-W1	10.80	31.0	0.0662	0.1440	b.d.l.	0.36	0.65	0.08	0.0032	0.23	b.d.l.
	HP11-CarKao-W2	10.80	31.0	0.0656	0.0000	b.d.l.	0.38	0.58	0.08	0.0025	0.19	b.d.l.
Site ST11	HP11-Site11-W1	10.64		0.0502	0.5376	2.516	2.28	40.52	0.00		45.78	1.54
	HP11-Site11-W4	9.58		0.0394	1.1156	31.69	7.28	270.85	5.09		327.74	16.16
	HP11-Site11-W5	8.76		0.0116	1.8450	45.44	9.45	386.58	7.36		451.96	22.56
	HP11-Site11-W10	9.06		0.0344	1.5244	31.37	7.19	263.71	4.87		319.18	15.79
	HP11-Site11-W11	9.38		0.0432	1.2131	22.75	6.31	199.40	3.69		248.69	12.13
Site ST12	HP11-Site12-W1	11.00		0.0298	0.3824	b.d.l.	0.55	40.05	0.14		41.74	0.38
	HP11-Site12-W3	8.92		0.0249	1.3080	44.02	8.27	384.52	7.56		432.70	21.50
	HP11-Site12-W4	9.50		0.0207	0.6818	22.18	4.43	191.24	3.91		247.31	11.53
	HP11-Site12-W5	8.85		0.0121	1.6377	45.18	8.69	385.32	7.49		440.03	21.79
	HP11-Site12-W6	9.34		0.0158	0.9075	37.44	6.32	323.69	6.47		371.92	18.20
	HP11-Site12-W7	8.60		0.0108	1.8849	45.34	8.90	393.22	7.79		447.21	22.17
	HP11-Site12-W8	8.15		0.0077	0.4070	54.65	10.28	475.50	9.28		515.39	25.89
Site ST7	HP11-Site7-W1	9.73		b.d.l.	0.5830	23.32	4.91	190.96	3.69		252.37	12.24
	HP11-Site7-W2	9.66		b.d.l.	0.6666	22.92	4.96	187.73	3.62		250.44	12.14
	HP11-Site7-W3	9.67		b.d.l.	0.6721	18.69	4.43	154.31	2.93		212.43	10.17
	HP11-Site7-W4	9.61		b.d.l.	0.6342	20.42	4.59	167.61	3.40		225.87	10.82
	HP11-Site7-W5	9.72		b.d.l.	0.7691	24.43	5.17	198.08	3.74		262.81	12.78
	HP11-Site7-W6	9.61		b.d.l.	0.7067	28.73	5.74	235.44	4.53		297.38	14.59
	HP11-Site7-W3Ti	9.44		0.0138	0.6870	41.14	7.16	346.46	7.33		400.87	19.95
	HP11-Site7-W7	10.00		0.0001	0.5377	27.95	5.46	232.61	4.81		290.68	14.20
	HP11-Site7-W8	10.13		b.d.l.	0.4945	20.08	4.41	163.75	3.32		223.03	10.64
	HP11-Site7-W9	9.91		0.0006	0.4971	29.36	5.76	249.17	4.95		309.31	15.19
	HP11-Site7-W10	10.14		b.d.l.	0.4482	12.43	3.49	97.32	2.00		163.85	7.53
	HP11-Site7-W11	9.96		b.d.l.	0.5121	28.02	5.45	228.89	4.58		292.43	14.25
Site ST9	HP11-Site9-W1	10.45		0.0119	0.5290	3.60	2.95	36.67	1.51		81.52	3.31
	HP11-Site9-W3	10.62		0.0127	0.3985	2.768	2.76	6.46	0.98		50.58	1.72
	HP11-Site9-W4	10.46		0.0118	0.4094	4.80	2.98	44.96	1.76		91.62	3.82
	HP11-Site9-W6	9.18		0.0079	1.6885	38.77	8.56	337.76	7.34		380.77	18.85
	HP11-Site9-W7	10.51		0.0137	0.6342	4.96	3.22	47.54	1.78		95.84	4.03

Gas composition of New caledonian springs and others hydrothermal systems associated with serpentinization

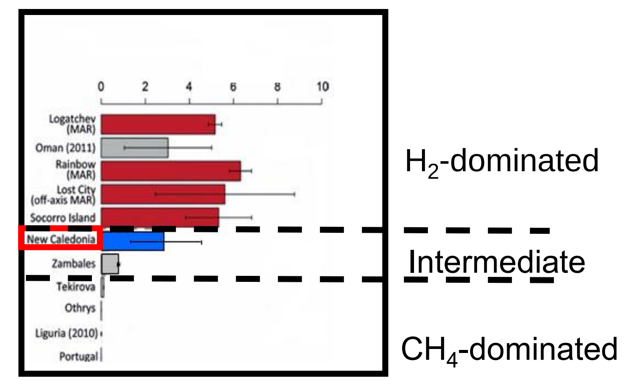
Temporal variation

H_2 CH_4



Monnin *et al.*, JGR Biogeosciences, 2021

H_2/CH_4 ratio

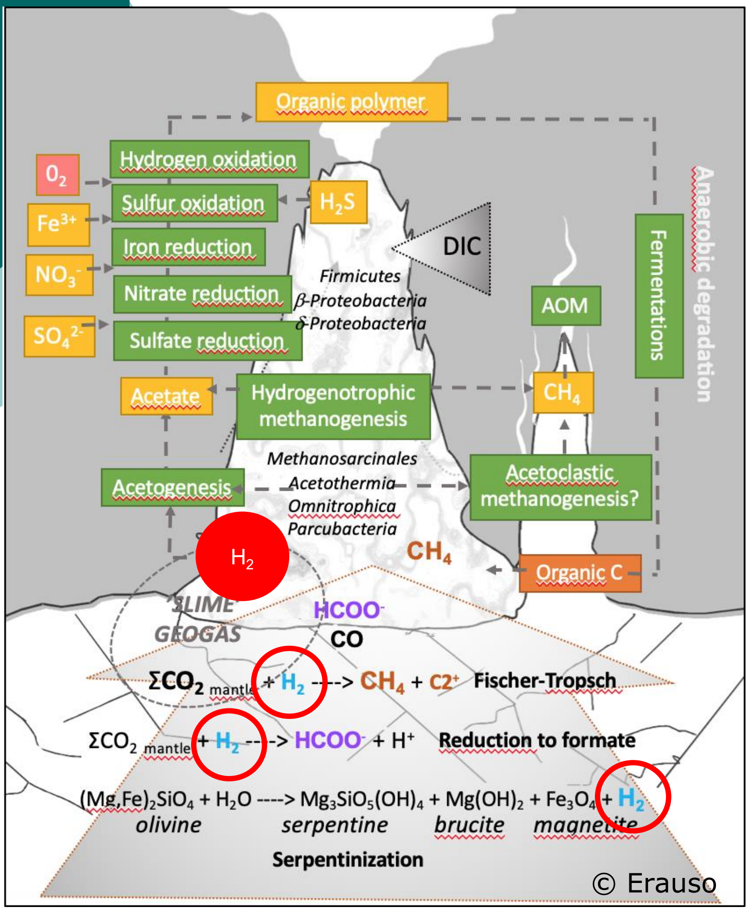


Modified from Boulart et al., 2013

Project MICROPRONY (ANR 2020-2024)

(PI : Gael Erauso, Méditerranean Institute of Oceanography)

Main objectives



Detected or hypothetical metabolisms in microbial ecosystems on the Prony Hydrotermal Field

- **To describe microbial diversity associated to Prony Hydrothermal Field**
  - To Identify, isolate and study model microorganisms or microorganisms with biotechnological interest
- **To determine the role of different microbial groups in biochemical cycles**
  - What are key abiotic and biotic factors? Biological consumption and production of H2 and CH4 ?
- **To investigate the microbial groups and processes involved in the formation and in the colonization of the carbonated chimneys**
  - To understand the emergence of life on Earth

Methodology

Molecular and culture approaches

Fluids (0.5-2L)

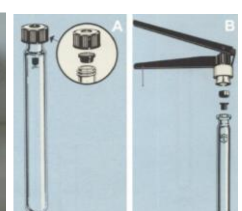
Chimneys (0.5-1g)

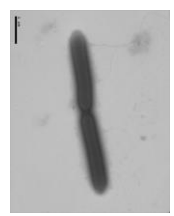
DNA extraction

Illumina Miseq metabarcoding and quantifications of DNA PCR-amplified or metagenomics
Identification and abundance of microorganisms

Microscopy (FISH)
Detection and abundance of microorganisms

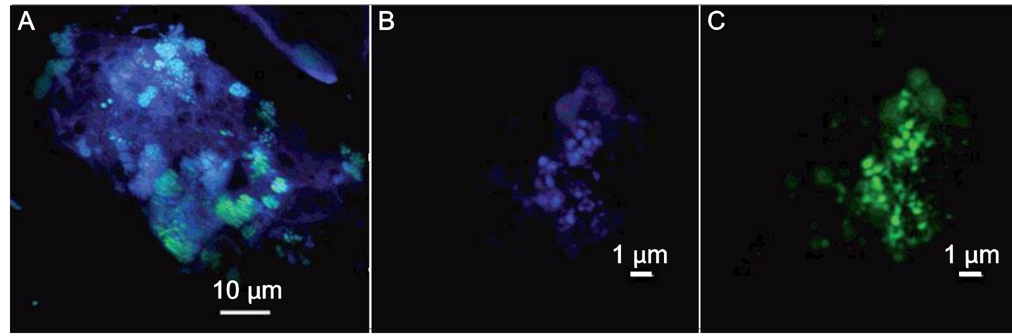
Alkaline and anaerobic media





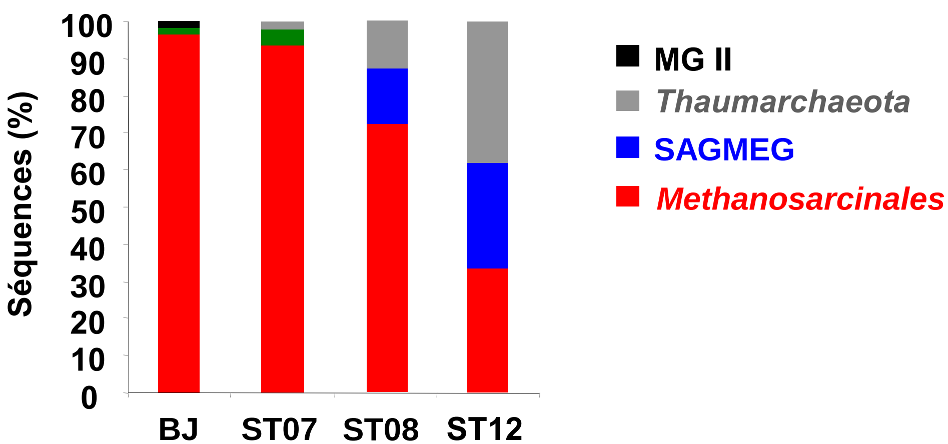
Enrichments and isolation of microorganisms
Monitoring of growth and metabolites
Consumption or production of H_2 (or CH_4)

Archaea dominated by *Methanosarcinales*
(oxidizing CH_4 or producing CH_4 using H_2)

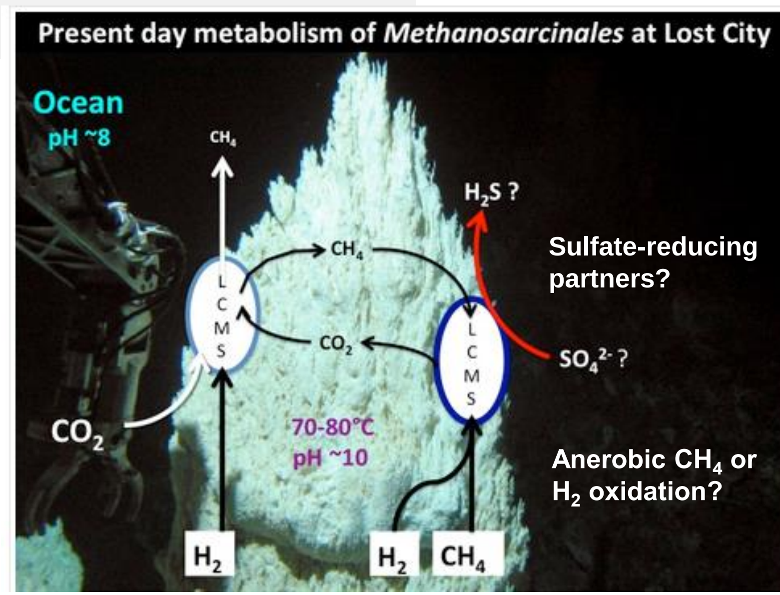


Postec et al., Frontiers in Microbiology, 2015

Low diversity of Archaea dominated by incultivated *Methanosarcinales*
Specific phylotype previously detected at Lost City or The Cedars potentially oxidizing or producing CH_4



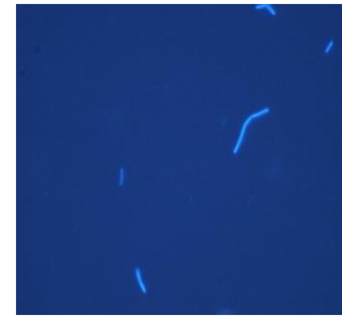
Quéméneur et al., EMR, 2014; Frouin et al., Frontiers in Microbiology, 2018



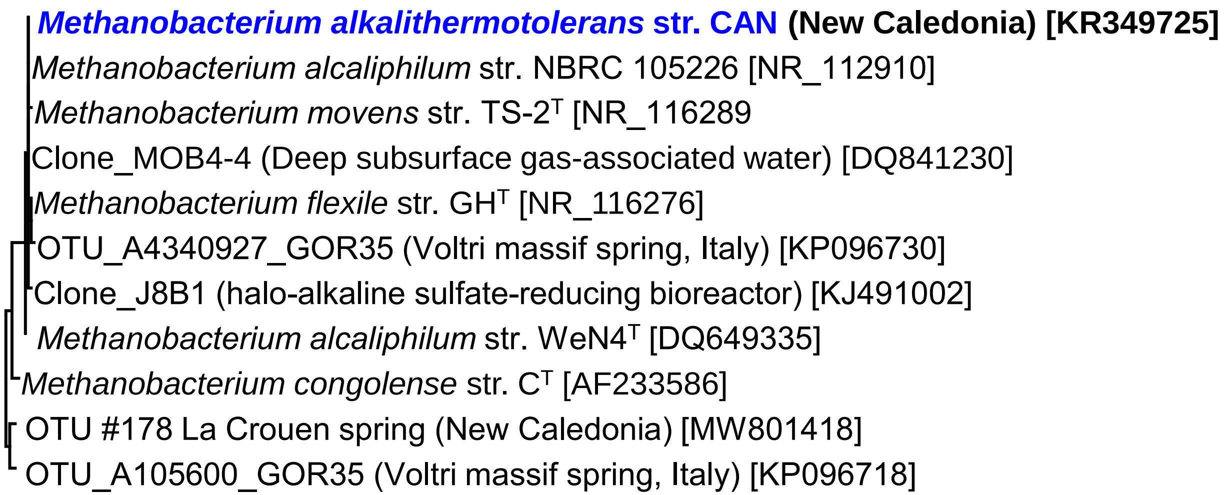
Isolation and characterization of an alkaliphilic and hydrogenotrophic methanogen

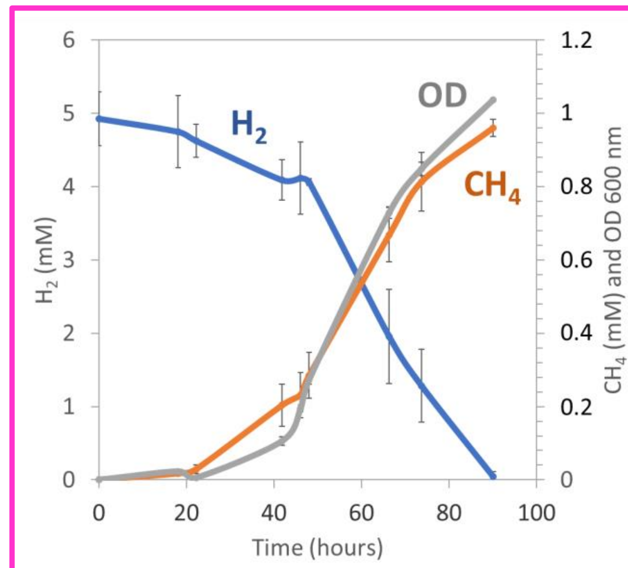
Methanobacterium alkalithermotolerans

- Archaea adapted to pH > 9
- CH_4 production by reduction of CO_2 and **H_2 oxidation**
- *Methanobacterium* often detected in on-land springs associated with serpentinization (Oman, Italy)



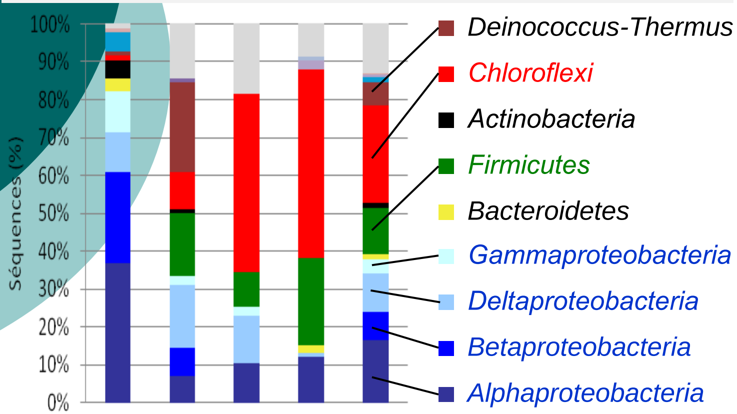
Quéméneur et al., Microorganisms, 2021; Mei et al., IJSEM, 2022



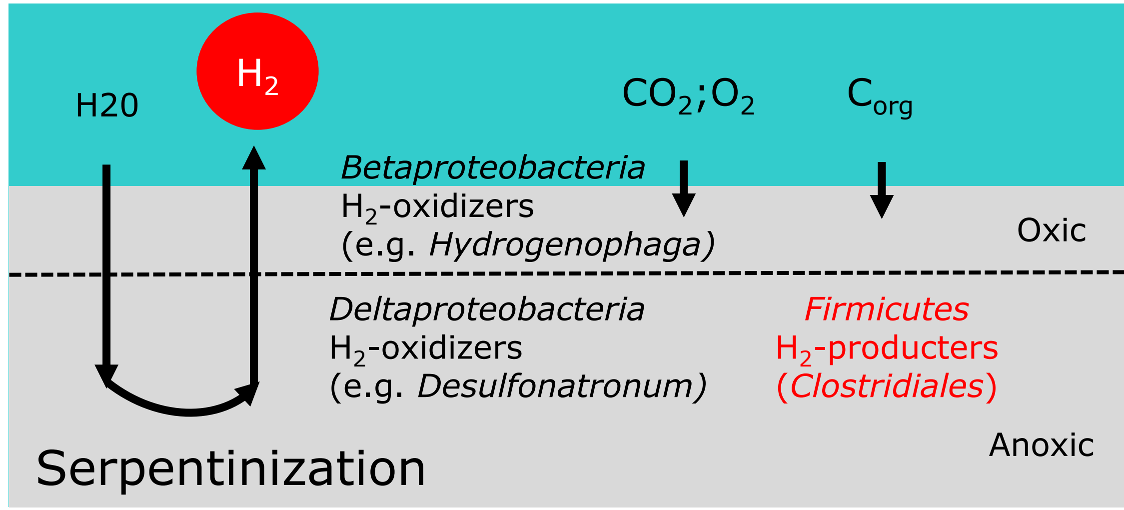


Biological hydrogen consumption and production by diverses bacteria

ADNr 16S Metabarcoding



Quéméneur et al., EMR, 2014



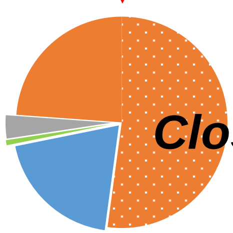
Quéméneur et al., EMR, 2014; Mei et al., Frontiers in Microbiology, 2016; Frouin et al., Frontiers in Microbiology, 2018

Metagenomic analyses

High diversity of *Firmicutes* and *Proteobacteria*

Firmicutes

Proteobacteria → **Role in H_2 oxydation**

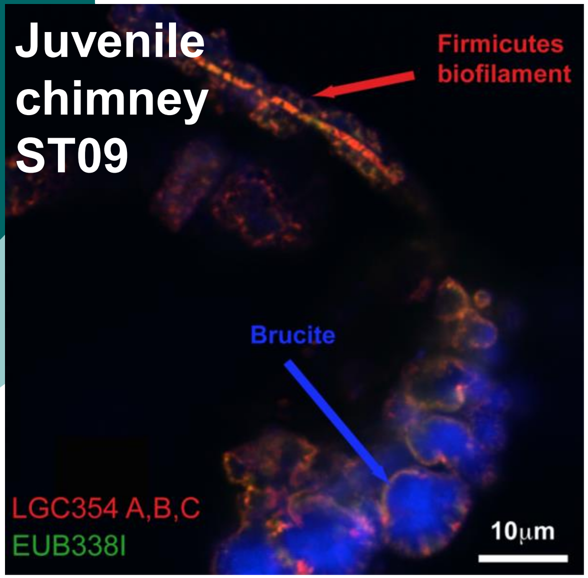


Clostridiales → **Role in H_2 production**

Contain the majority of Fe-Fe hydrogenases

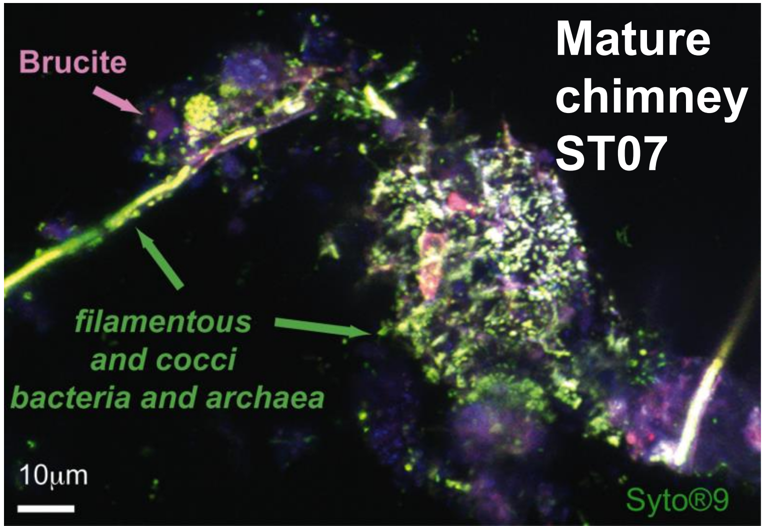
Mei et al., Frontiers in Microbiology, 2016

Bacteria of the phylum Firmicutes identified as the first colonizers

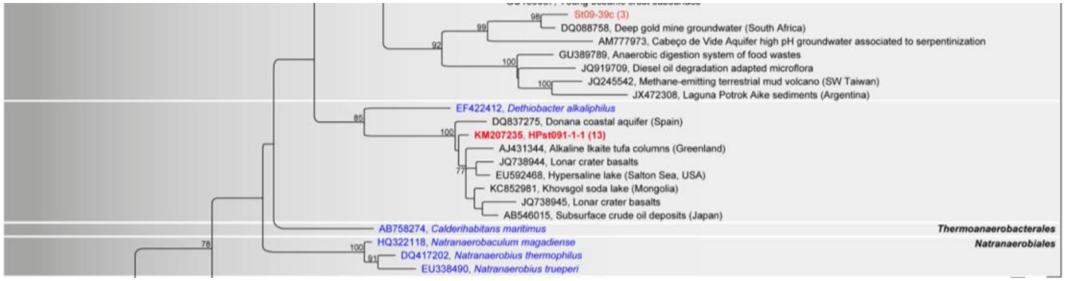


o *Firmicutes* (biofilament) identified as the first colonizers on fresh chimneys, followed by archaeal *Methanosarcinales* present only in mature chimneys

o *Firmicutes* are involved in the formation and early consolidation of the carbonated structures via organomineralization processes

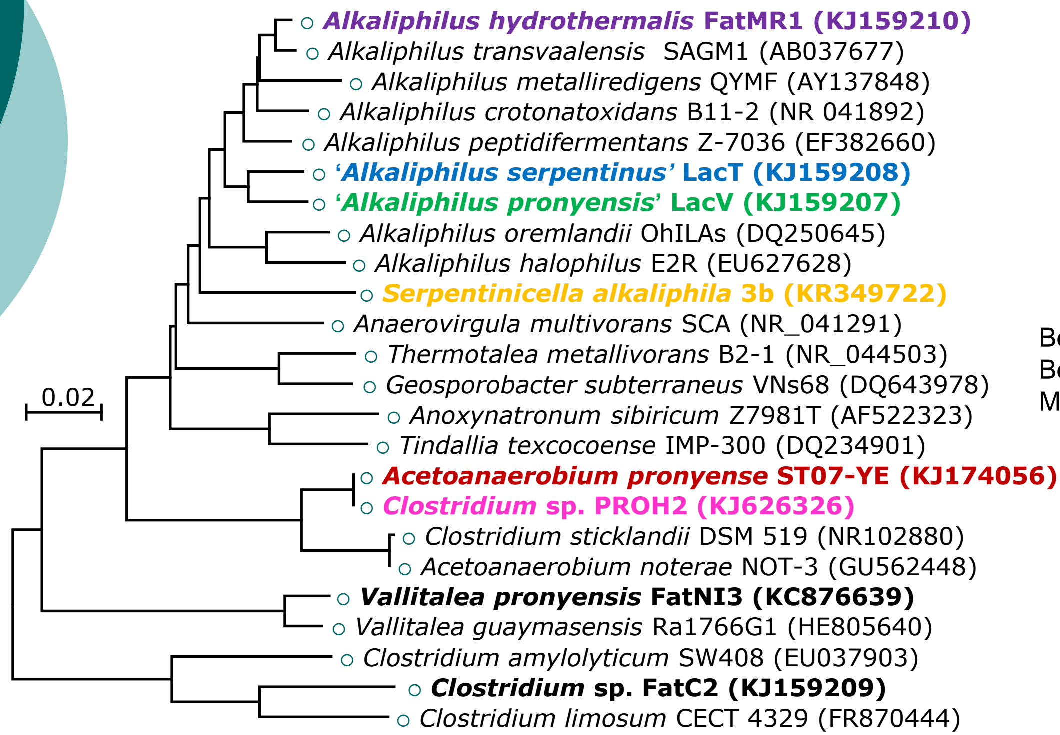


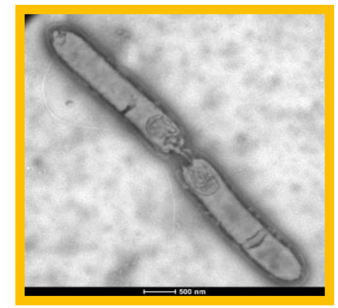
Uncultivated bacteria belonging to the *Firmicutes* play a central role in the ecology of the PHF.

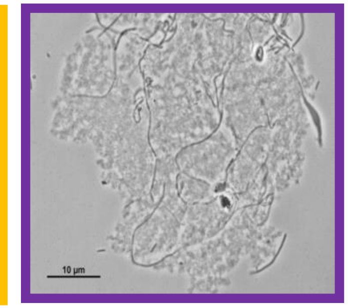


Pisapia et al., Front. Microbiol., 2017

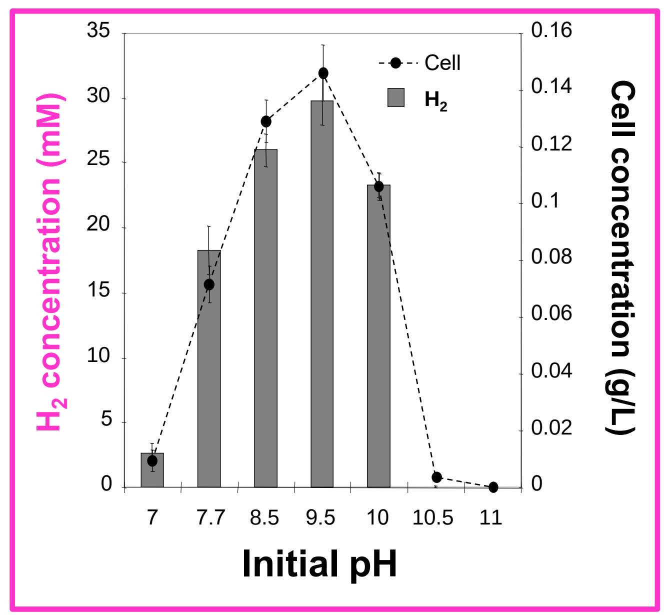
Isolation and characterization of alkaliphilic and anaerobic bacteria belonging to the phylum *Firmicutes*







Ben Aissa et al., IJSEM, 2014; Mei et al., IJHE, 2014;
Ben Aissa et al., Extremophiles, 2015; Bes et al., IJSEM, 2015,
Mei et al., IJSEM, 2016; Postec et al., SAM, 2021



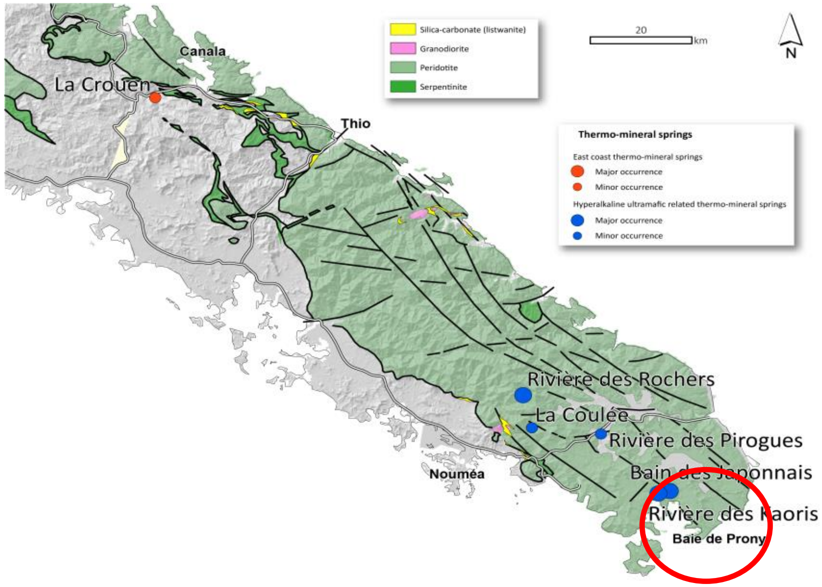
- Five novel anaerobic species
- *Clostridiales* species adapted to alkaline pH (pH > 9)
- Fermentation of sugars, organic acids and aminoacids
- High production of H_2 at alkaline pH by strain PROH2

Projet H2NAT 2014 : Production d'hydrogène à basse température dans les environnements hyperalcalins naturels

(PI : Christophe Monnin, financé par l'Observatoire Midi-Pyrénées)

Objectif général : Evaluer les rôles respectifs des processus abiotiques et biogéniques dans la production naturelle d'hydrogène

Objectifs spécifiques d'écologie microbienne :
-Évaluer les variations de la structure et de la diversité des communautés microbiennes en fonction des facteurs environnementaux des sources alcalines
-Identifier et isoler les microorganismes producteurs ou consommateurs d'hydrogène dans les sources alcalines de Nouvelle-Calédonie



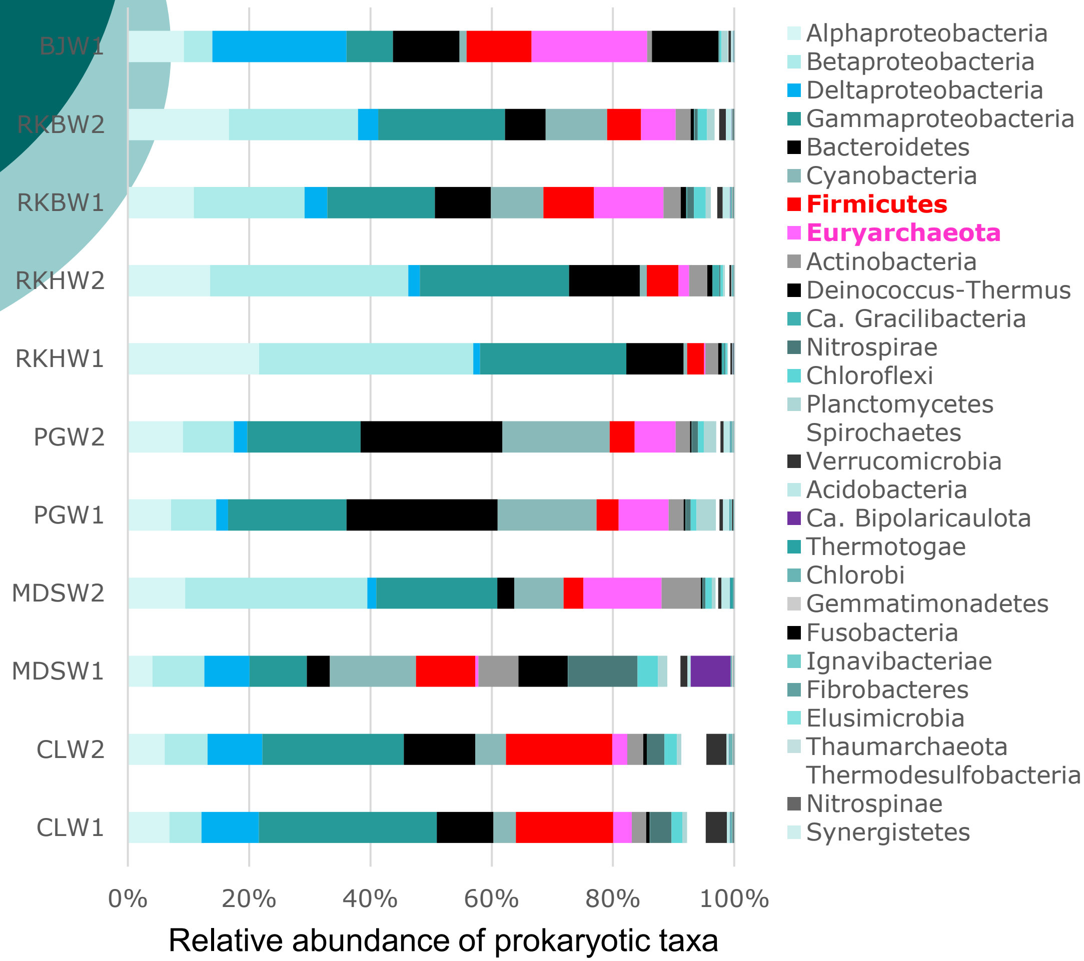
Modifié à partir de Maurizot et al., Geological Society, London, Memoirs, 2020

Hyperalkaline springs of New Caledonia

Quéméneur et al., Frontiers in Microbiology, 2023

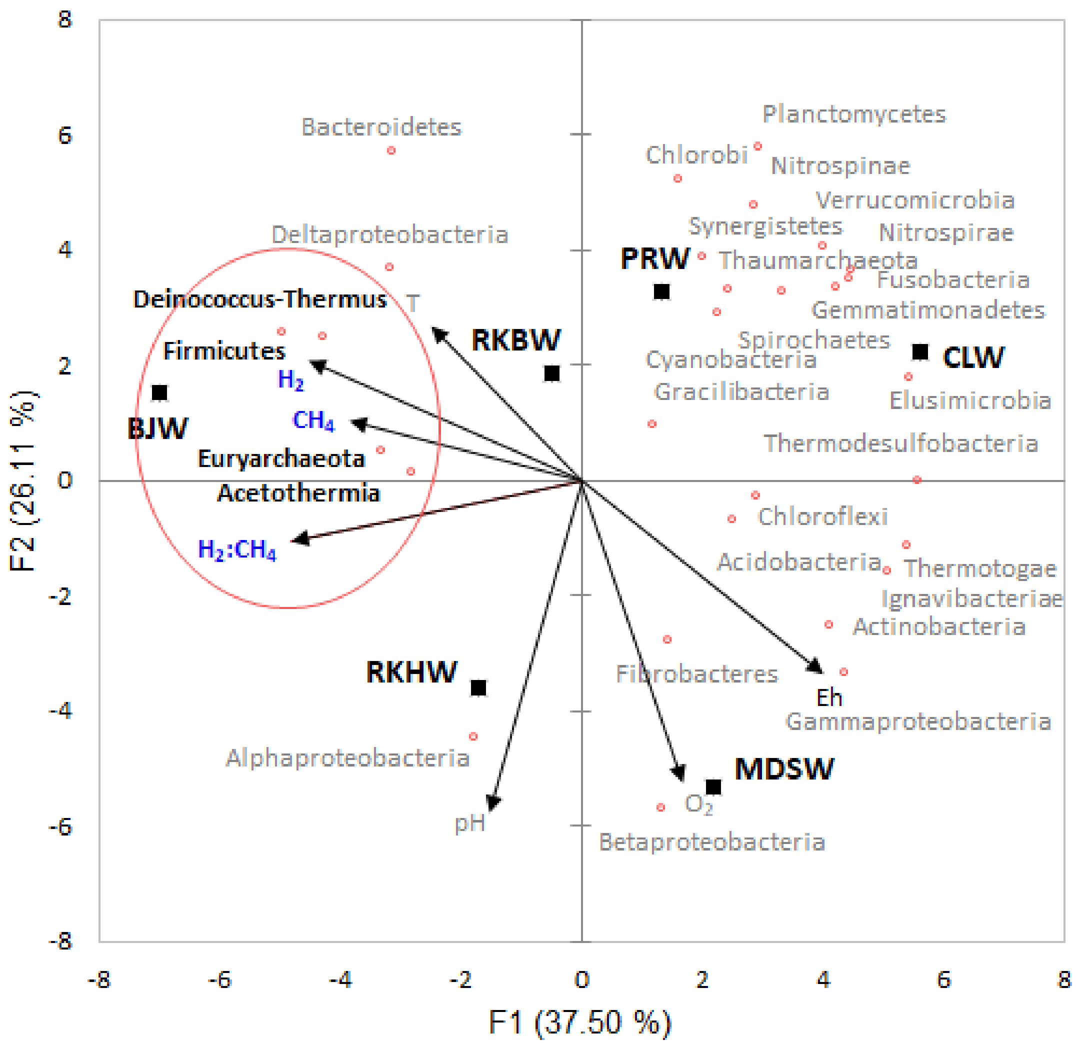
Prokaryotic diversity in high-pH waters of hyperalkaline springs

16S rDNA metabarcoding



- o *Archaea* (Lost City *Methanosarcinales*) >1% of procaryotes; max ~20% BdJ
- o Omnipresence of *Firmicutes*
- o *Betaproteobacteria* oxidizing H_2 (*Hydrogenophaga/Serpentinomonas*)
- o Sulfate-reducing bacteria in the intertidal BdJ site (*Desulfonatronum*)
- o *Beta-* et *Gammaproteobacteria* oxydant le CH_4 et les composés soufrés

Variations in prokaryotic communities in high-pH waters of hyperalkaline springs



- Link between microbial taxa and environmental factors (temperature, O_2, H_2, CH_4)
- Positive correlation between Archaea (*Methanosarcinales*) and CH_4
- Positive correlation between *Firmicutes* (Clostridiales) and H2

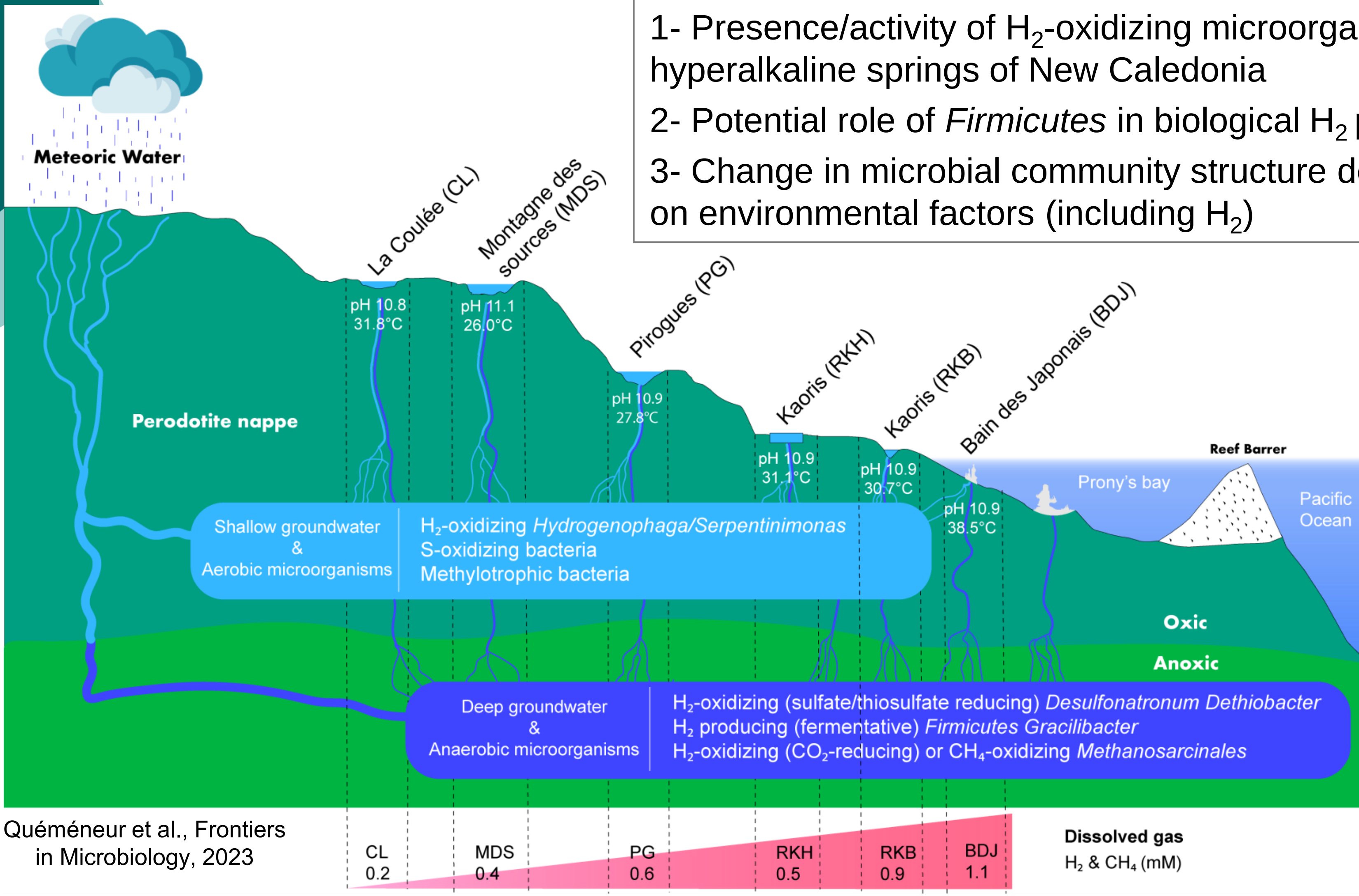
Conclusions and Perspectives

Subsurface microbial ecosystems: Sinks or sources of hydrogen ?

1- Presence/activity of H_2-oxidizing microorganisms in hyperalkaline springs of New Caledonia

2- Potential role of *Firmicutes* in biological H_2 production

3- Change in microbial community structure depending on environmental factors (including H_2)



Quéméneur et al., Frontiers in Microbiology, 2023

Conclusions and Perspectives

Subsurface microbial ecosystems: Sinks or sources of hydrogen ?

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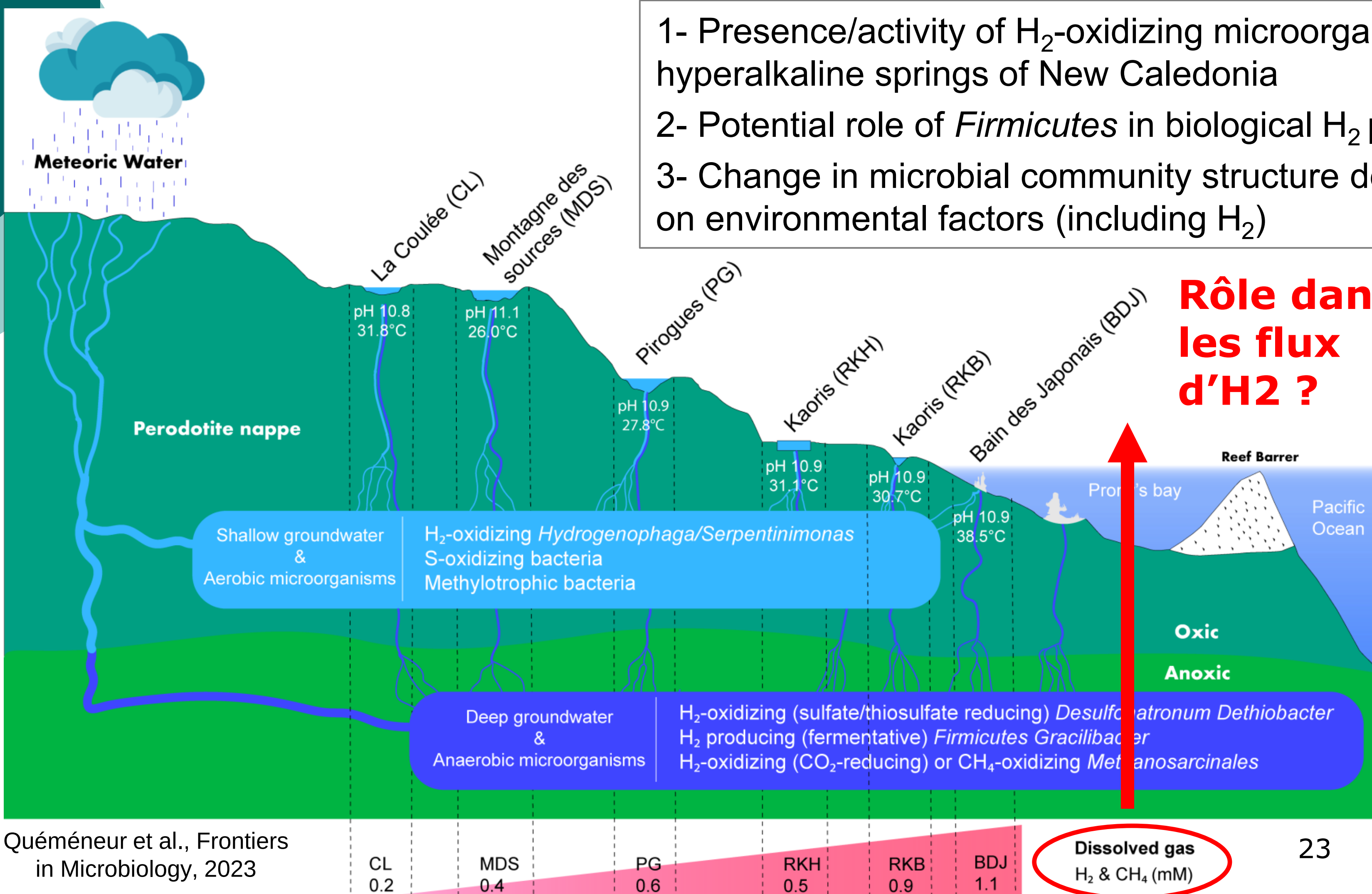
Rôle dans les flux d'H2 ?



Quéméneur et al., Frontiers in Microbiology, 2023

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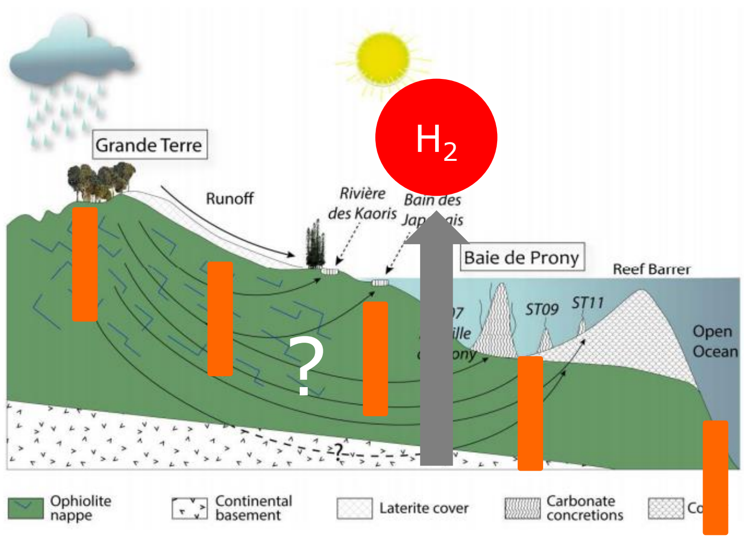
Conclusions and Perspectives

Subsurface microbial ecosystems: Sinks or sources of hydrogen ?

Ecosphère Continentale et Côtière

MICRO PRONY



Modified from Monnin et al., Biogeosciences, 2014

Projet EC2CO/INSU HYDROMIC (2023-2024)

Partenaires: MIO, GET, ISTERRE, DIMENC

- What is the role of microorganisms in the H_2 concentration measured at the surface? What are the key factors influencing their activities?
- How does H_2 shape the community composition and functioning in subsurface?
- What are the main microbial groups oxidizing H_2 ? Are there bioindicators?
- Project ANR MICROPRONY (2020-2024)
- **N**ew **C**aledonia Ophiolite Land-to-Sea **D**rilling **P**roject (**NCDP**) - Proposal submitted to IODP/ICDP

Thank you for your attention

and

Thank you, colleagues of the MIO team and our partners

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https://microprony.org/

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